Clinical Trials in Organ Transplantation

CTOT-24

Regulatory T Cell Modulation in Kidney Transplantation with Biologic Blockade of Dual Effector Pathways, CD28 and IL-6

Treg Modulation with CD28 and IL-6 Receptor Antagonists

VERSION NUMBER 5.0/ October 26, 2021

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PRINCIPAL INVESTIGATOR	**PROTOCOL CHAIR**	**MEDICAL MONITOR**
Flavio Vincenti, MD Clinical Professor of Medicine & Surgery Depts. Medicine & Surgery University of California at San Francisco 505 Parnassus Avenue, Rm #M884 San Francisco, CA 94118-0780 Phone: 415-353-1322 E-mail: flavio.vincenti@ucsf.edu	Sindhu Chandran, MD Associate Professor of Medicine Division of Nephrology Department of Medicine University of California at San Francisco 400 Parnassus Avenue, Seventh Floor San Francisco, CA 94143 Phone: 415-353-8374 E-mail: sindhu.chandran@ucsf.edu	Megan Morsheimer, MD, MPH Medical Officer Clinical Transplantation Branch Division of Allergy Immunology and Transplantation, NIAID, NIH 5601 Fishers Lane, 6B19 Bethesda, MD 20852-9827 Phone: 301-761-7579 E-mail: megan.morsheimer@nih.gov
BIOSTATISTICIAN	**PROJECT MANAGER**	**REGULATORY OFFICER**
Karen Kessler Senior Research Scientist Rho, Inc. 6330 Quadrangle Drive Chapel Hill, NC 27517 Phone: 919-595-6244 E-mail: karen_Kesler@rhoworld.com	Tina Sledge, RN Nurse Consultant/Project Manager Clinical Transplantation Branch Division of Allergy, Immunology, and Transplantation, NIAID, NIH 5601 Fishers Lane, 6B28 Bethesda, MD 20852-9827 Phone: 240-627-3561 E-mail: sledget@niaid.nih.gov	Julia Goldstein, MD Office of Regulatory Affairs Division of Allergy, Immunology, and Transplantation, NIAID, NIH 5601 Fishers Lane, 7B29 Bethesda, MD 20852 Phone: 240-627-3509 E-mail: goldsteinj@niaid.nih.gov

INVESTIGATOR SIGNATURE PAGE	
Protocol: CTOT-24	**Version/Date:** 5.0/ October 26, 2021
Title: *Regulatory T Cell Modulation in Kidney Transplantation with Biologic Blockade of Dual Effector Pathways, CD28 and IL-6*	
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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 Good Clinical Practice: Consolidated Guidance* dated April 1996. 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.

**The site Principal Investigator should sign and date at the indicated location below. A written signature/date is acceptable (e.g., scanned and sent via email as a PDF version). An electronic signature is also acceptable (e.g., sent via email as a PDF version).*

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Site Principal Investigator (Print)

_____ _____

Site Principal Investigator (Signature) **Date**

Protocol Synopsis

Title	Regulatory T Cell Modulation in Kidney Transplantation with Biologic Blockade of Dual Effector Pathways, CD28 and IL-6
Short Title	Treg Modulation with CD28 and IL-6 Receptor Antagonists
Clinical Phase	Phase I/II
Participating Sites	Cleveland Clinic Duke University Northwestern University University of Alabama at Birmingham University of California at San Francisco University of Colorado University of Nebraska
IND Sponsor/Number	NIAID/ IND# 139185
ClinicalTrials.gov Number	NCT04066114
Study Objective	This trial will be a pilot, proof of concept trial to evaluate the safety and mechanisms of transplant immune regulation via Tregs.
Study Design	Multi-center, non-randomized, single arm, prospective trial with an investigational product (lulizumab pegol (BMS-931699) in the context of a novel immunosuppression regimen
Primary Endpoint	The primary safety endpoint is the proportion of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at 6 months after transplantation. Clinical rejection occurring prior to 6 months, defined as treated rejection without biopsy confirmation will be included as acute rejection with respect to the endpoint.
Secondary Endpoint	The secondary safety endpoint is the proportion of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at or before 12 months after transplantation. Clinical rejection occurring prior to 12 months, defined as treated rejection without biopsy confirmation will be included as acute rejection with respect to the endpoint.
Accrual Objective	10 subjects
Study Duration	27-month accrual, 24-month follow-up

Treatment Description	Participants will receive the following study drugs, with day 0 defined as the date of kidney transplantation: • ATG (IV) 6 mg/kg given in divided doses on days 0 to 3 • Methylprednisolone (IV) 500 mg on day 0, 250 mg on day 1, 125 mg on day 2 • Prednisone (PO) 60 mg on day 3, 30 mg on day 4, then taper to 5 mg daily by day 28 (See Section 7.2.4) • Lulizumab pegol 25 mg on day 1, then 12.5 mg (SC) weekly until day 77 • Tocilizumab 8 mg/kg (IV) on day 2, then 162 mg (SC) every 2 weeks until day 168 • Belatacept (IV) 5 mg/kg every 4 weeks starting day 84 • Mycophenolate mofetil 1000 mg bid starting on day 1, stopped when everolimus level is within therapeutic range. • Everolimus (PO) starting at 0.75 mg BID on day 14, titrated to maintain trough level 3-8 ng/ml
Inclusion Criteria	1. Able to understand and provide informed consent 2. Agreement to use highly effective (<1% failure rate) methods of contraception (see study definitions). Female participants of child-bearing potential must consult with their physician and determine the most suitable method(s) from this list to be used for 12 months while on study drug regimen. 3. Male or female, 18 to 70 years 4. Recipient of primary, non-HLA identical living donor kidney transplant 5. No donor specific antibodies prior to transplant that are considered to be of clinical significance by the site investigator 6. EBV positive serology 7. CMV positive serology, unless donor-recipient pair are both CMV negative 8. Negative testing for latent TB infection within 3 months prior to transplant. Testing should be conducted using either a PPD or interferon-gamma release assay (i.e. QuantiFERON-TB, T-SPOT.TB). Patients with a positive test for latent TB infection must complete appropriate therapy for LTBI. A subject is considered eligible only if they have a negative test for LTBI within 3 months prior to transplant OR they have appropriately completed LTBI therapy prior to transplant. Latent TB infection treatment regimens should be among those endorsed by the CDC (Division of TB Elimination, 2016). 9. In the absence of contraindication, vaccinations must be up to date for hepatitis B, influenza, pneumococcal, varicella and herpes zoster, and MMR. 10. Hepatitis C Virus Ab positive subjects with negative HCV PCR are eligible if they have spontaneously cleared infection or are in sustained virologic remission for at least 12 weeks after treatment (see exclusion 13). 11. Negative SARS-CoV2 PCR testing within 2 weeks of transplant.

Exclusion Criteria	1. Prisoners or subjects who are compulsorily detained. 2. Inability or unwillingness of a participant to give written informed consent or comply with study protocol 3. Candidate for a multiple solid organ or tissue transplants 4. Prior history of organ or cellular transplantation 5. Known to have idiopathic FSGS as the underlying cause of ESRD 6. Requirement for uninterrupted anticoagulation therapy, including Plavix 7. Known hypersensitivity to mTOR inhibitors or contraindication to everolimus (including history of wound healing complications) 8. History of severe allergic and/or anaphylactic reactions to humanized or murine monoclonal antibodies 9. Hypersensitivity to rabbit proteins or rabbit Anti-thymocyte Globulin (ATG) 10. Known hypersensitivity to ACTEMRA® (tocilizumab) or lulizumab pegol (BMS-931699), 11. HIV infected subjects, including those who are well controlled on anti-retrovirals 12. Positive HBsAg or HBcAB serology 13. Hepatitis C virus antibody positive (HCVAb+) subjects who have failed to demonstrate sustained viral remission for more than 12 weeks (after anti-viral treatment) 14. Patients with a previous history of active Tuberculosis (TB) 15. Known active current viral, fungal, mycobacterial or other infections (including, but not limited to tuberculosis and atypical mycobacterial disease, Hepatitis B and C, and herpes zoster) 16. Donor or recipient residing in areas where annual incidence is ≥21 cases/100,000 for coccidioidomycosis according to current CDC map: (https://www.cdc.gov/fungal/diseases/coccidioidomycosis/images/valley-fever-map-2017.jpg). Donor or recipients residing in zones with annual incidence of <21 cases/100,000 will not require additional screening. 17. History of malignancy except treated basal cell cancer of the skin 18. History of hemolytic-uremic syndrome/ thrombotic thrombocytopenia purpura 19. History of demyelinating disorders (e.g., multiple sclerosis, chronic inflammation demyelinating polyneuropathy) 20. History of gastrointestinal perforations, active inflammatory bowel disease or diverticulitis 21. Any previous treatment with alkylating agents such as chlorambucil, or with total lymphoid irradiation; 22. Receipt of a live vaccine within 30 days prior to transplantation 23. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study. 24. Severe hyperlipidemia (total cholesterol >350 mg/dL, LDL >190 mg/dL, or triglycerides >500 mg/dL)

	25. Transaminase levels elevated more than 1.5 times the upper limit of normal (ULN) within 7 days prior to enrollment. 26. ANC less than 2,000 per mm^3 within 7 days prior to enrollment 27. Platelet count less than 100,000 per mm^3 within 7 days prior to enrollment 28. More than 50% CD8+/ CD28- T-cells in peripheral blood 29. Positive pregnancy test in women of child bearing potential, currently breastfeeding, or planning to become pregnant during the timeframe of the study or follow-up period. 30. Participation in any other studies with investigational drugs or regimens in the preceding year 31. cPRA greater than or equal to 20%, as determined by each participating site's laboratory 32.
Study Stopping Rules	The study may be prematurely terminated for the following reasons: 1. When 5 or more treated participants receive less than 80% of the expected total number of doses of either lulizumab pegol (BMS-931699) or tocilizumab 2. Banff 2A or higher grade acute cellular rejections in any one of the first 5 participants in the first 6 months post-transplant. 3. Antibody-mediated rejection (of any grade) in any one of the first 5 participants in the first 6 months post-transplant (Table 6) 4. Any TB or invasive fungal infection 5. Any diagnosis of PTLD or PML 6. Any graft loss 7. Any death 8. Grade 4 infections in two participants Additionally, an expedited safety review will also be conducted by the DSMB if there are ≥2 episodes of acute rejection in 2 subjects at any time during the study. During the process of data collection for the safety review and analysis by the DSMB, enrollment will be paused in the study, but others study operations can continue including the recruitment of potential participants, provision of study drug to previously enrolled participants.

Study Contacts: Participating Centers

SITE PRINCIPAL INVESTIGATOR

Emilio Poggio, MD
Director, Clinical Research of Kidney Transplant Program
Department of Nephrology & Hypertension
Cleveland Clinic
9500 Euclid Avenue
Cleveland, OH 44195
Phone: 216-444-6771
Email: poggioe@ccf.org

SITE PRINCIPAL INVESTIGATOR

Allan Kirk, MD, PhD
Department of Surgery Chairman
Professor of Surgery
Duke University
2301 Erwin Road
DUMC 3704
Durham, NC 27710
Phone: 919-681-3445
E-mail: allan.kirk@duke.edu

SITE PRINCIPAL INVESTIGATOR

John Friedewald
Professor of Medicine & Surgery
Northwestern University
Feinberg School of Medicine
Arkes Family Pavilion Suite 1900
676 N Saint Clair
Chicago, IL 60611
Phone: 312-695-8900
Email:
jfriedewald@northwestern.edu

SITE PRINCIPAL INVESTIGATOR

Scott Davis, MD
Assistant Professor, Medicine-Renal Med Diseases/Hypertension
University of Colorado
Anschutz Transplant Services
1635 Aurora Ct
7th Floor
Phone: 720-848-0005
Email:
SCOTT.DAVIS@CUANSCHUTZ.EDU

SITE PRINCIPAL INVESTIGATOR

Clifton Kew II, MD
Professor of Medicine & Surgery
Director of Research, Alabama Transplant Center
University of Alabama, Birmingham
1900 University Boulevard
THT 611G
Phone: 205-934-7200
E-mail: ckew@uab.edu

SITE PRINCIPAL INVESTIGATOR

Flavio Vincenti, MD
Clinical Professor of Medicine & Surgery
Depts. Medicine & Surgery
University of California at San Francisco
505 Parnassus Avenue, Rm #M884
San Francisco, CA 94118-0780
Phone: 415-353-1322
E-mail: flavio.vincenti@ucsf.edu

SITE PRINCIPAL INVESTIGATOR

Roslyn Mannon, MD
Professor of Medicine
Associate Chief of Research
Division of Nephrology
University of Nebraska MC
983040 Nebraska Medical Center
Omaha, NE 68198-3040
Phone: 402-559-9227
Email: Roslyn.mannon@unmc.edu

Study Contacts: Core Laboratories

CELLULAR ASSAY CORE

Qizhi Tang, PhD
Professor
Director, Transplantation Research Lab
University of California, San Francisco
San Francisco, CA 94143-0780
Phone: 415-476-1739
Email: Qizhi.Tang@ucsf.edu

GRAFT IMMUNOFLUORESCENCE STAINING/ AND GENE EXPRESSION

Zoltan Laszik, MD, PhD
Professor of Clinical Pathology
Department of Pathology, Box 0102
University of California, San Francisco
513 Parnassus Avenue, Room S566
San Francisco, CA 94143-0102
Phone: 415-502-8230
Email: zoltan.laszik@ucsf.edu

PERIPHERAL BLOOD GENE EXPRESSION/ URINE CYTOKINE ANALYSIS

Minnie Sarwal, MD
Professor
Department of Surgery
513 Parnassus Ave. Med Sci
San Francisco, CA 94143
Phone: 415-502-7921
Email: minnie.sarwal@ucsf.edu

HLA ANTIBODY TESTING FOR DSA

Rajalingam Raja, PhD, D (ABHI)
Main Hospital Level B,
CPMC Davies Campus
University of California, San Francisco
45 Castro Street
San Francisco, CA 94114
Phone: 415-476-3883
Email: Rajalingam.Raja@ucsf.edu

1. Table of Contents

Glossary of Abbreviations

ACR	Acute Cellular Rejection
AMR	Antibody Mediated Rejection
CFR	Code of Federal Regulations
COVID-19	Coronavirus Disease 2019
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
FACS	Fluorescence-Activated Cell Sorting
FDA	Food and Drug Administration
FFPE	Formalin Fixed Paraffin Embedded
FSGS	Focal Segmental Glomerulosclerosis
GCMS	Gas Chromatography-Mass Spectrometry
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonization
IL6	Interleukin 6
IND	Investigational New Drug
IRB	Institutional Review Board
ISH	In Situ Hybridization
MHC	Major Histocompatibility Complex
MMF	Mycophenolate Mofetil
MOP	Manual of Procedures
MPA	Mycophenolic Acid
NIAID	National Institute of Allergy and Infectious Diseases
PBMC	Peripheral Blood Mononuclear Cells
PD	Pharmacodynamic
PK	Pharmacokinetic
PI	Principal Investigator
PRA	Panel Reactive Antibody

RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Suspected Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Suspected Adverse Reaction
TCR	T Cell Receptor
Treg	T Regulatory Cells
WOCBP	Women of Childbearing Potential

Study Definitions Page

Acute T cell Mediated Rejection	Banff 2007 Type 1A or higher and clinical treatment for acute rejection. Central reading will be utilized when accounting for study stopping rule and for safety endpoint.
Acute Antibody Mediated Rejection	Diffusely positive staining for C4d, presence of circulating anti-donor antibodies, and morphologic evidence of acute tissue injury.
Contraception, Highly Effective	Women of Childbearing Potential: Highly effective methods of contraception have a failure rate of <1% when used consistently and correctly. • Progestogen only hormonal contraception associated with inhibition of ovulation • Hormonal methods of contraception including oral contraceptive pills containing a combination of estrogen + progesterone, vagina ring, injectables, implants and intrauterine devices (IUDs) • Non-hormonal IUDs • Bilateral tubal occlusion • Vasectomized partner • Intrauterine hormone-releasing system (IUS) • Complete abstinence Male Participants: Male participants will be required to always use a latex or other synthetic condom during any sexual activity (e.g. vaginal, anal, oral) with WOCBP; even if the participants have undergone a successful vasectomy or if their partner is already pregnant or breastfeeding. Males should continue to use a condom until one month after the last dose of lulizumab. Withdrawal (coitus interruptus) and/or the use of a spermicide without a condom are not acceptable methods of contraception or fetal protection.
Graft Failure	90 consecutive days of dialysis dependency
Investigational Agent	lulizumab pegol (BMS-931699)
Lost to Follow-up	Subject who cannot complete study visits due to inability to reach the subject, subject relocation, etc.
Protocol Mandated Procedures	Any procedure performed solely for this research study (not site-specific SOC).
Site Principal Investigator	Lead investigator listed on the FDA 1572 at a participating center who is responsible for the conduct of the study at that center.
Study Termination	Subjects who complete the study, are lost to follow up, withdraw consent, or die during the study. Data and specimens will no longer be expected from subjects who are terminated from the study.
Study Therapy	The investigational agents and all protocol required interventions and medications.

Withdrawal from Therapy	Subject who stops study therapy prior to protocol described duration.
Women of Childbearing Potential	A woman is considered fertile following menarche and until becoming post-menopausal* unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy, and bilateral oophorectomy. **A post-menopausal state is defined as 12 months of amenorrhea in a woman over age 45 years in the absence of other biological or physiological causes. In addition, females under the age of 55 years must have a serum follicle stimulating hormone, (FSH) level > 40 mIU/mL to confirm menopause.*

1. Study Hypotheses/Objectives

This trial will be a pilot, proof of concept trial with emphasis on safety and mechanistic assays examining transplant immune regulation via Tregs.

1.1 Hypothesis

The CTOT-24 investigators hypothesize that interleukin 6 (IL6) blockade is synergistic with co-stimulation blockade, decreasing the potential for activation of effector T cells while at the same time minimizing or reversing the negative effect of CD28 inhibition on Tregs.

1.2 Primary Objective

This study will evaluate the safety of the investigational agent lulizumab pegol (BMS-931699), administered with an immunosuppressive regimen that includes tocilizumab, in adult living-donor kidney transplant recipients in the first 6 months after transplantation.

1.3 Secondary Objective

This study will also evaluate the safety of the study therapy regimen in adult living donor kidney transplant recipients 6 to 12 months after transplantation.

