Protocol Title: Use of Belatacept during Post Depletional Repopulation to Facilitate Tolerance in Renal Allograft Recipients

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SYNOPSIS

Title of Study: Use of Belatacept During Post Depletional Repopulation to Facilitate Tolerance in Renal Allograft Recipients
Study Phase: II
IND Sponsor: Allan Kirk, MD PhD
Participating Site: Emory University
Study Design: Single-center, open-label, proof-of-concept study in non-HLA-identical live and deceased donor renal transplants.
Duration of Study: Subjects will be enrolled over a one-year period and followed for up to five years.
Number of Subjects: Approximately 40
Study Population: Adult, EBV seropositive patients who do not have evidence of DSA pretransplant and who are scheduled to receive a kidney transplant at Emory University. Candidacy for transplantation will be determined using the standard methods of evaluation active at Emory during the study period.
Primary Objective: To determine whether lymphocyte depletion with alemtuzumab followed by belatacept and sirolimus will permit renal allograft function without rejection, and will allow for a general population of renal transplant recipients to be weaned to belatacept monotherapy for maintenance immunosuppression. The primary endpoint will be the number of patients successfully withdrawn from sirolimus for 1 year after their last dose of sirolimus.

Secondary Objectives:

1) Determination of the prognostic ability of assays based on surface phenotype changes seen during homeostatic repopulation, including Ki67 expression, T_{REG} and B_{TRANS} cell surface phenotypes, and T_M to T_{REG}/B_{TRANS} balance, with regard to the anticipation of successful immunosuppressive drug withdrawal.

2) Assessment of the proposed therapies to prevent biopsy proven acute rejection at 1, 3 and 5 years post-transplant compared to the standard reported in the SRTR database for similar patients.

3) Assessment of the incidence of biopsy proven chronic allograft nephropathy at 1, 3 and 5 years post-transplant compared to that reported in the SRTR database for similar patients.

4) Assessment of the composite of occurrence of malignancies, and opportunistic infections by 1, 3 and 5 years compared to that reported in the SRTR database for similar patients.

5) Long term assessment of donor-specific immune responsiveness after prolonged therapy with belatacept (with or without sirolimus), and during and following drug withdrawal as determined by in vitro alloresponsiveness in CFSE mixed lymphocyte reactivity and ICCS.

6) Characterization of the repopulating immune system with regard to cell surface phenotype using MFC compared to prior experience with alemtuzumab alone or combined with other related regimens and,

7) Comparison of the function and survival of allografts transplanted using this protocol to historical controls transplanted using conventional immunosuppression to establish an initial assessment of this approach in a general transplant population.

Clinical Therapy:

Alemtuzumab. At the time of transplantation patients will be given a single dose of methylprednisolone 500 mg intravenously over 30 minutes followed by acetaminophen 650 mg orally or rectally and diphenhydramine 50 mg intravenously, followed in one hour by alemtuzumab, 30mg, given intravenously over three hours.

Transplantation. Transplantation will be performed using standard surgical techniques for organ engraftment. Patients will be cared for perioperatively on the transplant unit. Technical aspects of the postoperative management will be according to the standard of care.

Belatacept. Belatacept will be given within 24 hours of transplantation via a peripheral intravenous catheter at a dose of 10mg/kg (actual body weight) infused over 30 mins. The dose will be repeated on study days 4 (post op day 3) and 8 (post op day 7), then every 2 weeks for 5 additional doses. Thereafter, belatacept will be given once every 4 weeks (+/- 3 days) at 10mg/kg through 6 months then at 5mg/kg indefinitely.

Sirolimus. Sirolimus will be started on postoperative day 1 at a dose of 2 mg per day orally. Doses will be adjusted to maintain 24-hour trough levels of 8-10ng/ml until the drug is weaned as described below.

Toxicity attributable to sirolimus (e.g., mouth ulcers, arthralgias) will prompt dose reduction to address clinical concerns in this regard. If sirolimus trough levels need to be reduced below 4ng/ml to control drug side effects, the patient will be considered intolerant to the drug and will be changed to MMF (1.0 gm twice daily) and prednisone (starting at 30mg - 20mg daily tapered to off over 1-3 months depending on the time from transplantation).

Prophylaxis. With the exception of the immunosuppressive drugs used, perioperative care, including viral, bacterial and fungal infection prophylaxis will be administered in keeping with the current standard of care.

Weaning Schedule: Weaning of immunosuppression will begin for all patients meeting pre-defined criteria. Patients will be first weaned off sirolimus over at least a 2 – 6 month period by halving the dose and/or increasing the dosing interval. After sirolimus is weaned, patients will remain on belatacept monotherapy indefinitely.

Statistical Analyses: It is anticipated that an acute rejection rate prior to initiation of sirolimus withdrawal of up to 20% will exist, excluding up to four patients from eligibility for sirolimus withdrawal. It also is anticipated that four additional patients will either not meet other criteria for withdrawal or will refuse to be withdrawn from immunosuppression. The number of patients has therefore been chosen to have approximately 12 patients who are eligible for sirolimus withdrawal and in whom withdrawal is attempted, in order to establish a reasonable proof-of-concept. This target of 12 subjects in whom sirolimus withdrawal is attempted will allow for the detection approximately 6 subjects successfully withdrawn from sirolimus for one year, assuming the probability of a subject in whom withdrawal is attempted being successfully withdrawn from sirolimus for one year is 50%, a rate determined by the current cohort. Since there are no trials exploring monotherapy belatacept reported in the literature or registered with ClinicalTrials.gov, this cohort of patients withdrawn successfully will represent a significant finding.

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1 INTRODUCTION

1.1 Précis

Renal transplantation is the treatment of choice for most causes of end stage renal disease. However, current methods for transplantation rely upon the chronic use of immunosuppressive drugs to prevent immune rejection of the transplanted organ. These drugs are not completely effective such that many recipients eventually lose allograft function due to either acute or chronic rejection. They are also associated with metabolic, infectious and neoplastic side effects. Importantly, standard immunosuppressive approaches are also thought to inhibit adaptive immune processes promoting organ acceptance. This later effect makes transplant recipients dependent on immunosuppression for life.

Recent clinical trials have shown that a patient's dependence on chronic immunosuppression can be reduced by peri-transplant treatment with alemtuzumab, a monoclonal antibody that transiently depletes T-cells. Pre-clinical data have also indicated that, unlike conventional immunosuppressive therapy, drugs that inhibit T-cell costimulation, e.g. by interfering with the molecules CD80 and CD86, promote adaptation of the immune system such that immunosuppressive requirements diminish with time. In some animal models this has led to allograft tolerance, a condition in which a transplanted organ is accepted without the need for chronic immunosuppression and without recipient immuno-incompetence. This process has been shown in animal models to be facilitated by the mTOR inhibitor sirolimus, and to be aided by the infusion of donor blood or bone marrow.

This study will combine lymphocyte depletion, mTOR inhibition, and costimulation blockade, in 20 renal allograft recipients of either living or live donor kidneys to determine whether together, these therapies will promote allograft acceptance. Peri-transplant T-cell depletion induced by alemtuzumab will be used in combination with chronic therapy with sirolimus and belatacept a novel fusion protein specific for the costimulation molecules CD80 and CD86.

All patients will be given a single dose of alemtuzumab on the day of transplantation. All patients will then be treated with belatacept and sirolimus for 1 year. Patients who remain rejection-free for 1 year and meet in vitro flow cytometric criteria indicating that their immune system has repopulated (Ki67 expression returns to baseline) will be gradually withdrawn from sirolimus, to determine if they can maintain a rejection-free state on belatacept monotherapy. In addition to clinical outcome, immune reconstitution following T-cell depletion and the development of donor-specific immune unresponsiveness will be evaluated. If successful, the trial will present an immediately applicable method for organ transplantation without dependence on chronic immunosuppressive drugs.

1.2 Background

Transplantation is the treatment of choice for most causes of end stage renal disease.[4-6] Approximately 17,600 patients undergo renal transplantation in the United States each year with one-year graft survival exceeding 92% and 96% for deceased donor and live donor transplants respectively.[4] In 2009, the last year for which complete data are

available, 144,180 recipients were being treated with a functioning kidney graft in the United States,[4] making transplantation a significantly common condition, but meeting the FDA definition of an orphan indication per the Orphan Drug Act Amendment P.L. 98-551, October 1984.[47]

Although kidney transplantation provides superior renal replacement to dialysis in most cases, all renal allografts succumb to rejection without some modification of the recipient's immune system. To prevent this, patients must take immunosuppressive drugs for life.[4,7,8] In general, these drugs target some aspect of T cell activation, as T cells are the principal protagonists in cellular allograft rejection. A significant amount of allograft rejection, particularly that occurring late after transplantation, is attributable to the effects of B-cell mediated alloantibody production.[48-50] However, alloantibody production is also generally considered to be T cell dependent, relying upon the effects of T cell help. Thus, effective control of allospecific T cells generally prevents both antibody and cellular mechanisms of allograft destruction.[51] Indeed, a substantial cause of graft loss is attributable to late antibody mediated rejection driven by non-adherence with T cell-specific drugs.[52] Typical maintenance immunosuppressive regimens include a combination of steroids, a CNI, (cyclosprine A or tacrolimus) and/or an antiproliferative agent (azathioprine, MMF or sirolimus).[4,7,8,58] A trend toward decreased steroid use has emerged in the past 5 years with approximately 30% of centers avoiding or weaning steroids during the first year post transplant aiming to reduce the morbidity of steroid use, but accepting an incremental risk of rejection.[4,7]

In an effort to reduce the need for maintenance immunosuppression, over 80% of North American transplant centers now employ induction therapy[59] with a brief course of an anti-T cell antibody preparation (daclizumab, basiliximab, alemtuzumab or rabbit anti-thymocyte globulin, RATG). Indeed, most centers (58%) opt for T cell depletion with RATG or alemtuzumab,[4,7] driven by evidence suggesting this better reduces the need for subsequent maintenance therapy. One induction agent that is gaining attention is alemtuzumab.[32,33] Alemtuzumab is a CD52-specific humanized MAb indicated for the treatment of some lymphogenous malignancies and has been increasingly used off-label in transplant indications.[4,7] Its advantages include a singular target (CD52) and monoclonal composition, both of which greatly reduce the batch-to-batch variation and side effects seen with polyclonal RATG. However, its use has been limited by challenges typical of the orphan drug status of transplantation, in that companies have opted for development in more profitable indications despite considerable evidence of its effectiveness in transplantation.[60]

Historically, CNIs have emerged as the centerpiece drug of most maintenance immunosuppressive regimens. In the US, the predominant CNI is tacrolimus, and it is typically paired with MMF and steroids.[4] CNIs inhibit T cell receptor (TCR) signaling in naïve, memory and effector T cells, and T cell dependent B-cell help through inhibition of calcineurin-calmodulin-dependent pathways,[61-64] thereby inhibiting allograft rejection. CNI-based regimens are by far the most common in clinical transplantation, being used in over 94% of patients through 2009.[4] However, CNIs also prevent viral immunity[65,66] and impair adaptation to persistent antigens such that even after prolonged rejection-free survival of an allograft, removal of a CNI precipitates rejection.[25,67-70]

CNI-based immunosuppression effectively prevents rejection, but its use tempers the

substantial benefit obtained from transplantation.[9-21,61-66] CNIs must be administered daily and are expensive, costing over $17,000 per patient per year.[71-73] Both of these factors impair patient adherence. More importantly, they increase the risk of opportunistic infection and malignancy, and have many non-immune side effects that hamper their tolerability. Specifically, CNIs are nephrotoxic and induce secondary renal failure in up to 20% of chronic users, even those whose transplant is extra-renal.[74] In most centers, CNIs continue to be paired with steroids.[4,7] Steroids exacerbate osteoporosis and hyperlipidemia, and cause avascular osteonecrosis. Both classes of agent worsen glucose tolerance and hypertension and are associated with cosmetic effects fostering substantial non-adherence with prescribed therapies. Indeed, the long-term complications from anti-rejection therapies become increasingly apparent over time, such that the use of secondary medications, such as antihypertensives, lipid lowering agents, and therapies to counteract advanced osteoporosis, are common. Importantly, with advances in immunosuppression counterbalanced by increased immunosuppressive morbidity, the cost effectiveness of additive maintenance immunosuppression has reached a point of diminished cost-effectiveness.[73] As such, methods of transplantation that lessen the dependence on and side effects of maintenance immunosuppression stand to reduce the risk and expense of transplantation.

Belatacept is a new alternative to CNI-based maintenance therapy. Belatacept is a B7-specific fusion protein that mediates CD28-B7 costimulation blockade (CoB).[34-41] Naïve T cells require CD28 costimulation in addition to TCR signaling to optimally activate; failure to receive costimulation at the time of TCR signaling not only inhibits activation, but also promotes antigen-specific cell anergy and/or apoptosis.[22-24,34,75-78] This attenuates cells undergoing initial antigen engagement, but does not affect cells that are not engaged with antigen or those that have already been activated and moved beyond the requirement for costimulation. Thus, CoB has the potential to more selectively and more permanently influence allospecific T cells compared to CNIs. Furthermore, its precise targeting of costimulatory pathways avoids many of the off-target side effects associated with CNIs, MMF and steroids.[36-41] Unfortunately, belatacept appears less able to prevent early acute rejection than CNIs when used as indicated: in combination with basiliximab, steroids and MMF.[36-41] The mechanisms of this are only now becoming understood, and indeed, we have been actively engaged in the science of what as been termed CoB resistant rejection (CoBRR).[79-83] It is clear that patients for whom belatacept maintenance therapy is effective have a significantly reduced incidence of off-target side effects, particularly those generally associated with prolonged CNI use: nephrotoxicity, hypertension, hyperlipidemia and new onset type 2 diabetes.[36-41] Accordingly, belatacept has recently (June, 2011) been approved by the FDA as a CNI replacement. However, belatacept's optimal use remains undefined. Its patients remain encumbered with the side effects of adjuvant agents, and accept a higher risk of rejection compared to CNI-treated patients.

Depletional induction approaches have been shown to reduce the risk of early rejection post-transplant, and perhaps to reduce the needs for maintenance immunosuppression. Most notably, T cell depletion with alemtuzumab has been shown in several phase 2 clinical trials to allow for transplantation using only a single immunosuppressive agent.[26-33] Specifically, studies by Calne, Knechtle, Starzl and our group have demonstrated that kidney transplantation can be performed in selected patients using alemtuzumab at the time of transplantation, followed by monotherapy with CsA, sirolimus, or tacrolimus,

with 1, 3 and 5 year survival rates statistically equivalent to more conventional approaches reported in registry data. Acceptable results also have been obtained using polyclonal T cell depleting agents such as rabbit anti-thymocyte globulin in combination with monotherapy sirolimus, albeit with more side effects related to the RATG.[84] Over the past 4 years, additional trials have emerged indicating the general efficacy, ease of use and tolerability or alemtuzumab-based regimens.[85] Unfortunately, no development plans in transplantation have emerged, given the limited number of patients served and the remarkably low dose (generally a single dose) required in this indication. However, despite the ability of depletion to facilitate markedly reduced maintenance immunosuppression, it is clear that T cell depletion alone is not sufficient to induce tolerance, as patients treated with alemtuzumab alone, or who have stopped taking their maintenance therapy following depletion have uniformly experienced acute, albeit reversible, allograft rejection.[27] Thus, while T cell depletion reduces immunosuppressive requirements, additional therapies are necessary to induce lasting graft acceptance without the need for immunosuppressive therapy, or allograft tolerance.

