Mechanisms of belatacept effect on alloimmunity and antiviral response after kidney transplantation

An Investigator-Initiated Research Proposal
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SYNOPSIS

Title of Study: Mechanisms of belatacept effect on alloimmunity and antiviral response after kidney transplantation

Estimated Number of Study Centers and Countries/Regions: 1
Study Phase: 4
Research Hypothesis: Switching to a belatacept-based immunosuppression regimen after kidney transplant will have a significant impact on antibody and T cell-mediated immune response as compared with control patients as measured in peripheral blood at 6 months after belatacept switch and comparable safety and efficacy as compared with calcineurin inhibitor (CNI) based regimens.

Primary Objective: To analyze the effect of switching from CNI to belatacept in patients with evidence of CNI toxicity on the development and maintenance of immune memory in response to both alloantigen and viral antigens commonly encountered after transplant, and to assess the safety and efficacy of conversion to belatacept as maintenance immunosuppression in combination with prednisone and mycophenolate mofetil.

Study Design: Open label, single center clinical trial. Patients with evidence of CNI toxicity will be eligible for switch to belatacept-based regimen within the first three months after transplant. All enrolled subjects will also receive concomitant maintenance immunosuppression with mycophenolate mofetil and corticosteroids.

Duration of Study: 6 months, with option to extend to 12 months of belatacept treatment
Number of Subjects: 20
Study Population: Kidney transplant recipients within the first three months of their first transplant with evidence of CNI toxicity.

Test Product, Dose and Mode of Administration, Duration of Treatment: Study patients will receive intravenous belatacept at 5mg/kg every two weeks at day 1 and weeks 2, 4, 6, and 8, and then monthly at months 3, 4, and 5. At month 6 patients may elect to continue for an additional six month period of belatacept administration. Peripheral blood mononuclear cells (PBMCs) and sera for antibody testing will be collected at time of study entry and at 4, 8, 12, and 24 weeks after belatacept start, frozen and banked at our center, and analyzed in batch fashion for development of humoral and cell mediated immunity. Patients who elect to receive an additional 6 months of belatacept treatment will undergo additional immunologic analysis at 1 year after study entry.

Reference Therapy, Dose and Mode of Administration, Duration of Treatment: Immunologic evaluations performed on study patients will be compared with a matched group of 20

control patients receiving conventional immunosuppressive therapy and participating in an ongoing observational trial regarding immunologic mechanisms contributing to rejection in kidney transplant patients.

Criteria for Evaluation:

Primary Outcome Measures:

- Donor specific antibody testing at Month 6
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Month 6
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Month 6
- Immunophenotyping to determine overall immune profile at Month 6
- Patient and functional graft survival in stable renal transplant recipients

Secondary Outcome Measures:

- Donor specific antibody testing at Months 1 and 3 (and month 12 if continuing in study)
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)

Statistical Methods: A sample size of 20 subjects will be enrolled. Because of the exploratory nature of this study is not possible to estimate power for this study size.
Comparison between groups will be performed by Mann Whitney U test. In addition, a comparison will be made between patients' baseline and week 24 levels. ANOVA repeated measurement analysis will be used to evaluate immune measurements taken over time as compared with control patients.

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1 INTRODUCTION

1.1 Research Hypothesis

Primary Hypothesis:

Conversion from CNI-based therapy to belatacept will be safe and effective for prevention of rejection, with a significant impact on antibody and T cell-mediated immune response as compared with control patients as measured in peripheral blood at 6 months after belatacept conversion.

Outcomes to be tested:

- Belatacept-treated subjects will not have a greater incidence of SAEs as compared with control patients
- Belatacept-treated subjects will have similar rates of acute rejection as compared with control patients
- Belatacept-treated subjects will have a lower donor-specific antibody level at 6 months compared with control patients
- Belatacept-treated subjects will have a decrease in the frequency of alloantigen specific T cells over time as compared with control patients
- Belatacept-treated subjects will have a decrease in the frequency virus specific T cells over time as compared with control patients
- Belatacept-treated subjects will have a greater percentage of naïve and central memory T cells and a lower percentage of activated T cells as compared with control patients

1.2 Study Rationale

As belatacept comes into increasing clinical use, it is important to reach a better understanding of its immunologic mechanism of action. As the drug was engineered as a co-stimulation pathway inhibitor, we would hypothesize that its main effects would be on the activation of naïve T cells; however, an impact on pre-existing memory cells may also be seen. Both direct and indirect immunity play a role in allograft rejection after transplant, and it is important to differentiate between these two mechanisms as belatacept may affect each pathway differently: Direct recognition of alloantigen occurs when donor antigen presenting cells (APCs) present antigen to host T cell, as seen in early rejection, in contrast indirect recognition occurs when host APCs present either allo-antigen or viral antigen to host T cells.

It is known from Phase III trials that decreased formation of donor specific antibodies (DSA) are seen in patients treated de novo with belatacept compared with cyclosporine with both

standard and extended-criteria donors, [1,2] however, the mechanism behind this observation is unknown, and it is unclear whether this same affect might be anticipated in patients switched to belatacept one to three months post transplant. Finally, an increased frequency of post-transplant lymphoproliferative disorder in patients naïve for EBV with EBV positive donors (D+/R-) was also seen in these studies; this may reflect inhabitation of the development of antiviral immunity, but it is unclear how important the presence or lack of virus-specific memory T cells is in this response. Our laboratory is uniquely suited to perform a study of this nature given our experience and expertise in studies of immunity in transplant recipients as the designated investigative laboratory for the NIH-sponsored Clinical Trials in Organ Transplantation Study 2 (CTOT-2).

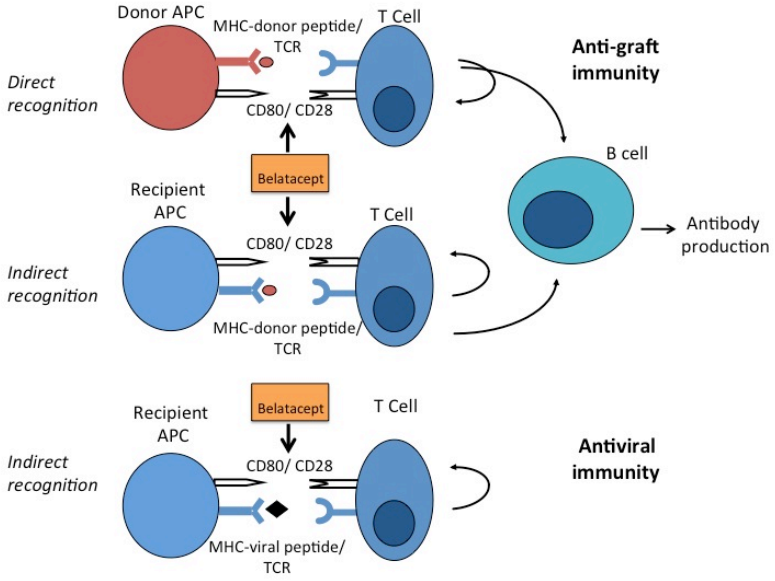


Figure 1: Model of belatacept action in alloimmunity and viral antigen recognition

Belatacept was designed as a co-stimulation inhibitor that is able to bind to CD80 molecules on the surface of antigen presenting cells, inhibiting the CD80-CD28 interaction that is required for T cell activation (figure 1).[3] Rodent models cannot be used for its study as the drug does not exhibit strong binding to murine CD80; preliminary efficacy and safety studies were therefore performed in primate models (see Investigator Brochure for additional details). No safety signal was seen in human Phase I trials, and Phase III studies have shown equivalence with or superiority to cyclosporine in prevention of death, graft loss, and chronic rejection, and an improved GFR as compared with cyclosporine treatment.[1,2] Overall safety profile was similar to cyclosporine, with the exception of PTLD which was seen with more frequency in the belatacept group, especially in patients naïve to EBV receiving transplants from an EBV seropositive donor.[4,5]

The calcineurin inhibitors, namely cyclosporine and tacrolimus, are widely used for immunosuppression in kidney transplantation. They have improved short term outcomes compared with earlier regimens such as azathioprine and steroids, but long term outcomes continue to be decreased by the nephrotoxicity related to long term calcineurin inhibitor use.[6]

1.3 Rationale for Dosing

Based on the IM 103-116 study of conversion from CNI to belatacept for maintenance of immunosuppression in kidney transplant recipients, a similar dosing schedule will be used with belatacept 5mg/kg every two weeks for a total of 5 doses followed by monthly administration of belatacept at the 5mg/kg dose.