2. Background and Rationale

2.1 Background and Scientific Rationale

A significant problem that continues to challenge transplant physicians is the need to develop immunosuppression strategies that can usher in a tolerant state without the risks associated with conditioning regimens or the threat of graft versus host disease (GVHD) associated with infused stem cells. Other challenges include maintenance of the stability of the tolerant state and the availability of reproducible biomarkers for tolerance.

Combining CD28 inhibition with blockade of other co-stimulation tracts or other co-activation pathways has been shown to induce long-term graft acceptance in experimental transplants (Kirk, 1997) (Larsen, 2005) (Zhao, 2012). Co-stimulation inhibition with CTLA4Ig or its second-generation sister drug, belatacept, inhibits T cell activation in vitro and induces anergy but neither agent by itself has been shown to induce tolerance in experimental or clinical organ transplantation (Larsen, 2005) (Kirk, 2014). While tolerance has been demonstrated only in small animals, based on the mechanism of action of IL6 and CTLA4-Ig, their combined inhibition may result in a Treg-mediated tolerogenic environment.

CTOT-24 will use a novel therapeutic approach to combine anti-CD28 therapy using lulizumab pegol (BMS-931699) initially, followed by Belatacept, along with inhibition of the IL6 pathway by tocilizumab, an anti-IL6R monoclonal antibody.

2.2 Rationale for Anti-CD28 Therapy (lulizumab pegol (BMS-931699))

A major disadvantage of CTLA4-Ig or belatacept is that binding to CD80/CD86 not only blocks CD28 mediated T cell activation but also prevents activation of the inhibitory pathways through CTLA4 and PDL1 (Linsley, 1993). Furthermore, belatacept blocks the suppressor activity of Tregs mediated by CTLA4 (Riella, 2012) (Zhang, 2013). The absence of the dual CTLA4 mediated “beneficial” as well as the detrimental effect on Tregs effects have been suggested to contribute to the high rejection rate and increased severity of rejections in kidney transplantation associated with belatacept (Vincenti, 2012). Higher rates of acute rejection were observed in the BENEFIT Trial in the more intense regimen of belatacept vs. the lower intensity regimen (lower dose and exposure) and are believed to be related to greater inhibition of the CTLA4 pathway at higher dose (Vincenti, 2012). Another intriguing finding was observed with the abatacept (CTLA4-Ig) treated psoriasis patients: at very high dose, CTLA4-Ig reversed the suppression of antibody response to KHL vaccine, while maintaining efficacy (Abrams, 1999). One explanation for this observation is that at lower doses of CTLA4-Ig, there is predominant inhibition of the CD28 pathway but at higher dose, the inhibitory CTLA4 pathway is blocked; similar to the better efficacy observed in the less intense regimen in BENEFIT Trial than the higher intensity regimen. The development of non-agonist anti-CD28 monoclonal antibodies that bind and block CD28 activation but allow CD80/CD86 to interact freely with receptors of the inhibitory pathways on T effector and Tregs is an important advance in co-stimulation blockade that overcomes this disadvantage (Poirier, 2010) (Salomon, 2000) (Suchard, 2013) (Vanhove, 2003). Indeed, selective CD28 blockade unlike CTLA4-Ig/belatacept has been shown to preserve regulatory function through CTLA4 mediated signaling (Linsley, 1993) (Riella, 2012) (Poirier, 2010).

This study uses a novel anti-CD28 receptor antagonist immunoglobulin light chain variable region (V_K) domain antibody developed by Bristol-Myers Squibb (BMS 931699 or lulizumab pegol). Lulizumab pegol (BMS-931699) is a potent inhibitor of T-cell activation, lacking costimulatory or agonist activity as measured by T-cell proliferation or cytokine release. In cynomolgus monkeys, the ability of BMS-908613-P40Br (surrogate of lulizumab pegol (BMS-931699)) to inhibit the antibody response to T-cell dependent antigens in was evaluated as a measure of PD activity. There was a strong relationship between CD28 receptor occupancy (RO) and in vivo PD responses. After a single SC dose of BMS-

908613-P40Br, the IgG response (area under the average IgG response-time curve up to Day 29) to keyhole limpet hemocyanin (KLH) was inhibited by ~0%, 79%, and 97%, with corresponding average CD28 RO of 26%, 62%, and 86%, at 0.05, 0.5, and 5 mg/kg, respectively. Following weekly repeated doses, the bioavailability of lulizumab was approximately 63% to 105%.

CD28 target engagement by lulizumab pegol (BMS-931699) in human whole blood has also been measured. Blood treated ex vivo with Lulizumab pegol (BMS-931699) demonstrated RO potency values that correlate closely with the in vitro functional assays, suggesting that saturation of CD28 is strongly correlated with inhibition of T-cell activation. CD28 RO and inhibition of a T-cell dependent antibody response were evaluated to assess the PD of lulizumab pegol (BMS-931699) in the SAD Study IM128001 in healthy humans following a single IV or SC dose of lulizumab pegol (BMS-931699). RO confirmed target engagement in a concentration-dependent manner with the majority of subjects reaching at least 80% RO for an average duration of at least 2 weeks at doses of 9 mg and above. Inhibition of a neo-antigen (KLH)-induced T-cell dependent antibody response was evaluated only in the 9, 25, and 100 mg IV dose groups. In the control placebo group, anti-KLH IgG antibodies were detectable in approximately half of the individuals by 2 weeks after KLH immunization. In all active treatment groups tested (9, 25, and 100 mg), the IV administration of Lulizumab pegol (BMS-931699) inhibited or delayed the generation of detectable anti-KLH IgG antibodies. The nonclinical data suggest that 80% RO for 2 weeks is needed for maximum immunosuppression as measured by anti-KLH IgG suppression following antigen challenge and as levels of RO fall below 80%, the immunosuppressive activity lessens and anti-KLH antibody formation rises. Results from the SAD study substantiated this premise.

Peripheral whole blood RO was assessed in the MAD Study IM128003 at baseline and after each dose of lulizumab pegol (BMS-931699) or placebo. A time- and dose-dependent effect on the maximal amount and duration of occupancy after multiple doses of lulizumab pegol (BMS-931699) was observed. Data from the MAD RO measurements are consistent with SAD, nonclinical animal experiments, and ex vivo human blood experiments. CD28 RO by lulizumab pegol (BMS-931699) on both CD4 and CD8 T cells was similar with respect to dose and time or exposure. Irrespective of dose level, after the first dose, maximum mean RO occurred 48 hours post dose and was similar, ranging from 93.32% to 95.43%, and was sustained above 90% (range: 90.90% to 94.25%) 7 days after the first dose. Seven days (168 hours) after the administration of the last dose (third dose for the 6.25-mg biweekly treatment and fifth dose for the 12.5- and 37.5-mg weekly treatments), mean RO ranged from 88.00% to 94.67%. Fourteen days (336 hours) after the last dose, mean RO was > 80%, which gradually tapered to approximately < 3%, 7%, and 30% for the 6.25-mg biweekly, 12.5-mg weekly, and 37.5-mg weekly treatments, respectively, 56 days (1,344 hours) post last dose. For this reason, we have selected the first dose of lulizumab pegol (BMS-931699) to be 25 mg, followed by weekly doses of 12.5 mg SC in this study, with the goal of consistently maintaining RO>80% and thereby aiming for maximal clinical efficacy. The drug had a favorable safety profile with no evidence of cytokine release in both phase 1 studies noted above (Shi, 2017).

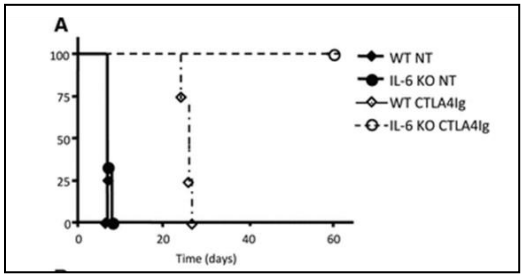
2.3 Rationale for tocilizumab, a Humanized Antibody to the IL6R

IL6 is a pleotropic cytokine produced by multiple cells and plays an important role in alloimmune responses and allograft injury (Jordan, 2017). IL6 regulates inflammation and the development, maturation and activation of T cells, B cells and plasma cells. In experimental transplantation, IL6 has been shown to regulate allograft rejection and tolerance (Zhao, 2012).

IL6 deficient mice have decreased lymphocyte proliferation, increased percentage of Treg cells and greater suppression function as compared to wild type mice. Although wild type 57 B56 recipients treated with CTLA4-Ig rejected fully MHC mismatched BALB/c heart transplants, treatment of IL6 deficient mice with CTLA4-Ig resulted in graft acceptance (see Figure 1) (Zhao, 2012). In the same study, indefinite graft survival was observed only in animals treated with CTLA1g and

anti-IL6 (Figure 2) (Kirk, 1997). IL6 inhibition promoted transplant tolerance by limiting effector T cell expansion, and by promoting Treg function.

A.



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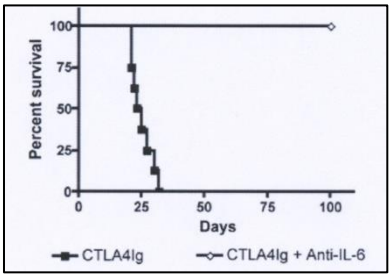


Figure 1. Graft Survival in IL-6 deficient Mice

A. *Effect of IL-6 deficiency on allograft tolerance. IL-6−/− and WT B6 recipients of fully MHC-mismatched BALB/c hearts were treated with a single dose of CTLA4Ig (0.25mg) on the day of transplantation. IL-6 deficiency facilitates allograft tolerance. Survival of BALB/c cardiac allografts is significantly prolonged in IL-6−/− recipients, when compared to WT controls (n = 4–7 per group) [3].* ***B.*** *In vivo neutralization of IL-6 inhibits rejection and facilitates allograft survival with costimulation blockade in wild-type recipients. Survival of fully MHC-mismatched BALB/c cardiac allografts in WT C57BL/6 recipients treated with CTLA4Ig and either anti-IL-6 mAb (n = 5) or control IgG (n = 8). Data are presented as a Kaplan–Meier curve [3].*

In preliminary analysis of FFPE cores of kidney biopsies by Nanstring from renal transplant recipients in the CTOT-21 (NCT02711826) trial, subjects with acute cellular and humoral rejection had increased level of intragraft IL6 transcripts (Figure 2). An increase in IL6 message was also noted in patients with subclinical inflammation on kidney biopsy.

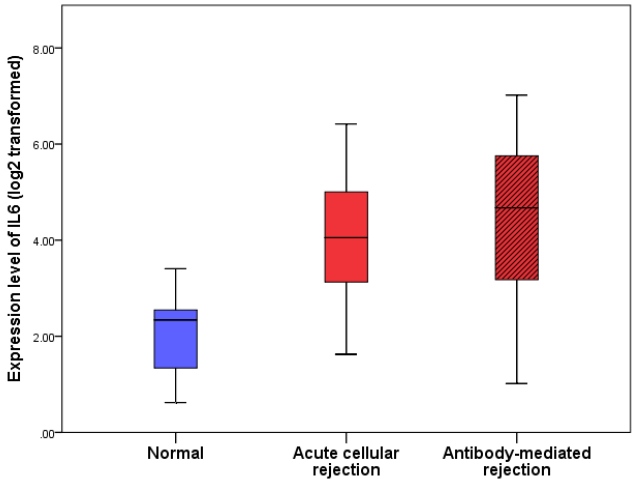


Figure 2. IL-6 Expression in Kidney Transplant

Box distribution of IL-6 expression in kidney transplant recipients with and without rejection

2.4 Rationale for belatacept and everolimus Based Maintenance Immunosuppression

At 6 months, tocilizumab will be discontinued, and participants will be henceforth on belatacept (every 4 weeks), everolimus, and prednisone, a regimen which we have shown previously to be safe and effective for long term maintenance (Wojciechowski, 2017). As in our previously described experience, everolimus will be used to with belatacept based on data indicating that mTOR inhibition may provide synergistic immunosuppression when coupled

with costimulation blockade. In non-human primate models of renal and islet transplantation the combination of CTLA4-Ig and sirolimus successfully prevented rejection and prolonged graft survival (Lo D. A., 2013) (Lowe, 2013). In addition to our clinical experience cited above, other clinical studies have demonstrated the safety and efficacy of belatacept in combination with lymphodepletion and mTOR inhibition (Ferguson, 2011) (Kirk, 2014).

2.5 Preclinical Experience with lulizumab pegol (BMS-931699)

Lulizumab pegol (BMS-931699) binds to human and cynomolgus monkey CD28 but does not bind to mouse CD28. This domain antibody is specific for CD28 and does not bind to the closely related CTLA-4. It is a potent inhibitor of T-cell activation and is a pure antagonist as determined by in vitro agonist, costimulation, and cross-linking experiments.

Surrogate CD28 dAbs that recognize mouse CD28, with potencies similar to lulizumab pegol (BMS-931699), were used to evaluate the impact of direct inhibition of CD28 in murine models of lupus, experimental allergic encephalomyelitis, and collagen induced arthritis and were found to be efficacious. Intended PD effects in cynomolgus monkeys have been demonstrated in vivo with BMS-908613-P40Br (inhibition of T-cell-dependent antibody response) and with lulizumab pegol (BMS-931699) (decreases in peripheral blood Tregs, B cells, serum IgG, and cortical lymphocytes in various lymph nodes, which is reflective of decreased germinal center activity). In vitro, lulizumab pegol (BMS-931699) and BMS-908613-P40Br showed similar binding affinities for human CD28 and similar potency in in vitro mixed-lymphocyte reaction assays. In monkeys, there was a strong relationship between CD28 receptor occupancy and in vivo PD responses.

The cynomolgus monkey was selected as the toxicology species because lulizumab pegol (BMS-931699) binds comparably to macaque CD28, is pharmacologically active in monkeys, and does not cross-react with rodent CD28. The preclinical safety assessments that support the clinical development of lulizumab pegol (BMS-931699) include:

1. Single-dose pharmacokinetic PK/PD studies conducted with BMS-908613-P40Br in monkeys to support the rationale for selection of the minimal anticipated biological effect level (MABEL) dose. There were no adverse drug related findings including any effects on plasma cytokines or peripheral blood T cell counts with doses up to 5 mg/kg

2. Single-dose exploratory toxicity study in mice with BMS-1m74-14982-S60C-P40Br to assess potential toxicity of CD28 antagonism in a rodent model. Doses up to 18 mg/kg were not associated with any adverse drug related findings.

3. Good Laboratory Practice (GLP) 1- and 6-month repeat-dose toxicity studies of lulizumab pegol (BMS-931699) in cynomolgus monkeys. In the 1-month repeat dose study, reductions in Tregs and cortical lymphocytes in lymph nodes were expected pharmacologic effects, while minimal/slight vacuolation in various tissues was attributed to the PEG moiety of lulizumab pegol (BMS-931699). All lulizumab pegol (BMS-931699)-related effects showed partial to complete resolution following an 8-week recovery period with the exception of vacuolation in the choroid plexus epithelium and pituitary gland. In the 6-month repeat dose study, findings were similar with the addition of lymphoma was noted in 1 female at 1 mg/kg/week that was considered secondary to lulizumab pegol (BMS-931699)-induced immunosuppression in cynomolgus monkeys latently infected with lymphocryptovirus (LCV). Based on this finding, a no-observed-adverse-effect-level (NOEL) was not determined in this study.

4. Exploratory in vitro study of potential lulizumab pegol (BMS-931699)-related effects (cytokine release, T-cell activation/proliferation) on human T cells. Purified human T cells were incubated with lulizumab pegol (BMS-

931699) or the superagonist anti-CD28 monoclonal antibody trans-Golgi network (TGN) 5.11A1. There were no lulizumab pegol (BMS-931699)-related effects, while TGN 5.11A1 induced both cytokine release and cellular activation.

5. GLP human tissue cross-reactivity study to demonstrate target distribution and inform of any potential unexpected epitope/tissue binding. Binding of lulizumab pegol (BMS-931699) was limited to mononuclear cells in most human tissues. As CD28 is expressed primarily by T cells, the staining of blood lymphocytes and mononuclear cells throughout the human tissue panel was expected reactivity.

Of note, in both the single-dose exploratory PK and PD studies, and repeat-dose monkey toxicity studies, immunogenicity occurred with low incidence, and the presence of the anti-drug antibodies did not affect the PK, PD, or toxicokinetics of lulizumab pegol (BMS-931699) in monkeys. No adverse irritation or local intolerance was observed at the lulizumab pegol (BMS-931699) IV or SC injection sites in either the 5-week or the 6-month studies in monkeys using lulizumab pegol (BMS-931699) concentrations and injection rates greater than or equal to those recommended for human use.

All the available preclinical evidence demonstrates that lulizumab pegol (BMS-931699) is an anti-CD28 antagonist and T-cell costimulation inhibitor lacking any agonist activity and was safe and well tolerated in cynomolgus monkeys.

2.6 Clinical Studies with lulizumab pegol (BMS-931699)

There have been two randomized controlled clinical trials of lulizumab pegol (BMS-931699) in patients with disease:

1. Study IM128027 in patients with active systemic lupus: n=346, route SC; the study was terminated based on lack of efficacy observed in the interim analysis.
2. Study IM128035 in patients with moderate to severe primary Sjogren's syndrome: n=18, route SC; the study was terminated due to unfavorable safety profile with the dose of the comparator drug (a BTK inhibitor) as determined from an interim analysis of another study in subjects with rheumatoid arthritis.

In the first study, IM128027, 346 subjects were randomized and treated with one of the following treatments utilizing a 1:1:1:1:1 randomization scheme: lulizumab pegol (BMS-931699) administered 12.5 mg weekly, 12.5 mg every 2 weeks, 5 mg every 2 weeks, 1.25 mg every 2 weeks, or placebo. The study was comprised of a placebo-controlled short-term period (Part 1 and Part 2) and a long-term extension (LTE) period. Treatment duration was up to 24 weeks (169 days) in the short-term period. The LTE period of the study remained blinded but did not have a placebo arm. Of the 346 subjects who were randomized and treated, 258 subjects completed the placebo controlled short-term period, and 212 subjects entered the LTE period of the study.