Depletional induction approaches have been investigated and moved into the mainstream over the same time period as CoB;[4] however, the development paths of depletional agents and CoB have been parallel, not complementary. The clinical trial sponsored in the first funding cycle of this grant has been the first to pair belatacept with alemtuzumab. An additional trial has paired belatacept with RATG.[86] As discussed in the Preliminary Studies, both trials have demonstrated that depletional induction plus belatacept is well tolerated and efficacious, and made it apparent that an approach that takes advantage of the long-term benefits of a CoB-based maintenance regimen can be gained without increased early rejection.[1,86] More importantly, we have now shown that many patients treated with a combined depletional/CoB therapy have diminishing donor-directed alloimmune responses over time. As such, we have been able to successfully reduce the immunosuppressive burden in many patients, avoiding CNIs and steroids from the outset, and eventually arriving at a maintenance regimen that consists solely of a once monthly dose of belatacept. These data (described below) are the first in man to systematically pair readily available agents and arrive upon a simple induction and maintenance regimen that prevents rejection (cellular and antibody medicated), avoids use of both CNIs and steroids, and delivers many patients to a regimen based solely on CoB. Importantly, our initial data suggest that the trajectory for these patients is towards lessening immunosuppressive requirements. Indeed, we have developed a means of assessing this process (see below) and now propose to incorporate this into the gradual move away from systemic immunosuppression.

The biology of CoB-based immune adaptation to an existing allograft has been extensively studied in animal models of transplantation, but is yet to be translated into humans. Pre-clinical work has shown that interruption of the interaction between CD28 and its ligands at the time of antigen exposure can prevent allograft rejection and that continued exposure to antigen in the presence of CoB facilitates a gradual, donor-specific elimination of allospecific T cells.[22-24,34,75-78] Several groups, including our own, have shown in rigorous non-human primate transplant models that CoB can prevent rejection for months to years after the withdrawal of all medications.[35,87-89] The durability of this effect depends in large part upon the precursor frequency and general activation state of

the recipient T cell repertoire, and is synergized by mTOR inhibitors and antagonized by CNIs.[22,23,80,82,90] In general, CoB's influence on the immune response makes it likely to be most effective as a protracted therapy. Importantly, CoB is not effective in the absence of donor antigen.[91] How mTOR inhibitors accelerate the reduction in allospecific T cells when used with CoB remains unproven. However, a likely mechanism relates to inhibition of cell maturation in the presence of donor antigen that induces impaired T cell activation leading to apoptosis.[22-24] Stimulated cells exposed to antigen but simultaneously deprived of costimulation or cytokines that signal through the mTOR pathway typically undergo apoptosis. Over time, donor cell mediated AICD eliminates donor specific recipient effector T cells while leaving most of the non-allospecific T cell repertoire intact. In the first round of this trial, we have for the first time rigorously investigated the requirements regarding the source and amount of donor antigen; prospectively testing whether donor bone marrow is a mandatory requirement for this effect to occur in humans. As discussed below, we find that the allograft itself appears to be a sufficient source of donor antigen to mediate the desired effects. This novel finding, derived from the only randomized assessment of this effect as it relates to CoB in humans, greatly simplifies the logistical challenges to implementation of the proposed regimen, and eliminates the theoretical concerns regarding bone marrow induced GVHD.

This trial, forged upon the prevailing concepts shaping our understanding of alloimmunity, will continue to build upon the promising clinical results obtained during the first funding cycle of this grant, use mechanistic insights gleaned from the study of existing patients, and test a refined and more generalizable form of this therapeutic approach to transplantation. It will continue the clinical and mechanistic follow-up of the first 20 patients enrolled in the study, implement the study of a second cohort of patients that includes broader inclusion criteria (a critical factor when considering the design of a adequately powered registration trial), and prospectively test a rational and biologically based means of guiding immunosuppressive reduction, ending in a reproducible approach to maintain kidney transplant recipients on a once monthly CoB-based monotherapy maintenance regimen, that progressively eliminates the risk of rejection and late DSA formation, and avoids the prevailing off-target side effects plaguing the field today. The resulting approach will be 1) feasible, based solely on available agents; 2) cost effective, eliminating drug burden with time; and 3) safer than chronic immunosuppressive drug exposure or induction regimens paired with rapid, empiric drug withdrawal.

Each agent in this regimen has been chosen for its specific properties and mechanism of action.

Alemtuzumab is a humanized MAb specific for CD52 with demonstrated consistent and reproducible lymphocyte depleting properties.[32,33] CD52 is a 12-amino acid glycosylphosphatidylinositol (GPI) anchored glycoprotein with several characteristics promoting its use as a therapeutic target.[92] It is a high-density molecule on T cells, B cells, monocytes and macrophages, but is absent on lymphoid progenitors. This facilitates effective but transient depletion of mature lymphocytes without myeloablation. CD52 is not internalized or modulated upon cross-linking and is thus a consistent target. Alemtuzumab recognizes the C-terminal peptide and a portion of the GPI anchor, a site in close proximity to the cell surface. This promotes efficient complement deposition and membrane attack complex formation for prompt cell lysis.[93] In addition to complement-

mediated cytotoxicity, alemtuzumab also is thought to support antibody-dependent cellular cytotoxicity by virtue of its IgG Fc region.[94] Recent reports suggest that alemtuzumab may enhance lymphocyte apoptosis *in vitro* in the absence of complement or immune effector cells, leading to cell death through a non-classical caspase-independent pathway.[95] Alemtuzumab also may trigger caspase-dependent cell death.[94] Thus, alemtuzumab facilitates target cell depletion through multiple redundant mechanisms and this redundancy is likely a factor in its consistent clinical depleting effect. Its use markedly reduces the risk of early acute rejection following kidney transplantation.[85]

Alemtuzumab is approved by the FDA for the treatment of B-cell chronic lymphocytic leukemia (CLL) in patients who have been treated with alkylating agents and who have failed fludarabine therapy.[96] It also has been increasingly used for the prevention of kidney transplant rejection, and now is used off-label for this purpose in approximately 7% of kidney transplant recipients in the United States.[4] Alemtuzumab is well tolerated, particularly in the low doses planned for this trial, and in contrast to other depleting agents derived from mouse or rabbit sources, does not induce serum sickness or a anti-xenogeneic protein immune response. It does not require a central line for infusion, and it provides rapid (<1 hour), reproducible, and gradually reversible T cell depletion in humans.[27,28] It has been used specifically in monotherapy combination with sirolimus. It also has been shown to have a reduced risk of post transplant lymphoproliferative disease PTLD) compared to other induction strategies.[97]

Sirolimus is an oral immunosuppressive agent that is approved by the FDA for the prophylaxis of organ rejection in patients aged 13 years or older receiving renal transplants.[98] Phase 2 studies by the PI and others have shown that it provides sufficient immunosuppression in selected patients following induction with a T cell depleting agent.[27,28,84] Unlike CNIs, sirolimus does not block T cell receptor signaling and as such, it does not impair the antigen signals required to mediate the salutary antigen-specific effects of CoB.[22,23,99,100] Specifically, it does not prevent allospecific AICD.[22] Experimentally in primates, sirolimus facilitates progressive donor-specific adaptation particularly when used with CoB.[90,101]

Belatacept is a newly approved inhibitor of T cell costimulation with superior binding affinity compared to other B7 specific agents.[35] It has been well tolerated in the trials to date and importantly it, and related compounds, have been suggested in preclinical studies to synergize with sirolimus to promote AICD and tolerance.[90,101,102] It is approved for the prevention of renal allograft rejection in EBV-seropositive individuals when used in combination with the steroids and MMF.

This study addresses a specific need for the transplantation community and is created in the spirit of the Orphan Drug Act of 1983.[47] Given the relatively low number of transplants performed annually, and the low dose of alemtuzumab required to achieve a therapeutic effect relative to that required for its indication, CLL, there is no economic incentive for a registration trial in organ transplantation. Similarly, regimens involving sirolimus, belatacept or other maintenance immunosuppressive drugs are typically developed for long-term use; there is little incentive for commercial development of regimens that are specifically designed to eliminate long-term drug use, and as such, no reasonable expectation of development without assistance. Nevertheless, all agents for

this study are available, and are approved products available for off-label use.

1.3 Summary of Results of Belatacept Investigational Program

Given belatacept was an investigational agent when the first version of this trial began, comprehensive information regarding its use to date is provided in Appendix C. A summary of this information is provided, in brief, below.

1.3.1 Preclinical Data

Both belatacept and its parent molecule CTLA4-Ig have been studied extensively in vitro and in non-human primates by the PI and others including Emory transplant investigators. A comprehensive list of the pertinent findings from pre-clinical studies is available in Appendix C and in the IB. These data are summarized below.

Pre-Clinical Safety

Although belatacept has increased binding affinity for B7 molecules in primates and humans compared to CTLA4Ig, the binding affinity for murine CD80 and CD86 is lower. Since CTLA4Ig binds more avidly than does belatacept in mice and it is very similar to belatacept in both its mechanism of action and its structure, CTLA4Ig is considered an acceptable homologue for belatacept for rodent toxicology assessments to support registration requirements. CTLA4Ig was not found to be mutagenic or clastogenic in mice but was shown to increase the risk of virally driven malignancies consistent with its known immunosuppressive properties.

Non-clinical safety assessments of belatacept were performed in cynomolgus monkeys and showed that it was well tolerated when given intravenously to monkeys at doses up to 50 mg/kg every other day for 1 month (15 doses) or once weekly for 6 months. Belatacept was not found to be mutagenic or clastogenic in cynomolgus monkeys. The major non-clinical findings in monkeys with belatacept were reversible, related to the pharmacology of the drug, and consisted of minimal decreases in serum IgG levels and minimal-to-moderate lymphoid depletion of germinal centers in the spleen and/or lymph nodes at all dose levels. There were no solid or lymphoid malignancies noted in the 6 month toxicity study with belatacept or in a 1 year toxicity study with CTLA4-Ig. Additional pertinent findings are summarized in Appendix C; a more thorough description of these studies is available in the IB.

Pre-Clinical Efficacy

Belatacept shows greater binding avidity than its parent molecule CTLA4Ig for human CD80 and CD86. It is approximately 10-fold more effective in vitro on a per-dose basis than CTLA4Ig at inhibiting T-cell proliferation, T-cell cytokine production, and CD28-dependent killing of target cells by natural killer-like cells. In an in vivo primate immune response model, belatacept potently blocked a primary antibody response against sheep red blood cells. At equivalent doses, belatacept was 3- to 11-fold more potent (by ID_{90}) than CTLA4Ig at blocking specific humoral responses.

Belatacept was found to be efficacious for the prevention of graft rejection in a cynomolgus monkey model of renal transplantation.[37] Belatacept monotherapy was

superior to a regimen of CsA + corticosteroids in the prolongation of graft survival in this stringent model. A regimen of belatacept + MMF + corticosteroids and a regimen of belatacept + basiliximab prolonged graft survival superior to belatacept monotherapy as well as that historically observed in monkeys treated with CsA + corticosteroids. Analysis of pharmacokinetic (PK) data in monkeys suggested a relationship between trough serum drug concentrations and immunosuppressive efficacy. Additional pertinent findings are summarized in Appendix C; a more thorough description of these studies is available in the IB.

1.3.2 Clinical Pharmacology

Single-dose Phase 1 studies with belatacept were performed in 40 healthy volunteers using doses ranging from 0.1 to 20 mg/kg. No deaths or serious adverse events (SAEs) were reported. All reported clinical AEs were of mild or moderate intensity. No histamine-like peri-infusional AEs were reported. There was no evidence for the development of anti-belatacept antibodies. Belatacept kinetics were found to be dose-proportional between 1 and 20 mg/kg. The half-life was adequately characterized between the 5 and 20 mg/kg dose levels, and ranged between 176 – 210 hours (7-9 days). The steady-state volume of distribution (VSS) values were small, indicating that the drug (with high molecular weight) was confined mainly in the plasma and extracellular space. Overall, the PK of belatacept appears to be linear following i.v. administration in healthy subjects. Additional pertinent findings are summarized in Appendix C; a more thorough description of these studies is available in the IB.

1.3.3 Phase 2 Study in Rheumatoid Arthritis

Study IM103002 was a Phase 2 pilot study that assessed the efficacy, safety, and immunogenicity of multiple i.v. doses of belatacept, CTLA4Ig, and placebo in 214 subjects with RA. Eligible subjects had a diagnosis of RA for ≤ 7 years had failed at least 1 disease-modifying anti-rheumatic drug therapy, including etanercept, and had active disease (≥ 10 swollen joints, ≥ 12 tender joints, an erythrocyte sedimentation rate ≥ 28 mm/h, and morning stiffness ≥ 45 minutes). Overall, belatacept demonstrated dose-dependent efficacy in this subject population, as evidenced by American College of Rheumatology scores. With respect to safety, no deaths were reported during the treatment or follow-up period (through Day 169), and no SAEs were considered drug related by the investigators.

1.3.4 Phase 2 Study in Solid Organ Transplantation

Study IM103100 was a 1-year, partially-blinded, randomized, active-controlled, multiple-dose, multicenter (including Emory) non-inferiority study in de novo renal transplant recipients.[36] All subjects received basiliximab induction and background maintenance immunosuppression with MMF and corticosteroids. Subjects were randomized in a 1:1:1 ratio to treatment with belatacept (more intensive [MI] or less intensive [LI] regimens) or CsA. Belatacept was administered in a double-blind fashion, with the investigator and subject blinded to the identity of the belatacept dose regimen. The MI regimen most closely approximates that proposed in this trial.

The dosing schedule, evaluation criteria, and efficacy results for Study IM103100 study are presented in Appendix C. Overall, the mean duration of exposure was comparable across all 3 treatment groups. Specifically, mean duration of exposure was 300, 308, and 294 days in the belatacept MI and LI groups and the CsA group, respectively. The overall histological severity grade of acute rejection episodes and the average number of rejection episodes per subject appeared to be similar across the 3 treatment groups. By month 12, chronic allograft nephropathy (CAN) was approximately 30%-50% less common with belatacept that with CsA. Death and/or graft loss occurred infrequently in all treatment groups, and was least frequently reported in the belatacept LI group. Most graft losses occurred for technical, rather than immunological reasons. With respect to safety, the rate of AEs, including AEs resulting in discontinuation, was similar across the 3 treatment groups. The rate of SAEs was somewhat higher for both belatacept treatment groups than for the CsA treatment group. As described in Appendix C, this difference is due to an increased number of reports of AEs of transplant rejection that were not subsequently confirmed as transplant rejection, in the belatacept treatment groups.