1.4 Overall Risk/Benefit Assessment

Belatacept is an immunosuppressive drug with a novel mechanism of action compared with existing therapies that has been shown to be safe and efficacious in previous trials as described above. By enrolling patients exhibiting toxicity with standard CNI-based therapy, we will maximize the potential benefit to enrollees by allowing them the option to switch to a non-CNI based regimen with likely renal function improvement compared with standard therapy. The potential risk of increased acute rejection and PTLD will be meliorated by the close supervision that subjects will receive by the study team. Therefore, this study offers a favorable risk-benefit relationship to potential enrollees.

2 STUDY OBJECTIVES

2.1 Primary Objectives

Primary Outcome Measures:

- Donor specific antibody testing at Month 6
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Month 6
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Month 6
- Immunophenotyping to determine overall immune profile at Month 6
- Survival and rejection in patients switched to belatacept at 12 months post transplant as compared with patients treated with conventional therapy at our center.

2.2 Secondary Objectives

Secondary Outcome Measures:

- Donor specific antibody testing at Months 1 and 3 (and month 12 if continuing in study)
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)

3 STUDY DESIGN AND EVALUATION

3.1 Study Design

This study is an open label, single center clinical trial. All subjects will also receive ongoing immunosuppression with mycophenolate mofetil and corticosteroid maintenance therapy.

3.1.1 Duration of Study

The duration of the study is 6 months, with the possibility to extend an additional 6 months. The decision to participate in an additional 6 months of belatacept administration will be based on patient preference.

3.1.2 Overview

This is an open label, single center clinical trial. All subjects will have undergone kidney transplantation with induction immunosuppression and will be on maintenance therapy with a CNI (tacrolimus or cyclosporine) plus mycophenolate mofetil and corticosteroid maintenance therapy at the time of enrollment. 20 subjects with evidence of CNI toxicity (see definition below) in the first three months after transplant will be enrolled to switch to the belatacept regimen. These enrolled subjects will be compared with 20 matched patients from an ongoing observational study of kidney transplant patients at our center.

All subjects will also receive induction with basiliximab and a background maintenance immunosuppressive regimen of MMF and corticosteroids.

Study patients will receive intravenous belatacept at 5mg/kg every two weeks at day 1 and weeks 2, 4, 6, and 8, and then monthly at months 3, 4, and 5. At month 6 patients may elect to continue for an additional six-month period of belatacept administration

3.1.3 Primary Outcome Measures and Justification

Patients switched to the belatacept-based regimen will be compared to a group of matched patients on standard CNI-based immunosuppression regimen on the following primary outcome measures: (1) the donor specific antibody level, (2) the frequency of alloantigen and viral antigen reactive cells as measured by cell surface cytokine analysis, and (3) the frequency of immunophenotypes described below at Month 6. Matching will be performed based on age within 10 years, sex, deceased versus living transplant, and DSA level at time of enrollment.

The intent is to investigate differences in these measures as a means to determine the immunologic mechanism of action of belatacept. These endpoints were selected based on previous research showing that these measures are key determinants of immune function in transplant patients. In addition, baseline levels at time of enrollment of these endpoints will be compared with results from months 1, 3, and 6, as well as month 12 if patients elect to continue for an additional 6 months of belatacept therapy. Month 6 will be used as the primary measure of outcome for the immunologic assays, with months 1, 3 and 12 if applicable as the secondary measures.

To perform these mechanistic studies, blood from subjects will be collected at each study time point as described below and peripheral blood mononuclear cells (PBMCs) will be isolated and frozen for future analysis.

For analysis of response to alloantigen stimulation, PBMCs will be incubated with either intact donor cells or membrane preparations from donor cells to test either direct or indirect immunity, respectively. Following incubation, a cocktail of fluorescent-conjugated monoclonal antibodies will be added to detect relevant immunologic subclasses of cells as well as relevant surface cytokines and the percentage of each lymphocyte subset will be analyzed using flow cytometry according to previously published protocols developed at the UCLA Immunogenetics Center [7].

For analysis of response to viral antigens, PBMCs will be extracted and stored as described above using well-characterized CMV or EBV antigens for stimulation during the incubation period using a similar protocol adapted for detection of relevant antiviral immune lymphocytes.

Overall immune profile will also be analyzed by immunophenotyping using multiparameter flow cytometry using the panels described below:

	Defined Cell Population	FITC	PE	PerCP	APC	Pac Blue	PE-Cy7
1	Central/effector memory, naïve T cells	CD57	CCR7	CD3	CD45RA	CD8	CD4
2	Activation, memory	CD45RA	CCR5	CD3	CD95	CD8	CD4
3	Activation, effectors	CD45RA	HLA-DR	CD3	CD38	CD8	CD4
4	NK cells	CD161	CD94	CD3	CD56	CD16	
5	B cells	CD19	CD27	CD20	CD38	CD3	
6	T regs	FoxP3	CD103	CD4	CD25	CD3	

In addition we will analyze rates of rejection and patient and graft survival at 6 and 12 months post transplant as compared with patients receiving conventional immunosuppression regimens at our center.

3.1.4 Safety Monitoring

A Data Safety Monitoring Board will not be used for this study given that it is a small scale, single center study. However, safety including SAEs will be monitored by study staff in an ongoing fashion during patient study participation.

3.2 Study Population

The study population includes recipients of renal allografts in the first three months after first episode of transplantation that demonstrate side effects from the standard calcineurin inhibitor based regimen. CNI toxicity is delineated as follows:

1. Neurologic toxicity, defined as tremor, altered mental status, or seizure
2. Renal toxicity, defined as GFR <60
3. Metabolic toxicity, defined as a new requirement for medication to control hyperglycemia
4. Hematologic toxicity, defined as development of thrombotic microangiopathy

History of induction therapy with either basiliximab or antithymocyte globulin is permissible for study entry; as our center primarily uses basiliximab this will be the predominant induction therapy for study participants.

3.3 Criteria for Evaluation

Primary Measures:

- Donor specific antibody testing at Month 6
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Month 6
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Month 6
- Immunophenotyping to determine overall immune profile at Month 6
- Patient and functional graft survival in stable renal transplant recipients

Secondary Outcome Measures:

- Donor specific antibody testing at Months 1 and 3
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Months 1 and 3 (and month 12 if continuing in study)

Definitions

Acute and chronic rejection will be defined using the Banff 97 working classification of kidney transplant pathology.[8] Standard clinical and histologic criteria for calcineurin inhibitor associated nephrotoxicity will be used.[9] Full Banff criteria analysis will be provided for each biopsy performed.

3.4 Sample Size Determination

Because of the exploratory nature of this analysis, it is not possible to estimate power for this study, a goal of 20 patients was used similar to other immunologic pilot studies and is a reasonable goal for rapid accrual at our center.

3.5 Interim Analyses

No interim analyses will be performed. However, safety and any SAEs will be monitored on a continuous basis.

4 STATISTICAL METHODOLOGY

4.1 Data Set Descriptions

Data sets will be generated for all patients enrolled in the study with recorded frequency of alloantigen specific cells at weeks 0, 4, 12, and 24 after study start after both direct and indirect stimulus, frequency of anti CMV and anti EBV specific cells at weeks 0, 4, 12, and 24, and frequency of cell populations as determined by immunophenotyping analysis at weeks 0, 4, 12, and 24. A representative group of 20 control patients receiving standard immunosuppressive medications will be identified from an ongoing observational study at our center using matching criteria described above. Patient matching will be performed in a blinded fashion. The ongoing observational study at UCLA is led by Dr. Reed and all patients undergoing kidney transplantation at UCLA are eligible for enrollment for an ongoing evaluation of immunologic predictors of rejection. PBMCs have been aliquoted and stored from these patients at time points post transplantation similar to those described in this protocol so they will be an ideal source for comparative analysis.