2.6.1 Lulizumab pegol (BMS-931699) Dose Selection for CTOT-24

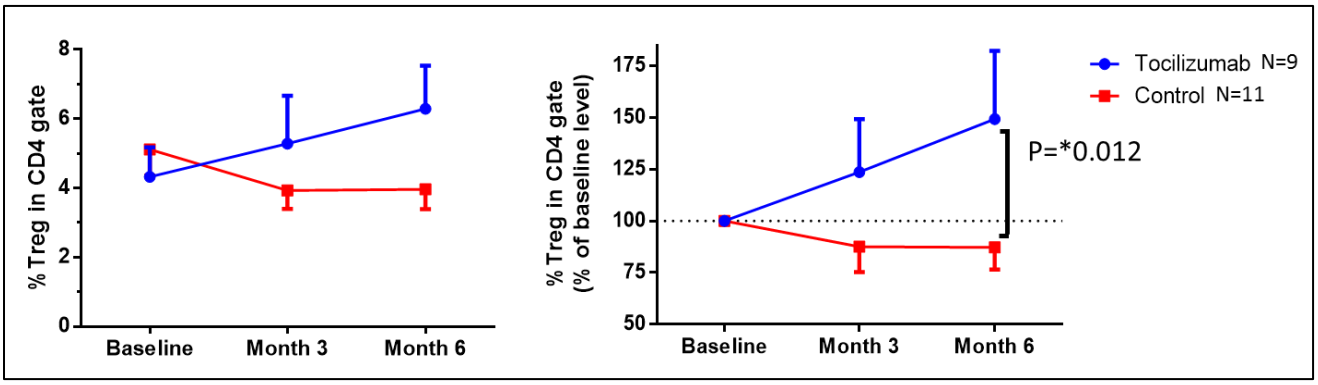
In CTOT-24, subjects will receive lulizumab pegol (BMS-931699) 25 mg (SC) on study day 1 (post-operative day 1), followed by 12.5 mg (SC) weekly through day 77. Dose selection was based on PK/PD data as presented in section 2.5, which includes favorable safety data in non-human primates with doses up to 5 mg/kg. Peri-transplant induction with twice the maintenance dose has been chosen due to the importance of preventing early alloimmunity. Assuming an average 70kg adult, the 25mg induction dose is 0.36mg/kg/dose, more than an order of magnitude less than that safely tolerated in the non-human primate model. The weekly maintenance dosing is consistent with the dose utilized in the aforementioned two clinical studies, IM128027 and IM128035. Adverse events in the placebo-controlled dose escalation IM128027 trial is presented in Table 2 in 5.1.2

2.7 Clinical Studies with Tocilizumab in Kidney Transplantation

An ongoing, randomized controlled study at UCSF (NCT02108600) assigns subjects with intragraft inflammation at 6 months to either monthly treatments with tocilizumab (8 mg/kg IV) or control (no additional therapy), with a follow-up kidney biopsy 6 months later. At the time of CTOT-24 protocol development, 27/48 have been enrolled in the study.

Dr. Jordan at Cedars Sinai Hospital has treated 36 patients with chronic antibody mediated rejection with tocilizumab (8 mg/kg monthly, maximum 800 mg per dose, for 6-25 months) without any major complications (Choi, 2017). Tocilizumab both in IV and subcutaneous preparations are currently approved for therapy in rheumatoid arthritis. So far, it appears from our ongoing trial as well as Dr. Jordan's study to be safe when added to standard of care therapy. It has been observed that patients with rheumatoid arthritis who received tocilizumab had a significant increase in circulating Tregs (Kikuchi, 2015) (Thiolat, 2014). Preliminary results of the UCSF trial in the first 18 patients who have completed at least 6 months in the study indicate that this increase is seen in kidney transplant patients on immunosuppression, as well (Figure 3). Also, patients who received tocilizumab showed a significant reduction of IFN-γ and IL17 in stimulated CD4 T cells when compared to the control group at month 6 (Figure 3). This modulatory effect of IL6 blockade may counteract the adverse effect of CD28 blockade on Tregs. An additional advantage for the study is that tocilizumab can be administered subcutaneously, rendering the combination therapy easier to implement.

A.



B.

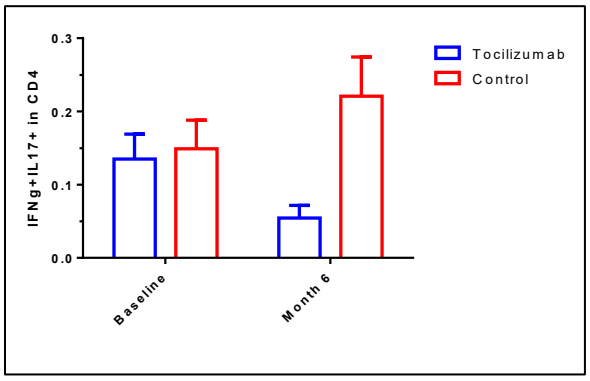


Figure 3. Circulating Treg Frequency in Kidney Transplant Recipients after Tocilizumab

A. *Circulating Treg frequency in kidney transplant recipients on immunosuppression who received tocilizumab for 6 months compared to controls who did not receive tocilizumab.* ***B.*** *IFN-γ + IL-17 secretion in CD4+ T cells stimulated by PMA/ Ionomycin for 4 hours in tocilizumab treated patients and control at 6 months.*

2.7.1 Tocilizumab Dose Selection for CTOT-24

In CTOT-24, the dose of tocilizumab used will be 8 mg/kg for the first dose followed by 162 mg SC every 2 weeks. The dose of 8 mg/kg IV every 4 weeks or 162 mg SC every other week is the approved dose for tocilizumab according to the label for the treatment of rheumatoid arthritis. This dosing regimen is based on clinical studies with the 4 mg per kg and

8 mg per kg IV doses or the 162 mg weekly and every other weekly SC doses of tocilizumab which showed decreases in levels of C-reactive protein to within normal ranges were seen as early as week 2. Greatest improvements in the pharmacodynamic parameters were observed with 8 mg/kg. The dose of 8 mg/kg IV every 4 weeks was used by Dr. Jordan in patients with chronic AMR as well as in our study of subclinical graft inflammation and appears to be relatively safe and well tolerated. The second and subsequent doses will be given subcutaneously at 162 mg every 2 weeks based on efficacy demonstrated in clinical studies of rheumatoid arthritis.

3. Study Design

3.1 Description of Study Design

CTOT-24 is a multi- center, open-label, pilot, single arm clinical trial which aims to enroll 10 living donor renal transplant recipients to evaluate the safety of lulizumab pegol (BMS 931699) in the context of a novel immunosuppressive regimen using anti-thymocyte globulin (rabbit) (ATG), steroids,) Nulojix® (belatacept), Actemra® (tocilizumab), mycophenolate mofetil (MMF) and Zortress®(everolimus) (Table 1 and Figure 4). Study participants will be enrolled prior to transplantation. The primary endpoint will be evaluated at 6 months and study participants will continue to be followed for 24 months after transplantation. The study plans to complete enrollment within a 27 month period.

Participants receive induction with ATG and methylprednisolone and are initially maintained on tocilizumab and prednisone. Lulizumab pegol (BMS-931699) is administered on the day after transplantation and weekly until 3 months; after which lulizumab pegol (BMS-931699) will be replaced with belatacept every 4 weeks. MMF will be started on the day after transplant to reduce the risk of acute rejection during the initial weeks when tocilizumab has not achieved its full effect on reducing effector T cell function and increasing Tregs. Zortress® (everolimus) is added at 14 days post-transplant to reduce the impact on wound healing during the first two weeks. MMF will be discontinued once the everolimus level is within the therapeutic range. Tocilizumab will be discontinued at 6 months, and participants will be maintained on belatacept, everolimus, and prednisone.

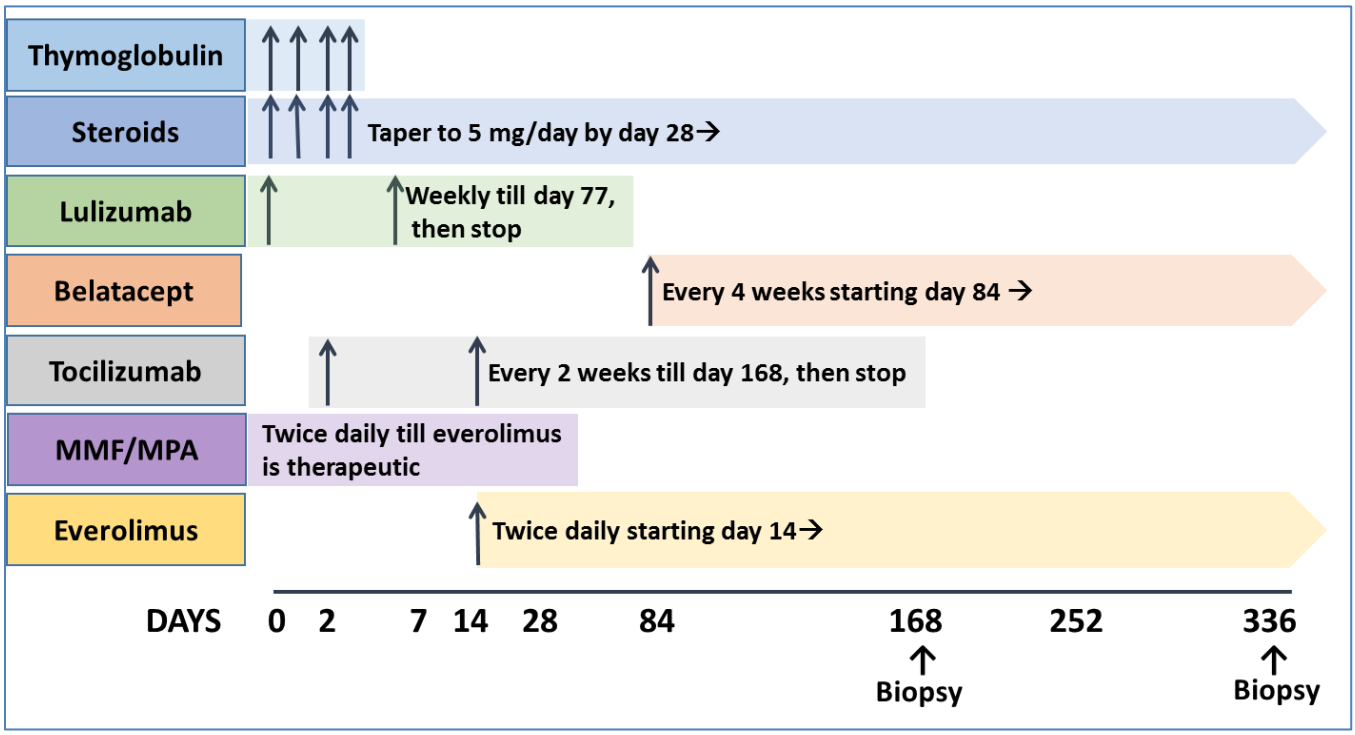
Participants undergo protocol biopsies at 6 months after transplant. At 3, 6, 9 and 12 months they will also undergo a battery of mechanistic assays to identify changes in the immune profile and correlate their emergence and evolution with different components of the study regimen.

Table 1. Study drugs

Study Drug	**Dosing**
Anti-thymocyte globulin, rabbit (ATG)	6 mg/kg (IV) total dose, given in divided doses on days 0-3
Methylprednisolone Prednisone	500 mg (IV) on day 0, 250 mg on day 1, 125 mg on day 2 60 mg on day 3, 30 mg on day 4, then taper to 5 mg daily by day 28 (See Section 7.2.4)
Lulizumab pegol (BMS 931699)	25 mg (SC) on day 1 12.5 mg (SC) weekly till day 77
Actemra® (tocilizumab)	8 mg/kg (IV) on day 2 162 mg (SC) every 2 weeks till day 168
Nulojix® (belatacept)	5 mg/kg (IV) every 4 weeks starting day 84
Mycophonelate mofetil	1000 mg PO bid started on day 1, then discontinued once everolimus level is within therapeutic range
Zortress®(everolimus)*	Start at 0.75 mg (PO) bid on day 14, titrate to maintain level 3-8 ng/ml

*Participants who do not tolerate everolimus will be switched to mycophenolate mofetil 1000 mg BID, PO

Figure 4. Study Scheme



3.2 Primary Endpoint

The primary safety endpoint is the proportion of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at 6 months after transplantation. Clinical rejection occurring prior to 6 months, defined as treated rejection without biopsy confirmation, will be included as acute rejection with respect to the endpoint.

3.3 Secondary Endpoint

The secondary safety endpoint is the proportion of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at 12 months after transplantation. Clinical rejection occurring prior to 12 months, defined as treated rejection without biopsy confirmation, will be included as acute rejection with respect to the endpoint.

3.4 Mechanistic Endpoints

1. Frequency of circulating Tregs at 3, 6 and 12 months post-transplant
2. Treg suppressive activity at 3, 6 and 12 months post-transplant using irradiated donor PBMC as stimulators and measuring cytokine production in response to anti-CD3 and anti-CD28
3. Alloreactive T cell frequency at 3, 6 and 12 months post-transplant
4. Expression of T cell checkpoint inhibition related genes at 3, 6 and 12 months post-transplant

Comparisons of mechanistic data will be made within the cohort of participants enrolled in this study across different time points.

4. Selection of Participants and Clinical Sites/Laboratories

4.1 Rationale for Study Population

This study aims to enroll 10 recipients of HLA mismatched living donor kidney recipients with PRA <50% and no DSA. Since this study is testing a novel CNI-free immunosuppression regimen, only those at low risk for rejection will be enrolled to maximize participant safety. This population is similar to the population that was enrolled in the BENEFIT study (NCT00256750) to test a novel belatacept-based immunosuppressive regimen. It has been shown that that CD28- T-cells exert their late stage functions without reliance on ongoing CD28/B7 costimulation, and will therefore not be susceptible to CD28 blockade (Lo D. W., 2011) (Xu, 2014) (Shoji, 2018). Therefore, patients with a high proportion of CD28-1 cells among the circulating CD8T cells will be excluded from this study due to their increased risk of costimulation blockade resistant rejection. On the other hand, HLA matched living donor recipients have excellent outcomes irrespective of immunosuppressive regimens and therefore will also be excluded from the study.

4.2 Inclusion Criteria

Individuals who meet all the following criteria are eligible for enrollment as study participants:

1. Able to understand and provide informed consent
2. Agreement to use highly effective (<1% failure rate) methods of contraception (see study definitions). Female participants of child-bearing potential must consult with their physician and determine the most suitable method(s) from this list to be used for 12 months while on study drug regimen.
3. Male or female, 18 to 70 years
4. Recipient of primary, non-HLA identical living donor kidney transplant
5. No donor specific antibodies prior to transplant that are considered to be of clinical significance by the site investigator
6. EBV positive serology
7. CMV positive serology, unless donor-recipient pair are both CMV negative
8. Negative testing for latent TB infection within 3 months prior to transplant. Testing should be conducted using either a PPD or interferon-gamma release assay (i.e. QuantiFERON-TB, T-SPOT.TB). Subjects with a positive test for latent TB infection must complete appropriate therapy for LTBI. A subject is considered eligible only if they have a negative test for LTBI within 3 months prior to transplant OR they have appropriately completed LTBI therapy prior to transplant. Latent TB infection treatment regimens should be among those endorsed by the CDC (Division of TB Elimination, 2016).
9. In the absence of contraindication, vaccinations must be up to date for hepatitis B, influenza, pneumococcal, varicella and herpes zoster, and MMR.
10. Hepatitis C Virus Ab positive subjects with negative HCV PCR are eligible if they have spontaneously cleared infection or are in sustained virologic remission for at least 12 weeks after treatment (see exclusion 13).
11. Negative SARS-COV2 PCR testing within 2 weeks of transplant.

4.3 Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Prisoners or subjects who are compulsorily detained.
2. Inability or unwillingness of a participant to give written informed consent or comply with study protocol
3. Candidate for a multiple solid organ or tissue transplants
4. Prior history of organ or cellular transplantation

5. Known to have idiopathic FSGS as the underlying cause of ESRD
6. Requirement for uninterrupted anticoagulation therapy, including Plavix
7. Known hypersensitivity to mTOR inhibitors or contraindication to everolimus (including history of wound healing complications)
8. History of severe allergic and/or anaphylactic reactions to humanized or murine monoclonal antibodies
9. Hypersensitivity to rabbit proteins or rabbit Anti-thymocyte Globulin (ATG)
10. Known hypersensitivity to ACTEMRA® (tocilizumab) or lulizumab pegol (BMS-931699),
11. HIV infected subjects, including those who are well controlled on anti-retrovirals
12. Positive HBsAg or HBcAb serology
13. Hepatitis C virus antibody positive (HCVAb+) subjects who have failed to demonstrate sustained viral remission for more than 12 weeks (after anti-viral treatment)
14. Subjects with a previous history of active Tuberculosis (TB)
15. Known active current viral, fungal, mycobacterial or other infections (including, but not limited to tuberculosis and atypical mycobacterial disease, Hepatitis B and C, and herpes zoster)
16. Donor or recipient residing in areas where annual incidence is ≥21 cases/100,000 for coccidioidomycosis according to current CDC map (https://www.cdc.gov/fungal/diseases/coccidioidomycosis/images/valley-fever-map-2017.jpg). Donor or recipients residing in zones with annual incidence of <21 cases/100,000 will not require additional screening.
17. History of malignancy except treated basal cell cancer of the skin
18. History of hemolytic-uremic syndrome/ thrombotic thrombocytopenia purpura
19. History of demyelinating disorders (e.g., multiple sclerosis, chronic inflammation demyelinating polyneuropathy)
20. History of gastrointestinal perforations, active inflammatory bowel disease or diverticulitis
21. Any previous treatment with alkylating agents such as chlorambucil, or with total lymphoid irradiation;
22. Receipt of a live vaccine within 30 days prior to transplantation
23. Past or current medical problems or findings from physical examination or laboratory testing that are not listed above, which, in the opinion of the investigator, may pose additional risks from participation in the study, may interfere with the participant's ability to comply with study requirements or that may impact the quality or interpretation of the data obtained from the study.
24. Severe hyperlipidemia (total cholesterol >350 mg/dL, LDL >190 mg/dL, or triglycerides >500 mg/dL)
25. Transaminase levels elevated more than 1.5 times the upper limit of normal (ULN) within 7 days prior to enrollment
26. ANC less than 2,000 per mm^3 within 7 days prior to enrollment
27. Platelet count less than 100,000 per mm^3 within 7 days prior to enrollment
28. More than 50% CD8+/ CD28- T-cells in peripheral blood
29. Positive pregnancy test in women of child bearing potential, currently breastfeeding, or planning to become pregnant during the timeframe of the study or follow-up period.
30. Participation in any other studies with investigational drugs or regimens in the preceding year
31. cPRA greater than or equal to 20%, as determined by each participating site's laboratory

4.4 Selection of Clinical Sites

The primary study site will be UCSF. Other sites will include Cleveland Clinic, Duke University, University of Alabama at Birmingham (UAB), and the University of Colorado. All sites perform 40-120 living donor kidney transplants per year

allowing easily for the target enrollment of 10 subjects within the projected enrollment period of 12 months. All centers also have long experience with the use of belatacept based immunosuppression in kidney transplant recipients.