Topics of special interest, including the assessment of renal function, cardiovascular and metabolic effects, and an exploratory analysis in an extended criteria donor population or in subjects with impairment of renal function post-transplant, is presented in Appendix C.

1.3.5 Clinical Results from the Ongoing Trial.

In keeping with the study plan proposed in the initial funding cycle, we have conducted and preliminarily reported[1] a prospective, randomized trial to determine whether a single dose of alemtuzumab permits patients to avoid both CNIs and steroids, and instead be transplanted on a belatacept/sirolimus-based regimen. The trial was designed to offer patients the opportunity to wean from sirolimus at 1 year, and if stable, consider weaning off all immunosuppression thereafter. Randomization occurred with regard to whether patients received a single dose of unfractionated donor bone marrow (10^8 nucleated cells/kg) on day 7. Enrollment was completed, with 20 participants participating. All were non-sensitized (crossmatch negative, PRA<20%) recipients of live donor kidneys enrolled in an IRB-approved (IRB# 5064), prospectively registered (ClinicalTrials.gov Identifier: NCT00565773) trial. Induction immunosuppression consisted of intraoperative alemtuzumab (30mg IV), with three daily doses of methylprednisolone (500mg) followed by steroid elimination on day 3. All patients received and tolerated the intended induction dose. Maintenance immunosuppression consisted of belatacept (10mg/kg IV on days 1, 3, 7, 14, q2w x 4, q month x 3, 5mg/kg monthly) and sirolimus (adjusted to trough levels of 8-12ng/ml x 6 months, then 5-8ng/ml). All patients tolerated belatacept. Four patients developed arthalgias, edema or mouth ulcers requiring conversion from sirolimus to MMF.

Twenty patients (median 45 years, range 20-69; 12 male:8 female, 16 Caucasian:4 African American, all EBV seropositive) have been transplanted. In general, alemtuzumab induction followed by a belatacept-based maintenance regimen has been well tolerated and prevented rejection. With a mean follow-up of >700 days (102-1345 days), all patients have excellent graft function Mean creatinine in mg/dl (range; n having reached that time point) at 6, 12 and 24 months is 1.1 (0.7-1.9; n=19), 1.1 (0.9-1.9; n=19) and 1.2 (0.6-1.8; n=11) respectively. No patient was chimeric after bone marrow infusion, and there have been no differences in outcome attributable to the infusion

(discussed below). There have been no malignancies, significant infectious illnesses, nor CMV viremia or disease (see discussion below regarding preservation of protective immunity). Asymptomatic BK viremia has been detected in nine patients and in all cases this responded to reductions in sirolimus and/or reduced belatacept dosing. Transient, low level, clinically insignificant EBV viremia was detected in four patients. One patient has developed recurrent IgA nephropathy without a significant change in renal function. One patient has developed ulcerative colitis.

Twelve patients have elected to wean to monotherapy belatacept; of these, five have been successfully weaned from sirolimus and maintained from 3 to 15 months on monotherapy belatacept. Three patients failed sirolimus weaning. Two of these patients had subclinical rejection detected on protocol biopsy after sirolimus weaning and by protocol received a oral prednisone taper and were returned to belatacept/sirolimus maintenance. Follow-up biopsy at 3 months confirmed resolution of sub-clinical rejection in both cases. One patient developed a new DSA after being weaned from sirolimus without renal dysfunction or biopsy findings. The patient received a single dose of IVIg (2 grams/kg), and was returned to sirolimus/belatacept therapy. The DSA is currently undetectable. Five patients are in the process of sirolimus weaning. This includes two patients deemed ineligible for weaning due to recurrent IgA nephropathy or ulcerative colitis. Seven patients have declined weaning, opting rather to continue the well tolerated belatacept and low dose sirolimus indefinitely. One patient is less than 1 year post transplant, and thus has not reached a point where weaning is offered. These preliminary data suggest that a subset of selected patients can be maintained on monotherapy belatacept. Indeed, we have mechanistic data, discussed below, that form the basis of a specific testable hypothesis addressing patients' individual capacities to wean to monotherapy belatacept, and the optimal timing to do so. These data will serve as a major theme of the continuation of this protocol.

Two patients were weaned from all immunosuppression after successful elimination of sirolimus for over 6 months and re-consenting to the weaning process. Remarkably, both patients remained rejection free off all immunosuppressive medication for 4 and 7 months, respectively. One patient developed an asymptomatic DSA at 4 months. A biopsy showed no rejection. She was returned to belatacept maintenance and treated with a single dose of IVIg (2 grams/kg). The DSA resolved and she remains well >1 year after this event with normal graft function equivalent to her baseline serum creatinine of 0.7mg/dl. The other patient developed a DSA and a concomitant clinical mixed T cell mediated rejection (TCMR) and antibody-mediated rejection (ABMR) 7 months after withdrawal of all immunosuppression. The patient was treated with RATG, plasmapheresis and IVIg, resolved the rejection and was, by protocol, returned to a standard CNI based immunosuppressive regimen. He remains stable on tacrolimus, MMF and prednisone with a serum creatinine of 1.5 mg/dl (baseline creatinine off all medication was 1.3mg/dl) over 1 year after this rejection event. From these data, we conclude that although patients can be weaned from immunosuppression for significant periods of time, that the state of tolerance in insufficiently durable, at least at this relatively early time point, to avoid alloimmunity. Interestingly, both DSA events occurred immediately following a viral upper respiratory infection, suggesting that they were evoked as a heterologous response to an environmental pathogen. Regardless, this

indicates to us that complete immunosuppressive weaning offers an unacceptable risk and we have accordingly altered our protocol to eliminate this as an explicit goal of the trial. It is important to point out that our approach in this situation is substantially different from that taken by the investigators of a recently reported tolerance trial.[2,109-111] In this trial performed at the Massachusetts General Hospital (MGH), several patients have been weaned from all immunosuppression. However, ABMR led to graft loss in 2 of 10 patients, and DSA formed in several other patients that have been followed expectantly. Endothelial injury has been observed in many patients. From a statistical standpoint, it is not clear if the results from our regimen differ from those of this trial, as we have not pressed forward aggressively to continue pursing a drug-free state in the face of an asymptomatic DSA. We have viewed the appearance of DSA as an unacceptable risk for late graft loss that should be avoided, and opted not to consider immunosuppression elimination without some evidence that monotherapy belatatcept has some risk. We reason that the ultimate goal is graft function without side effects, and that risking graft loss in pursuit of an immunosuppressive-free state without evident benefit over a state maintained with a once monthly CoB-based regimen imposes risk without compensatory benefit. Particularly in the absence of a clear metric identifying patients at risk for ABMR and graft loss, we view complete drug withdrawal to be unsupportable at this time.

2 STUDY OBJECTIVES

2.1 Primary Objective

This study will determine whether T-cell depletion followed by belatacept and daily maintenance therapy with sirolimus will permit recipients of renal allografts to avoid rejection and eventually be weaned to belatacept monotherapy maintenance immunosuppression. The primary endpoint will be the number of patients successfully withdrawn from sirolimus for 1 year after their last dose of sirolimus.“Successfully withdrawn” will be defined as a patient off sirolimus without clinical or biopsy evidence of rejection.

2.2 Secondary Objectives

1. Determination of the prognostic ability of assays based on surface phenotype changes seen during homeostatic repopulation, including Ki67 expression, T_{REG} and B_{TRANS} cell surface phenotypes, and T_M to T_{REG}/B_{TRANS} balance, with regard to the anticipation of successful immunosuppressive drug withdrawal.
2. Assessment of the proposed therapies to prevent biopsy proven acute rejection at 1, 3 and 5 years post-transplant compared to the standard reported in the SRTR database for similar patients;
3. Assessment of the incidence of biopsy proven chronic allograft nephropathy at 1, 3 and 5 years post-transplant compared to that reported in the SRTR database for similar patients;
4. Assessment of the composite of occurrence of malignancies, and opportunistic infections by 1, 3 and 5 years compared to that reported in the SRTR database for similar patients;
5. Long term assessment of donor-specific immune responsiveness after prolonged therapy with belatacept (with or without sirolimus), and during and following drug

withdrawal as determined by in vitro alloresponsiveness in CFSE mixed lymphocyte reactivity and ICCS;
6. Characterization of the repopulating immune system with regard to cell surface phenotype using MFC compared to prior experience with alemtuzumab alone or combined with other related regimens; and,
7. Comparison of the function and survival of allografts transplanted using this protocol to historical controls transplanted using conventional immunosuppression to establish an initial assessment of this approach in a general transplant population.

3 STUDY DESIGN AND EVALUATION

3.1 Study Design

This will be a single-center, open-label, non-randomized, proof-of-concept study in recipients of living or deceased donor renal transplants (DDRTs). Twenty renal transplant patients will be enrolled, at least 10 of which will be recipients of DDRTs. At the time of transplantation, participants will be given alemtuzumab, 30mg intravenously over three hours. Participants will receive combination maintenance therapy with sirolimus and belatacept for at least one year. Patients' absolute lymphocyte counts (ALCs) and percent expression T cell of Ki67 will be assessed monthly. After one year of therapy, participants may consent to and begin sirolimus withdrawal or continue therapy through study close. Patients wishing to withdraw from sirolimus will begin sirolimus weaning when their ALC has reached at least 80% of baseline and had two consecutive monthly assessments of their percent expression of Ki67 fall within at least one standard deviation of the Ki67 expression of a healthy control population. Weaning will be triggered of the Ki67 expression, with other surface phenotype assessments being followed observationally. Patients meeting these parameters will be weaned off of sirolimus by halving the dose and/or increasing the dosing interval over at least a three-month period. Each dose reduction will require stable (within 1 standard deviation) peripheral Ki67 expression. After sirolimus is discontinued, patients will remain on belatacept monotherapy indefinitely. Clinical and mechanistic observation will continue for the duration of the study to assess the efficacy and safety of this approach. Subjects will be followed for at least five years.

Duration of Study

Subjects will be followed for at least five years. Belatacept is now approved by the FDA. Therefore, subjects remaining on belatacept at the conclusion of their five-year participation would be eligible to continue this therapy. If at the conclusion of this study belatacept is not commercially available to patients, the Principal Investigator will discuss other treatment options utilizing approved immunosuppressive therapies with the subject. If subjects are successfully weaned from sirolimus as a result of their participation in this protocol, there will be no need for consideration of alternative therapy.

3.2 Study Population

Adult, EBV seropositive patients who do not have evidence of DSA pretransplant and who are scheduled to receive a kidney transplant at Emory University. Candidacy for

transplantation will be determined using the standard methods of evaluation active at Emory during the study period.

3.3 Criteria for Evaluation

1. Number of patients successfully withdrawn from sirolimus for 12 months after last dose of sirolimus
2. Biopsy proven acute rejection or chronic allograft nephropathy by 1, 3 and 5 years
3. Evidence of donor-specific hyporesponsiveness by MLR and ICCS
4. Evidence of regulatory T cell and transitional B cell activity in the periphery and allograft
5. Donor specific and third party alloantibody formation

3.4 Sample Size Determination

Our prior experience with alemtuzumab, belatacept and sirolimus, in recipients of live donor kidneys suggests that this regimen is very effective in preventing acute rejection, with only one steroid sensitive, clinical rejection episode seen in the first year. However, with the liberalization of the inclusion criteria, it is anticipated that an acute rejection rate prior to initiation of sirolimus withdrawal of up to 20% will exist, excluding up to four patients from eligibility for sirolimus withdrawal. It also is anticipated that four additional patients will either not meet other criteria for withdrawal or will refuse to be withdrawn from immunosuppression. The number of patients has therefore been chosen to have approximately 12 patients who are eligible for sirolimus withdrawal and in whom withdrawal is attempted, in order to establish a reasonable proof-of-concept. This target of 12 subjects in whom sirolimus withdrawal is attempted will allow for the detection approximately 6 subjects successfully withdrawn from sirolimus for one year, assuming the probability of a subject in whom withdrawal is attempted being successfully withdrawn from sirolimus for one year is 50%, a rate determined by the current cohort. Since there are no trials exploring monotherapy belatacept reported in the literature or registered with ClinicalTrials.gov, this cohort of patients withdrawn successfully will represent a significant finding.

3.5 Interim Analyses

No interim analysis is planned.

4 STATISTICAL METHODOLOGY

4.1 Data Set Descriptions

A summary of all patient data will be kept in a study database developed for this study. In addition to the experimental data, this database will include recipient and donor demographics and transplant relevant medical history (comorbidities, HLA type, etc.), graft function, immunosuppressive medications and levels, and a running summary of the patient's post transplant course. Data will be recorded and reported in accordance with the standards required by the United Network for Organ Sharing (UNOS). The data set will consist of all patients who are randomized and transplanted and will be used for all

efficacy analyses. The as-treated data set will consist of all patients who receive at least one dose of belatacept and will be used for all safety analyses. Subgroup analyses will be performed for selected endpoints in subjects whom withdrawal was attempted and not attempted in order to provide supporting information to the main analyses. Demographic and baseline characteristics of patients will be summarized descriptively by treatment group, by means and standard deviations (SDs) for continuous variables and frequency distribution for categorical variables. Summaries will be performed based on the data set.

4.2 Analyses

Demographics and Baseline Characteristics

Demographic and baseline characteristics of patients will be summarized descriptively by treatment group, by means and standard deviations (SDs) for continuous variables and frequency distribution for categorical variables. Summaries will be performed based on the data set.

Efficacy Analyses

No formal statistical hypothesis testing will be performed for the primary endpoint. All efficacy analyses will generally be descriptive in nature. The primary efficacy analysis will be to assess the proportion of patients successfully withdrawn from sirolimus for one year. The point estimate of this proportion and the exact two-sided 95% confidence interval (CI) will be provided for each treatment group. A patient will be considered to be successfully withdrawn from immunosuppression for one year if the patient has been administered only belatacept for a period of 365 days, following successful completion of sirolimus, and has not experienced biopsy-proven acute rejection, formation of DSA, or graft loss for this same period of 365 days. Secondary efficacy outcome measures involving incidences and proportions will be descriptively summarized with point estimates and exact, two-sided 95% CIs by treatment group. Other secondary efficacy outcome measures will be descriptively summarized by means and SDs for continuous outcomes and frequency distributions for categorical outcomes.

Safety Analyses

All AEs and SAEs will be summarized by treatment group. Laboratory values will also be descriptively summarized.

5 SUBJECT SELECTION CRITERIA

For entry into the study, the following criteria MUST be met.

5.1 Inclusion Criteria

1. Recipients, age 18 or older, of a kidney transplant at Emory University Hospital who are seropositive for the IgG antibodies specific for EBV and seronegative for IgM antibodies for EBV.
2. Willingness and legal ability to give signed written informed consent.