4.2 Analyses

Immunologic mechanisms

Primary endpoints will be patient immune analysis at 24 weeks (end of study) as compared with control patients at a comparable time point for DSA, frequency of alloantigen and viral antigen reactive cells, and frequency of the immunophenotypes described above, comparison to be performed by Mann Whitney U test. In addition, a comparison will be made between patients' baseline and week 24 levels. ANOVA repeated measurement analysis will be used to evaluate immune measurements taken over time as compared with control patients

Efficacy and safety

As an additional primary analysis, subject and graft survival at 6 months will be determined within each group. GFR measured at baseline and months 1, 3, 6, and 12 months will be descriptively summarized. Cumulative rates of acute and chronic rejection, and chronic allograft nephropathy, will be summarized, as well as incidence of post-transplant diabetes.

All AEs and SAEs since transplantation will be summarized for the patients receiving belatacept. For the control patients, rates of rejection, PTLD, and death will be summarized.

5 SUBJECT SELECTION CRITERIA

For entry into the study, all subjects must meet the following criteria.

5.1 Inclusion Criteria

Signed Written Informed Consent

1) The subject is willing to provide signed written informed consent

Target Population

2) The subject is a first-time recipient of a living or deceased donor kidney transplant
3) Evidence of calcineurin inhibitor side effects during the first 3 months after transplant as defined as

1. Neurologic toxicity, defined as tremor, altered mental status, or seizure
2. Renal toxicity, defined as GFR <60
3. Metabolic toxicity, defined as a new requirement for medication to control hyperglycemia
4. Hematologic toxicity, defined as development of thrombotic microangiopathy

Age and Gender

4) Men and women, ages 18 and older, inclusive
5) Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the study in such a manner that the risk of pregnancy is minimized . See section 9.7 below for details regarding handling of WOCBP subjects.

WOCBP includes any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥ 12 consecutive months; or women on hormone replacement therapy with documented serum follicle stimulating hormone level > 35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where the partner is sterile (e.g., vasectomy), should be considered to be of child bearing potential. WOCBP who are taking MMF must in addition use methods of birth control as stipulated in the package insert, namely:

Either intrauterine device, or partner with vasectomy, or one hormone (oral contraceptive pill, transdermal patch, vaginal ring, or progesterone injection or implant) and one barrier method (diaphragm or cervical cap with spermicide, contraceptive sponge, or male or female condom), or two barrier methods as described above.

WOCBP must have a negative serum pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin [HCG]) within 72 hours prior to the start of study medication then every 3 months during the period of study participation.

6) Men must use an adequate method of contraception throughout the study, and for up to 8 weeks after the last infusion, so that the risk of pregnancy to their partners is minimized.

7) MMF must be dosed at 500 mg by mouth twice daily or greater at the time of study entry

8) Prednisone must be dosed at >=10 mg by mouth daily for patients less than 6 weeks post-transplantation, and at >=5mg by mouth daily for patients greater than 6 weeks post-transplantation at the time of study entry.

5.2 Exclusion Criteria
Gender and Reproductive Status

1) WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for up to 8 weeks after the last infusion.

2) Women who are pregnant or breastfeeding

3) Women with a positive pregnancy test on enrollment or prior to study drug administration

4) Males unwilling or unable to use an adequate method of contraception for the entire study period and for up to 8 weeks after the last infusion of study medication

Immunologic status

5) Subjects with PRA ≥ 30% at time of transplant

6) Subjects with zero HLA antigen mismatched donors (either from related or unrelated donor)

7) Subjects with any prior solid organ transplant (including kidney)

8) Subjects receiving a concurrent solid organ (heart, liver, pancreas) or cell (islet, bone marrow, stem cell) transplant

9) Subjects with a history of biopsy-proven acute rejection post-transplant (humoral or cellular) in the first three months post transplantation

Infection related risks

10) Subjects who are hepatitis C antibody-positive or polymerase chain reaction

(PCR)-positive for hepatitis C

11) Subjects who are hepatitis B surface antigen-positive or PCR-positive for hepatitis B

12) Subjects with known human immunodeficiency virus (HIV) infection

13) Subjects with active tuberculosis (TB) requiring treatment within the previous 3 years or any subject who previously required triple (or more) combination therapy for TB.

14) Subjects who are EBV antibody negative and have received grafts from EBV antibody positive donors.

Prohibited Therapies and/or Medications

15) Subjects who have used any investigational drug within 30 days prior to the Day 1 visit

16) Subjects previously treated with belatacept

18) Use of mTOR inhibitors at any time after transplantation

Other Exclusion Criteria

18) Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) for treatment of either a psychiatric or physical (e.g., infectious disease) illness must not be enrolled into this study.

19) Subjects who have difficult i.v. access or other reasons that would likely preclude medication administration

6 STUDY CONDUCT

6.1 Ethics
This study will be conducted in accordance with the ethical principles that have their origin in the current Declaration of Helsinki, and will be consistent with International Conference on Harmonization Good Clinical Practice (ICH GCP) and applicable regulatory requirements.

The study will be conducted in compliance with the protocol. The protocol and any amendments and the subject informed consent will receive Institutional Review Board (IRB) approval prior to initiation
of the study.

Freely given written informed consent must be obtained from every subject or their legally acceptable representative prior to clinical trial participation, including informed consent for any screening procedures conducted to establish subject eligibility for the trial.

For further details on informed consent, see Section 10.2.

The rights, safety and well-being of the trial subjects are the most important considerations and should prevail over interests of science and society.

Study personnel involved in conducting this trial will be qualified by education, training, and experience to perform their respective task(s).

This trial will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure, debarment).

Systems with procedures that assure the quality of every aspect of the study will be implemented.

6.2 Study Therapy
6.2.1 Treatment and Subject Identification
Study subjects will be switched to belatacept using the regimen described above. These treatments will be administered with concurrent maintenance therapy with MMF and corticosteroids. At the time of switch to belatacept, ongoing CNI therapy will be tapered to off over a 30 day course, as follows: 100% on day 1, 40-60% on day 15, 20-30% on day 23, and none on day 29 and beyond, following the previously published protocol[10]. An exception to this taper schedule would be made in the case of CNI toxicity with thrombotic microangiopathy, where tapering may be done over a two week time course.

MMF dosing will be maintained at a minimum of 500 mg by mouth twice daily.

For patients less than 6 weeks post-transplantation, prednisone dosing must be 10mg by mouth daily at the time of study entry and can be tapered to 5mg by mouth daily after 6 weeks post-transplantation. For patients who are greater than 6 weeks post-transplantation, prednisone must be at a minimum of 5mg by mouth daily and will be maintained at this level throughout the duration of the study.

At the time of enrollment, immediately after written informed consent is obtained and

before performing any study-related procedures, each subject will be assigned a unique sequential subject number for identification throughout the study. This subject number must not be reused for any other participant for the study.

6.2.2 Administration of Belatacept
Day 1 is defined as the day of study drug administration (screening is Day 0).
Infusion doses will be based on the subject's actual body weight at study Day 1, and will not be modified during the course of the study, unless there is a change of body weight ± 10% (see
Section 8.3.1). Study drug should be administered to the subject at a relatively constant rate over 30 minutes. See Section 8.3.1 for details on the preparation and administration of the study medication. See Section 7.2.6 for details on infusion visits and visit windows.

On study visit days, when the subject is to have peripheral blood collected, these measures are to be completed prior to belatacept
dosing.

Belatacept Regimen

Subjects will receive intravenous belatacept at 5mg/kg every other week starting from day 1 and continuing with weeks 2, 4, 6, and 8, and then monthly at months 3, 4, 5, and 6. At month 6 patients may elect to continue for an additional six-month period of belatacept administration.

6.2.3 Dose Modifications
In the absence of AEs deemed at least possibly related to study drug treatment, subjects will complete their scheduled infusions as prescribed by protocol. In the event of new, serious, and unexpected toxicity potentially related to belatacept, study drug administration should be interrupted..