5. Known and Potential Risks and Benefits to Participants

5.1 Risks of lulizumab Pegol (BMS 931699)

The known risks of lulizumab pegol (BMS-931699) are primarily derived from four randomized blinded placebo-controlled studies: two in healthy volunteers (Shi, 2017) and two in patients with disease.

5.1.1 Risks of lulizumab pegol (BMS-931699) from Studies in Healthy Volunteers

There have been two randomized blinded placebo-controlled studies in healthy volunteers:

1. SAD (single ascending dose study), BMS study IM128001: n=156, with 108 subjects receiving the active drug (0.01-100 mg IV or 9, 25, or 50 mg SC).
2. MAD (multiple ascending dose study), BMS study IM128003: n=24, with 18 subjects receiving the active drug (6.25 mg every 2 weeks, 12.5 mg weekly, or 3.75 mg weekly SC for 5 weeks).

In the SAD study, the most frequently reported AE was headache (n = 17 [15.7%] for lulizumab pegol (BMS-931699) vs. n = 4 [8.3%] for placebo). Acute infusion reactions occurred in 7 subjects. Four subjects were discontinued from the study due to AEs of acute infusion reaction within 60 minutes of initiation of IV infusion of lulizumab pegol (BMS-931699). The most commonly reported symptoms were flushing, feeling hot, erythema, and ocular hyperemia. All events were of moderate (grade 2) intensity. The symptoms were reversible on cessation of administration and initiation of standard treatment. There were no clinically relevant changes in vital signs following administration of lulizumab pegol (BMS-931699). Also, no clinically meaningful changes were observed in pro-inflammatory cytokines following a single dose of lulizumab pegol (BMS-931699), confirming the lack of CD28 receptor agonistic activity in humans.

Two subjects experienced SAEs. One subject in part 1 experienced acute renal failure due to severe dehydration on day 16 following administration of 25 mg lulizumab pegol (BMS-931699) SC; this condition was not attributed to study drug and fully resolved. One subject in part 2 had a perforated appendix 7 days after receiving a partial dose of lulizumab pegol (BMS-931699) on day 1. This event was not attributed to lulizumab pegol (BMS-931699).

Isolated, asymptomatic ALT increases (with 41 U/L considered ULN) were seen in 20 (18.5%) subjects following administration of lulizumab pegol (BMS-931699) and 6 (12.5%) subjects following administration of placebo in the SAD study.

During the MAD study, infections and infestations occurred in 5 (27.8%) subjects following administration of lulizumab pegol (BMS-931699) (versus 0 in the placebo group). No correlation was observed between exposure and infection rate. One subject in the 12.5-mg lulizumab pegol (BMS-931699) weekly group experienced 2 infective episodes: oral herpes on day 40 followed, after 7 days, by an upper respiratory infection; in both episodes the severity was classified as mild. One subject receiving 12.5 mg lulizumab pegol (BMS-931699) weekly presented with a furuncle of mild severity on day 69. One subject receiving 37.5 mg lulizumab pegol (BMS-931699) weekly presented on day 89 with a peritonsillar abscess of moderate severity, which required antibiotic treatment with 500 mg amoxicillin 3 times a day for 10 days. One subject receiving 37.5 mg lulizumab pegol (BMS-931699) weekly had a mild viral infection on day 81. One SAE occurred that was considered possibly related to lulizumab pegol (BMS-931699). A subject receiving 6.25 mg lulizumab pegol (BMS-931699) every 2 weeks required hospitalization on day 49 for cellulitis that developed in his right hand after damage of the skin at the base of his third finger. The hospitalized patient was treated with IV antibiotics, and the lesion was surgically drained. The traumatic skin damage in his right hand is a potential inciting factor for the cellulitis. However, it could not be excluded that lulizumab pegol (BMS-931699) might have made the subject more susceptible to the subsequent infection. Therefore, the SAE was considered possibly related to the study drug.

Three discontinuations due to AEs were reported. One discontinuation occurred in the placebo group due to a mild increase in transaminases to < 2 times ULN. One subject in the 12.5-mg and 1 in the 37.5-mg lulizumab pegol (BMS-931699) weekly group discontinued due to moderate injection-site reactions. The most frequently occurring AEs were headache and nausea, and were mild to moderate in severity.

5.1.2 Risks of lulizumab pegol (BMS-931699) from Studies in Patients with Disease

Safety data for the IM128027 placebo-controlled short-term Lupus trial (a period of 24 weeks and up to 42 days post last dose of the short-term treatment period or the start of LTE treatment, whichever occurred first, are summarized below. Data from the LTE are still being analyzed.

Table 2. Summary of subjects with AEs reported during the short-term period, all treated subjects, Study IM128027

	Placebo	lulizumab pegol (BMS-931699)			
	N=71	1.25 mg every 2 weeks N=70	5 mg every 2 weeks N=68	12.5 mg every 2 weeks N=68	12.5 mg weekly N=69
Deaths	0	2 (2.9)	0	0	0
SAEs	6 (8.5)	8 (11.4)	9 (13.2)	5 (7.4)	5 (7.2)
Related SAEs	1 (1.4)	0	5 (7.4)	3 (4.4)	3 (4.3)
Discontinued due to SAEs	1 (1.4)	5 (7.1)	4 (5.9)	2 (2.9)	3 (4.3)
AEs	62 (87.3)	59 (84.3)	60 (88.2)	56 (82.4)	59 (85.5)
Related AEs	19 (26.8)	19 (27.1)	29 (42.6)	30 (44.1)	33 (47.8)
Discontinued due to AEs	3 (4.2)	9 (12.9)	9 (13.2)	5 (7.4)	8 (11.6)

During the short-term period, there were 2 deaths in the 1.25 mg every 2 weeks group: 1 death due to cerebral hemorrhage and 1 death due to complications of a SLE flare, both deemed unrelated to investigational product by the investigator. Overall, the number of AEs and SAEs that were related to treatment was higher in the 5 mg every 2 weeks, 12.5 mg every 2 weeks, and 12.5 mg weekly dose groups. A total of 33 subjects experienced SAEs during the short-term period. The only SAE experienced by ≥ 2 subjects in one of the lulizumab pegol (BMS-931699)-treated groups, by preferred term, was SLE; in the placebo group, cellulitis and lupus nephritis were experienced by ≥ 2 subjects. The following SAEs were considered related to study medication by the investigator: cellulitis (placebo), herpes zoster (12.5 mg every 2 weeks), hypertension (12.5 mg every 2 weeks), lung infection (12.5 mg weekly), lupus enteritis (5 mg every 2 weeks), optic neuritis (12.5 mg weekly), pleurisy (5 mg every 2 weeks), respiratory tract infection (5 mg every 2 weeks), serum sickness (5 mg every 2 weeks), SC abscess (12.5 weekly), SLE (5 mg every 2 weeks), and systemic inflammatory response syndrome (12.5 every 2 weeks).

With respect to infusion and injection site reactions, 35 subjects (4 of 71 subjects who received placebo and 31 of 275 subjects who received lulizumab pegol (BMS-931699)) experienced 123 AEs (12 in subjects who received placebo and 111 in subjects who received lulizumab pegol (BMS-931699)) of local injection site reactions. These events were mostly mild and considered related to study medication. There were 2 SAE injection reactions reported. First, there was an SAE that was reported as serum sickness (Grade 2 / moderate) in the lulizumab pegol (BMS-931699) 5 mg every 2 weeks group occurring approximately 4 weeks after initiating study therapy and 1 day after the most recent dose, which was considered to be related to study drug by the investigator and Sponsor. The subject reported feeling unwell, dizzy, and feverish with muscle aches. The event resolved with treatment. The second case was a subject in the lulizumab pegol (BMS-931699) cohort that received 12.5 mg every 2 weeks. The subject was hospitalized for systemic inflammatory response syndrome (Grade 3/severe) shortly after receiving the second dose of lulizumab pegol (BMS-931699); the event was considered related to study drug. This subject presented with fevers and diarrhea, was found to have SIRS,

underwent hospitalization for 3 days and was treated empirically with antibiotics (no microbial etiology identified). The event was considered resolved, and study drug was discontinued.

In the second study, IM128035, subjects with Primary Sjögren's Syndrome were randomized 1:1:1 to receive either BMS-986142 350 mg PO daily and SC placebo injection, lulizumab pegol (BMS-931699) 12.5 mg SC once weekly and oral placebo, or oral and SC placebo for 12 weeks. A total of 45 subjects were enrolled into the study, and 18 subjects were randomized to treatment. Five subjects were treated with lulizumab pegol (BMS-931699), 6 subjects were treated with BMS-986142, and 7 subjects received placebo. Three of these 18 subjects completed the double-blind treatment period; 2 placebo-treated subjects and 1 lulizumab pegol (BMS-931699)-treated subject. There were no deaths in this study. No serious or severe AEs were noted in the lulizumab pegol (BMS-931699) arm of the study. Collectively, the data from the above studies demonstrate that lulizumab pegol (BMS-931699) has an acceptable safety profile.

5.2 Risks of Actemra® (tocilizumab)

Most common adverse reactions (incidence of at least 5%) are upper respiratory tract infections, nasopharyngitis, headache, hypertension, increased ALT, injection site reactions.

Serious Infections

The package insert includes a "black box" warning about serious infections, including tuberculosis. Serious and sometimes fatal infections due to bacterial, mycobacterial, invasive fungal, viral, protozoal, or other opportunistic pathogens have been reported in patients receiving immunosuppressive agents including Actemra® for rheumatoid arthritis. The most common serious infections included pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis. Among opportunistic infections, tuberculosis, cryptococcus, aspergillosis, candidiasis, and pneumocystosis were reported with Actemra®. Other serious infections, not reported in clinical studies, may also occur (e.g., histoplasmosis, coccidioidomycosis, listeriosis). Patients have presented with disseminated rather than localized disease, and were often taking concomitant immunosuppressants such as methotrexate or corticosteroids which in addition to rheumatoid arthritis may predispose them to infections.

Patients with known active current viral, fungal, mycobacterial or other infections (including, but not limited to tuberculosis and atypical mycobacterial disease, Hepatitis B and C, and herpes zoster) will be excluded from participation in this study.

Patients will be closely monitored for the development of signs and symptoms of infection during and after treatment with Actemra®, as signs and symptoms of acute inflammation may be lessened due to suppression of the acute phase reactants. Actemra® will be held if a patient develops a serious infection, an opportunistic infection, or sepsis. A patient who develops a new infection during treatment with Actemra® will undergo a prompt and complete diagnostic workup appropriate for an immunocompromised patient, initiate appropriate antimicrobial therapy, and be closely monitored

Tuberculosis

Patients will be evaluated for tuberculosis risk factors and have a test for latent infection within 3 months prior to enrollment in the study, conducted using either a PPD or interferon-gamma release assay (i.e. QuantiFERON-TB, T-SPOT.TB). Those with a history of active TB will be excluded from participation. Those with a positive test for latent TB infection must have completed appropriate therapy for LTBI; treatment regimens should be among those endorsed by the CDC (Division of TB Elimination, 2016). Only those patients will be enrolled who have a negative test for LTBI within 3 months prior to transplant OR have appropriately completed LTBI therapy prior to transplant.

Viral Reactivation

Viral reactivation has been reported with immunosuppressive biologic therapies and cases of herpes zoster exacerbation were observed in clinical studies with Actemra®. No cases of Hepatitis B reactivation were observed in the trials; however, patients who screened positive for hepatitis were excluded. Study participants will be required to have evidence of immunity to varicella.

Gastrointestinal Perforations

Events of gastrointestinal perforation have been reported in clinical trials, primarily as complications of diverticulitis in RA patients. Patients with a history of gastrointestinal perforations, active inflammatory bowel disease or diverticulitis will be excluded from participation in the study. Patients presenting with new onset abdominal symptoms will be promptly evaluated for early identification of gastrointestinal perforation.

Laboratory Parameters

Neutropenia

Treatment with Actemra® was associated with a higher incidence of neutropenia. Infections have been uncommonly reported in association with treatment-related neutropenia in long-term extension studies and post-marketing clinical experience. See section 7.2.1.4, Toxicity Prevention and Management for Tocilizumab.

Thrombocytopenia

Treatment with Actemra® was associated with a reduction in platelet counts. Treatment-related reduction in platelets was not associated with serious bleeding events in clinical trials. See section 7.2.1.4, Toxicity Prevention and Management for Tocilizumab.

Elevated Liver Enzymes and Hepatotoxicity

Treatment with Actemra® was associated with a higher incidence of transaminase elevations. These elevations did not result in apparent permanent or clinically evident hepatic injury in clinical trials. Increased frequency and magnitude of these elevations was observed when potentially hepatotoxic drugs (e.g., MTX) were used in combination with Actemra®. In one case, a patient who had received Actemra® 8 mg/kg monotherapy without elevations in transaminases experienced elevation in AST to above 10x ULN and elevation in ALT to above 16x ULN when MTX was initiated in combination with Actemra®. Transaminases normalized when both treatments were held, but elevations recurred when MTX and Actemra® were restarted at lower doses. Elevations resolved when MTX and Actemra® were discontinued. See section 7.2.1.4, Toxicity Prevention and Management for Tocilizumab.

The Marketing Authorization Holder identified eight cases as tocilizumab related moderate to severe drug-induced liver injury including acute liver failure, hepatitis and jaundice. This serious hepatic injury occurred between 2 weeks to more than 5 years after initiation of tocilizumab with median latency of 98 days. In these eight cases, two cases of acute liver failure required liver transplantation. In the context of total world-wide tocilizumab exposure of approximately 1,066,849 patients (882,370.3PY) up to 10th April 2018, serious hepatotoxicity events are considered rare. The benefit-risk profile of tocilizumab in the approved indications remains favorable.

Lipid Abnormalities

Treatment with Actemra® was associated with increases in lipid parameters such as total cholesterol, triglycerides, LDL cholesterol, and/or HDL cholesterol. Lipid parameters will be assessed approximately 4 to 8 weeks following initiation of

Actemra® therapy, then at approximately 24-week intervals. Participants will be managed according to the local standard of care.

Immunosuppression

The impact of treatment with Actemra® on the development of malignancies is not known. During the 24 week controlled period of the studies, 15 malignancies were diagnosed in patients receiving ACTEMRA-IV compared to 8 malignancies in the control groups. Exposure adjusted incidence of malignancies was similar in the ACTEMRA-IV group (1.32 events per 100 patient-years and in the placebo + DMARD group (1.37 events per 100 patient-years). Actemra® is an immunosuppressant, and treatment with immunosuppressants may result in an increased risk of malignancies.

Hypersensitivity Reactions, Including Anaphylaxis

Hypersensitivity reactions, including anaphylaxis, have been reported in association with Actemra® and anaphylactic events with a fatal outcome have been reported with intravenous infusion of Actemra®. Anaphylaxis and other hypersensitivity reactions that required treatment discontinuation were reported in 0.1% (3 out of 2644) of patients in the 6-month controlled trials of intravenous Actemra®, 0.2% (8 out of 4009) of patients in the intravenous all-exposure RA population, 0.7% (8 out of 1068) in the subcutaneous 6-month controlled RA trials, and in 0.7% (10 out of 1465) of patients in the subcutaneous all-exposure population. In the SJIA controlled trial with intravenous Actemra®, 1 out of 112 patients (0.9%) experienced hypersensitivity reactions that required treatment discontinuation. In the PJIA controlled trial with intravenous Actemra®, 0 out of 188 patients (0%) in the Actemra® all-exposure population experienced hypersensitivity reactions that required treatment discontinuation. Reactions that required treatment discontinuation included generalized erythema, rash, and uticaria. Injection site reactions were categorized separately. In the post-marketing setting, events of hypersensitivity reactions, including anaphylaxis and death have occurred in patients treated with a range of doses of intravenous Actemra®, with or without concomitant arthritis therapies. Events have occurred in patients who received premedication. Hypersensitivity, including anaphylaxis events, have occurred both with and without previous hypersensitivity reactions and as early as the first infusion of Actemra®.

Those with known hypersensitivity to Acetmra® will be excluded from this study. The first dose of Actemra® will be administered IV in the hospital with appropriate medical support to manage anaphylaxis and the participant will be monitored for at least 24 hours afterward. Subsequent doses will be given SC and participant will be advised to seek immediate medical attention if they experience any symptoms of a hypersensitivity reaction. If anaphylaxis or other hypersensitivity reaction occurs, the administration of Actemra® will be stopped immediately and Actemra® will be discontinued permanently.

Demyelinating Disorders

The impact of treatment with Actemra® on demyelinating disorders is not known, but multiple sclerosis and chronic inflammatory demyelinating polyneuropathy were reported rarely in RA clinical studies. Patients with a history of demyelinating disorders (e.g., multiple sclerosis, chronic inflammation demyelinating polyneuropathy) will be excluded from participation in this study. Participants will be monitored during the study for signs and symptoms potentially indicative of demyelinating disorders.

5.3 Risks of Other Protocol Specified Medications

5.3.1 Risks of ATG, steroids, belatacept, everolimus, and mycophenolate mofetil

ATG, steroids, belatacept, everolimus, and MMF are considered standard of care maintenance IS medications in kidney transplantation and the risks of these drugs are well described. The risks of the specific regimen in this protocol, which includes lulizumab pegol (BMS-931699) and Actemra®, are unknown.

5.4 Reproductive Risks

Investigators shall counsel women of child bearing potential, and male participants who are sexually active with WOCBP, on the importance of pregnancy prevention and the implications of an unexpected pregnancy and when applicable, the potential of fetal toxicity occurring due to transmission of study drug, present in seminal fluid, to a developing fetus, even if the participant has undergone a successful vasectomy or if the partner is pregnant. Investigators will also counsel study participants about the risks of embryo toxicity and teratogenicity with everolimus and Mycophenolate. Investigators shall advise on the use of highly effective methods of contraception (see Study Definitions).