3. For recipients of live donor kidneys, a willing renal donor who consents for donation of donor blood for testing throughout the follow-up period, and consents to have their kidney used in this experimental study. For recipients of DDRTs, access to donor lymphocytes for subsequent mechanistic study.
4. Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to eight weeks after the study in such a manner that the risk of pregnancy is minimized. WOCBP must have a negative serum or urine pregnancy test within 72 hours prior to the start of study medication.

5.2 Exclusion Criteria

1. Immunosuppressive drug therapy within one year prior to enrollment. Specifically, candidates may not be taking or have taken prednisone, CsA, tacrolimus, azathioprine, MMF, cyclophosphamide, methotrexate, infliximab, etanercept, or other agents whose therapeutic effects are immunosuppressive in the year prior to transplantation and may not have conditions in which there is an anticipated need for these drugs during the study period. Recipients may not have taken depletional anti-lymphocyte agents at any time.
2. Any active malignancy or history of a malignancy within five years of enrollment, or any history of any hematogenous malignancy or lymphoma at any time. Patients with primary, cutaneous basal cell or squamous cell cancers may be enrolled providing the lesions are appropriately treated prior to transplant.
3. Any known immunodeficiency syndrome (including subjects with known human immunodeficiency virus (HIV) infection) or other condition that, in the opinion of the investigators, would likely increase the risk of protocol participation or confound the interpretation of the data.
4. Inability or unwillingness to comply with protocol monitoring and therapy, including, among others, a history of noncompliance, circumstances where compliance with protocol requirements is not feasible due to living conditions, travel restrictions, access to urgent medical services, or access to anti-rejection drugs after the research protocol is completed.
5. Absence of EBV-specific IgG antibodies
6. Absence of CMV specific antibodies in cases where the donor has evidence of CMV infection (CMV-specific antibodies)
7. WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for up to eight weeks after the study, or who are pregnant or breastfeeding on enrollment or prior to study drug administration
8. Subjects with underlying renal disease of: Primary focal segmental glomerulosclerosis, Type I or II membranoproliferative glomerulonephritis, Hemolytic uremic syndrome (HUS)/thrombotic thrombocytopenic purpura syndrome. If a subject has ESRD of unknown etiology and/or has no histologically-confirmed diagnosis, the subject may be enrolled into the study as long as there are no clinical signs or symptoms consistent with excluded clinical diagnoses.

9. Subjects with current or historical evidence of DSA as assessed by solid phase flow cytometry
10. Subjects who are hepatitis C antibody-positive or polymerase chain reaction (PCR)-positive for hepatitis C, or who are hepatitis B surface antigen-positive or PCR-positive for hepatitis B
11. 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. Subjects with a known positive purified protein derivative (PPD) must have completed treatment for latent TB and have a negative chest x-ray at the time of enrollment. All patients will undergo PPD testing within 3 months of enrollment, have a negative CXR, and no symptoms indicative of TB
12. Subjects with any active infection or other contraindication that would normally exclude transplantation including a life expectancy that is severely limited by their underlying medical condition
13. Subjects with a history of substance abuse (drug or alcohol) or psychotic disorders that are in the opinion of a consulting psychiatrist incompatible with adequate study follow-up
14. Subjects with active peptic ulcer disease, chronic diarrhea, or gastrointestinal malabsorption that would preclude oral drug absorption
15. All women 40 years or older and women of any age who have first degree relatives with a history of breast carcinoma or who have other risk factors of breast carcinoma, must have a screening mammogram, or provide results of a screening mammogram performed within 6 months of enrollment. Subjects with a mammogram that is suspicious for malignancy and in whom the possibility of malignancy cannot be reasonably excluded following additional evaluations will be excluded.
16. Subjects who have used any investigational drug within 30 days prior to their transplant
17. Prisoners or subjects who are compulsorily detained (involuntarily incarcerated) will not be enrolled.

6 Study Therapy

6.1 Transplant-specific therapies

Alemtuzumab

At the time of transplantation (in pre-op holding or the operating room) patients will be given a single dose of methylprednisolone 500 mg intravenously over 30 minutes followed by acetaminophen 650 mg orally or rectally and diphenhydramine 50 mg intravenously, followed in 1 hour by alemtuzumab, 30mg, given intravenously over 3 hours.

Transplantation

Transplantation will be performed using standard surgical techniques for organ

engraftment. An 18-gauge needle core biopsy will be obtained from the allografted kidney 30 minutes to 1 hour after reperfusion. These will be divided for routine histology, immunohistochemistry (2/3 of biopsy) and RT-PCR analysis (1/3 of biopsy) snap frozen on site. Patients will be cared for perioperatively on the transplant unit. Technical aspects of the postoperative management (Foley catheterization, ureteral stenting, etc) will be according to the standard of care as determined by the attending surgeon.

Belatacept

Belatacept will be given within 24 hours of transplantation via a peripheral intravenous catheter at a dose of 10mg/kg infused over 30 minutes. The dose will be repeated on study days 4 (post op day 3) and 8 (post op day 7), then every 2 weeks for 5 additional doses. Thereafter, belatacept will be given once every 4 weeks (+/- 3 days) at 10mg/kg through 6 months then at 5mg/kg indefinitely.

Sirolimus

Sirolimus will be started on postoperative day 1 at a dose of 2 mg per day orally. Doses will be adjusted to maintain 24 hour trough levels of 8-10ng/ml until the drug is weaned as described below. Sirolimus has been associated with delayed graft function in organs affected by acute tubular necrosis. Therefore, in the unlikely event that patients experience delayed graft function (<5% of live donor transplants experience delayed graft function), defined as inadequate renal function to avoid dialysis by post-operative day 7, maintenance therapy will be changed to mycophenolate mofetil (1.5 gm twice daily) and prednisone (30mg daily) until graft function improves. Patients will then be converted to sirolimus.

Toxicity attributable to sirolimus (e.g. mouth ulcers, arthralgias) will prompt dose reduction to address clinical concerns in this regard. If sirolimus trough levels need to be reduced below 4ng/ml to control drug side effects, the patient will be considered intolerant to the drug and will be changed to mycophenolate mofetil (1.0 gm twice daily) and prednisone (30mg – 20mg daily tapered to off over 1-3 months depending on the time from transplantation). Patients on prednisone and mycophenolate will be considered for weaning based on the criteria described below.

Prophylaxis

With the exception of the immunosuppressive drugs used, perioperative care will be administered in keeping with the current standard of care. This will include post-operative viral (oral valganciclovir in keeping the standard of care based on pre-operative donor and recipient CMV status), fungal (oral nystatin or clotrimazole), and bacterial prophylaxis (perioperative surgical prophylaxis transitioning to oral Pneumocystis prophylaxis) dosed for 6 months post transplantation. All prophylaxis agents will be withdrawn at 6 months unless there are specific clinical indications otherwise.

6.2 Maintenance Immunosuppressive Reduction

Beginning any time after year 1 (week 53), participants who are deemed eligible for sirolimus withdrawal as determined by clinical and flow cytometric parameters

(described below) may be withdrawn from sirolimus. Withdrawal will proceed at a maximum rate of 66% of the original dose for 4 weeks, 33% of the original dose for another 4 weeks, then discontinued. However, each reduction in dose will be predicated on stable flow cytometric profile.

Criteria for Sirolimus Withdrawal. Participants will be deemed eligible for withdrawal of sirolimus if they meet all of the following criteria:

1. No episodes of steroid resistant acute rejection since transplant, and no episodes of steroid sensitive rejection for 12 months.
2. Stable renal function, as defined by both of the following:
   a. GFR, using calculated CrCl (Cockcroft-Gault) of > 40ml/min.
   b. Creatinine, which in the 12 weeks preceding withdrawal has not increased more than 20% with respect to the lowest creatinine observed during the same period.
   c. Absence of histologic evidence of acute or chronic rejection on pre-withdrawal renal biopsy according to the Banff criteria for acute, chronic and humoral rejection.
3. Absence of DSA reactivity, as measured by de novo anti-donor-HLA antibodies.
4. ALC at least 80% of the baseline ALC prior to alemtuzumab.
5. Ki67 expression on CD3+ T cells that is within 1 standard deviation of the normal expression in untreated controls. Ki67 is typically seen in <3% of lymphocytes in health controls and a value >5% exceeds the value accepted for purposes of this trial.

For determining eligibility, a renal biopsy will be obtained and creatinine clearance will be estimated. Requisite blood samples will be obtained within two weeks before starting sirolimus withdrawal. Results of laboratory assessments of immune status will be made available to, and reviewed by, the investigator. These data will be reviewed with the knowledge of previous results of immunosuppression withdrawal obtained in the current trial up to the time of review.

Maintenance Immunosuppressive Therapy

Patients who are weaned from sirolimus will be maintained on belatacept 5mg/kg once per month +/- 3 days indefinitely. In the event that patients are not successfully weaned from sirolimus, long-term maintenance therapy will be established on an individual basis. Patients with subclinical or steroid sensitive rejections can be continued using sirolimus and belatacept, or altered based on the individualized requirements of each patient. This may include the use of CNIs, anti-metabolites, steroids, biologics or other available medications dose to achieve the required therapeutic effect. Patients experiencing steroid resistant TCMR or ABMR will be converted to a CNI-based regimen.

6.3 Treatment Administration

Infusion of Alemtuzumab shall begin within 24 hours of surgery, and shall be given prior to belatacept.

Infusion of the first belatacept dose should begin no later than 24 hours of surgery but not prior to the surgeon having made an initial intraoperative assessment and has concluded that the subject remains a transplant candidate and the transplant will proceed. Infusion doses will be based on the subject's body weight at enrollment, and will not be modified during the course of the study, unless there is a change of body weight ± 10%. Belatacept should be administered to the subject at a relatively constant rate over 30 minutes.

Sirolimus will begin orally within 48 hours of surgery and after both belatacept and alemtuzumab.

6.3.1 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. The investigator must immediately notify the medical monitor. The subject will be considered eligible to receive further study drug treatment only after discussion with the medical monitor.

Subjects who require ongoing dialysis therapy may receive the Week 4 dose of belatacept at the discretion of the investigator, but should receive no additional belatacept dosing, unless the need for dialysis has resolved by the Week 6 visit. Such subjects in whom belatacept dosing is discontinued should be placed on a conventional immunosuppressive regimen.

Subjects who receive therapeutic plasmapheresis for the treatment of suspected humoral acute rejection *may* receive an additional dose(s) of belatacept. This decision must be individualized on a case-by-case basis and the investigator must discuss any dose modification with the BMS medical monitor in advance of such modification.

6.4 Discontinuation of Therapy

Belatacept therapy MUST be immediately discontinued for the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- Any clinical adverse event, 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
- Missing 2 consecutive belatacept infusions, unless the subject is receiving lymphocyte-depleting therapy or has approval by the medical monitor to remain in the study
- Subjects receiving 56 consecutive days of dialysis and the investigator has determined that the subject no longer requires immunosuppression as the graft is functionally lost

- Imprisonment or the compulsory detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

Subjects who are discontinued from study therapy must be followed for safety for 8 weeks post-study drug discontinuation.

6.5 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 must be reported on the appropriate case report form (CRF) pages, and any deviations from specified administration should be clearly documented.

Data regarding concomitant medications will be collected only if they are related to the treatment of serious adverse or adverse events that are probably, possibly, or definitely related to study medications.

6.6 Treatment of Rejection

Biopsy proven acute rejection episodes will be treated based on the discretion of the transplant physician or surgeon. It will be permissible but not mandatory to continue protocol participation for patients who experience an early steroid sensitive rejection as described above. Presumed rejection episodes will be confirmed by biopsy as soon as possible after the initiation of symptoms. Biopsies will be performed at the Emory Transplant Clinic if at all possible. If patients are greater than a 24-hour trip from the center, the PI may elect to have the biopsy performed by a qualified transplant physician or surgeon. The Banff grade of rejection will be documented in the clinical case file and the date of the first episode of acute rejection will be the date the rejection is confirmed by biopsy. Management of rejections must be based on biopsy and clinical findings.

Patients with suspected rejection will undergo physical exam, laboratory evaluation, ultrasound examination of the kidney, and percutaneous renal biopsy accompanied by blood collection for peripheral phenotype analysis, transcriptional analysis and evaluation of donor specific alloreactivity. Biopsies will be evaluated by the attending transplant physician and reviewed with a pathologist skilled in the evaluation of renal transplant biopsy material. Biopsies will be evaluated using the Banff criteria for determination of allograft rejection.[130-132] Patients with biopsy proven rejection will be treated with methylprednisolone 500mg x 1-3 days. Failure to respond to methylprednisolone in no more than 3 days, or rapid worsening of graft function despite methylprednisolone therapy will prompt use of an approved antilymphocyte agent or other therapies deemed appropriate by the attending transplant physician or surgeon. After treatment of a steroid resistant rejection episode, patients will be discontinued from study treatment/immunosuppressive weaning and returned to a maintenance regimen established at the discretion of the attending transplant physician or surgeon in keeping with standard of care immunosuppression for renal transplantation. After treatment of a steroid sensitive rejection episode, patients may be restarted on the previous level of immunosuppression. The dosing and intensity of the regimen for maintenance

immunosuppression shall be guided by the attending transplant physician or surgeon, to re-establish clinical stability of renal function. This may result in patients receiving different doses, or restarting previously discontinued medications. Any rescue therapy will prompt a reinstitution of viral, fungal and PCP prophylaxis. The need for additional antilymphocyte agents will be carefully scrutinized by the PI prior to their administration. Alternative therapies will be utilized as appropriate (e.g., plasmapheresis, IVIG) unless it is clear (by biopsy) that the rejection is being mediated by lymphocytes.

6.7 Prohibited Therapies

Use of immunosuppressive agents and corticosteroids will be limited to those specified in the protocol. Vaccinations will be recommended based on the prevailing standard of care for transplant patients.[8] Due to the risk of infection, use of live vaccines will be contraindicated during the course of the study.

6.8 Withdrawal of Subjects from Study

Subjects will be removed from the study for the following reasons:

1. Withdrawal of informed consent (subject's decision to withdraw for any reason)
2. Any clinical adverse event, 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
3. Pregnancy
4. Missing 2 consecutive belatacept infusions, unless the subject is receiving lymphocyte-depleting therapy or has approval by the medical monitor to remain in the study
5. Subjects requiring dialysis for 56 consecutive days and the investigator has determined that the subject no longer requires immunosuppression as the graft is functionally lost
6. Imprisonment or the compulsory detention for treatment of either a psychiatric or physical (e.g., infectious disease) illness.

Subjects who are discontinued from study therapy will be followed for safety for 8 weeks post-discontinuation.

6.9 Stopping Rules

Specific criteria for discontinuing the trial at the time of the IRB review will be: treatment failure, defined as biopsy-proven, steroid-resistant acute rejection, severe infection, malignancy, death, or allergic response occurring in three of the first ten patients or five patients at any time. If two of five consecutive subjects who have received protocol therapy experience steroid resistant acute rejection, graft loss, malignancy, severe infection, and/or death in a period of three months, enrollment will be stopped and the study will be reassessed by the investigators and the study monitor.