6.2.4 Discontinuation of Therapy
Study therapy MUST be immediately discontinued for the following reasons:

• Withdrawal of informed consent (subject's decision to withdraw for any reason)
• Any clinical AE, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy is not in the best interest of the subject
• Pregnancy
• Imprisonment or the compulsory detention for treatment of either a psychiatric or physical illness (e.g., infectious disease).

6.2.5 Treatment Compliance
All medications specified in this protocol must be administered as described within the

protocol. Study medication usage is to be reviewed, and compliance is to be discussed with subjects at each visit to assure compliance. All study medication and concomitant medication usage will be collected, and any deviations from specified administration should be clearly documented.

6.2.6 Other Guidance: Prior and Concomitant Therapy
6.2.6.1 Corticosteroids
All subjects in this study will be treated with daily corticosteroids.

6.2.6.2 Mycophenolate Mofetil
All subjects in this study will be treated with MMF. Daily MMF should be administered in 2 divided doses on a consistent schedule in relation to time of day and meals.

6.2.6.3 Basiliximab Dosing
Basiliximab dosing is up to the discretion of the treating physician using common practice at the study institution, typically given at two doses of 20 mg each.

6.2.6.4 Prophylaxis medications

Medications commonly in use for antibiotic prophylaxis will be continued following normal transplant protocols in place at our center. These medications include sulfamethoxazole/trimethoprim and valganciclovir or acyclovir. To summarize, patients who are antibody negative for CMV (R-) receiving transplants from CMV antibody positive donors (D+) will receive valganciclovir prophylaxis for the first 6 months post transplant at 900 mg daily by mouth, or as adjusted for their renal function. Patients with other CMV serology combinations (D-/R-, D+/R+, and D-/R+) will receive acyclovir 400 mg daily by mouth for the first 3 months post transplant. In addition, single-strength trimethoprim-sulfamethoxazole is given daily by mouth for the first 3 months post transplant.

6.2.6.5 Diagnosis and Treatment of Rejection

Screening for acute and chronic rejection will be performed using standard protocols in place at our center. Biopsy performance will be per center standard protocols. Treatment will be up to the discretion of the treating transplant nephrologist. The severity of acute rejection will be determined using the Banff 97 classification. Full Banff subscores will be obtained and reported.

6.3 Blinding/Unblinding

As this is an open label study, no arrangements need to be made for blinding or potential unblinding.

6.4 Prohibited and Restricted Therapies During the Study
6.4.1 Prohibited Therapies
No prohibited therapies.

6.4.2 Precautions
6.4.2.1 Vaccinations
There is limited information available regarding the effectiveness of immunizations in non-human primates and humans that have been treated with belatacept. No data are available on the effect of therapeutic vaccinations in subjects receiving belatacept.

Due to the risk of infection, vaccination of subjects with any live vaccine is absolutely contraindicated during the course of the study, as is the administration of LIVE oral polio vaccine to household contacts. The Centers for Disease Control recommend that subjects should not be administered a live virus vaccination for at least 3 months after discontinuing high dose corticosteroid therapy (defined as > 20 mg/day of prednisone for > 2 weeks). In view of the long half-life of belatacept, study subjects should not be administered a live virus vaccine for a minimum of 3 months following the last dose of study medication.

6.4.2.2 Monitoring for EBV reactivation
EBV seronegative patients who have received organs from EBV seropositive donors are excluded from participation to decrease the risk of developing PTLD. In addition, evidence of EBV replication will be assessed using PCR testing throughout the period of study drug administration.

6.5 Non-therapy Precautions and Restrictions
6.5.1 Precautions
None.

6.6 Withdrawal of Subjects from Study
All efforts should be made to follow all subjects for the entire duration of the study. Withdrawal of a subject from study medication (Section 6.2.4) does not imply that the subject is withdrawn from the study. Section 7.2.4 details study-related procedures for subjects who discontinue study medication.

Subjects MUST be discontinued from study therapy AND withdrawn from the study for the following reasons:

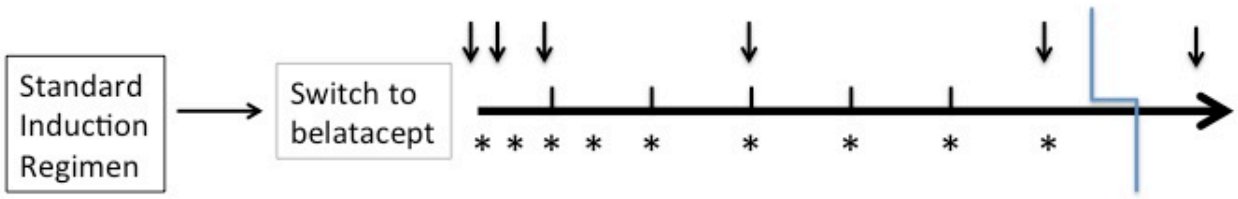
• Withdrawal of informed consent (subject's decision to withdraw for any reason)
• Any clinical AE, laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued treatment with study therapy and further participation in the study (including obtaining vital status of the subject and allograft) is not in the best interest of the subject
• Termination of the study
• Subjects who become prisoners or become involuntary incarcerated for treatment of either a psychiatric or physical (e.g., infectious disease) illness.
For discontinuation of study therapy, see Section 6.2.4.

6.6.1 Discontinued Subjects
The investigator should accept only those subjects who give a reasonable indication that they will complete the study. An evaluation that reflects the status of the subject at premature termination, with a final assessment and reasons for termination, must be provided by the investigator.

See Section 7.2.4 for a list of the procedures to be completed for subjects who discontinue study medication.

7 STUDY PROCEDURES AND OBSERVATION
7.1 Time and Events Schedule

See figure 2 below and Table for outline of events.



Large arrow indicates 12 months of study duration. Study visits indicated by small arrows, study-provided belatacept administration by asterisks. Grey line indicates interruption in scale between 6 month and 12 month time point. Extension from 6 to 12 months of therapy is optional.

Figure 2: Schematic of study design

Table: Study Procedures

	Baseline	Day 1	Week 2	Week 4	Week 6	Week 8	Week 12	Week 16	Week 20	Week 24	Week 48#
Obtain informed consent	X										
Confirm inclusion/exclusion criteria	X										
Full medical history	X										
Physical exam	X						X			X	
Weight/Vital signs	X	X	X	X	X	X	X			X	
Drug infusion*		X	X[a]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[c]
Donor specific Ab testing	X			X			X			X	X
Study labs	X			X			X			X	X
Pregnancy test**	X						X			X	X
Review concomitant medications	X	X	X	X	X	X	X			X	X
AE monitoring	X	X	X	X	X	X	X	X	X	X	X
Local laboratory testing**	X	X	X	X	X	X	X	X	X	X	X
EBV PCR testing	X			X			X			X	X

*Belatacept will be administered at 5mg/kg
**If applicable
*** Standard of care laboratory testing will be performed at each visit following standard care practices at our center
#For patients who elect to continue additional 6 months of belatacept therapy
[a] +/- 2 days
[b] +/- 3 days
[c] +/- 7 days for patients who elect to continue additional 6 months of belatacept therapy

7.2 Procedures by Visit

Study procedures for the screening, baseline, and post-belatacept administration period are detailed in chart form in the table above and described further below.:

7.2.1 Screening/Baseline Evaluations

a) Screening may occur at any time for subjects 0 to 3 months after kidney transplantation who may meet study criteria

b) If patients appear to be possible candidates for enrollment, obtain written informed consent and enroll the subject. Consent must be obtained prior to any change in medical therapy or any procedure performed solely for the purpose of this study (A subject is considered enrolled in the study upon signing informed consent. Some standard-of-care

procedures routinely obtained for transplant subjects, regardless of study involvement, are likely to be obtained prior to study participation consent date, and should be documented as such.)

c) Assign a unique study number to the patient

d) Obtain complete medical history and physical examination.

e) Obtain body weight (clothes on, shoes off), and measure vital signs (blood pressure (BP), heart rate, temperature, and respiration).

f) Study labs will be drawn by obtaining whole blood and sending it via courier to the Immunogenetics Center for separation into PBMC and serum and storage. PCR testing will be performed.

g) DSA testing

h) Check and record all concomitant medications

7.2.2 Day 1

a) Confirm all inclusion/exclusion criteria.