5.5 Risks of Study Procedures

5.5.1 Risks of Blood Draws

Risks of blood draw or venipuncture are typically minimal with temporary local discomfort. More serious risks would include ecchymosis and, rarely, localized infection. The anticipated amount of blood for mechanistic assays in this study is not more than 67.5 ml for a single visit. The amount of blood that may be drawn from adult participants for research purposes will not be more than 10.5 mL/kg or 550 mL, whichever is smaller, over an eight-week period. The participant's clinical condition will be taken into consideration to determine if research blood tests can be performed.

5.5.2 Risks of Kidney Transplant Biopsy

This study requires one research biopsy approximately 6 months after transplant. There is a risk of bleeding associated with transplant kidney biopsies. Transient hematuria occurs in 3 to 10% of patients and may prolong hospitalization, require bladder catheterization for clot drainage, or require blood transfusion in approximately 1% of patients. Ureteral obstruction from blood clot may require percutaneous nephrostomy in <1% of patients. Massive hemorrhage requiring surgical exploration, transplant nephrectomy, or arterial embolization occurs in ~0.1 % of patients. Death from massive hemorrhage is rare.

5.6 Risks of Coronavirus Disease 2019 (COVID-19)

Early reports indicate an increased risk of critical illness and mortality in kidney transplant recipients infected with SARS-CoV-2, possibly due to chronic immunosuppression and co-existing conditions (Akalin, 2020); (Pereira, 2020). Given that both lulizumab pegol (BMS-931699) and Actemra® are associated with increased risk of infection in general, it is possible that study therapy may increase the risk of developing symptomatic severe COVID-19 illness in subjects who would have otherwise been asymptomatic.

All subjects will be screened for active infection with 2019-nCoV RT-PCR prior to study entry and prior to receiving the first dose of the study therapy. Those with a history of positive result for 2019-nCoV RT-PCR at any time in the past will be excluded from participation.

Subjects will be asked to report any fever, cough or other new symptoms immediately to the study team. The study therapy will be discontinued immediately in any subject (symptomatic or asymptomatic) with a positive result for 2019-nCoV RT-PCR during the study. Results of standard of care screening for COVID-19 and testing for SARS-CoV-2 which

occur in any subject during the study will be collected. Subjects who are in quarantine (Akalin, 2020) for possible exposure to SARS-CoV-2 will not receive the study therapy or undergo study-related procedures until they complete the quarantine period and have a negative result for 2019-nCoV RT-PCR; such subjects may be asked to conduct study follow-up visits remotely during the quarantine period, if possible.

5.7 Potential Benefits

This study might not provide direct or immediate benefit to study participants.

6. Investigational Agent

6.1 lulizumab Pegol (BMS 931699)

6.1.1 Formulation, Packaging, and Labeling

A previous publication described the discovery and preclinical characterization of a domain antibody (dAb), lulizumab pegol, that binds to the CD28 receptor and blocks this signaling pathway (Suchard, 2013). Lulizumab pegol (BMS-931699) was generated using phage display and affinity maturation through the diversification of a selected subset of amino acid residues. Monomeric anti-CD28 domain antibodies were formatted with polyethylene glycol (PEG). Lulizumab pegol (BMS-931699) is a potent inhibitor of T-cell proliferation and cytokine production. Unlike the first-generation T-cell stimulation inhibitor abatacept (a cytotoxic T lymphocyte–associated antigen-4-immunoglobulin [CTLA-4Ig] fusion protein that binds with different affinities to CD80 and CD86 on antigen-presenting cells) (Linsley, 1993), lulizumab pegol (BMS-931699) is equipotent at inhibiting both CD80- and CD86-driven T-cell proliferation. No agonist activity, as measured by preclinical T-cell proliferation or cytokine release, has been observed with lulizumab pegol (BMS-931699).

6.1.2 Dosage, Preparation, and Administration

Dosage Form

Lulizumab injection, 12.5 mg/Vial (12.5 mg/mL)

Lulizumab injection for SC administration is a clear to slightly opalescent, colorless to pale yellow solution, essentially free of particulate matter on visual inspection. The drug product is a single use, preservative-free, ready-to-use solution, contained in a 3-cc type I glass vial, closed with a 13-mm stopper and sealed with a 13-mm aluminum seal.

Each vial of the drug product contains the labeled amount of lulizumab, sorbitol, sodium phosphate monobasic, sodium phosphate dibasic, and water for injection. Sodium hydroxide solution may be added to adjust the solution pH to 5.9. A 30% overfill is included in each vial to account for vial, needle, and syringe (VNS) holdup.

Drug Product Preparation and Administration

To assure the sterility of the prepared solutions, the vials are single use. Because solutions of lulizumab may foam, shaking or excessive agitation of the vials should be avoided. Additionally, care must be taken to ensure the sterility of the prepared solution, as the drug product does not contain antimicrobial preservatives or bacteriostatic agents.

Lulizumab injection is administered via SC route either undiluted or diluted in 0.9% Sodium Chloride Injection (normal saline [NS]) to concentration range of 0.5-5 mg/ml. Dosing solutions of lulizumab concentration 5 mg/mL are prepared by direct injection of NS into the original drug product vial. Dosing solutions with lulizumab concentration lower than 5 mg/ml are prepared by injection of the drug product into an IV bag containing NS. A conventional, commercially available, disposable sterile plastic syringe of appropriate size should be used for the preparation and dilution of dosing solutions. Care must be taken to maintain the sterility of the prepared solutions. A 21-gauge, 1.5-inch sterile needle is recommended for withdrawal of the product from the vial or IV bag containing diluted lulizumab. A 27-gauge, 0.5-inch sterile needle is recommended for subsequent SC dosing. A sterile, commercially available, disposable 1-cc luer-lock polycarbonate syringe should be used for withdrawal and administration of the SC doses.

Subjects will be administered lulizumab pegol (BMS-931699) 25 mg SC on day 1 after transplantation then 12.5 mg SC weekly until day 77.

Recommended Storage and Use Conditions

lulizumab pegol (BMS-931699) injection should be stored refrigerated at 2°—8° C (36°—46° F) with protection from light and freezing. Prepared vials and syringes may be stored for up to 24 hours in a refrigerator, 2°to 8°C (36° to 46°F), and up to 4 hours of the 24-hour period may be at room temperature, 15°C to 25°C (59°F to 77°F). The prepared syringes and vials should be protected from light. Prior to administration, the drug product should reach room temperature by storing unrefrigerated for approximately 30 minutes before use.

6.2 Drug Accountability

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

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

All records regarding the disposition of the investigational product will be available for inspection.

6.3 Toxicity Prevention and Management

Infusion reactions are not expected, since the drug administration will be via the SC route. In the MAD study as well as study IM128027, injection site reactions were observed to be infrequent, low-grade and self-limiting (Shi, 2017). Participants will be monitored for 24 hours after the 1st dose for injection site reactions.

Two serious adverse events related to lulizumab infusion were noted in IM128027: serum sickness and systemic inflammatory response syndrome. These events were delayed reactions occurring hours after receiving several doses of the investigational agent. Both subjects required hospitalization and the reactions resolved with treatment.

There has been no clinical experience with overdose of lulizumab pegol (BMS-931699). Treatment of overdose of lulizumab pegol (BMS-931699) should consist of general supportive care. There is no known antidote for overdose with lulizumab pegol (BMS-931699).

Lulizumab therapy will be discontinued in participants with persistent or worsening abnormalities in liver function tests despite discontinuation of tocilizumab (see Section 7.2.1).

6.4 Premature Discontinuation of Investigational Agent

Study therapy may be prematurely discontinued for any participant for any of the following reasons:

1. Severe injection site reaction with the 1st dose
2. Severe acute cellular rejection (Banff 1b or higher) (see Section 13.2, Participant Stopping Rules and Withdrawal Criteria) or any acute clinical or subclinical antibody mediated rejection
3. Any diagnosis of malignancy other than basal cell carcinoma
4. Any diagnosis of tuberculosis, or invasive fungal infection
5. Grade 4 infection

Study therapy may also be prematurely discontinued for any participant if the investigator believes that the study treatment is no longer in the best interest of the participant.

6.5 Study Drug Access

The investigational drug product, lulizumab pegol (BMS-931699), will be supplied by the product manufacturer through a clinical trial agreement between the drug manufacturer (Bristol-Meyers Squibb) and the study sponsor DAIT NIAID. At the end of the study period, BMS will not continue to supply study drug. The site investigator is responsible to ensure the subject receives appropriate standard of care or other appropriate treatment in the independent medical judgement of the investigator to treat the condition under study.

7. Immunosuppressive Regimen

7.1 Induction Immunosuppression

7.1.1 Thymoglobulin

Study participants will be administered four doses of rabbit anti-thymocyte globulin (Thymoglobulin), total dose 6 mg/kg given in divided doses on the day of transplantation and days 1-3.

The first dose should be infused over at least 6 hours; doses on subsequent days should be infused over at least 4 hours. Premedication with methylprednisolone (see below), acetaminophen, and/or an antihistamine prior to each infusion is recommended. If the platelet count falls below 50,000 cells/mm^3 after receiving Thymoglobulin, the subsequent dose will be delayed until the platelet count is >/= 50,000 cells/mm^3.

7.1.2 Methylprednisolone

All study participants will receive Methylprednisolone 500 mg IV on the day of transplantation, 250 mg IV on day 1, and 125 mg IV on day 2 (prior to Thymoglobulin on days 1 and 2).

7.2 Maintenance Immunosuppression

7.2.1 Actemra® (tocilizumab)

7.2.1.1 Tocilizumab Formulation, Packaging, and Labeling

Tocilizumab is a recombinant humanized anti-human interleukin 6 (IL-6) receptor monoclonal antibody of the immunoglobulin IgG1κ (gamma 1, kappa) subclass with a typical H2L2 polypeptide structure. Each light chain and heavy chain consists of 214 and 448 amino acids, respectively. The four polypeptide chains are linked intra and inter-molecularly by disulfide bonds. Actemra® has a molecular weight of approximately 148 kDa. The antibody is produced in mammalian (Chinese hamster ovary) cells.

Intravenous Infusion

Actemra® injection is supplied as a sterile, preservative-free solution for further dilution prior to intravenous infusion at a concentration of 20 mg/mL. Actemra® is a clear, colorless to pale yellow liquid, with a pH of about 6.5. Single-dose vials are available for intravenous administration containing 80 mg/4 mL, 200 mg/10 mL, or 400 mg/20 mL of Actemra®. Injectable solutions of Actemra® are formulated in an aqueous solution containing disodium phosphate dodecahydrate and sodium dihydrogen phosphate dehydrate (as a 15 mmol per L phosphate buffer), polysorbate 80 (0.5 mg per mL), and sucrose (50 mg per mL).

Subcutaneous Injection

Actemra® injection is supplied as a sterile, clear, colorless to slightly yellowish, preservative free liquid solution for subcutaneous administration with a pH of approximately 6.0. It is supplied in a 1 mL ready-to-use, single-use prefilled syringe (PFS) with a needle safety device. Each prefilled syringe delivers 0.9 mL (162 mg) of Actemra®, in a histidine buffered solution composed of Actemra® (180 mg/mL), polysorbate 80, L-histidine and L-histidine monohydrochloride, L-arginine and L-arginine hydrochloride, L-methionine, and water for injection.

7.2.1.2 Tocilizumab Dosage, Preparation, and Administration

Package Insert Instructions for Intravenous Infusion

Actemra® for intravenous infusion should be diluted by a healthcare professional using aseptic technique as follows:

- Patients at or above 30 kg weight: use a 100 mL infusion bag or bottle, and then follow steps 1 and 2 below.

Step 1. Withdraw a volume of 0.9% or 0.45% Sodium Chloride Injection, USP, equal to the volume of the Actemra® injection required for the patient's dose from the infusion bag or bottle.

Step 2. Withdraw the amount of ACTEMRA for intravenous infusion from the vial(s) and add slowly into the 0.9% or 0.45% Sodium Chloride Injection, USP infusion bag or bottle. To mix the solution, gently invert the bag to avoid foaming.

- The fully diluted Actemra® solutions for infusion using 0.9% Sodium Chloride Injection, USP may be stored at 2° to 8°C (36° to 46°F) or room temperature for up to 24 hours and should be protected from light.
- The fully diluted Actemra® solutions for infusion using 0.45% Sodium Chloride Injection, USP may be stored at 2° to 8°C (36° to 46°F) for up to 24 hours or room temperature for up to 4 hours and should be protected from light.
- Actemra® solutions do not contain preservatives; therefore, unused product remaining in the vials should not be used.
- Allow the fully diluted Actemra® solution to reach room temperature prior to infusion.
- The infusion should be administered over 60 minutes and must be administered with an infusion set. Do not administer as an intravenous push or bolus.
- Actemra® should not be infused concomitantly in the same intravenous line with other drugs. No physical or biochemical compatibility studies have been conducted to evaluate the co-administration of Actemra® with other drugs.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If particulates and discolorations are noted, the product should not be used.
- Fully diluted Actemra® solutions are compatible with polypropylene, polyethylene and polyvinyl chloride infusion bags and polypropylene, polyethylene and glass infusion bottles.

<u>Package Instructions for Subcutaneous Injections</u>

Actemra® for subcutaneous injection is not intended for intravenous drip infusion.

- Assess suitability of patient for SC home use and instruct patients to inform a healthcare professional before administering the next dose if they experience any symptoms of allergic reaction. Patients should seek immediate medical attention if they develop symptoms of serious allergic reactions. Actemra® subcutaneous injection is intended for use under the guidance of a healthcare practitioner. After proper training in subcutaneous injection technique, a patient may self-inject Actemra® or the patient's caregiver may administer Actemra® if a healthcare practitioner determines that it is appropriate. Patients, or patient caregivers, should be instructed to follow the directions provided in the Instructions for Use (IFU) for additional details on medication administration.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Do not use Actemra® prefilled syringes (PFS) exhibiting particulate matter, cloudiness, or discoloration. Actemra® for subcutaneous administration should be clear and colorless to pale yellow. Do not use if any part of the PFS appears to be damaged.
- Patients using Actemra® for subcutaneous administration should be instructed to inject the full amount in the syringe (0.9 mL), which provides 162 mg of Actemra®, according to the directions provided in the IFU.

- Injection sites should be rotated with each injection and should never be given into moles, scars, or areas where the skin is tender, bruised, red, hard, or not intact.

7.2.1.3 Tocilizumab Study Dosing

Participants in CTOT-24 will receive 8 mg/kg of intravenous tocilizumab on day 2 after transplantation, then 162 mg subcutaneously every 2 weeks till day 168.

Actemra® is provided for study subjects enrolled in the trial. Actemra® designated for the study will be maintained in the research pharmacy and accountability is expected as according to Section 6.2.

7.2.1.4 Toxicity Prevention and Management for Tocilizumab

Neutropenia

As recommended by the manufacturer in the package insert, participants with ANC less than 2,000 per mm^3 will be excluded from participation. Neutrophil counts will be monitored weekly for 12 weeks after start of therapy and at least every 4 weeks thereafter until the end of therapy..

During the study, Actemra® dose will be maintained for participants with ANC >1000 /mm^3, temporarily held for participants with ANC 250-1000/mm^3 till the ANC recovers to >1000/mm^3, and discontinued for participants with ANC <250/mm^3.

In addition, the following mitigation strategy (**Table 3**) for subjects with neutropenia will be followed:

Table 3. Neutropenia Mitigation Strategy

ANC	STRATEGY	MEDICATION	RECOMMENDED DOSE ADJUSTMENT	MONITORING OF RESPONSE
1500 to <2000/mm^3	Adjust dose of anti-proliferative and stop concomitant medications that may be contributing to neutropenia	Everolimus	Reduce current dose by 25-50%	Weekly ANC When ANC improves to >2000/mm^3, slowly increase dose of anti-proliferative agent
		MMF (or Myfortic)	If on 1000 (720) mg bid: reduce to 500 (360) mg bid 750 (540) mg bid: reduce to 500 (360) mg bid 500 (360) mg bid: reduce to 250 (180) mg bid 250 (180) mg bid: hold	
		Valganciclovir	Discontinue	
1000 to <1500/mm^3	Adjust dose of anti-proliferative	Everolimus	Reduce current dose by 50%	Weekly ANC When ANC improves to >1500/mm^3, slowly resume anti-proliferative agent
		MMF (or Myfortic)	If on 1000 (720) mg bid: reduce to 500 (360) mg bid If on 750 (540) mg bid: reduce to 250 (180) mg bid If on 500 (360) mg bid: reduce to 250 (180) mg bid If on 250 (180) mg bid :hold	
250 to <1000/mm^3	Hold anti-proliferative **AND** Hold tocilizumab dosing until ANC improves	Everolimus	Hold	Weekly ANC When ANC improves to >1000/mm^3, resume tocilizumab When ANC improves to >2000/mm^3 for 2 weeks after resuming tocilizumab, slowly resume anti-proliferative
		MMF (or Myfortic)	Hold	
<250/mm^3	Adjust dose of anti-proliferative AND Discontinue tocilizumab	Everolimus	Hold	Weekly ANC When ANC improves to >500/mm^3, resume MMF at 250 mg bid or Everolimus at 0.75 mg bid
		MMF (or Myfortic)	Hold	

Thrombocytopenia

As per directions from the package insert, participants with a platelet count <100,000/mm^3 will be excluded from participation and platelet counts will be monitored at least every 4 to 8 weeks after start of therapy and at least every 3 months thereafter for all subjects.

During the study, Actemra® dose will be maintained for participants with platelet count >100,000 /mm^3, temporarily held for participants with platelet counts 50,000-100,000/mm^3 till the count recovers to >100,000/mm^3, and discontinued for participants with platelet counts <50,000/mm^3.

Elevated Liver Enzymes

As per directions from the package insert, participants with transaminase levels > 1.5 times ULN will be excluded from participation in the study. ALT and AST levels will be monitored at least 4 to 8 weeks after start of therapy and at least every 3 months thereafter. When clinically indicated, other liver function tests such as bilirubin will be considered.

During the study, Actemra® dose will be temporarily held for participants with AST or ALT 2-5 times ULN until the AST or ALT recover to <2 times ULN, and discontinued for participants with AST or ALT >5 times ULN.