7. STUDY CONDUCT

7.1 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)/Independent Ethics Committee (IEC) approval/favorable opinion 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.

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.

7.2 Benefits

If avoidance of steroids and reduced maintenance immunosuppression can be achieved, the patient may be freed from many of the long-term morbidities of standard immunosuppressive therapy. This may result in a significant improvement in quality of life and reduced expense for immunosuppressive medications as compared to patients on conventional regimens. Close monitoring of allograft recipients increases the likelihood of identifying potential adverse drug events or deterioration in graft function and thus increases the potential for intervening in a beneficial way to improve graft and patient survival.

7.3 Costs

All standard of care x-rays, diagnostic tests, medical services, hospital and clinic visits related to the transplant procedure and established post-transplant clinical pathway follow-up care, and immunosuppressive medications will be the responsibility of the transplant recipient and will be billed to the subject's insurance as such. Any additional study specific visits, procedures, and/or blood collections for mechanistic assays will be

provided by the study and will not be the responsibility of the study participant.

All anti-rejection medications used in this study are available to persons outside of research studies. The patient will be strongly advised to seek access to support from existing federal, state and /or private insurance after their transplant.

7.4 Risks and Discomforts

Renal allotransplantation as it is currently practiced is associated with many known risks. These include but are not limited to risks inherent in any major vascular procedure such as bleeding, wound infection, pneumonia, pulmonary embolus, stroke, myocardial infarction and death. The current standard of care includes potent but imperfect immunosuppressive agents that significantly increase the risks of malignancy (particularly lymphomas and cutaneous malignancies), wound break-down and infection, cataracts, osteoporosis, hypertension, hemolytic uremic syndrome, hyperlipidemia, insulin requiring diabetes, and renal insufficiency and do not completely eliminate the risk of graft rejection. Organs transplanted from other individuals may carry infectious agents that may cause disease in the recipient. Organs are screened for HIV, hepatitis B and C viruses, EBV and CMV. Organs are not used if they are found to be positive for HIV or hepatitis. Prophylactic agents are used to limit infectious disease.

All transplantation using treatment with alemtuzumab/belatacept/sirolimus instead of conventional immunosuppression will continue to be associated with the operative and general risks listed above. The experimental data suggest that the risk of wound complications may be less with steroid sparing protocols than with conventional therapy, but this remains to be proven and could still be significant or even increased in association with sirolimus.

7.5 Risks for Women of Child-bearing Potential

As of July of 2010, two healthy volunteers became pregnant at 12 months and 15 months, respectively, after a single dose of subcutaneous belatacept given in Phase 1 trial. Both pregnancies resulted in normal births. Only one woman with a kidney transplant had become pregnant after 7 years of belatacept treatment in a clinical trial. She has an induced abortion at approximately 8 weeks of pregnancy without complications.

All other pregnancies in the kidney transplantation trials were in partners of subjects, with a total of 15 pregnancies in 12 partners treated with belatacept (10 normal births, 2 spontaneous abortions, and 3 without neonatal outcomes reported).

7.6 Immunosuppressive Therapy-specific risks

<u>Alemtuzumab</u>
Alemtuzumab is known to have a significant risk of cytokine release syndrome. This syndrome can usually be minimized by premedication with steroids but can include fever, chills, malaise, hypotension, tachycardia, and pulmonary edema. Lymphopenia may be associated with increased risk of infection.

Sirolimus

Phase III studies of sirolimus in cyclosporine based regimens have revealed mild dose related thrombocytopenia and increases of serum triglycerides and cholesterol. This latter finding is probably its most consistent effect and was seen in 40-50% of patients. Hypertriglyceridemia predominates. HMG-CoA reductase inhibitors and fibric acid have been used and well tolerated to counteract the hyperlipidemia associated with sirolimus. Additionally, the unexpected finding of nephrotoxicity has been encountered but is likely attributable to sirolimus' potentiation of CNI nephrotoxicity and thus is likely to be inapplicable in this trial. Other common events reported in trials include leukopenia, hypertension, anemia, nausea, vomiting, elevated liver enzymes, rash and acne [37,38] Other risks from the package insert for sirolimus include possible development of lymphoma and other malignancies, particularly of the skin, increased susceptibility to infection including opportunistic infections, fatal infections, and sepsis, and lymphocele, a known surgical complication of renal transplantation, occurring significantly more often in a dose-related fashion in sirolimus-treated patients.

Belatacept in Kidney Transplant Study

Specific risks associated with belatacept may include those typically associated with transplant related immunosuppression including infection and malignancy.

Over 1000 transplant patients have received belatacept in clinical trials. Patients in these trials received belatacept or cyclosporine (a marketed drug for comparison), in addition to basiliximab, mycophenolate mofetil (MMF also known as CellCept), which is an immunosuppressant, and steroids to prevent transplant rejection. In general, patients treated with belatacept had the same overall rate of side effects, including infections and cancers, as patients who received cyclosporine. In the first 36 months of follow-up in the three clinical trials done in kidney transplant patients, the frequency of side-effects was similar comparing belatacept and cyclosporine. The most commonly reported side-effects (in $\geq$ 20% of subjects) among belatacept subjects were urinary tract infection, diarrhea, constipation, nausea, swelling, decrease in transplanted kidney function, fever, cough, high blood pressure, and low white blood cells count. Some side effects were serious and required hospitalization. Some were fatal. Some side effects were serious and required hospitalization. Some were fatal.

Post-transplant Lymphoproliferative Disorder

Post-transplant lymphoproliferative disorder (PTLD), a tumor of white blood cells that can occur after kidney transplantation, developed more frequently in patients who received belatacept (14 cases out of 949; 1.5% of subjects) than those who received cyclosporine (3 cases out of 476; 0.6%). In addition, more than half of the PTLD cases in belatacept patients involved the brain (9 cases out of 14; 65% of belatacept patients), which is a higher proportion than expected. A total of 11 of 17 subjects with PTLD died; 8 out of 14 in the belatacept group and 3 out of 3 in the cyclosporine group.

PTLD is known to occur after transplant in approximately 1% of adults and is almost always associated with the Epstein-Barr virus (EBV). NULOJIX® (belatacept) is contraindicated in transplant recipients who are Epstein-Barr virus (EBV) sero negative

or with unknown EBV serostatus due to the risk of post-transplant lymphoproliferative disorder (PTLD), predominantly involving the central nervous system (CNS). As the total burden of immunosuppression is a risk factor for PTLD, higher than the recommended doses or more frequent dosing of Nulojix and higher than recommended doses of concomitant immunosuppressive agents are not recommended. Physicians should consider PTLD in patients reporting new or worsening neurological, cognitive, or behavioral signs or symptoms.

The risk of PTLD was higher in EBV sero negative patients compared to EBV seropositive patients. EBV seropositive patients are defined as having evidence of acquired immunity shown by the presence of IgG antibodies to viral capsid antigen (VCA) and EBV nuclear antigen (EBNA). Epstein-Barr virus serology will be ascertained before starting administration of Nulojix, and only patients who are EBV seropositive will receive Nulojix. Transplant recipients who are EBV sero negative, or with unknown serostatus, should not receive Nulojix.

Other known risk factors for PTLD include cytomegalovirus (CMV) infection and T-cell-depleting therapy. T-cell-depleting therapies to treat acute rejection should be used cautiously. CMV prophylaxis is recommended for at least 3 months after transplantation. Patients who are EBV seropositive and CMV seronegative may be at increased risk for PTLD compared to patients who are EBV seropositive and CMV seropositive. Since CMV seronegative patients are at increased risk for CMV disease (a known risk factor for PTLD), the clinical significance of CMV serology for PTLD remains to be determined; however, these findings should be considered when prescribing Nulojix.

The physician will perform tests to see if the patient has antibodies to EBV. If the patient does not have antibodies to the virus, they will not be allowed to enroll in the study. If they do have antibodies to the virus, the physician will counsel them about their risk of developing PTLD. Additional monitoring and the use of anti-viral medications may be prescribed by their physician to help reduce their risk.

Post-transplant lymphoproliferative disease (PTLD) is known to be associated with the degree of immunosuppression. It occurs in approximately 0.7% of adults and up to 4.5% of children following transplantation [39-42]. This condition can give rise to lymphoma in over 50% of those patients in which it occurs. The relative risk of this condition compared to conventional therapy in humans is not known but should be reduced substantially by exclusion of EBV positive to negative mismatches.

PTLD Safety Surveillance

Special surveillance for PTLD will be considered for all subjects in this study. The PTLD safety surveillance should be implemented as soon as IRB/IEC approval and subject consent are obtained.

Subjects with Suspected PTLD

CNS imaging and/or neurologic consultation should be considered for any subject with a new or worsening neurologic finding. For subjects who undergo biopsy for suspicion of

PTLD, we recommend evaluating the biopsy specimen for CD3, CD20, CD79, and EBER. Management of belatacept and other immunosuppressants in subjects with suspected PTLD should be discussed with the BMS Medical Monitor. BMS should be informed of all confirmed cases of PTLD.

Progressive Multifocal Leukoencephalopathy (PML)

Progressive multifocal leukoencephalopathy (PML) is an often rapidly progressive and fatal opportunistic infection of the CNS that is caused by the JC virus, a human polyoma virus. In clinical trials with Nulojix, two cases of PML were reported in patients receiving Nulojix at higher cumulative doses and more frequently than the recommended regimen, along with mycophenolate mofetil (MMF) and corticosteroids; one case occurred in a kidney transplant recipient and the second case occurred in a liver transplant recipient. As PML has been associated with high levels of overall immunosuppression, the recommended doses and frequency of Nulojix and concomitant immunosuppressives, including MMF, should not be exceeded.

Physicians should consider PML in the differential diagnosis in patients with new or worsening neurological, cognitive, or behavioral signs or symptoms. PML is usually diagnosed by brain imaging, cerebrospinal fluid (CSF) testing for JC viral DNA by polymerase chain reaction (PCR), and/or brain biopsy. Consultation with a specialist (e.g., neurologist and/or infectious disease) should be considered for any suspected or confirmed cases of PML.

If PML is diagnosed, consideration should be given to reduction or withdrawal of immunosuppression taking into account the risk to the allograft.

7.7 Therapy Failure

Patients may have an increased risk of graft loss due to rejection should the primary therapy fail. It is not clear if the rate of rejection onset will be significantly faster for patients in this protocol. However, the Sponsor-investigator has extensive experience in managing immunosuppressive reduction trials including those with complete immunosuppressive withdrawal. The Sponsor-investigator and the principal investigator are in close and frequent contact related to the management of this trial.The monitoring of patients in this trial greatly exceeds that in standard practice specifically to minimize the likelihood of a late rapid rejection.

7.8 Phlebotomy

Peripheral blood draws (venipuncture); during this study, blood drawn for research will not exceed 450ml per six-week period. The patient may experience some discomfort at the site of the needle entry, and there is a risk of bruising at the site. There is a remote risk of fainting or local infection.

7.9 Renal Biopsy

Renal biopsy is a standard procedure that carries a low risk for the patient of bleeding. The risk is minimized by guidance with real time ultrasound and decreases with time from the transplant procedure. Biopsies performed within one week of transplant carry a 1-2% risk of significant bleeding (defined as requiring some intervention, either transfusion or surgery). This decreases to less than 0.5% after the 4th week as the kidney scars into place. Transplanted kidneys are easier to biopsy than native kidneys due to their location in the iliac fossa as opposed to in the flank. Biopsy will not be performed on patients with a platelet count <80 unless clinically indicated. A biopsy shall be considered clinically indicated when obtained to evaluate any abnormality and required by the attending physician to guide clinical care.

During a biopsy, a patient will feel mild burning for several seconds during instillation of a local anesthetic agent. Thereafter, the patient will feel a pushing sensation, but usually little pain, as the needle enters the kidney. There may be some mild residual pain. In the event that significant bleeding is suspected, either through clinical observation or a significant decrease in hemoglobin, an ultrasound will be performed. Transfusion will be administered as necessary or the patient will be taken to the operating room for direct control of the bleeding.

8. STUDY OBSERVATIONS

8.1 Flow Charts/Time and Events Schedule

Month 1

Tests / Evaluations	On Enrollment		Day 7 Post-transplant (study day 8	Day 14 (+/- 2 days) post-transplant	Day 21 (+/- 2 days) post-transplant	Day 28 (+/- 3 days) post-transplant
Informed Consent	X					
Medical History	X					
Demographics	X					
Physical Examination	X		X	X	X	X
Vital Signs	X		X	X	X	X
Confirm Inclusion/ Exclusion criteria	X					
CBC with Differential	X		X	X	X	X
Blood Chemistry/lipids	X		X	X	X	X
Urinalysis	X		X	X	X	X
Serum Pregnancy Test	X					
Sirolimus trough (study)			X	X		
Donor-specific Antibody	X					X
Panel Reactive Antibody	X					X
Donor-specific and third party mixed lymphocyte responsiveness/ ICCS	X					
Lymphocyte Subsets-Ki67	X[a]					X[a]
Peripheral blood transcription	X[a]					X[a]
Informed Consent	X					
HIV Ab	X					
HBsAg and HCV Ab	X					
CMV and EBV PCR	X					X
BK viremia/viruria PCR	X					X
Adverse Events	X		X	X	X	X
QoL	X					
Immunogenicity	X[a]					

[a] These are specific study related assays for reporting purposes.

Months 2-6

Tests/Evaluations	Every 2 Weeks	Monthly	Every 3 Months	At 6 Months	With Clinical Biopsies
Physical Examination		X			X
Vital Signs		X			X
CBC with Differential	X	X			X
Blood Chemistry/lipids	X	X			X
Urinalysis	X	X			X
Sirolimus trough	x				X
Lymphocyte Subsets Ki67				X[a]	X
Peripheral transcription				X[a]	X
Donor-specific and third party mixed lymphocyte responsiveness/ other studies of hyporesponsiveness				X	X
PRA				X	X
Renal Biopsy				X	X
Intra graft mRNA analysis				X	X
				X	
CMV and EBV PCR		X			
BK viremia/viruria PCR		X			X
Adverse Events	X	X			X
QoL				X	
Neurologic Exam			X		
Immunogenicity				X[a]	

=[a] These are specific study related assays for reporting purposes.

Long-Term Follow-Up Cohort 1

Tests / Evaluations	Every 6months	Yearly
Telephone Reporting	X	
Serum Chemistry/ lipids /CBC	X	
Sirolimus trough		X
Lymphocyte subsets	X	
Peripheral transcription		X
CMV and EBV PCR	X	
BK viremia/viruria PCR	X	
PRA		X
Anti-Donor Antibody		X
Donor-specific and third party mixed lymphocyte responsiveness/ ICCS		X
Renal Biopsy		X[b]
Intragraft mRNA Analysis		X[b]
Adverse Events	X	X
QoL	X	
Neurologic Exam	X	
PK		
Immunogenicity	X	

=

[b] To be performed prior to weaning patient to monotherapy (generally at 12 months) and again after stable on monotherapy as clinically indicated.