b) Obtain height and body weight (clothes on, shoes off), and measure pre-dose vital signs (BP, heart rate, temperature and respiration). Height and weight recorded for Day 1 can be obtained 1 day prior, if necessary.

c) Assess and record AEs pretreatment.

d) Check and record any changes in concomitant medications.

e) Ensure all pretreatment (baseline) laboratory assessments and procedures have previously been obtained, or obtain at Day 1 prior to start of any study dosing (i.e., a negative serum pregnancy test must be confirmed within 72 hours of initiation of study dosing, if previous test is not within 72 hours, repeat prior to dosing at Day 1). Draw baseline whole blood for immunology analysis if not previously drawn

f) Administer study medication (belatacept 5 mg/kg over 30 minutes i.v.)

g) Continue daily dosing of prednisone, MMF, doses to be determined by the subject's treating physician, to continue throughout the duration of the study following practices of standard medical care

7.2.3 Infusion-only Visit Procedures

a) To occur on Days 1 as above, as well as weeks 2, 6, 8, 16, and 20

b) Obtain weight and vital signs

c) Review for changes in concomitant medication and AEs

d) Administer study medication

7.2.4 Standard Study Visits

a) To occur on Weeks 4, 12, and 24 as well as week 48 for those electing to continue in the study

b) Assess and record AEs pretreatment.

c) Check and record any changes in concomitant medications

d) Obtain weight and vital signs

e) Draw whole blood for immunology analysis, to be sent by courier to the Immunogenetics Center

f) DSA testing

g) EBV PCR testing

h) WOCBP will undergo pregnancy testing at weeks 12 and 24 as well as week 48 for those continuing in the study

i) Administer study medication

7.2.5 Early Termination Procedures

For randomized, transplanted, and treated subjects who received at least 1 dose of study medication and discontinue from study medication for any reason, if informed consent continues to be provided we will continue to perform study procedures scheduled for weeks 4, 12, and 24 including an additional study lab draw at the time of drug discontinuation if it is not within 14-days of one of the above listed study visits

7.2.6 Visit Windows
To facilitate scheduling the infusion-only visits, the following windows are permitted for subsequent doses:

Visit	Visit Window
Week 2	Target date ± 2 days
Week 4 - Month 6	Target date ± 3 days
Month 12	Target date ± 7 days

7.3 Details of Procedures
All assessments should be performed or administered prior to study drug administration unless otherwise indicated.

7.3.1 Study Materials
Local laboratory will be used for all testing

7.3.2 Safety Assessments
Safety assessments include AEs, clinically significant changes in vital signs, physical examination, and laboratory test abnormalities. The investigator will determine the severity of each AE as mild, moderate, severe, or very severe. In addition, the investigator will determine the relationship of the AE to the administration of the study drug.

Physical examinations may be performed by a Doctor of Medicine, Doctor of Osteopathy, Physician's Assistant, or Nurse Practitioner.

Urine or serum pregnancy tests will be performed prior to the first dose for all WOCBP. If any female subject becomes pregnant, she will be immediately discontinued from study medication.

In addition, for women who become pregnant while using MMF or within 6 weeks of discontinuing therapy, study personnel or the patient's healthcare practitioner will report the pregnancy to the Mycophenolate Pregnancy Reference Registry (1-800-617-8191) and will strongly encourage the patient to enroll in the pregnancy registry. The patient will also be apprised of the potential hazard of mycophenolate products to the fetus. The risks and benefits of MMF will be discussed with the patient. In certain situations, the patient and her healthcare practitioner may decide that the maternal benefits outweigh the risks to the fetus.

7.3.2.1 Events of Special Interest

- Subject death or graft loss (Confirmation of graft loss by a SCr criterion [see Section 3.3 for definition] requires determination of central SCr ≥ 6 mg/dL for ≥ 4 weeks. Therefore, an additional central laboratory test(s) may be required.)
- Acute rejection
- PTLD
- Infections

7.3.3 Other Assessments

7.3.3.1 Kidney Allograft Biopsy
Allograft biopsies are to be performed as determined by the patient's treating physician following standard practice at the study institution. The Banff 97 working classification of kidney transplant pathology will be used for grading of rejection.

7.3.4 Laboratory Test Assessments
Study labs for immunologic monitoring should be obtained prior to infusions. In addition, standard lab testing will be performed as part of the patient's ongoing clinical care under the direction of their treating physician. Laboratory personnel will be blinded to the patient's demographic characteristics and clinical status. Any laboratory test result that the investigator considers clinically relevant will be recorded as an AE.

List of tests:

Immunology testing:

Whole blood will be collected at baseline and at Weeks 4, 12, and 24.
Samples must be taken prior to beginning the infusion of belatacept. Additionally, a sample for immunologic testing should be obtained at the time of any suspected rejection episode(s).

Anti-donor Antibody Determination:

Anti-donor antibody testing will be performed at baseline, and at Weeks 4, 12, and 24

All samples should be taken prior to beginning infusion of belatacept. Additionally, a sample for anti-donor antibodies (pre-dose) should be obtained at the time of any suspected rejection episode(s).

Pregnancy Tests:

A serum pregnancy test (minimum sensitivity 25 IU/L of ß-HCG) must be performed at a local laboratory for all WOCBP within 72 hours prior to the first dose of study medication (baseline or Day 1). Additional testing will occur at weeks 12 and 24, and week 48 for those continuing in the study.

If a female subject becomes pregnant, she will be discontinued from the study.

7.3.5 Immunologic Assessments

7.3.5.1 Primary Assessments

- Donor specific antibody testing at Month 6
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Month 6

- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Month 6
- Immunophenotyping to determine overall immune profile at Month 6

7.3.5.2 Secondary Assessments

- Donor specific antibody testing at Months 1 and 3, and month 12 if electing to continue in study
- Cell surface cytokine secretion in response to donor cell antigen by direct and indirect stimulation at Months 1 and 3, and month 12 if electing to continue in study
- Cell surface cytokine secretion in response to CMV and EBV antigens by direct and indirect stimulation at Months 1 and 3, and month 12 if electing to continue in study

7.3.5.3 Brief summary of methods immunologic assessment

Cell surface cytokine secretion will be performed using both alloantigen and viral antigen stimuli following our previously published protocol [7]. Cells previously banked from kidney transplant donors will be used to analyze allospecific cells for the presence of both direct and indirect immune response. A cocktail of fluorescent conjugated monoclonal antibodies against cell surface markers and secreted cytokines will be used to distinguish between alloreactive naïve versus memory T cells using multiparameter flow cytometry. Results will be expressed as percentage of cells expressing either IFN-γ or IL-10 per each of the various lymphocytes subsets.

Analysis of virus-specific cytokine secretion will be performed in a similar fashion for analysis of indirect immune response to virus. PBMCs will be incubated with commercially available overlapping peptide pools for major CMV and EBV antigens and incubated in the presence of anti-CD28 antibody.

Immunophenotyping will be performed to further dissect the influence of belatacept on the immune response**.** PBMCs at each time point will be assessed by multiparameter flow cytometry using panels aimed at characterization of T cell immunophenotypes including central memory, effector memory, and naïve cells, activated cells, B cells, NK cells, and T regulatory cells.

See also section 3.1.3 above for table of antibody panels to be used and justification of approach.

7.3.6 Potential for premature termination of clinical trial

The incidence of acute rejection during the first year post-transplant is typically about 10% for a cyclosporine based immunosuppression regimen [4]. If a two-fold or greater (>=20%) incidence of acute rejection is observed, the patient demographics and data collected will be reviewed and the trial will be considered for premature termination. Because of the small

number of patients participating in this trial, an up to two-fold deviation from expected rates was established.

8 Drug Product

8.1 Study Product
Bristol-Myers Squibb will supply Belatacept.:

Identification

Belatacept is supplied as a sterile, white or off-white lyophilized powder for intravenous administration. Prior to use, the lyophile is reconstituted with a suitable fluid to obtain a clear to slightly opalescent, colorless to pale yellow solution, with a pH in the range of 7.2 to 7.8. Each 250 mg single-use vial of belatacept also contains: monobasic sodium phosphate (34.5 mg), sodium chloride (5.8 mg), and sucrose (500 mg).