7.2.2 Nulojix® (belatacept)

Nulojix® is indicated for prophylaxis of organ rejection in adults receiving a kidney transplant. As previously described, CTOT-24 participants will receive Nulojix®with Zortress®after induction with thymoglobulin and a 1-month corticosteroid taper.

7.2.2.1 Nulojix® Study Dosing

For CTOT-24 participants, Nulojix® (5mg/kg) will be administered every 4 weeks starting at day 84. Nulojix® will be started 1 week after the last study dose of lulizumab on day 77. Nulojix® will be administered for 52 weeks during the study. After 52 weeks, the site investigator will determine the best IS regimen for the subject.

Nulojix® is provided for study subjects enrolled in the trial. Nulojix® designated for the study will be maintained in the research pharmacy and accountability is expected as according to Section 6.2.

7.2.3 Zortress® (everolimus)

Zortress® is indicated for the prophylaxis of organ rejection in adult patients at low-moderate immunologic risk receiving a kidney transplant.

7.2.3.1 Zortress® Study Dosing

Zortress®will be started at 0.75 mg BID 14 days after transplantation. Zortress® will be titrated to target trough levels 3-8 ng/mL. Participants who do not tolerate everolimus will be switched to MMF. Participants with rare hereditary problems of galactose intolerance, the Lapp lactase deficiency or glucose-galactose mal absorption should not take everolimus as this may result in diarrhea and malabsorption; these participants should take MMF/MPA (Section7.2.5). Administration of medications known to interact with everolimus is allowed but trough levels should be carefully monitored and dosing titrated to maintain the target levels to minimize toxicity while maintaining efficacy. Everolimus will be administered for 52 weeks during the study. After 52 weeks, the site investigator will determine the best IS regimen for the subject.

7.2.3.2 Zortress® Toxicity Prevention and Management

In participants who develop any of the following conditions after initiation of Zortress® therapy, everolimus will be discontinued and mycophenolate mofetil will be started at 1000 mg twice daily.

1. New onset or worsened proteinuria with urine pr/cr >1 g/g
2. Severe hypercholesterolemia (LDL>190 mg/dL or triglycerides >500 mg/dL) not responsive to therapy with lipid lowering agents
3. Severe edema or fluid accumulations such as pleural or pericardial effusions

4. Interstitial pneumonitis
5. Severe mouth ulcers
6. Severe cytopenia (WBC <1,000/mm^3) (also see **Table 3. Neutropenia Mitigation Strategy**)
7. Any condition which in the determination of the investigator requires discontinuation of Zortress® therapy such as planned surgery.

7.2.4 Maintenance Corticosteroids

After the initial methylprednisolone doses, participants will commence a taper with oral prednisone: 60 mg on day 3, 30 mg on days 4-10, 20 mg on days 11-17, 10 mg on days 18-24, and then taper to final maintenance dose of 5 mg daily by day 28.

7.2.5 Mycophenolate Mofetil (MMF)/Mycophenolic Acid (MPA)

MMF/MPA will be started no later than one day after transplant at 1000/ 720 mg PO twice daily, provided the WBC count permits (see Table 3. Neutropenia Mitigation Strategy). Participants will stay on MMF/MPA till the everolimus level is within the therapeutic range. Participants who do not tolerate everolimus can also stay on or switch back to mycophenolate mofetil 1000 mg twice daily or 720 mg twice daily of Myfortic (mycophenolic acid) and remain in the trial (see 7.2.3.2 Zortress Toxicity Prevention and Management). As per standard of care, all Mycophenolate prescribers in the study will be required to enroll in the FDA mandated mycophenolate REMS (risk evaluation and mitigation strategy) program.

7.3 Treatment of Rejection

Study participants who develop mild acute cellular rejections (Banff type 1a) in this study will be treated with steroids and maintained on the study regimen. In participants with higher grades of acute cellular rejection (Banff type 1b and above) or any acute antibody mediated rejection, the study regimen will be discontinued and replaced with a tacrolimus-based regimen. These participants will go into safety follow up.

8. Other Medications

8.1 Anti-Infective Prophylactic Medications

All participants in the study will be required to receive CMV prophylaxis with valganciclovir for a minimum of 3 months, as tolerated. Please refer to **Table 3. Neutropenia Mitigation Strategy** for management of medications known to contribute to neutropenia. If valganciclovir is stopped to mitigate neutropenia, weekly CMV PCR monitoring will be performed until 12 weeks after transplant.

Other anti-infective prophylaxis medications for the prevention of urinary tract infections, oral candidiasis and pneumocystis pneumonia will be administered per standard of care for kidney transplant recipients at each clinical center.

8.2 Prohibited Medications/Products

8.2.1 Non-Leukoreduced Blood Products

For participants requiring treatment, leukoreduced blood products should be used whenever possible.

8.3.2 Vaccinations

The use of live vaccines will be proscribed during trial participation, as per standard of care for kidney transplant recipients. Examples include (but are not limited to) the following: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, live varicella, and TY21a typhoid vaccines.

8.3.3 Cytochrome P450 3A4 and P-gp

Grapefruit and grapefruit juice inhibit cytochrome P450 3A4 and P-gp activity and should therefore be avoided with concomitant use of everolimus.

9. Study Mandated Procedures

9.1 Renal Transplant Biopsy

Study participants will undergo biopsies at 6 months after transplantation. Research tissue specimens will be collected during these procedures for mechanistic assays. In the case of clinically indicated ("for-cause") biopsies, unstained slides from FFPE kidney tissue will be sent to the central lab for mechanistic studies. In addition, digital images will be provided to the central pathologist in order to obtain a central read for all for-cause biopsies.

9.2 Blood Draws

Blood draws are necessary after kidney transplantation to monitor allograft function. Whenever possible, study participants will have additional blood drawn for research purposes at time points when standard of care testing is already being done. Study participants will also have non-standard of care blood draws for research purposes.

10. Study Visits

10.1 Enrollment

The research study will be explained in lay terms to each potential research participant prior to transplantation. The potential participant will sign an informed consent form before undergoing any study procedures. Once the informed consent has been signed, the participant is considered enrolled in the study and will be assigned a unique participant number.

10.2 Donor Visit

Donors will be asked to consent for one study visit where blood will be collected to allow for mechanistic assays. See Appendix 1 for the donor schedule of events. Minimal donor medical information will be collected (e.g. demographics, HLA typing, CMV serology status).

10.3 Screening/Baseline Visit

The purpose of the screening period is to confirm eligibility to continue in the study.

If not already available in the participant's medical record (e.g. testing performed for transplant evaluation), testing should be performed according to the schedule of events (Appendix 2) to complement available results and confirm study eligibility.

10.4 Study Visits

10.4.1 Medication Schedule

Study participants will receive protocol directed medications on Day 0, 1, 2, 3 and 7 after transplant. Thereafter, participants will return to the transplant clinic for study medications (i.e. lulizumab, belatacept, and tocilizumab) weekly until 12 weeks after transplant; biweekly until 26 weeks after transplant; and then monthly until study completion. See Appendix 3 Medication Schedule.

10.4.2 Follow-Up Visits

In addition to study medication administration, participants will have study assessments (i.e. physical exam, review of medical history and AE, laboratory testing and mechanistic blood collections) throughout the study (Appendix 2). Study follow-up visits will be aligned with medication administration visits whenever possible.

10.5 Unscheduled Visits/ Clinically Indicated Biopsies

If creatinine increases or other concerns arise between scheduled study visits, participants will be instructed to return to the study site for an "unscheduled" visit. Study assessments will be performed, and research specimens collected if a participant has a clinically indicated biopsy. Please see Appendix 2 for details.

10.6 Visit Windows

Study visits should take place within the time limits specified in the schedule of events.

11.Mechanistic Assays

The mechanistic goal of the study is to determine the impact of the proposed novel immunosuppression regimen on the alloimmune profile with a particular emphasis on mechanisms of tolerance. Specific assays to be performed are described below.

11.1 Peripheral Blood Assays

Peripheral blood samples will be collected from the donor and recipient pre-transplant and from the recipient at 1, 3, 6, 9 and 12 months post-transplant to evaluate the impact on the allo-immune profile of the key components of the novel immunosuppression regimen. We are principally interested in comparing the effect of lulizumab pegol (BMS-931699) to belatacept. Comparisons will be made within the cohort across these time points.

Time point	Therapeutic agents
1 and 3 months	Lulizumab + tocilizumab
6 months	Belatacept + tocilizumab
9 and 12 months	Belatacept

11.1.1 Multiparameter Flow Cytometry (MFC):

Several panels of MFC will be used to profile potential changes in peripheral blood leukocyte subsets during the study. The panel design is shown in below in **Table 4.** These panels will allow in-depth phenotyping of T cells, Tregs, and B cells along with profiling of monocytes and NK cells.

Table 4. MFC Panel

Panel name	Markers
Leukocyte subsets	CD45, CD3, CD7, CD19, CD14, CD16, CD56, HLA-DR
T cell phenotype	CD45, CD3, CD4, CD8, CD28, CD2, CD45RO, CD57, PD-1, CCR7
Treg	CD45, CD3, CD4, CD127, CD25, FOXP3, HELIOS, CTLA-4, CD38, CD39
B cell	CD45, CD19, CD24, CD27, CD38, IgD, MTG

11.1.2 Alloreactive T cell Frequency (ATF) assay

This assay was developed in the UCSF Transplantation Laboratory by Q. Tang, PhD to measure the frequency of circulating donor-reactive CD4 conventional T cells, CD8 T cells and Tregs. The Tang laboratory has used this assay to analyze 300+ samples collected from kidney, liver, lung, and islet transplants and maternal fetal pairs (see **Figure 5**).

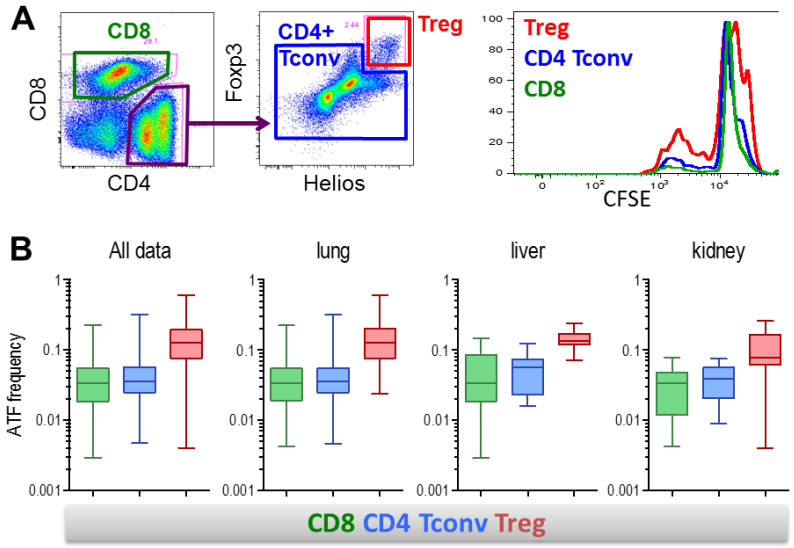


Figure 5.

ATF Assay. A. An example of ATF assay flow profile. B. ATF results on transplant patients accumulated thus far n=265.

This assay uses CD40L-stimulated donor B cells to stimulate CFSE-labeled recipient PBMC, with proliferation of T cell subsets determined on day 4 using flow cytometry. Using this assay in kidney transplant recipients, the Tang Laboratory has found significantly higher donor-specific CD8 T cells in rejectors vs. non-rejectors in HIV+ kidney transplant recipients. To determine the contribution of anergy, exhaustion, and deletion/terminal senescence, we will also add cytokines (IL-2, IL-7 and/or IL-15), antibodies to checkpoint molecules PD-1, LAG3, and TIM3, or both during stimulation with donor antigen presenting cells. The rationale is that cytokines and checkpoint inhibitors will rescue anergic and exhausted cells, respectively whereas unresponsiveness due to deletion or terminal senescence cannot be rescued. Controls stimulated with antibodies to CD3 and CD28 will be included to determine if T cell dysfunction is donor specific. This will allow us to categorize each sample and each T cell subset as normal, anergic, exhausted, and deleted or terminally senescent. We will then compare CD4 T conv, CD8 and Treg responses across time in the study.

11.1.3 Donor Antigen Stimulated Gene Expression

By analyzing gene expression in PBMC stimulated with donor antigen presenting cells, the Tang Laboratory has found that genes implicated in T cell checkpoint inhibition (CTLA-4, SFASL, NFATC1, NFATC2, LAG3 and HAVCR2) were expressed at higher level in a never-rejector vs. an early rejector among HIV+ liver transplant recipients (see **Figure 6**). In this assay, recipient PBMC are stimulated with donor antigen presenting cells (either activated B cells or monocyte-derived dendritic cells), and the cells are collected on day 3 for gene expression analyses of a panel of 96 genes using qPCR. This assay not only measures frequency of responders, but also the type of response induced by donor antigens. We will use this assay to determine if the different components of the novel regimen alter donor-specific responses to favor tolerance.

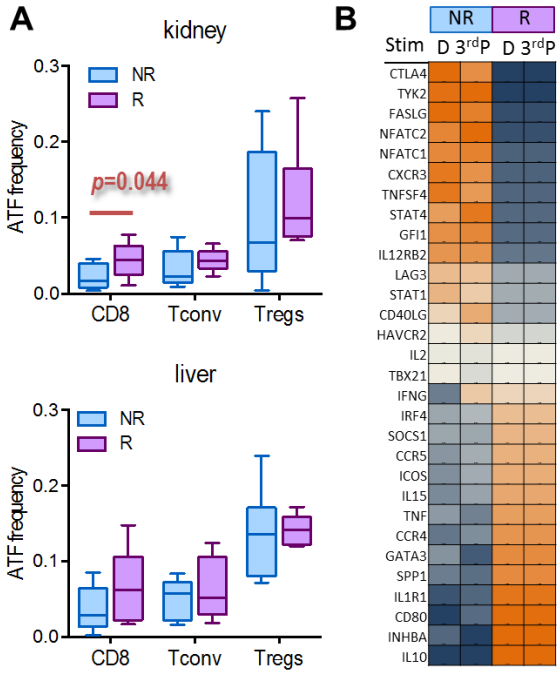


Figure 6. *Donor-specific T cell response of HIV+ transplant patients. A. ATF comparison of rejector and non-rejector HIV+ kidney (n=8R, 8NR, top) and liver (n=6R, 9NR, bottom) transplant patients. B. Donor (D) and 3rd party (3rdP) sBc stimulated gene expression in pre-transplant PBMC of a liver transplant early rejector and a non-rejector.*

11.1.4 In Vitro Treg Suppression

Donor-specific suppression activity of recipient Tregs will be measured by using irradiated donor PBMC as stimulators. General suppressive activity will be measured by stimulating Tregs with anti-CD3 and anti-CD28. Recipient PBMC will be collected and banked before transplant and at specified time points during therapy. PBMC collected before transplant will be used as standard responders so that changes observed in the amount of suppression can be attributed to the activity of Tregs collected at different points during the study.

11.1.5 Kidney Solid Organ Response Test (KSORT)

The Sarwal Laboratory has developed the kSORT blood QPCR assay as a noninvasive tool to detect high risk of acute rejection of renal transplants as early as 3 months prior to detection by the biopsy (Roedder S, 2014). The KSORT gene signature comprises the following genes (CFLAR, DUSP1, ITGAX, RNF130, PSEN1, NKTR, RYBP, NAMPT, MAPK9, IFNGR1, CEACAM4, RHEB, GXMK, RARA, SLC25A37, EPOR, RXRA) developed into an assay called kSORT (kidney solid organ response test) (Table 5). kSORT provides an AR risk score where a score >0 indicates acute rejection and a score <0 indicates no acute rejection. The SORT genes will be analyzed in our trial to define their association with inflammation and borderline rejection on kidney biopsies and to examine whether how they are modified in different phases of the study investigational regimen.

Gene ID	Protein name	Function
DUSP1	Dual specificity protein phosphatase-1	Dephosphorylates MAP kinase to regulate cell cycle
CFLAR	CASP8 and FADD-like apoptosis inhibitor	Cytosolic protein that inhibits tumor necrosis factor related apoptosis
ITGAX	Integrin alpha–X	Membrane receptor for fibrinogen that mediates cell-cell interaction during inflammatory responses; important in monocyte adhesion and chemotaxis
NAMPT	Nicotinamide phosphoribosyltransferase	Catalyzes formation of NAD; also exists as nonsecreted form that acts as cytokine and adipokine; plays a role in regulating circadian clock
MAPK9	Mitogen-activated protein kinase 9	Activated by cytokines and/or stress response to promote cell proliferation, differentiation, migration, transformation and programmed cell death
RNF130	E3 ubiquitin-protein ligase RNF130	Cytoplasmic zinc-binding protein
IFNGR1	Interferon-receptor gamma-1	Membrane protein that binds interferon gamma; mutation causes Immunodeficiency 27A
PSEN1	Presenilin-1	Stimulates cell-cell adhesion; may play role in signaling and apoptosis; mutations associated with familial early-onset Alzheimer's
RYBP	RING1 and YY1-binding protein	Nucleic and cytosolic protein that inhibits transcription, promotes apoptosis
NKTR	NK-tumor recognition protein	Membrane protein that is a component of a putative tumor recognition complex on the surface of NK cells; binds cyclosporin A
SLC25A37	Mitoferrin-1	Mitochondrial iron transporter that takes up iron for developing erythroid cells
CEACAM4	Carcinoembryonic antigen-related cell adhesion molecule 4 (CD66a)	Surface protein found on lymphocytes for cell to cell adhesion
RARA	Retinoic acid receptor alpha	Suppresses transcription when unbound, loss of suppression when bound, associated with spermatocyte survival when bound
RXRA	Rxra protein	Nuclear protein that regulates transcription
EPOR	Erythropoietin receptor	Promotes erythroblast proliferation and differentiation when bound by EPO
GZMK	Granzyme K	Serine protease released by NK or T cells for killing of cancerous cells, viruses, or bacteria
RHEB	GTP-binding protein Rheb	Promotes proliferation and inflammation through activation of mTOR complex

Table 5. kSORT genes

11.1.6 HLA Antibody Testing for DSA

HLA antibody testing will be conducted using singe antigen bead testing at 1, 3, 6 and 12 months post-transplant to track the development of any donor specific antibody. The testing will be done at the UCSF Immunogenetics Laboratory.