Long-Term Follow-Up Cohort 2

Tests / Evaluations	Monthly after Year 1	Every 3 months from 6 - 24Months	Every 6months	Yearly
Telephone Reporting			X	
Serum Chemistry/ lipids /CBC	X[c]		X	
Sirolimus trough[d]	X			X
Lymphocyte subsets Ki67			X	
Peripheral transcription				X
CMV and EBV PCR		X	X	
BK viremia/viruria PCR		X	X	
PRA				X
Anti-Donor Antibody				X
Donor-specific and third party mixed lymphocyte responsiveness/ ICCS				X
Renal Biopsy				X[b]
Intragraft mRNA Analysis				X[b]
Adverse Events	X[e]			
QoL			X	
Neurologic Exam			X	
Immunogenicity			X	

[c]Study participants may have these labs drawn more or less frequently as deemed clinical necessary by the investigator.

[d]Study participants who are not weaning sirolimus and are on a stable, low dose can have trough levels monitored as frequently as a monthly basis or as clinically indicated.

[e]If study participant is on a stable dose of sirolimus, not weaning, and receiving commercial belatacept as an outpatient, then adverse events may be assessed every three months.

Year 1 Medication Flowchart

	Day of Transplant 1st Dosing Day 1	Day 2	Day 4	Day 8	Day 14	Mo 1 / Wk 4	Wk 6	Wk 8	Wk 10	Mo 3 / Wk 12	Wk 16	Wk 20	Mo 6 / Wk 24	Wk 28	Wk 32	Wk 36	Wk 40	Wk 44	WK 48	Mo 12 / Wk 52
					Wk 2															
belatacept 10 mg/kg	X		X	X	X	X	X	X	X	X	X	X	X							
belatacept 5 mg/kg														X	X	X	X	X	X	X
sirolimus	Sirolimus will be started on post-op day 1 at a dose of 2 mg per day orally. Doses will be adjusted to maintain 24-hour trough levels of 8-10 ng/ml until the drug is weaned as described below.																			
methylprednisolone 500 mg	X																			
alemtuzumab 30 mg	X																			
Prophylaxis	Post-operative viral (oral valganciclovir of duration in keeping the standard of care based on pre-operative donor and recipient CMV status), fungal (oral nystatin or clotrimazole), and bacterial prophylaxis (perioperative surgical prophylaxis transitioning to oral Pneumocystis prophylaxis) dosed for 6 months post transplantation. All prophylaxis agents will be withdrawn at 6 months unless there are specific clinical indications otherwise.																			
Immunosuppression Withdrawal	During year 2 (weeks 53-104), weaning of immunosuppression will begin for all patients meeting pre-defined criteria. Patients will be first weaned off sirolimus over at least a 2 – 6 month period. After sirolimus, is weaned, patients will remain on belatacept monotherapy indefinitely.																			

9 Details of Research Procedures

9.1 Pre-transplant Tissue Acquisition

Patients will be enrolled prior to transplantation to facilitate informed consent and baseline sample procurement. All patients will be specifically informed that protocol participation will not influence their receipt of the kidney being offered. Upon enrollment, patients will have standard hematology, chemistry, virology, and coagulation samples drawn. In addition, samples will be drawn for baseline serum (from 10 ml whole blood) and lymphocyte harvest (from 100ml whole heparinized blood). Serum and cells will be separated and used for baseline assays with excess cells cryopreserved. A separate 5 ml heparinized sample will be drawn for T cell subset phenotyping. The total amount of blood drawn for research purposes will not exceed 450ml in any six-week period.

At the time of transplantation, donor samples will be obtained from 120 ml heparinized whole blood from live donors, and a similar amount of blood or spleen or lymph node from deceased donors. Lymphocytes will be procured and cryopreserved by the Emory Transplant Center Biorepository (see Resources). Lymphocytes will only be used for studies related to this protocol. After the transplant, cryopreserved serum and cell samples will be utilized by the Sponsor-investigator for mechanistic analysis, storage, or distribution to collaborating laboratories. Samples may also be sent to collaborating sites for study-related mechanistic analysis.

9.2 Post-transplant Monitoring

Post-transplant Blood Monitoring. In general, functional monitoring will proceed in keeping with the standard of care for kidney transplantation at the Emory Transplant Center. Blood for testing of renal function (creatinine, BUN, hemoglobin/hematocrit) and electrolytes (including sodium, potassium, chloride, bicarbonate, magnesium, calcium and phosphorous) will be drawn daily until discharge and at least two times weekly for one month. Patients will be monitored weekly for at least 3 months or until the ALC exceeds 500 cells/mm^3, whichever is longer. Thereafter, renal function will be assessed at least every two weeks until they reach their one-year evaluation. Lymphocyte repopulation and specific Ki67 and T_{REG} and B_{TRANS} phenotypes will be assessed monthly until 1 year after sirolimus elimination, and then every 3 months through 5 years by analysis of 7ml of heparinized blood by flow cytometry.

Biopsy Monitoring. A protocol biopsy will be performed following allograft reperfusion. Additional protocol biopsies will be performed at 6 months, and when the criteria for weaning to monotherapy belatacept are met (generally this will be during the second post transplant year) and again when the patient has been weaned to monotherapy belatacept for three months. An additional biopsy will be performed one year after weaning off sirolimus. It is assumed that histological acute allograft rejection is indicative of under-immunosuppression. Since histological rejection has been documented to occur in the absence of clinical renal dysfunction (so-called sub-clinical rejection) in up to 30% of persons with stable allograft function, it is reasonable to assume that some patients studied in this trial will have sub-clinical rejection, and thus be inappropriate for further immunosuppressive reduction. Protocol biopsies are thus clinically indicated to pre-empt inappropriate immunosuppressive weaning, as well as to detect other occult pathology

known to arise in transplanted kidneys that could confound the interpretation of the study.[133-134] In the event that subclinical rejection is detected, the patient will receive anti-rejection therapy in keeping with the standard of care, generally an oral steroid taper over 30 days and reevaluation of the patient's need for chronic immunosuppression. A follow-up biopsy will be performed in 3 months to assess resolution of subclinical rejection. Subclinical rejection, once resolved, will not in and of itself remove the patient from consideration of weaning, if it occurs during a period of homeostatic repopulation.

Renal biopsies also will be performed if any sustained rise in serum creatinine of greater than or equal to 30% of the baseline creatinine is detected. Baseline creatinine will be defined as the mean of five stable serum creatinine values within the first three months post-transplant. Stable is defined as a variance of +/- 20% or less of any value. Clinical suspicion of allograft pathology based on other grounds (urine cytology, pyuria, proteinuria, etc.) also will be considered grounds for biopsy at the discretion of the attending physician. An attending transplant physician or surgeon will perform all biopsies. The risks of transplant kidney biopsy will be included in the informed consent process for the protocol. At the time of each biopsy (protocol or clinically indicated), blood will be drawn for peripheral phenotyping and lymphocyte harvest (50ml heparinized).

Patients will be evaluated for cellular alloreactivity, donor-specific hyporesponsiveness and viral specific responsiveness pre-operatively, at the time that sirolimus withdrawal commences, 3 months after sirolimus withdrawal, at the time of clinical rejections, and at study termination. This will require 50ml of heparinized blood to be drawn at these time points and cryopreserved for analysis.

9.3 Specific Tests and Evaluations

Donors

All live donors will provide written informed consent for participation in this trial. Donor characteristics (deceased or living, age, race, sex, medical history, laboratory tests, and ischemic time) will be documented. Live donors will be specifically informed of the experimental nature of their recipient's protocol. The living donor may decline to consent for the blood donation aspect of this protocol without affecting the recipient's ability to participate in this study. However if the donor declines to sign the informed consent for participation in all aspects of the protocol, the recipient may not be enrolled.

Recipient Evaluations Prior to Immunosuppressive Dosing

After informed consent is obtained, the following tests and evaluations are to be conducted within 72 hours prior to randomization/study treatment unless otherwise specified:

- Demographics
- Medical history
- Physical examination
- Vital signs
- Urinalysis

- Serum pregnancy test for females of childbearing potential
- CBC with differential
- Lymphocyte subsets, and baseline Ki67
- Peripheral blood transcription
- Donor specific MLR/ ICCS and antibody titers
- Blood chemistry: blood urea nitrogen (BUN), AST, ALT, total bilirubin, creatinine, sodium, potassium, calcium, bicarbonate, chloride, glucose
- HIV antibody, HBsAg, HCV Ab
- EBV and CMV IgG and IgM, and EBV and CMV PCR
- BK viremia and viruria
- Panel Reactive Antibody/ alloantibody assessment
- Donor characteristics including ischemic time

Evaluations During Treatment Period

See flowcharts in Section 8 for specific tests and the timing of test to be drawn during the study period. In addition, a general health related quality of life measure will be collected from all patients. We will utilize the SF-36 questionnaire. The instrument will be administered pre-transplant and at post-transplant visits in month6, 12, 18, 24, then annually thereafter.

A neurologic assessment (exam and history) will be done every 3 months up to the year 2 annual visit. After year two, these assessments will be done every 6 months until the end of participation in the study.

The neurologic exam will include:

A review of any new neurological symptoms of changes, such as:

- Personality
- Memory
- Headaches
- Pain
- Seizures
- Impairment of consciousness
- Swollowing
- Vision, hearing, and language function
- Coordination
- Gait
- Weakness
- Sensory alterations
- Sphincter disturbances
- Involuntary movements

The neurologic exam will include testing of:

- Mental status
- Gait
- Speech
- Cranial nerves
- Cerebral function
- Deep tendon reflexes
- Sensory function
- Motor function

The patient will be watched closely for any neurological changes that may develop throughout the study. If the patient develops a new finding or if a condition worsens, there will be a follow-up neurologic exam. If needed, more tests and procedures might be ordered and the patient might be referred to a Neurology specialist.

10. INVESTIGATIONAL PRODUCTS

Investigational product is defined as a pharmaceutical form of an active ingredient or placebo being tested or used as a reference in the study, whether blinded or unblinded.

This trial will be conducted under an FDA Investigational New Drug application held by the Sponsor-investigator. All medications are approved for human use

10.1 Belatacept Preparation

A pharmacist or qualified personnel at the site will prepare 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.

All dilutions and transfers of Belatacept must be performed using polypropylene non-siliconized syringes (Norm-Ject®) manufactured by Henke Sass Wolf in Germany and filtered using a 1.2 μm FILTERFLOW® filter extension set.

NOTE: It is recommended that a separate needle and syringe be used to withdrawal the drug product solution from each vial.

Glass bottles are acceptable for belatacept infusions.

The Belatacept solution should then be diluted in D_5W or NSS to a final Belatacept concentration as low as 1 mg/mL. The volume of the fully diluted infusion solution should be approximately 100 mL.

The final Belatacept solution should be visually inspected for particulate matter prior to administration.

The continuous infusion solution must be filtered upon administration using an in-line, sterile, non-pyrogenic, low protein-binding filter with a pore size of 1.2 μm (to be

provided by BMS). This infusion should be administered over a period of approximately 30 minutes. Any unused portion of the infusion solution should not be stored for reuse.

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.

10.2 Other Agents

Alemtuzumab will be administered in an inpatient setting and the patient will be monitored with pulse oximetry.. A well-described first dose effect is seen with all anti-lymphocyte preparations though more aggressive cardiopulmonary monitoring is not typically necessary. Additional monitoring can be implemented as deemed necessary by the Principal Investigator in keeping with the standard of care. Belatacept will be administered as an inpatient for doses 1 and 2 and may be given as an outpatient thereafter. Alemtuzumab may be administered via a central venous catheter, a vascular dialysis access, or peripherally and will be administered over a 3 hour period by the nursing or anesthetic staff. Premedication will be given as described above for alemtuzumab prior to drug dosing. Drug will be administered while a member of the attending staff is physically on-site. A member of the attending staff will physically examine the patient prior to the first 2 doses of Belatacept. All patients receiving alemtuzumab or Belatacept will have adequate vascular access to support resuscitative efforts should they be required.

Patients requiring dialysis will be dialyzed prior to receiving alemtuzumab or Belatacept. The transplant pharmacy staff will prepare alemtuzumab and Belatacept within 6 hours of administration. See package insert (alemtuzumab) or Investigators' Brochure (Belatacept) for how drug is supplied and prepared.

No special precautions are warranted in handling alemtuzumab or Belatacept or solutions containing these agents. Empty and partially used ampules or vials should be disposed of according to each institution's standard policies for disposal of biological waste.

Sirolimus will be administered orally initially by the nursing or physician staff, and eventually by the patient in keeping with the package insert.

11 ADVERSE EVENT REPORTING

11.1 Importance of Adverse Event Reporting

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

11.2 Data Safety Monitoring Plan

The safety of interventions and treatments associated with this protocol will be under continuous review by the investigative team. A formal Data Safety Monitoring Board will be established to monitor the conduct and progress of the trial and to insure the prompt implementation of patient safeguards. The Board will be assembled including three non-trial affiliated individuals: a nephrologist, a transplant surgeon, and at least one other medical personnel with sufficient knowledge of transplantation to interpret the ongoing progress of the trial. The Principal Investigator will notify the Board of all Serious Adverse Events, and data pertinent to patient recruiting and outcome. The Board will meet annually and convey its recommendations in writing to the Sponsor-investigator and Principal Investigator.. DSMB reports will be submitted to the Institutional Review Board per IRB requirements and meeting minutes will be disseminated to all necessary parties.

All adverse events will be reviewed weekly in a multidisciplinary format that will involve review by a transplant surgeon, a transplant physician, and other members of the transplant staff. All Serious Adverse Events will be reviewed within the accepted guidelines and submitted to the appropriate regulatory bodies governing this study.

Renal failure has many known complications. Pre-existing conditions, which progress in keeping with known history, will not be expeditiously reported. This protocol is not designed to influence those pre existing complications in any way. Therefore, only acute exacerbations that deviate from their natural history will be reported.

11.3 Collection of Safety Information

An ***Adverse Event (AE)*** is defined as any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have to 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 a investigational (medicinal) product (IP), whether or not considered related to the IP.

During clinical trials, 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 adverse events.)

Following the subject's written consent to participate in the study, all serious AEs should be collected, including those thought to be associated with clinical trial procedures. The collection of non-serious AE information should begin at initiation of investigational product. Non-serious AE 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. Following study completion, any SAE thought to be related to study drug or clinical trial procedures should also be reported.

Certain adverse events occur commonly in this study population and will not be recorded as an adverse event, unless it is believed to be associated with the study medications (Alemtuzumab, Sirolimus, Belatacept) and/or it meets the definition of a serious adverse

event. This list is inclusive of, but not limited to events noted below, and will be at the discretion of the Principal Investigator.