8.2 Packaging and Labeling

Belatacept for Injection will be supplied in a 250 mg/vial, and is administered by IV injection.

Belatacept for Injection, 250 mg/Vial

Belatacept for Injection, 250 mg/Vial, is a sterile non-pyrogenic lyophilized powder. Each vial contains 275 mg of belatacept, 550 mg of sucrose, 38.0 mg of sodium phosphate monobasic monohydrate, 6.4 mg of sodium chloride, and 1 N sodium hydroxide/1 N hydrochloric acid solution sufficient to adjust pH to 7.5. A 10% overfill is included in each vial to account for VNS holdup.

8.3.1 Drug Product Preparation
Belatacept :

A pharmacist or qualified personnel at the site, not otherwise associated with the conduct of the study, will reconstitute the drug for i.v. administration.

Care must be taken to assure sterility of the prepared solution, as the drug product does NOT contain any antimicrobial preservatives or bacteriostatic agents.

Recommended safety measures for preparation and handling include protective clothing, gloves, and safety cabinets. Unused drug product solution should be discarded as per the site procedures. Constituted solutions of belatacept may foam; therefore, shaking should be avoided.**Error! Bookmark not defined.**

Deleted:

Belatacept is provided as a lyophilized powder in preservative-free, single-use vials. Each vial provides 250 mg of belatacept for intravenous administration. Each vial must be reconstituted with 10.5 mL of a suitable reconstitution fluid, **using a silicone-free syringe**.

Suitable fluids for reconstitution include sterile water for injection, 0.9% sodium chloride injection or 5% dextrose injection. The reconstituted solution must be further diluted with either 0.9% sodium chloride injection or 5% dextrose injection to belatacept concentrations between 2 mg/mL and 10 mg/mL, before administration by intravenous infusion. The diluted reconstituted solution must be administered as an intravenous (IV) infusion at a relatively constant rate over 30 minutes.

Prior to administration, the belatacept infusion solution should be inspected visually for particulate matter or discoloration, and the solution should be discarded if any particulate matter or discoloration is observed. The entire, fully diluted belatacept infusion should be administered over a period of 30 minutes and must be administered with an infusion set and a sterile, non-pyrogenic, low protein binding filter (pore size of 0.2 μm to 1.2 μm). Following administration, it is recommended that the intravenous line be flushed with infusion fluid to ensure administration of the complete dose Any unused portion of the infusion solution should not be stored for reuse.

Other medicines: belatacept should not be infused concomitantly in the same intravenous line with other agents. The investigational product should be stored in a secure area according to local regulations. The investigator is responsible for ensuring that it is dispensed only to study subjects and only from official study sites by authorized personnel, as dictated by local regulations.
See Section 6.2.2 for additional details on study medication administration.

No data are available on the compatibility of Belatacept with other i.v. substances.
Other drug substances should not be added or infused simultaneously through the same

i.v. line. Assure adequate, appropriate flushing between each drug substance if multiple drugs are administered through the same line sequentially.
Other protocol-required drug products:

Other protocol-required drug products should be administered to subjects according to the dosing details provided in Section 6.2.6.

8.3.2 Recommended Storage Conditions

The investigator is responsible for ensuring that the product is stored under the appropriate environmental conditions (temperature, light, and humidity), and will be stored and reconstituted as per the belatacept (Nulojix) package insert.

8.4 Drug Product Records at Site
It is the responsibility of the investigator to ensure that a current record of drug product disposition is maintained at each study site where drug product is inventoried and disposed.

Records or logs must comply with applicable regulations and guidelines, and should include:

- Amount received and placed in storage area
- Amount currently in storage area
- Label identification number or batch number and use data or expiry date
- Dates and initials of person responsible for each drug product inventory entry/movement
- Amount dispensed to each subject, including unique subject identifiers
- Non-study disposition (e.g., lost, wasted, broken)

8.5 Return and Destruction of Investigational Product
Not applicable

8.6 Retained Samples for Bioavailability/Bioequivalence Studies
Not applicable.

9 ADVERSE EVENT REPORTING IN CLINICAL TRIALS

9.1 Importance of Adverse Event Reporting
Timely and complete reporting of safety information assists BMS in identifying any untoward medical occurrence, thereby allowing: (1) protection of the safety of study subjects; (2) a greater understanding of the overall safety profile of the drug product; (3) recognition of dose-related toxicity; (4) appropriate modification of study protocols; (5) improvements in study design or procedures; and (6) adherence to worldwide regulatory requirements.

9.2 Collection of Safety Information

An ***Adverse Event (AE)*** is defined as any new untoward medical occurrence or worsening of a pre-existing medical condition in a patient or clinical investigation subject administered an investigational (medicinal) product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of investigational product, whether or not considered related to the investigational product.

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. (In order to prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more AEs.)

A ***Serious Adverse Event (SAE)*** is any untoward medical occurrence that at <u>any dose</u>:

- results in death

- is life-threatening (defined as an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe)
- requires inpatient hospitalization or causes prolongation of existing hospitalization (see note below for exceptions)
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect

    is an important medical event, defined as a medical event that may not be immediately life-threatening or result in death or hospitalization but, based on appropriate medical and scientific judgment, may jeopardize the subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed above. Examples of such events include but are not limited to intensive treatment in an emergency department or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization. Potential drug induced liver injury (DILI) us also considered an important medical event (see Section 7.6 for the definition of potential DILI.

Suspected transmission of an infectious agent (e.g., any organism, virus or infectious particle, pathogenic or non-pathogenic) via the study drug is an SAE.

Although pregnancy, overdose and cancer are not always serious by regulatory definition, these events must be handled as SAEs (See Section 7.5 for reporting pregnancies).

NOTE: The following hospitalizations are not considered SAEs:

- a visit to the emergency room or other hospital department lasting less than 24 hours that does not result in admission (unless considered an "important medical event" or a life-threatening event)
- elective surgery planned before signing consent
- admissions as per protocol for a planned medical/surgical procedure
- routine health assessment requiring admission for baseline/trending of health status (e.g., routine colonoscopy)
- medical/surgical admission for purpose other than remedying ill health state that was planned before study entry. Appropriate documentation is required in these cases.
- admission encountered for another life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g., lack of housing, economic inadequacy, caregiver respite, family circumstances, administrative).

Adverse events can be spontaneously reported or elicited during open-ended questioning, examination, or evaluation of a subject. To prevent reporting bias, subjects should not be questioned regarding the specific occurrence of one or more adverse events.

If known, the diagnosis of the underlying illness or disorder should be recorded, rather than its individual symptoms. The following information should be captured for all AEs: onset, duration, intensity, seriousness, relationship to investigational product, action taken, and treatment required. If treatment for the event was administered, it should be recorded in the medical record. The investigator must supply BMS and the IRB/IEC with any additional information requested, notably for reported deaths of subjects.

All SAEs, whether related or unrelated to belatacept, and all pregnancies must be reported to BMS (by the investigator or designee) within 24 hours.

All SAEs should be reported via confirmed facsimile (fax) transmission, or scanned and reported via electronic mail to:

SAE Email Address: Worldwide.Safety@BMS.com

SAE Fax Number: <<609-818-3804>>

For studies conducted under an **Investigator IND**, any event that is both serious and unexpected must be reported to the Food and Drug Administration (FDA) as soon as possible **and no later than 7 days** (for a death or life-threatening event) **or 15 days** (for all other SAEs) **after the investigator's or institution's initial receipt of the information.** BMS will be provided with a simultaneous copy of all adverse events filed with the FDA. SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/.

MedWatch SAE forms should be sent to the FDA at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)
http://www.accessdata.fda.gov/scripts/medwatch/

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology
Bristol-Myers Squibb Company
Fax Number: 609-818-3804
Email: Worldwide.safety@bms.com

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS using the same procedure used for transmitting the initial SAE report. All SAEs should be followed to resolution or stabilization.