11.2 Biopsy Assays

These assays will be performed on kidney tissue collected during protocol kidney biopsies at 6 months post-transplant and for any cause biopsies during the first year post-transplant.

11.2.1 Graft Histology

Graft tissue will be obtained per study protocol at 6 months post-transplant. Histologic examination will be done at the University of California at San Francisco in the Laszik Laboratory. Biopsy results will be reported using the Banff '07 schema for acute rejection and chronic allograft injury. The centers will send renal tissue (1 core in a formalin jar).

For sites with clinically indicated biopsies performed at other times during the study, digital images of the local case (i.e. H&E, PAS, Trichrome) will be sent to the Laszik Laboratory for a central pathology read. No extra cores will be collected specifically for the study at the time of clinically indicated biopsies, but available renal tissue will be sent to Laszik Laboratory for mechanistic assays.

Primary data will be sent to the Statistical and Clinical Coordinating Center for final analysis. High-resolution images of entire sections of all stains from each biopsy will be archived. Slide images will be stored for additional analysis, if needed.

11.2.2 Multi-parameter Immunofluorescence and In Situ Hybridization

The Laszik Laboratory has developed various sets of IF stains on FFPE kidney biopsies using a combination of inflammatory cell markers to measure inflammation (1) quantitatively and (2) qualitatively. The total inflammatory load is measured by using leukocyte common antigen (LCA) as a marker while the composition of the inflammation in kidney biopsies performed during the study.

The Laszik Laboratory at UCSF has also adapted the RNAScope® in situ hybridization (ISH) platform to analyze FFPE biopsies for mRNA expression. This is a novel and highly sensitive ISH technology with very high specificity. To determine the cellular source of IL-6 mRNA expression, we have successfully applied multiplex IF stains to the same sections that the ISH was performed on. The quality of both the ISH and multiplex IF signals is sufficient to apply computer-assisted image analysis tools for quantitation of intragraft IL-6 levels.

Renal tissue will be collected at 6 months post-transplant for these analyses.

11.2.3 Gene Expression by Nanostring

The Laszik Laboratory at UCSF has also developed a novel method of conducting gene expression analyses on FFPE-embedded kidney biopsy tissue with the NanoString nCounter platform using a customized panel of 800 genes. Multivariate regression analysis was carried out to compare log2 transformed gene-expression levels in biopsies with acute cellular rejection and acute antibody mediated rejection compared to normal biopsies (12 biopsies in each group). They found that cases with ACR and AMR showed increased expression of IL6 compared to normal cases (see **Table 6.**) supporting a role for IL-6 blockade. This technology will be used in our study to measure the gene expression of IL-6 as well as other inflammatory cytokines in the IL6/Th17 and costimulation pathways.

Table 6. ACR and AMR mRNA Gene Expression

mRNA	Log2 fold change	95% confidence limits	corrected p-value
IL6 (AMR vs. Normal)	2.48	1.51- 3.44	<0.0005
IL6 (ACR vs. Normal)	2.00	1.06- 2.94	<0.0005

11.3 Urinary Biomarkers of Graft Inflammation and Rejection

11.3.1 Urine Cytokine Analysis

The Sarwal Lab has analyzed whole genome expression profiles in 13 independent solid organ transplant cohorts and defined a 12-gene common rejection module (CRM) in biopsy tissue for the prediction of acute rejection (Khatri P, 2013). They have now developed a non-invasive assay for mRNA analysis for the CRM genes in urine (uCRM) which will used in this study to assess the impact on graft inflammation of the novel immunosuppressive regimen used in this study. In addition, we will also use urinary CXCL-9 which has been previously validated as a biomarker of immune mediated graft injury (Hricik, 2013).

12. Biospecimen Storage

Biological specimens obtained under this protocol may be used in future assays to reevaluate biological responses as additional research tests are developed over time. These specimens will be collected at time points already scheduled for the core mechanistic studies, in order to allow specimens to be stored for use in new assays that have yet to be optimized or conceived, or assays performed by other CTOT members for cross-validation studies. Appropriate informed consent will be obtained for both the collection and storing of samples. The specimens from these evaluations may be stored beyond the funding period. During the funding period, samples will be identifiable, which means samples will be coded with a participant ID number that could be directly linked to the participant and the participant's medical record. When the funding period is over, samples will be anonymized, which means a sample that was previously identifiable, has had all identifiers removed and can no longer be linked back to the participant or the participant's medical record by any means.

Study participants will be informed that they may be approached about additional clinical evaluations or studies that have received the full approval of the NIAID as new evaluations are identified. If additional evaluations are determined to be desirable, this protocol (and other appropriate study documents, e.g., the informed consent and the statistical analysis plan) will be amended and submitted to the appropriate regulatory authorities, ethics committees, and IRBs for approval. Each participant's signature will be obtained on the revised informed consent form before additional evaluations are performed. The specimens from these evaluations may be stored up to the end of the contract—approximately 5 years, or longer if the contract is extended.

13. Criteria for Participant and Study Completion and Premature Study Termination

13.1 Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up.
2. The participant is "lost to follow-up" 3 months after the date of a missed study visit (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed).
3. The participant dies.
4. The Investigator no longer believes participation is in the best interest of the participant.
5. CTCAE Grade 3 or higher injection site reaction for lulizumab pegol (BMS-931699).
6. Development of delayed hypersensitivity reactions such as serum sickness.
7. Development of cytokine release syndrome or systemic inflammatory response syndrome.
8. Acute cellular rejection Banff Grade 2A or higher; acute clinical or subclinical antibody mediated rejection of any grade.
9. TB or any invasive fungal infection.
10. Development of acute diverticulitis or intestinal perforation
11. Newly diagnosed malignancy, including PTLD. A participant who develops completely resectable non-melanoma skin cancer can remain in the study.
12. Development of PML.
13. Development of COVID-19.

13.2 Participant Replacement

Participants who receive at least 80% of the doses of lulizumab pegol (BMS-931699) and tocilizumab described in the study protocol will not be replaced and will be included in the per protocol analysis. Those who receive less than 80% of either agent will be analyzed in the intent-to-treat group. Enrollment will continue until there are 10 subjects in the per protocol group.

13.3 Follow-up after Early Study Withdrawal

Participants who are withdrawn from the study for any reason will undergo safety follow up till 1 year post-transplant (Appendix 4 Safety follow up schedule). If a participant is withdrawn from the study and does not wish to follow up till 1 year post-transplant, then he/she will be asked to complete a final visit.

13.4 Study Stopping Rules

Any of these events will stop trial enrollment and require DSMB review (See Section 14.8.2). Participants who have already received the first dose of the investigational agent will remain on study protocol directed therapy during the data review period.

1. When 5 or more treated participants receive less than 80% of the expected total number of doses of either lulizumab pegol (BMS-931699) or tocilizumab
2. Banff 2A or higher grade acute cellular rejections in any one of the first 5 participants in the first 6 months post-transplant (**Table 7**)

3. Antibody-mediated rejection (of any grade) in any one of the first 5 participants in the first 6 months post-transplant (**Table 7**)
4. Any TB or invasive fungal infection
5. Any diagnosis of PTLD or PML
6. Any graft loss
7. Any death
8. Grade 4 infections in two participants

Additionally, an expedited safety review will also be conducted by the DSMB if there are ≥2 episodes of acute rejection in 2 different subjects at any time during the study. During the process of data collection for the safety review and analysis by the DSMB, enrollment will be paused in the study, but others study operations can continue including the recruitment of potential participants, provision of study drug to previously enrolled participants.

Table 7. Rejection Episodes, Threshold of 25%

Number of Subjects with Event	Number of Enrolled Subjects Total	Cumulative Incidence Rate (%)	Lower 95% Exact Confidence Limit (%)
2	2	100.00	22.36
3	**3**	**100.00**	**36.84**
2	4	50.00	9.76
3	**4**	**75.00**	**24.86**
3	5	60.00	18.93
4	**5**	**80.00**	**34.26**
3	6	50.00	15.32
4	**6**	**66.67**	**27.13**
4	7	57.14	22.53
5	**7**	**71.43**	**34.13**
4	8	50.00	19.29
5	**8**	**62.50**	**28.92**
4	9	44.44	16.88
5	**9**	**55.56**	**25.14**
5	10	50.00	22.24
6	**10**	**60.00**	**30.35**

**Highlighted and bolded rows meet the threshold for stopping the study; with a threshold of 25% the study would not be stopped until at least 3 subjects were enrolled.*

14. Safety Monitoring and Reporting

14.1 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 those data. Adverse events that are classified as serious according to the definition of health authorities must be reported promptly (per Section 14.5, *Reporting of Serious Adverse Events and Adverse Events*) to the sponsor (DAIT/NIAID). Appropriate notifications will also be made to site principal investigators, Institutional Review Boards (IRBs), and health authorities.

Information in this section complies with *ICH Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting, ICH Guideline E-6: Guideline for Good Clinical Practice*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 5: http://ctep.cancer.gov/reporting/ctc.html.

14.2 Definitions

14.2.1 Adverse Event (AE)

Any untoward or unfavorable medical occurrence 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 adverse events in the 1996 International Conference on Harmonization E-6 Guidelines for Good Clinical Practice) (from 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#Q2)

14.2.2 Suspected Adverse Reaction (SAR)

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

14.2.3 Unexpected Adverse Event

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator Brochure or package insert or is not listed at the specificity, severity or rate of occurrence that has been observed; or, is not consistent with the risk information described in the general investigational plan or elsewhere in the IND. Please also see Section 14.5.2.2.

"Unexpected" also refers to adverse events or suspected adverse reactions that are mentioned in the Investigator Brochure or package insert as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation (21 CFR 312.32(a)Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or Sponsor [add DAIT/NIAID or other Sponsor, *if applicable]*, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death.
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or Sponsor [add DAIT/NIAID or other Sponsor, *if applicable],* 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 events that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they 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 are not to be reported as an SAE unless hospitalization is prolonged due to complications.

14.2.4 Other Significant Adverse Events

Overdose, potential drug-induced liver injury (DILI), or cancer must be reported as serious adverse events. DILI is defined as:

- ALT or AST elevation >3 times upper limit of normal (ULN) AND
- Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase) AND
- No other immediately apparent possible causes of ALT or AST elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drugs(s) known to be hepatotoxic.

In addition, any confirmed cases of COVID-19 infection of any grade in a study subject must be reported as a Serious Adverse Event. If no other serious criteria are met for DILI or COVID-19 infection, the event should be considered of medical importance.

14.3 Grading and Attribution of Adverse Events

14.3.1 Grading Criteria

The study site will grade the severity of adverse events experienced by the study subjects according to the criteria set forth in the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE) Version 5. 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 adverse events. The NCI-CTCAE has been reviewed by the study team and has been deemed appropriate for the subject population to be studied in this protocol.

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

Grade 1 = mild adverse event.

Grade 2 = moderate adverse event.

Grade 3 = severe and undesirable adverse event.

Grade 4 = life-threatening or disabling adverse event.

Grade 5 = death.

Events grade 2 or higher will be recorded on the appropriate AE case eCRF for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent adverse event is defined as an increase in grade from

baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to baseline will also be recorded as adverse events, but are not treatment-emergent. 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 adverse event if changes in therapy or monitoring are implemented as a result of the event/result.

14.3.2 Attribution Definitions

The relationship, or attribution, of an adverse event to the study therapy regimen or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE SAE eCRF. Final determination of attribution for safety reporting will be determined by DAIT/NIAID. The relationship of an adverse event to study therapy regimen or procedures will be determined using the descriptors and definitions provided in **Table 8**.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: http://ctep.cancer.gov/reporting/ctc.html.

Table 8. Attribution of Adverse Events

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

Attribution assessment for the following study interventions and procedures will be made when a SAE is reported:

Investigational Products:

1. lulizumab pegol (BMS-931699)

Immunosuppressive Regimen:

1. Thymoglobulin
2. Corticosteroids
3. Tocilizumab
4. Belatacept
5. Everolimus
6. Mycophenolate Mofetil/Mycophenolic Acid

Study Mandated Procedures and Medications:

1. Kidney biopsy
2. Blood Draw

14.4 Collection and Recording of Adverse Events

14.4.1 Collection Period

Adverse events will be collected from the time of first study procedure, until a participant completes study participation or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study. Donor adverse events occurring within 24 hours after study mandated blood draw will be collected if the AE meets serious criteria.

14.4.2 Collecting Adverse Events

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

- Observing the subject.
- Interviewing the subject [e.g., using a checklist, structured questioning, diary, etc.].
- Receiving an unsolicited complaint from the subject.
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an adverse event, as defined in Section 14.3, *Grading and Attribution of Adverse Events.*

14.4.3 Recording Adverse Events

Throughout the study, the investigator will record adverse events and serious adverse events as described previously (Section 14.2, *Definitions)* on the appropriate AE/SAE eCRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or until the end of study participation, or until 30 days after the subject prematurely withdraws (without withdrawing consent)/or is withdrawn from the study, whichever occurs first.

14.5 Reporting of Serious Adverse Events and Adverse Events

14.5.1 Reporting of Serious Adverse Events to Sponsor

This section describes the responsibilities of the site investigator to report serious adverse events to the sponsor via the AE/SAE eCRF. Timely reporting of adverse events is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all serious adverse events (see Section 0, *Serious Adverse Event)*, regardless of relationship or expectedness within 24 hours of discovering the event.

For serious adverse events, all requested information on the AE/SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. As additional details become available, the AE/SAE eCRF will be updated and submitted.

14.5.2 Reporting to Health Authority

After an adverse event requiring 24-hour reporting (per Section 14.5.1, *Reporting of Serious Adverse Events to Sponsor*) is submitted by the site investigator and assessed by DAIT/NIAID, there are two options for DAIT/NIAID to report the adverse event to the appropriate health authorities:

14.5.2.1 Annual Reporting

DAIT/NIAID will include in the annual study report to health authorities all adverse events classified as:

- Serious, expected, suspected adverse reactions (see Section 14.2.2, *Suspected Adverse Reaction,* and Section 14.2.3, *Unexpected Adverse Event*).
- Serious and not a suspected adverse reaction (see Section 14.2.2, *Suspected Adverse Reaction*).
- Pregnancies.

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

14.5.2.1.1 Frequent Infections in SOT Not Listed as "Expected' in the Lulizumab IB

Though infections are frequently observed in immunosuppressed kidney transplant recipients (Abbott, 2004) (Abbott K., 2001) (Alangaden G. T., 2006) (Alangaden G. , 2007) (Karuthu, 2012) (Parasuraman, 2013) (Pelle, 2007) (Silva, 2010) (Veroux, 2008), the Reference Safety Information in BMS' lulizumab IB (BMS-931699) does not list the occurrence of infections as expected SARs. Given the high frequency of infections anticipated in the trial population, and the inability to identify infection causality (lulizumab vs other immunosuppressive drugs), the CTCAE v5.0 Grade 1 to 3 infections listed below of any etiology, (**Table 9**) will be reported in the IND Annual Report.

Table 9: Frequent Infections in Solid Organ Transplant Recipients

Most frequent infections in kidney transplant patients	• Bacterial Urinary Tract Infection (UTI) • Bacterial Pneumonia • Bacteremia • Bacterial Sepsis
Nosocomial and surgically related infections	• Infection with antimicrobial-resistant species: methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant enterococci, Candida species (non-albicans) • Aspiration pneumonia • Catheter, stent infection • Wound infection • Anastomotic leaks, complications and ischemia • *Clostridium difficile* colitis
Activation of Latent Infection (relapsed, residual, opportunistic)	• Polyomavirus BK infection, nephropathy • Hepatitis C virus infection • Hepatitis B virus infection • Herpes virus infections: Herpes simplex virus, Cytomegalovirus, Varicella-zoster virus, Epstein-Barr virus
Recipient-derived infections due to colonization	• Pseudomonas
Common Viral Respiratory Illnesses	• Adenovirus infection • Rhinovirus infection • Influenza infection • Parainfluenza • RSV • SARS-CoV-2 infection
Common Viral GI Illnesses	• Norovirus • Enterovirus • Adenovirus • Rotavirus • Astrovirus • Sapovirus

14.5.2.2 Expedited Safety Reporting

This option, with 2 possible categories, applies if the adverse event is classified as one of the following:

Category 1: **Serious and unexpected suspected adverse reaction** [**SUSAR]** (see Section 14.2.2, *Suspected Adverse Reaction* and Section 14.2.3, *Unexpected Adverse Event* and 21 CFR 312.32(c)(1)i).

The sponsor shall report any suspected adverse reaction that is both serious and unexpected in an expedited manner. Expedited reporting of infections will comprise only CTCAE v5.0 ≥ Grade 4 events. All infections will be reported in the AR. The sponsor shall report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study drug and the adverse event, such as:

1. A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, hepatic injury, or Stevens-Johnson Syndrome);
2. 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 (e.g., tendon rupture);
3. An 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.

Category 2: **Any findings from studies that suggests a significant human risk**

The sponsor shall report any findings from other epidemiological studies, analyses of adverse events within the current study or pooled analysis across clinical studies or animal or *in vitro* testing (e.g. mutagenicity, teratogenicity, carcinogenicity) 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 package insert or other aspects of the overall conduct of the study.

DAIT/NIAID or other Sponsor] shall notify the FDA and all participating investigators of expedited Safety Reports within 15 calendar days; unexpected fatal or immediately life-threatening suspected adverse reaction(s) shall be reported as soon as possible or within 7 calendar days.

14.5.3 Reporting of Adverse Events to IRBs/IECs

All investigators shall report adverse events, including expedited reports, in a timely fashion to their respective IRBs/IECs in accordance with applicable regulations and guidelines. All Safety Reports to the FDA shall be distributed by DAIT/NIAID to all participating institutions for site IRB/IEC submission.

14.6 Pregnancy Reporting

The investigator shall be informed immediately of any pregnancy in a study subject. A pregnant subject shall be instructed to stop taking study medication. These subjects will revert to standard of care immunosuppression. The investigator shall counsel the subject and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant subject shall continue until the conclusion of the pregnancy.

The investigator shall report to the SACCC all pregnancies within 1 business day of becoming aware of the event using the Pregnancy eCRF. All pregnancies identified during the study shall be followed to conclusion and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC when details about the outcome are available. The SACCC will notify the sponsor and provide medical summaries of all pregnancy reports.