Occurrence of:

- Post-op pain.
- Isolated upper respiratory infection, nasopharyngitis, cough, dyspnea and bronchitis.
- Isolated episodes of diarrhea, constipation, nausea, abdominal distention and vomiting.
- Hypokalemia/hyperkalemia, hyponatremia/hypernatreima, hypocalcemia,/ hypercalcemia, hypophosphatemia/hyperphosphatemia, /hypomagnesemia,/hypermagnesemia, hyperuricemia and hyperglycemia
- Peripheral edema
- Hematuria, proteinuria, glycosuria and dysuria.
- Headache, dizziness, tremor, insomnia, anxiety, fatigue and depression.
- AVF thrombosis and renal artery stenosis.
- Incontinence, hydronephrosis, hematoma, lymphocele, musculoskeletal pain, and atrial fibrillation.
- Acne, facial/body rash, and folliculitis.

Data on concomitant medications will be collected only if they are related to the treatment of a serious adverse or adverse event that is probably, possibly, or definitely related to study medications. All identified AEs must be recorded and described on the appropriate Non-serious or Serious AE page of the CRF. 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: date (and time) of onset and resolution, severity of the event (see definitions), Investigator's opinion of the relationship to investigational product (see definitions), treatment required for the AE, cause of the event (if known), and information regarding resolution/outcome. The Investigator shall supply the Sponsor and Ethics Committee with any additional requested information, notably for reported deaths of subjects.

The following categories and definitions of severity should be used:

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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 Version (CTCAE). 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

Adverse events will be graded on a scale from 1 to 5 according to the following standards

NCI-CTCAE manual:

- Grade 1 = mild adverse event. (Awareness of event but easily tolerate.)
- Grade 2 = moderate adverse event (Discomfort enough to cause some interferences with usual activity)
- Grade 3 = severe and undesirable adverse event. (Inability to carry out usual activity).
- Grade 4 = life-threatening or disabling adverse event..(Debilitating significantly incapacitates subject despite symptomatic therapy).
- Grade 5 =Death

The following categories and definitions of causal relationship to study drug should be used:

- Certain: There is a reasonable causal relationship between the study drug and the AE. The event responds to withdrawal of study drug (dechallenge), and recurs with rechallenge when clinically feasible.
- Probable: There is a reasonable causal relationship between the study drug and the AE. The event responds to dechallenge. Rechallenge is not required.
- Possible: There is reasonable causal relationship between the study drug and the AE. Dechallenge information is lacking or unclear.
- Not likely: There is a temporal relationship to study drug administration, but there is not a reasonable causal relationship between the study drug and the AE.
- Not related: There is not a temporal relationship to study drug administration (too early, or late, or study drug not taken), or there is a reasonable causal relationship between another drug, concurrent disease, or circumstance and the AE. Adverse Events Related to Study Conditions

If the Investigator believes that an SAE is not related to the investigational 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.

11.4 Overdose An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. For reporting purposes, BMS considers an overdose, regardless of adverse outcome, as an important medical event (see Serious Adverse Events).

11.5 AE Follow-up

AEs should be followed to resolution or stabilization, and reported as SAEs if they become serious. This also applies to subjects experiencing AEs that cause interruption or discontinuation of investigational product, or those experiencing AEs that are present at the end of their participation in the study; such subjects should receive post-treatment follow-up as appropriate. If an ongoing AE changes in its severity or in its perceived relationship to study drug, a new AE entry for the event should be completed.

11.6 Reporting of AE Information Following Study Completion

Collection of safety information following the end of investigational product administration is important in assisting in the identification of possible delayed toxicities or withdrawal effects. All SAEs must be collected which occur within 56 days of discontinuation of dosing or completion of the subject's participation in the study if the last scheduled visit occurs at a later time. In addition, the Investigator should report any SAE which may occur after this time period which they believe to be certainly, probably or possibly related to investigational product. Finally, all events of death, graft loss, malignancy, PTLD, and serious infections (i.e., otherwise meeting SAE reporting requirements) must be reported for all randomized subjects until the end of the study, irrespective of study drug discontinuation or investigator-deemed causality.

11.7 Handling of Serious Adverse Events (SAEs)

A ***serious AE*** **or reaction** is any untoward medical occurrence that at <u>any dose</u>:

- results in death,
- is life-threatening (defined as an event in which the patient or 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 prolongation of existing hospitalization, (refer to note 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(s) that may not be immediately life-threatening or result in death or hospitalization but, based upon appropriate medical and scientific judgment, may jeopardize the patient/subject or may require intervention (e.g., medical, surgical) to prevent one of the other serious outcomes listed in the definition above.)

NOTE:

- <u>Pregnancy:</u> Incidence of pregnancy is not considered a SAE; pregnancy must, however, be reported immediately using the BMS Pregnancy Surveillance Form
- <u>Cancer/Overdose:</u> All cases of cancer and overdose must be reported immediately using the SAE Report Form.
- <u>Hospitalizations (exceptions):</u> Criteria for hospitalizations not reported as SAEs include admissions for:
    - Planned as per protocol medical/surgical procedure
    - Routine health assessment requiring admission for baseline/trending of health status documentation (e.g., routine colonoscopy)
    - Medical/surgical admission for purpose other than remedying ill health state (planned prior to entry into study trial; appropriate documentation required)
    - Admission encountered for other life circumstance that carries no bearing on health status and requires no medical/surgical intervention (e.g. lack of

housing, economic inadequacy, care-giver respite, family circumstances, administrative)

An SAE report should be completed for any event where doubt exists regarding its status of seriousness.

Adverse events classified as "serious" require expeditious handling and reporting to BMS to comply with regulatory requirements.

All serious AEs whether related or unrelated to investigational product, must be immediately reported to BMS (or designee) by confirmed facsimile transmission. If only limited information is initially available, follow-up reports are required. The original SAE form must be kept on file at the study site.

For studies conducted under an Investigator IND, any event that is both serious and unexpected must be reported to the FDA as soon as possible and, in no event, later than 7 days (death or life-threatening event) or 15 days (all other SAEs) after the investigator's or institution's initial receipt of the information. Bristol-Myers Squibb will be provided with a simultaneous copy via facsimile of all adverse events filed with the FDA. SAEs should be reported on the MedWatch Form 3500A, which can be accessed at:

http://www.accessdata.fda.gov/scripts/MedWatch/

MedWatch forms should be sent to the FDA online at the above internet address or at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)

Cases of pregnancy must be reported on paper Pregnancy Surveillance Forms in lieu of SAE pages.

Collection of complete information concerning SAEs is extremely important. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

In accordance with local regulations, Investigators will be notified of all AEs that are serious, unexpected, and certainly, probably, or possibly related to the investigational product or serious reportable adverse events which could be associated with the trial procedures. This notification will be in the form of an Expedited Safety Report (ESR).

Upon receiving such notices, the Investigator must review and retain the ESR with the Investigator Brochure. Where required by local regulations or when there is a central Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study, the Sponsor will submit the ESR to the appropriate IRB/IEC. The Investigator and IRB/IEC will determine if the informed consent requires revision. The Investigator should also comply with the IRB/IEC procedures for reporting any other safety information. Where

required, submission of ESRs by the Investigator to Health Authorities should be handled according to local regulations.

Periodically, according to the Investigator Brochure SOP, the Investigator Brochure will be updated and include new and relevant safety information. Until such time that an AE becomes identified in the Investigator Brochure, it should be considered unexpected, regardless of whether the AE has been the subject of a previous ESR.

11.8 Laboratory Test Abnormalities

At a minimum, the following laboratory abnormalities should be captured on the non-serious or serious AE pages of the CRF as appropriate:

- Any laboratory test result that meets the criteria for a Serious Adverse Event
- Any laboratory abnormality that requires the subject to have investigational product discontinued or interrupted
- Any laboratory abnormality that requires the subject to receive specific corrective therapy, that is not seen commonly in the study population as previously defined.

It is expected that wherever possible, the clinical, rather than the laboratory term would be used by the reporting Investigator (e.g., anemia versus low hemoglobin value).

11.9 Other Safety Considerations

Any clinically significant changes noted during interim or final physical examinations, electrocardiograms, 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).

11.10 Pregnancy

Sexually active women of childbearing potential 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).

Before enrolling women of childbearing potential (WOCBP) in this clinical trial, Investigators must review the guidelines about study participation for WOCBP. The topics include the following:

- General Information
- Informed Consent Form
- Pregnancy Prevention Information Sheet
- Drug Interactions with Hormonal Contraceptives
- Contraceptives in Current Use
- Guidelines for the Follow-up of a Reported Pregnancy

Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. The subject must sign an informed consent form documenting this discussion.

All WOCBP MUST have a **negative** pregnancy test within 72 hours **prior** to receiving investigational product. The minimum sensitivity of the pregnancy test must be 25 IU/L or equivalent units of HCG. If the pregnancy test is positive, the subject must not receive investigational product and must not be enrolled in the study.

Pregnancy testing must also be performed periodically throughout the study and the results of all pregnancy tests (positive or negative) recorded on the case report form.

In addition, all WOCBP should be instructed to contact the Investigator immediately if they suspect they might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

If following initiation of study treatment, it is subsequently discovered that a trial subject is pregnant or may have been pregnant at the time of investigational product exposure, including during at least 6 half-lives after product administration, the investigational product will be permanently discontinued in an appropriate manner (e.g., dose tapering if necessary for subject safety). Exceptions to investigational product discontinuation may be considered for life-threatening conditions only after consultation with the BMS Medical Monitor or as otherwise specified in this protocol. The Investigator must immediately notify the BMS Medical Monitor of this event and record the pregnancy on the Pregnancy Surveillance Form. Pregnancy Surveillance Forms are forwarded to BMS.

Protocol-required procedures for study discontinuation and follow-up must be performed on the subject unless contraindicated by pregnancy (e.g., x-ray studies). Other appropriate pregnancy follow-up procedures should be considered if indicated. In addition, the Investigator must report to BMS, on the appropriate BMS pregnancy surveillance forms(s), follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of eight weeks.

12 ADMINISTRATIVE SECTION

12.1 Compliance with the Protocol and Protocol Revisions

The study shall be conducted as described in this approved protocol. 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.

12.2 Informed Consent

Informed consent will be obtained from each participant before enrollment. Additional informed consent will be obtained before beginning withdrawal of sirolimus.

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

The following sections contain procedures on obtaining informed consent from subjects or their legally acceptable representative prior to participating in a clinical trial.

Procedures are described for all subjects, including those who are unable to give informed consent. The relevant procedures must be used whenever they are applicable.

12.3 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 BMS and 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.

12.4 Subjects Unable to Give Informed Consent

12.4.1 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.

12.4.2 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.

12.4.3 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.

12.5 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 is an appropriate independent witness. 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.

12.6 Update of Informed Consent

The informed consent and any other information provided to subjects or the subject's legally acceptable representative, 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.

13 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 investigational product or entered as a control in the investigation. Data reported on the CRF, that are derived from source documents, must be consistent with the source documents or the discrepancies must be explained.

Paper CRFs must be completed legibly in ink. Subjects are to be identified by initials, birth date and subject number, if applicable. All requested information must be entered on the CRF in the spaces provided. If an item is not available or is not applicable, it must be documented as such; do not leave a space blank.

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).

The completed CRF including any paper SAE CRFs must be promptly reviewed, signed, and dated by a qualified physician who is an Investigator or Sub-investigator. The Investigator must retain a copy of the CRFs including records of the changes and corrections.

13.1 Institutional Review Board/Independent Ethics Committee (IRB/IEC)

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 Investigator Brochure or product labeling, information to be provided to subjects.

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.

13.2 Records Retention

The Investigator must retain investigational product disposition records, copies of CRFs (paper 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.

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.

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APPENDIX A: DIAGNOSIS OF GRAFT REJECTION

Clinical Assessment – Signs & Symptoms of Rejection	- A presumptive diagnosis of an acute episode of rejection will be based on the following finds Fever > 102, orally Graft swelling Graft tenderness > 0.3 mg/dl rise in serum creatinine or a 15% rise in serum creatinine Oliguria Reduced flow in perfusion, extraction or excretion profile on renal scan Ultrasound findings consistent with rejection
Clinical Assessment – Onset of Rejection	- The clinical diagnosis of rejection will be confirmed at the time additional immunosuppressive therapy is initiated. The date of the first episode of acute rejection will be the date additional immunosuppressive medication is initiated
Clinical Assessment – Therapeutic Response	- Complete Resolution – return of clinical parameters to baseline. - Partial Resolution –improvement in clinical parameters, but failure to return to baseline within 5 days following start of corticosteroid pulses. - No Response - no improvement in clinical parameters within 5 days following start of corticosteroid pulses.
Clinical Assessment – Severity Grade of Rejection	- Mild – complete resolution within 5 days following corticosteroid pulses. - Moderate - partially responsive to one treatment of corticosteroid pulses, but requires additional steroid therapy. - Severe - unresponsive to > 2 corticosteroid pulses, and requires aggressive therapy with OKT3.
Histologic Assessment – Renal Biopsy	- Results of renal biopsy provide histologic confirmation of diagnosis as per Banff criteria

NOTE: It is expected that activated lymphocytes may be encountered in the grafts of patients in the absence of a detrimental immune response. However, any evidence of immune injury will prompt more aggressive functional monitoring, and a heightened index of suspicion in considering additional immunosuppressive therapy.

APPENDIX B: ASSAYS FOR THE DETERMINATION OF ALLOREACTIVITY

The following assays will be performed to assess the recipients' general ability to mount an alloreactive response, and the specific ability to mount a response toward the donor. The development of assays for determining alloresponsiveness is an ongoing process and other assays may be considered as they become available.

We will use multiparameter flow cytometry (MFC) panels (see table below) that we have established and standardized as part of the original trial, and also harmonized in support of the NIH-sponsored CTOT (NCT00308802 and NCT01436305) and CTOTC-02 (NCT00951353) transplant consortia. Our standardization of these MFC panels allows our unique patients to be placed in the context of multiple other immunosuppressive regimens including standard CNI-based approaches. The panels are designed to determine the degree to which patients' T cells are predominantly naïve ($CD45RA^+$, $CCR7^+$), effector memory (T_{EM}; $CD45RA^-$, $CCR7^-$), central memory ($T_{C;}$ $CD45RA^-$, $CCR7^+$), terminal effector (T_{EFF}; $CD45RA^+$, $CCR7^-$), exhausted (T_{EX}; $CD57^-$, PD-1^+) or senescent (T_{SEN}; $CD57^+$, PD-1^-). In addition to phenotypic markers distinguishing naïve from more differentiated cells, our flow panels include surface and intracellular molecules that regulate T cell migration (CCR7 and CCR5), cytotoxic potential (perforin, granzyme), recent thymic emigration (RTE; CD103, CCR5), activation status (CD38, HLA-DR, CD45RA/RO, CD28), susceptibility to apoptosis (BCL-2), proliferation (Ki-67), association with regulatory functions (FoxP3 and CD103), and B cell maturation (IgD, IgM, CD19, CD27). T_{REGS} will be defined as $CD3^+CD4^+$,$CD25^+$, $FoxP3^+$ cells derived from the live cell, lymphocyte forward and side scatter gate. B_{TRANS} will be defined as $CD3^-$, $CD19^+$, IgD^+, $CD38^{hi}$ cells derived from the live cell, lymphocyte forward and side scatter gate. Percentage data will be derived directly and absolute cell counts will be extrapolated using the simultaneously drawn complete blood counts with differential accessed through merger of the Laboratory Information Management Software (LIMS) file with data from the Clinical Data Warehouse (see Resources). Assessments of baseline and subsequent values will be based on percentage data rather than absolute cell count, hypothesizing that ratios are more relevant after 1 year when the absolute cell count has normalized. This data stream is an established SOP and links all samples to clinical data through a HIPAA-compliant data merger. All flow panels are in daily use in support of this trial and the CTOT and CTOTC trials.