The collection of non-serious adverse event (NSAE) information should begin at initiation of study drug. NSAE information should also be collected from the start of a placebo lead-in period or other observational period intended to establish a baseline status for the subjects. NSAEs should be followed to resolution or stabilization, or reported as SAEs if they become

serious. Follow-up is also required for NSAEs that cause interruption or discontinuation of study drug, or those that are present at the end of study treatment as appropriate. All identified NSAEs must be documented appropriately.

9.3 Potential Drug-Induced Liver Injury (DILI)

Wherever possible, timely confirmation of initial liver-related laboratory abnormalities should occur prior to the reporting of a potential DILI event. All occurrences of potential DILIs, meeting the defined criteria, must be reported as SAEs (see Section 7.2.1 for reporting details).
Potential drug induced liver injury is defined as

1. AT (ALT or AST) elevation > 3 times upper limit of normal (ULN)

AND

2. Total bilirubin > 2 times ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase),

AND

3. No other immediately apparent possible causes of AT elevation and hyperbilirubinemia, including, but not limited to, viral hepatitis, pre-existing chronic or acute liver disease, or the administration of other drug(s) known to be hepatotoxic.

9.4 Adverse Events Related to Study Conditions
If the investigator believes that an SAE is not related to the drug product, but is potentially related to the conditions of the study, (such as withdrawal of previous therapy, or complication of a trial procedure), the relationship should be specified in the narrative section of the SAE page of the CRF.

9.5 Laboratory Test Abnormalities
All laboratory test values captured as part of the study should be recorded on the appropriate laboratory test results pages of the CRF, or be submitted electronically from a central laboratory. In addition, in order for BMS to collect additional information about clinically important laboratory abnormalities, at a minimum, the following laboratory abnormalities should be captured on the AE or SAE pages of the CRF as appropriate:

• Any laboratory test result that meets the criteria for a SAE
• Any laboratory abnormality that required the subject to have drug product discontinued or interrupted
• Any laboratory abnormality that required the subject to receive specific corrective therapy.
It is expected that wherever possible, the clinical, rather than the laboratory term would be used by the reporting investigator (e.g., ‘anemia’ vs. ‘low hemoglobin value’).

9.6 Other Safety Considerations

Any clinically significant changes noted during interim or final physical examinations, ECGs, x-rays, and any other potential safety assessments, whether or not these procedures are required by the protocol, should also be recorded on the appropriate AE page of the CRF (i.e., non-serious or serious).

9.7 Pregnancy
Sexually-active WOCBP must use an effective method of birth control during the course of the study, in a manner such that risk of failure is minimized. (See Section 5.1 for definition of WOCBP).

Precautions

WOCBP should be advised against becoming pregnant from the beginning of the study until 8 weeks or 5 half lives (whichever is greater) after administration of the last dose.

Subjects should be instructed to contact the Investigator immediately if pregnancy is suspected (e.g., missed or late menstrual period). Male subjects should be advised to avoid unprotected sex throughout the study and until sperm produced during the period of drug exposure has been cleared. Drug-exposed sperm may be present in the semen for 3 months following the passage of 5 half lives of the study drug.

Subjects should be advised to notify the Investigator of any change in birth control method during exposure to Study Drug or if they need to take any prescription drug or other medication not prescribed by the Investigator. The Investigator should be aware of changes in birth control method so that the use of a study-prohibited contraceptive method is identified.

Hormonal contraceptives could be depressed from drug-drug interactions with the study drug or other concomitant medications. Hormonal contraceptives should not be used to prevent pregnancy until a Drug-Drug Interaction (DDI) study has verified that the pharmacokinetics of the hormone contraception remain at therapeutic levels, and this can be substantiated by appropriate PK studies and the investigator believes that this is appropriate for the subject.

Testing Methods and Timing

Methods for Pregnancy Testing

Blood or urine testing is acceptable. Urine pregnancy tests must have a minimum test sensitivity of at least 25 IU/L. Kits measuring either total human chorionic gonadotropin (hCG) or the beta (β) fraction.

Timing of Pregnancy Testing

Outpatient Trials: WOCBP should be tested:

- At screening

- Every 3 months during period of study participation
- Whenever pregnancy is suspected

Pregnancy Reporting

In the event that a WOCBP becomes pregnant during a clinical trial, the implications (on the health of the mother and fetus) of treatment suspension must be reviewed and considered. If it is determined that continued treatment is desirable, the IRB must approve continued treatment and participation.

The investigator must immediately notify BMS of this event and complete and forward a *Pregnancy Surveillance Form* to BMS within 24 hours and in accordance with SAE reporting procedures described in Section 7.2.1. The pregnancy should also be reported to the National Transplant Pregnancy Registry. (U.S.)

Follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome and, where applicable, offspring information must be reported on the Pregnancy Surveillance Form.

Any pregnancy that occurs in a female partner of a male study participant should be reported to BMS. Information on this pregnancy will be collected on the *Pregnancy Surveillance Form*. If required by local regulations or IRB/IEC requirements, the female partner should be asked to sign a *Medical Information Release Form* to acknowledge agreement for disclosure of information if additional information is requested by BMS. (Some countries may require the acknowledgement prior to the initial report of pregnancy.)

Pregnancy Reporting Check List

- Confirm in the pregnancy report if study medication has been discontinued.
- Enter positive pregnancy test results on the Case Report Form.
- Submit a completed *Pregnancy Surveillance Form* to BMS and report the pregnancy to the National Transplant Pregnancy Registry (U.S.)
- Provide clarification or follow-up information that is requested by BMS.

Pregnancy Counseling

When a pregnancy is confirmed, the investigator should offer counseling to both parents regarding potential adverse health effects including teratogenic effects. A prenatal evaluation should be provided, along with a list of health care providers specialized in obstetrical care if this information is available. Available information should be reviewed on the potential for complications and possibility for birth effects due to exposures from

study drug(s). The pregnant subject (or the pregnant partner of a male subject) should be requested to sign a *Medical Information Release Form*. If required by local regulations or IRB/IEC requirements, the subject should be asked to sign a new Informed Consent Form (ICF) if BMS requests additional information

If the pregnant patient is not of legal age, the parent or legal guardian may need to be involved in the conversations.

10 ADMINISTRATIVE SECTION

10.1 Compliance with the Protocol and Protocol Revisions

The study shall be conducted as described in this approved protocol. All revisions to the protocol must be discussed with BMS. The investigator should not implement any deviation or change to the protocol without prior review and documented approval/favorable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects. Any significant deviation must be documented in the CRF.

If a deviation or change to a protocol is implemented to eliminate an immediate hazard(s) prior to obtaining IRB/IEC approval/favorable opinion, as soon as possible the deviation or change will be submitted to:

- IRB/IEC for review and approval/favorable opinion
- BMS
- Regulatory authority(ies), if required by local regulations.

Documentation of approval signed by the chairperson or designee of the IRB(s)/IEC(s) must be sent to BMS.

If the revision is an administrative letter, investigators must inform their IRB(s)/IEC(s).

If an amendment substantially alters the study design or increases the potential risk to the subject: (1) the consent form must be revised and submitted to the IRB(s)/IEC(s) for review and approval/favorable opinion; (2) the revised form must be used to obtain consent from subjects currently enrolled in the study if they are affected by the amendment; and (3) the new form must be used to obtain consent from new subjects prior to enrollment.

10.2 Informed Consent

Investigators must ensure that subjects or their legally acceptable representatives are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate.

10.2.1 Informed Consent Procedures

Preparation of the consent form is the responsibility of the investigator, and must include all elements required by ICH, GCP, and applicable regulatory requirements, and must adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. The consent form must also include a statement that regulatory authorities have direct access to subject records. Prior to the beginning of the study, the investigator must have the IRB/IEC's written approval/favorable opinion of the written informed consent form and any other information to be provided to the subjects.

The investigator must provide the subject or legally acceptable representative with a copy of the consent form and written information about the study in the language in which the subject is most proficient. The language must be non-technical and easily understood. The investigator should allow time necessary for subject or subject's legally acceptable representative to inquire about the details of the study, then informed consent must be signed and personally dated by the subject or the subject's legally acceptable representative and by the person who conducted the informed consent discussion. The subject or legally acceptable representative should receive a copy of the signed informed consent and any other written information provided to study subjects prior to subject's participation in the trial.