Information requested about the delivery shall include:

- Gestational age at delivery
- Birth weight, length, and head circumference
- Gender
- Appearance, pulse, grimace, activity, and respiration (APGAR) score at 1 minute, 5 minutes, and 24 hours after birth, if available
- Any abnormalities.

All pregnancy complications that result in a congenital abnormality, birth defect, miscarriage, and medically indicated abortion - an SAE shall be submitted to the SACCC using the SAE reporting procedures described above.

In addition, any pregnancy occurring during participation in CTOT-24 will be reported to the Mycophenolate, Actemra® and National Transplant pregnancy registries, as applicable.

14.7 Reporting of Other Safety Information

An investigator shall promptly notify the site IRB as well as the SACCC and sponsor when an "unanticipated problem involving risks to subjects or others" is identified, which is not otherwise reportable as an adverse event.

14.8 Review of Safety Information

14.8.1 Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive periodic reports from the SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the study site(s) on appropriate eCRFs.

In addition, the Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SACCC.

14.8.2 DSMB Review

14.8.2.1 Planned DSMB Reviews

The Data and Safety Monitoring Board (DSMB) shall review 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.

The DSMB will be informed of an Expedited Safety Report in a timely manner.

14.8.2.2 Ad hoc DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the DSMB may be called upon for *ad hoc* reviews. The DSMB will review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID. In addition, any of the events described under Study Stopping Rules (Section 13.4) will trigger an *ad hoc* comprehensive DSMB Safety Review.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

15. Statistical Considerations and Analytical Plan

15.1 Overview

This is a pilot, multi-center, single arm, prospective trial using the investigational product, lulizumab pegol (BMS-931699), in the context of a novel immunosuppression regimen in primary living donor kidney transplant recipients at low immunologic risk. The scientific objective is to examine the safety and mechanisms of transplant immune regulation via pharmacologic modulation of Tregs. 10 participants will be enrolled and receive the study directed therapy for 1 year. The primary safety endpoint (section 3.2) is the proportion (95% CI) of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at 6 months after transplantation.

The secondary safety endpoint (section 3.3) is the proportion (95% CI) of participants who remain free of biopsy-proven acute T-cell mediated or antibody-mediated rejection as defined by Banff criteria at 12 months after transplantation.

Mechanistic endpoints include describing the frequency of circulating Tregs at 3, 6 and 12 months post-transplant, Treg suppressive activity at 3, 6 and 12 months post-transplant using irradiated donor PBMC as stimulators and measuring cytokine production in response to anti-CD3 and anti-CD28, alloreactive T cell frequency at 3, 6 and 12 months post-transplant, and the expression of T cell checkpoint inhibition related genes at 3, 6 and 12 months post-transplant.

Study participants will continue to be followed for 24 months after transplantation to document safety events.

15.2 Measures to Minimize Bias

All biopsies will be read by a central pathologist and central reads will be used to assess all stopping rules and endpoint analyses. Although the study is open-label, the mechanistic analyses of recipient specimens at the central laboratories will be blinded with respect to the status of the participant in the study.

15.3 Analysis Plan

Statistical analyses of the safety and clinical outcomes will be descriptive for the analysis samples defined below in section 15.4.1., employing standard methods for the estimation of binomial proportions and their exact two-sided 95% confidence intervals. Statistical analyses of the mechanistic outcomes will be exploratory in nature. The plans for statistical analyses of study data will be described in more detail in a Statistical Analysis Plan (SAP).

15.3.1 Analysis Populations.

Intent-to-Treat (ITT) sample: all enrolled and transplanted participants.

Safety sample: all participants that receive at least one dose of either lulizumab pegol (BMS-931699) or tocilizumab.

Per Protocol (PP) sample: all participants that receive at least 80% of the doses of lulizumab pegol (BMS-931699) and tocilizumab as described in section 3.1.

15.3.2 Analysis of Primary Endpoint

The primary safety endpoint, defined in section 3.2, will be descriptively summarized with a point estimate and two-sided, 95% exact binomial confidence interval. The numerator will be the number of participants that meet the primary safety endpoint and will be analyzed using the safety sample. A sensitivity analysis of the primary endpoint will also be performed using the per protocol (PP) sample.

15.3.3 Analysis of Secondary Endpoint

The secondary safety endpoint, defined in section 3.3, will be descriptively summarized with a point estimate and two-sided, 95% exact binomial confidence interval. The numerator will be the number of participants that meet the secondary safety endpoint and will be analyzed using the safety sample.

15.3.4 Analyses of Exploratory Endpoint(s)/Outcome(s)

15.3.5 Descriptive Analyses

Disposition of participants will be summarized in all participants that receive at least one dose of lulizumab pegol (BMS-931699) or tocilizumab. Standard descriptive statistics for continuous and categorical variables will be used to summarize the following on all participants:

- baseline and demographic characteristics
- use of concomitant medications
- reasons for early termination
- all reported AEs/SAEs

15.4 Interim Analyses

No formal interim analyses of this study are planned.

15.5 Sample Size Considerations

This pilot study is designed to evaluate 10 participants. Therefore, no formal power and sample size analyses have been performed. However, with 10 evaluable participants receiving at least 80% of the doses of lulizumab pegol (BMS-931699) and tocilizumab as described in the study protocol, proportions of rejection-free participant and corresponding two-sided, 95% exact binomial confidence intervals are shown in Table 10.

Table 10. Proportions and Confidence Intervals for 10 Participants receiving at least 80% of the doses of lulizumab pegol (BMS-931699) and tocilizumab

Number of Participants Rejection-Free at 6 Months	Incidence Rate (%)	95% Confidence Interval (%)
0	0	(0.0, 30.85)
1	10	(0.25, 44.50)
2	20	(2.52, 55.61)
3	30	(6.67, 65.25)
4	40	(12.16, 73.76)
5	50	(18.71, 81.29)
6	60	(26.24, 87.84)
7	70	(34.75, 93.33)
8	80	(44.39, 97.48)
9	90	(55.50, 99.75)
10	100	(69.15, 100)

16. Quality Assurance and Quality Control

The sponsor will review site processes for quality management of the study prior to enrollment at each clinical center, to include processes for data and biological specimen collection. Expectations will be communicated to each site regarding study conduct. In addition, all study staff are required to have GCP and ICH training.

A quality control plan for electronic data capture and data management will be created by the data center and will be reviewed by the sponsor prior to study onset. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

The Sponsor will develop a risk-based monitoring plan to direct study monitoring. Sponsor monitors will follow written Standard Operating Procedures (SOPs) to verify that the clinical trial is conducted, data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonization Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

17. Identification and Access to Source Data

17.1 Source Data

Source documents and source data are considered to be the original documentation where participant information, visits consultations, examinations and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation and validation of clinical findings, observations and other activities during a clinical trial.

17.2 Access to Source Data

The site investigators and site staff will make all source data available to the DAIT/NIAID, as well as to relevant health authorities. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

18. Protocol Deviations

18.1 Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human participant protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local or institutional human participant protection regulations, policies, or procedures.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety or well-being, or the completeness, accuracy and reliability of the study data.

18.2 Reporting and Managing Protocol Deviations

The study site principal investigator has the responsibility to identify, document and report protocol deviations as directed by the study Sponsor. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site Principal Investigator, b) notify the NIAID Project Manager and c) will complete a Protocol Deviation form. The SACCC will prepare and submit Protocol Deviation reports to the appropriate review bodies (i.e. DSMB, FDA). The NIAID Medical Monitor will review and approve the action plan that will be implemented as a result of the Protocol Deviation.

19. Ethical Considerations and Compliance with Good Clinical Practice

19.1 Statement of Compliance

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

19.2 Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The principal investigator or physician designee listed on the FDA 1572 will review the consent and answer questions. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. All participants (or their legally acceptable representative) will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in participants' primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

19.3 Privacy and Confidentiality

A participant's privacy and confidentiality will be respected throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

20. Publication Policy

The CTOT policy on the publication of study results will apply to this trial.

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Appendix 1. Donor Schedule of Events

Visit Number	D1
Visit Window	
Study Assessments	
Donor Consent	x
Demographics (Age at Donation, Gender, Ethnicity)	x
Local Laboratory Assessments	
Donor HLA Typing (retrospective chart review)	x
CMV IgG (retrospective chart review)	x
Coccidioidomycosis Serology (for subjects from endemic areas)	x
Central Laboratory Assessments	
Alloreactive T Cell Frequency Assay (ATF)	x
Donor Antigen Stimulated Gene Expression (DAGE)	

Appendix 2. Recipient Schedule of Events

					Wk 1	Wk 2	Wk 3	Mo 1				Mo 2				Mo 3		Mo 4	Mo 5	Mo 6	Mo 7	Mo 8	Mo 9	Mo 12	Mo 24	
	Study Eligibility	Transplant/ Day 0	Day 1	Day 2	Day 7	Day 14	Day 21	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 14	Wk 18	Wk 22	Wk 26	Wk 30	Wk 36	Wk 42	Wk 52	Wk 104	Clinically Indicated Biopsy
								Day 28	Day 35	Day 42	Day 49	Day 56	Day 63	Day 70	Day 77	Day 84	Day 98	Day 126	Day 154	Day 182	Day 210	Day 252	Day 294	Day 364	Day 728	
Visit Number	Screen 1	1	2	3	4	5	5a	6	6a	7	7a	8	8a	9	9a	10	11	12	13	14	15	16	17	18	19	CIB
Visit Window	30 days prior to txp[1]	None	None		± 2 days															± 3 days						- 14 days prior to last study visit
General Study Assessments																										
Study Eligibility	x																									
Informed Consent	x																									
Demographics	x																									
Medical History	→	→	→	→	→	→		→		→		→		→		→	→	→	→	→	→	→	→	→	→	→
Physical Examination/ Vital Signs	x	x	x		x	x		x		x		x		x		x	x	x	x	x	x	x	x	x		x
Review/Collect Concomitant Medications		→	→	→	→	→		→		→		→		→		→	→	→	→	→	→	→	→	→	x	→
Adverse Event/Serious Adverse Event Assessment		→	→	→	→	→		→		→		→		→		→	→	→	→	→	→	→	→	→	x	→
Local Laboratory Assessments																										
Recipient HLA Typing (abstract from transplant evaluation)	x																									
Donor Specific Anti-HLA Antibodies Class 1 and 2 (abstract from transplant evaluation)	x																									
CMV, EBV, HIV1, HIV2, HBV (HBsAg, HBcAb), HCV (HCVAb) serology (abstract from transplant eval)	x																									
EBV by PCR																x				x			x	x		
CMV by PCR[4]					x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]				x[4]			x[4]	x[4]	x[4]		
BKV by PCR in serum								x				x				x		x	x	x			x	x		
TB Testing (PPD or interferon-gamma release assay) within 3 months of enrollment	x																									
SARS CoV-2 RT-PCR	x																									
CBC (with differential and platelets)[5]	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PT/INR	x																			x				x		
Basic Chemistry (Na, K, Cl, CO_2, BUN, Glucose, Creatinine)	x		x	x	x	x		x		x		x		x		x	x	x	x	x	x	x	x	x	x	x
Liver Tests (ALT, AST, T Bilirubin, D Bilirubin)[5]	x							x				x				x		x		x		x		x	x	x
Everolimus Level								x		x		x		x		x	x	x	x	x	x	x	x	x	x	x
Fasting Lipid Panel	x							x								x				x			x	x	x	
Urine Protein and Creatinine Ratio								x				x				x		x	x	x			x	x	x	x
Urine Pregnancy Test	x					x				x				x			x	x	x	x	x	*Prior to Belatacept				
Local Pathology Results - Graft Histology																				x				x[3]		x[3]
Central Laboratory Assessments																										
Blood Specimens																										
T Cell Subsets (to include CD8+ and CD28- counts)	x															x				x			x	x		x
Multiparameter Flow Cytometry (MFC) - shared specimen 2 x 10 mL Green Top NaHep Tubes																										
Alloreactive T Cell Frequency (ATF) - shared specimen 2 x 10 mL Green Top NaHep Tubes	x[2]															x				x			x	x		x
Donor Antigen Stimulated Gene Expression (DAGE) - 2 x 10 mL Green Top NaHep Tubes	x[2]															x				x				x		
In Vitro Treg Suppression (SUP)[2] - 4 x 10 mL Green Top NaHep Tubes	x[2]															x				x				x		
Kidney Solid Organ Response Test by QPCR (KSORT) - 2.5 mL PAXgene Tube								x								x				x				x		x
Donor Specific Antibody (DSA) - 5 mL Red Top Tube								x								x				x				x		x
Renal Biopsy Specimens																										
Graft Histology (HIS) - Digital Images or Local Case																				x						x[3]
Multi-Parameter Immunofluorescence/ In Situ Hybridization/ RNAScope (DII) - Tissue in Formalin (shared)																										
Gene Expression in Nanostring nCounter Platform (NANO) - Tissue in Formalin (shared)																				x						x[3]
Urine Specimens																										
Urine Supernatant for Archive (USP) - shared specimen 50-100 ml urine								x								x				x				x[3]		x[3]
Urine Cytokine Analysis - Gene Expression Profiles (UPR) - shared specimen 50-100 ml urine																										

[1] Screening laboratory results should be dated within 30 days prior to transplant unless otherwise specified. SARS-CoV-2 testing must be completed within 2 weeks of transplant.

[2] Mechanistic blood specimens can be collected at screening visit or any time prior to transplant.

[3] Tissue should be collected from surveillance and/or for-cause biopsies for DII and NANO whenever available. Slides should also be sent for central reading and local pathology recorded for the study.

[4] CMV by PCR must be obtained weekly through week 12 if subject is not on prophylaxis.

[5] All relevant laboratory values including WBC, ANC, AST, and ALT must be obtained and reviewed prior to study drug administration.

Appendix 3. Recipient Medication Schedule

Please remember to obtain and review relevant lab results (e.g. urine pregnancy and liver tests, etc.) prior to lulizumab, tocilizumab, and belatacept.

						Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9	Wk 10	Wk 11	Wk 12	Wk 14	Wk 16	Wk 18	Wk 20	Wk 22	Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	Wk 48	Wk 52
Day	**Day 0**	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**7**	**14**	**21**	**28**	**35**	**42**	**49**	**56**	**63**	**70**	**77**	**84**	**98**	**112**	**126**	**140**	**154**	**168**	**196**	**224**	**252**	**280**	**308**	**336**	**364**
Visit Window	None					± 2 days																		± 3 days						
Study Medications																														
Thymoglobulin (anti-thymocyte globulin, rabbit)	1.5 mg/kg	1.5 mg/kg	1.5 mg/kg	1.5 mg/kg																										
Methylprednisolone	500 mg	250 mg	125 mg	60 mg	30 mg	Taper to 5 mg/day →																								
Mycophenolate Mofetil/ Mycophenolic Acid	1,000 mg/ 720 mg (d/c when EVR target trough is reached)																													
Lulizumab pegol		25 mg				12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg	12.5 mg														
Actemra (tocilizumab)			8 mg/kg				162 mg		162 mg		162 mg		162 mg		162 mg		162 mg	162 mg	162 mg	162 mg	162 mg	162 mg	162 mg							
Nulojix (belatacept)																	5mg/kg		5mg/kg		5mg/kg		5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg	5mg/kg
Zortress (everolimus)							0.75-mg BID	Titrate to 3-8 ng/ml → → → →																						

Appendix 4. Schedule of Events for Reduced Follow Up

	Mo 1	Mo 2	Mo 3	Mo 4	Mon 5	Mo 6	Mo 9	Mo 12
	Wk 4	Wk 8	Wk 12	Wk 18	Wk 22	Wk 26	Wk 42	Wk 52
	Day 28	Day 56	Day 84	Day 126	Day 154	Day 182	Day 294	Day 364
Visit Number	RF1	RF2	RF3	RF4	RF5	RF6	RF7	RF8
Visit Window	± 3 days						± 4 days	± 5 days
General Study Assessments								
Physical Examination/ Vital Signs	x	x	x	x	x	x	x	x
Review/Collect Concomitant Medications	→	→	→	→	→	→	→	→
Adverse Event/Serious Adverse Event Assessment	→	→	→	→	→	→	→	→
Local Laboratory Assessments								
CMV, EBV by PCR			x			x	x	x
BKV by PCR in serum	x	x	x		x	x	x	x
CBC (with differential and platelets)	x	x	x	x	x	x	x	x
T Cell Subsets (to include CD8+ and CD28- counts)			x			x	x	x
PT/INR								x
Basic Chemistry (Na, K, Cl, CO_2, BUN, Glucose, Creatinine)	x	x	x	x	x	x	x	x
Liver Tests (ALT, AST, T Bilirubin, D Bilirubin)			x			x	x	x
Everolimus Level	x	x	x	x	x	x	x	x
Fasting Lipid Panel	x		x			x	x	x
Urine Protein and Creatinine Ratio	x	x	x		x	x	x	x
Local Pathology Results - Graft Histology (When available with standard of care biopsy)						x		x
Central Laboratory Assessments								
Blood Specimens								
Multiparameter Flow Cytometry (MFC) - shared specimen 2 x 10 mL Green Top NaHep Tubes								
Alloreactive T Cell Frequency (ATF) - shared specimen 2 x 10 mL Green Top NaHep Tubes						x	x	x
Donor Antigen Stimulated Gene Expression (DAGE) - 2 x 10 mL Green Top NaHep Tubes						x		x
In Vitro Treg Suppression (SUP)[2] - 4 x 10 mL Green Top NaHep Tubes						x		x
Kidney Solid Organ Response Test by QPCR (KSORT) - 2.5 mL PAXgene Tube						x		x
Donor Specific Antibody (DSA) - 5 mL Red Top Tube						x		x
Renal Biopsy Specimens								
Graft Histology (HIS) - Digital Images or Local Case						x		x[1]
Multi-Parameter Immunofluorescence/ In Situ Hybridization/ RNAScope (DII) - Tissue in Formalin (shared)								
Gene Expression in Nanostring nCounter Platform (NANO) - Tissue in Formalin (shared)						x		x[1]
Urine Specimens								
Urine Supernatant for Archive (USP) - shared specimen 50-100 ml urine								
Urine Cytokine Analysis - Gene Expression Profiles (UPR) - shared specimen 50-100 ml urine						x		x

[1] Tissue should be collected from surveillance and/or for-cause biopsies for DII and NANO whenever available. Slides should also be sent for central reading. No extra passes specifcally for CTOT-24.