10.2.2 Subjects Unable to Give Informed Consent
10.2.2.1 Minors
Investigators must follow standard procedures to ensure that the minor's parents or legally acceptable representatives are able to give fully informed written consent. (In the event that the parents or legal guardians are unable to read, then follow ICH Guideline 4.8.9). Whenever feasible, minors who are judged to be of an age of reason must also give their written assent by signing and dating the completed informed consent. All local laws, rules and regulations regarding informed consent of minors must be followed.

10.2.2.2 Subjects Experiencing Acute Events or Emergencies
A legally acceptable representative or legal guardian must provide informed consent when consent of the subject is not possible prior to clinical trial participation, e.g., for subjects experiencing an acute medical event such as myocardial infarction or stroke. Informed consent of the subject must additionally be obtained if they become capable of making and communicating their informed consent during the clinical trial. All local laws, rules and regulations regarding informed consent of adult subjects incapable of giving informed consent must be followed.

10.2.2.3 Mentally Impaired or Incapacitated Subjects
Investigators should determine whether or not a mentally impaired or incapacitated subject is capable of giving informed consent and should sign a statement to that effect. If the subject is deemed mentally competent to give informed consent, the investigator should follow standard procedures. If the subject is deemed not to be mentally competent to give informed consent, a fully informed legal guardian or legally acceptable representative can be asked to give consent for, or on behalf of, the subject. All local

laws, rules and regulations regarding informed consent of mentally impaired or incapacitated subjects must be followed.

Subjects who are involuntarily hospitalized because of mental illness must not be enrolled in clinical trials.

10.2.2.4 Other Circumstances
Imprisonment or the compulsory detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness must not be enrolled in clinical trials.

In circumstances where a subject's only access to treatment is through enrollment in a clinical trial, e.g., for subjects in developing countries with limited resources or for subjects with no marketed treatment options, the investigator must take special care to explain the potential risks and benefits associated with the trial and ensure that the subject is giving informed consent.

When a subject may be in a dependent relationship with the investigator, a well-informed physician who is not engaged in the clinical trial and is completely independent of the relationship between the subject and investigator should obtain the subject's informed consent.

10.2.3 Illiterate Subjects
If the subject or legally acceptable representative is unable to read, a reliable and independent witness should be present during the entire informed consent discussion. The choice of the witness must not breach the subject's rights to confidentiality. A reliable independent witness is defined as one not affiliated with the institution or engaged in the investigation. A family member or acquaintance are appropriate independent witnesses. After the subject or legally acceptable representative orally consents and has signed, if capable, the witness should sign and personally date the consent form attesting that the information is accurate and that the subject or legally acceptable representative has fully understood the content of the informed consent agreement and is giving true informed consent.

10.2.4 Update of Informed Consent
The informed consent and any other information provided to subjects or their legally acceptable representatives should be revised whenever important new information becomes available that is relevant to the subject's consent, and should receive IRB/IEC approval/favorable opinion prior to use. The investigator, or a person designated by the investigator, should fully inform the subject or the subject's legally acceptable representative of all pertinent aspects of the study and of any new information relevant to the subject's willingness to continue participation in the study. This communication should be documented.

During a subject's participation in the trial, any updates to the consent form and any updates to the written information will be provided to the subject.

10.3 Monitoring for Protocol Compliance
Representatives of BMS must be allowed to visit all study site locations periodically to assess the data, quality and study integrity. On site, they will review study records and directly compare them with source documents, discuss the conduct of the study with the investigator, and verify that the facilities remain acceptable.

In addition, the study may be evaluated by BMS internal auditors and government inspectors who must be allowed access to CRFs, source documents, and other study files. BMS audit reports will be kept confidential.

THE INVESTIGATOR MUST NOTIFY BMS PROMPTLY OF ANY INSPECTIONS SCHEDULED BY REGULATORY AUTHORITIES, AND PROMPTLY FORWARD COPIES OF INSPECTION REPORTS TO BMS.

10.4 Records and Reports
An investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the drug product.

The confidentiality of records that could identify subjects must be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

10.5 Institutional Review Board (IRB)
Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB/IEC for the protocol, consent form, subject recruitment materials/process (e.g., advertisements), and any other written information to be provided to subjects. The investigator or sponsor should also provide the IRB/IEC with a copy of the IB or product labeling and information to be provided to subjects and any updates.

The investigator or sponsor should provide the IRB/IEC with reports, updates, and other information (e.g., ESR, amendments, administrative letters) according to regulatory requirements or institution procedures.

10.6 Records Retention
The investigator must retain drug product disposition records, copies of CRFs (or electronic files), and source documents for the maximum period required by applicable regulations and guidelines, or institution procedures, or for the period specified by the sponsor, whichever is longer. The investigator must contact BMS prior to destroying any records associated with the study.

BMS will notify the investigator when the trial records are no longer needed.

If the investigator withdraws from the study (e.g., relocation, retirement), the records shall

be transferred to a mutually agreed upon designee (e.g., another investigator, IRB). Notice of such transfer will be given in writing to BMS.

11 GLOSSARY OF TERMS AND LIST OF ABBREVIATIONS

11.1 Glossary of Terms

Not applicable.

11.2 List of Abbreviations

Term Definition
ACR American College of Rheumatology
AE adverse event
ANOVA analysis of variance
APC antigen-presenting cell
BCG Bacille Calmette-Guerin
BMS Bristol-Myers Squibb
BMS-188667 CTLA4Ig (abatacept)
BMS-224818 LEA29Y (belatacept)
BP blood pressure
BPAR biopsy-proven acute rejection
BUN blood urea nitrogen
C2 plasma concentration 2 hours post-dose
CAN chronic allograft nephropathy
CI confidence interval
CIT cold ischemia time
CLT total body clearance
CMV Cytomegalovirus
CNI calcineurin inhibitor
CRF case report form
CsA cyclosporine
CSBPAR clinically-suspected and biopsy-proven acute rejection
CTC Common Toxicity Criteria
CTLA4Ig BMS-188667 (abatacept)
CVA cerebrovascular accident
DBP diastolic blood pressure
DCD donor with cardiac death
DGF delayed graft function
DSMB Data Safety Monitoring Board
D5W dextrose 5% in water for injection
Term Definition

EAC Event Adjudication Committee
EBV Epstein-Barr virus
ECD extended criteria donor

ECG electrocardiogram
ESR expedited safety report
ESRD end-stage renal disease
FPG fasting plasma glucose
GCP Good Clinical Practice
GFR glomerular filtration rate
HbA1c hemoglobin A1c
HCG-human chorionic gonadotropin
HDL high-density lipoprotein
HIV human immunodeficiency virus
HLA human leukocyte antigen
IB Investigator Brochure
ICH International Conference on Harmonization
Ig immunoglobulin
IL interleukin
IRB Institutional Review Board
ITT intent-to-treat
i.v. intravenous(ly)
IVRS interactive voice response system
KM Kaplan-Meier
LEA29Y BMS-224818 (belatacept)
LDL low-density lipoprotein
LI less intensive
MAP mean arterial pressure
MedDRA Medical Dictionary of Drug Regulatory Activities
MI more intensive
MMF mycophenolate mofetil (CellCept.)
NKF-K DOQI National Kidney Foundation Kidney Disease Outcomes Quality Initiative
NSS 0.9% normal saline solution
OR operating room
PCR polymerase chain reaction
PG plasma glucose
PK pharmacokinetic(s)
p.o. oral(ly)
PPD purified protein derivative (test)
PRA panel reactive antibodies
PTDM post-transplant diabetes mellitus
PTLD post-transplant lymphoproliferative disorder
QoL quality of life
Term Definition

RA rheumatoid arthritis
SAE serious adverse event
SBP systolic blood pressure

SCr serum creatinine
SD standard deviation
SeBP seated blood pressure
SF-36 Short Form-36
TB tuberculosis
TGs triglycerides
TLC therapeutic lifestyle changes
UNOS United Network of Organ Sharing
VSS steady-state volume of distribution
WBC white blood cell
WOCBP women of childbearing potential

12 REFERENCES

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