Laser	488 nm Blue			633 nm Red			407 nm Violet			
filter-PMT	530/30	575/26	780/60	660/20	730/50	780/60	450/50		660/40	
Tube #	FITC	PE	PE-Cy7	APC	Alexa 700	APC-Alexa780	V450	PacOrange	Qdot 655	
1	CD57	PD1	CCR7	CD127	CD3	CD8	CD4	CD14\20	CD45RA	Memory
2	CD2	CD28	CCR7	CD27	CD3	CD8	CD4	CD14\20	CD45RA	Memory
3	CD11a	CD38	CCR7	HLADR	CD3	CD8	CD4	CD14\20	CD45RA	Activation
4	CD103	CD31	CCR7	CCR5	CD3	CD8	CD4	CD14\20	CD45RA	Trecs
5	CD39	FOX P3	CD127	CD25	CD3		CD4	CD14\20	CD45RA	Treg
6	IgD	IgM	CD19	IgG	CD27	CD21	CD5		CD38	B cells
7	Ki67	BCL2	CCR7		CD3	CD8	CD4	CD14\20	CD45RA	intracellular
8	Perforin	Gran B	CCR7		CD3	CD8	CD4	CD14\20	CD45RA	intracellular

T cell assays assessing the peripheral T cell repertoire with regards to alloimmunity and protective immunity. Recognizing that diminished alloreactivity is relevant only if it is

specific for the allograft and does not come at the expense of protective immunity, we have and will continue to follow our patients for their capacity to protect themselves from opportunistic infection. In addition to clinical parameters (rejection, viremia), we will continue to evaluate alloreactive potential against donor and third party alloantigens, and viral specific T cell reactivity against CMV and EBV *in vitro* at time points indicated in the experimental plan above. This is vital to gain insight into the antigen specificity of any treatment effect. As discussed in the Preliminary Studies section, we are beginning to see elimination of donor allo-responsiveness but maintenance of non-donor responsiveness and CMV responsiveness. Quantitative in vitro measures of this repertoire migration are vital to understanding the durability of any observed clinical phenotype that emerges.

We will continue to measure the proliferation of CFSE-loaded CD4 and CD8 T cells responding to antigenic stimulation by donor and third party alloantigens (in the form of T cell depleted lymphocytes), and immuno-dominant peptide pools for CMV and EBV as shown in the Preliminary Studies. Briefly, frozen recipient PBMC will be thawed and labeled with CFSE (Renovar Inc., Madison, WI) as per the manufacturer's instructions. Responder CFSE-labeled PBMC will be co-cultured with mitomycin-c treated donor or 3rd party allogeneic stimulators. After 4-days, cells will be stained with fluorescence-labeled antibodies or tetramers and analyzed by MFC to identify the populations of interest. This technique, compared to thymidine incorporation, avoids radiation, better identifies proliferating subsets, and measures proliferative intensity (the number of rounds of proliferation to distinguish between limited versus extensive proliferation).[119] Using MFC, the proliferative potential of responding cells will be determined by quantifying the number of cell divisions that occur in immunosuppressed patients as compared to controls. We have established the CFSE proliferation assay in our lab in combination with Ki-67 staining, which marks proliferating cells, to identify the proliferative capacity of antigen-specific T cells.

For viral-specific responses, we use MHC defined tetramers containing immuno-dominant viral peptides for common CMV and EBV epitopes. We will continue to define the activation status of these cells, determine their cross-reactivity for donor and third party alloantigens, and relate this *in vitro* to proliferative capacity, effector function, and relationship to maintenance immunosuppressive regimen. We make use of two approaches to determine the phenotype and function of virus-specific T cells over time after renal transplantation. First, we have made CMV tetramers (A2-restricted NLVPMVATV and B7-restricted TPRVTGGGAM) and EBV-specific tetramers (A2-restricted GLCTLVAML, B7-restricted RPPIFIRRL, and B8-restricted FLRGRAYGL and RAKFKQLL peptides) to assess CD8 responses. By gating on the tetramer positive cells and making use of flow analysis, it is possible to determine the phenotype of the virus-specific T cells and quantify them to a sensitivity of 0.03% of CD8 T cells. Second, we analyze the function (CD107a/degranulation) and cytokine expression (IFN-γ, TNF-α and IL-2) of antigen-specific CD4 and CD8 T cells as we have previously described.[120,121] Prior studies have shown that T_{CM} proliferate better and are more protective against viral infection than T_{EM}. As such, we hypothesize that maintaining a high proliferative potential is critical for immunosuppressed patients to combat infection, and that the selective resistance of T_M will allow this to persist post alemtuzumab treatment. To determine if the fluctuations in T cell phenotype associate with quantitative

changes in alloreactivity or viral-specific immunity, we continue to quantify changes in the frequency, phenotype, and function of alloreactive T cells over time after transplantation, and merge these analyses with the viral specific approaches described below and data from the Clinical Data Warehouse denoting outcome specifically related to the development of acute rejection and viremia. Allospecific cells will be evaluated using both CFSE proliferation and ICCS using donor- or third-party allogeneic stimulators. This technique allows the assessment of proliferating subsets by division cycle and has been used extensively by our group.[83,122,123]

Transcriptional Studies. Peripheral blood (7.5 ml) will continue to be drawn into EDTA tubes at the times indicated in the protocol (Appendix A). Cells will be ficol separated and placed in 100 µl of RNAlater (Ambion) in a sterile, RNAase-free 2.5 ml freezing tube, placed on ice at 4°C and frozen at -80°C within four hours. We routinely process microarray-grade RNA from peripheral blood with a typical yield of 5-10 mcg/2.5mls blood. Renal biopsies will be obtained at the times indicated in the protocol as two 18-gauge needle core biopsies. The cores will be split with two halves sent for histological processing and two halves immediately transferred into 100 µl of RNAlater in a sterile, RNAase-free 2.5 ml freezing tube, placed on ice at 4°C and frozen at -80°C within four hours. These samples will be stored for batched analysis. Two halves of the core biopsies will be processed locally for diagnostic purposes. Two stained (H/E, PAS) slides will be forwarded to pathology for centralized light microscopic analysis and quantitative Banff scoring. Four unstained slides will be forwarded to the Emory pathology lab for immunohistochemistry (CD3, CD20, CD56, and C4d).

In the first iteration of the trial, we proposed transcriptional analysis of blood and biopsy material and indeed have collected samples that we will evaluate as described n the initial study. These assays have been batched for analysis at the completion of the study as batch variation markedly alters interpretation of array-type transcriptional analysis. We will continue collection of samples as per our established protocol. Briefly, clinical samples will be procured and processed to RNA and PCR LDAs will be performed in the PI's lab. These arrays will be prospectively evaluated for their ability to segregate clinical outcomes in protocol patients and related to previously studied patients transplanted under standard of care immunosuppression. We have ample published experience with RT-PCR (TaqMan) in transplantation.[124-126] Total RNA will be reverse transcribed to cDNA using TaqMan reverse transcription kit (Applied Biosystems) and amplified (50ºC for 2min, 99ºC for 10min, 40 cycles at 99ºC for 15sec and 60ºC for 1min) using 384-well LDAs (Applied Biosystems) or as individual target assays as necessary. Each amplification will be performed in quadruplicate and the mean values of quadruplicate assays will serve as the reported value. All samples will be compared to a pooled sample of cDNA from our established homogenous reference for normal kidney (pre-procurement live donor biopsy). Data will be analyzed and reported as described.[124-126]

We will construct a custom 384 well LDAs for these studies. These arrays will contain targets that we have shown to discriminate between subclinical and clinical acute rejection,[125] BK nephropathy,[126] and reperfusion injury.[124] These LDAs will allow for the validation of 98 genes in quadruplicate and will amply accommodate RT-PCR validation of 15 genes per condition and 20 known control genes for validation of results. Individual biopsy gene transcript data will be normalized by log transformation, and compared

between biopsy and blood samples with one-way analysis of variance (ANOVA). Post-hoc inter-group comparisons for different conditions will be made using a Bonferroni correction to appropriately account for multiple comparisons with significance defined as a two-tailed $p<0.05$.

APPENDIX C: SUMMARY OF RESULTS OF BELATACEPT INVESTIGATIONAL PROGRAM

Given that the investigational agent used in this trial is belatacept, comprehensive information is provided regarding its use to date.

Preclinical Data

Belatacept binds weakly to murine B7 molecules, and therefore, cannot be studied in rodent models of immune-mediated diseases or transplantation. The parent molecule of belatacept, CTLA4Ig, was therefore utilized in various rodent, non-human primate, and ex vivo T-cell proliferation assay human experiments to determine the potency of belatacept compared with CTLA4Ig. A more extensive description of these studies is available in the IB; the pertinent findings are summarized below.

Efficacy:

- Belatacept shows ~ 2-fold greater binding avidity than CTLA4Ig for human CD80 and ~ 4-fold greater binding avidity than CTLA4Ig for human CD86
- Belatacept is approximately 10-fold more effective in vitro on a per-dose basis than CTLA4Ig at inhibiting T-cell proliferation, T-cell cytokine production, and CD28-dependent killing of target cells by natural killer-like cells
- In an allogeneic mixed-leukocyte culture assay under secondary allostimulation conditions, belatacept was approximately 5- to 7-fold more potent than CTLA4Ig at blocking interleukin (IL)-2, IL-4, and interferon-□ T-cell cytokine production
- In an in vivo primate immune response model, belatacept potently blocked a primary antibody response against sheep red blood cells. At equivalent doses, belatacept was 3- to 11-fold more potent (by ID_{90}) than CTLA4Ig at blocking specific humoral responses
- Belatacept was found to be efficacious for the prevention of graft rejection in cynomolgus monkeys, a non-human primate model of renal transplantation
- Belatacept monotherapy was superior to a regimen of CsA + corticosteroids in the prolongation of graft survival in a stringent primate renal transplant model
- A regimen of belatacept + MMF + corticosteroids and a regimen of belatacept + basiliximab demonstrated efficacy (ie, prolongation of graft survival) superior to belatacept monotherapy as well as that historically observed in monkeys treated with CsA + corticosteroids
- Analysis of pharmacokinetic (PK) data in monkeys suggested a relationship between trough serum drug concentrations and immunosuppressive efficacy
- Treatment with belatacept, alone or in combination with basiliximab, did not result in long-term tolerance to the donor graft. Therefore, it appears likely that chronic intermittent dosing of belatacept is required for optimal long-term prophylaxis of rejection
- In an islet cell transplant model, treatment of 5 pancreatectomized macaques with a belatacept-based regimen yielded islet cell allograft survival of 56, 190, 204, 216, and

> 220 days. In contrast, a control regimen of sirolimus and anti-IL-2 receptor yielded islet cell allograft survival of only 7 days.

Safety:

The nonclinical safety assessment of belatacept was performed in the cynomolgus monkey. As noted previously, belatacept is a 2-amino acid variant of CTLA4Ig. Although the mutations in belatacept increase binding affinity in primates compared to CTLA4Ig, the binding affinity for murine CD80 and CD86 is lower with belatacept than it is with abatacept, and belatacept has approximately 10% of the activity of CTLA4Ig in an in vitro murine spleen cell bioassay. Since CTLA4Ig, which is active in mice, binds more avidly than does belatacept in mice and it is very similar to belatacept in both its mechanism of action and its structure, CTLA4Ig is considered an acceptable homologue for belatacept for rodent toxicology assessments to support registration requirements. A more thorough description of these studies is available in the IB; the pertinent findings are summarized below.

- No drug-related toxicity was observed in a single-dose intravenous (i.v.) toxicity study using cynomolgus monkeys given belatacept at doses of up to 90 mg/kg
- Belatacept was well tolerated when given i.v. to monkeys at doses up to 50 mg/kg every other day for 1 month (15 doses) or once weekly for 6 months (26 doses). The major nonclinical findings in monkeys with belatacept were reversible, related to the pharmacology of the drug, and consisted of minimal decreases in serum IgG levels and minimal-to-moderate lymphoid depletion of germinal centers in the spleen and/or lymph nodes at all dose levels. Similar observations were noted for CTLA4Ig when administered to monkeys at doses up to 50 mg/kg once weekly for 1 year (52 doses)
- No adverse cardiovascular, respiratory, or neurological effects have been detected in any of the single- or repeat-dose toxicity studies of belatacept when given to monkeys for up to 6 months. In addition, no belatacept-related changes were observed in plasma or serum levels of histamine, complement (C3a), tumor necrosis factor-α, or IL-6 (mediators associated with hemodynamic changes and anaphylactoid responses)
- CTLA4Ig was not found to be mutagenic or clastogenic when tested at maximum concentrations recommended by international guidelines or maximal achievable concentration
- In studies conducted with CTLA4Ig, the major safety concern identified relates to the potential for an increased incidence of virally-induced tumors in the presence of long-term immunomodulation. In a mouse carcinogenicity study, increases in the incidence of lymphoma and mammary gland tumors were observed at human exposure multiples of 0.8- and 1.9-fold, respectively. In mice, murine leukemia virus and mouse mammary tumor virus have been reported to cause lymphomas and mammary tumors, respectively. The presence of these viruses was confirmed in mice from this study based upon electron microscopy and immunohistochemistry. Data collected from these studies strongly support the conclusion that the increased malignancies in this study are secondary to long-term CTLA4Ig-induced immunomodulation and the immunological control of these specific viruses

- No evidence of lymphoma, other solid tumors, or preneoplastic morphological changes such as lymphoid hyperplasia were observed in the 6-month monkey toxicity study conducted with belatacept or in the 1-year monkey toxicity study conducted with CTLA4Ig despite the presence of lymphocryptovirus in the CTLA4Ig study, which could mediate these changes
- In i.v. embryo-fetal development studies with CTLA4Ig, no maternal or embryo-fetal toxicity was observed at doses up to 200 mg/kg/day in rats, 300 mg/kg/day in mice, or 200 mg/kg/3 days in rabbits (human exposure multiple of 30- and 29-fold for rats and rabbits, respectively; human exposure multiples not determined for mice). In the rat and rabbit studies, CTLA4Ig was shown to cross the placenta, indicating that fetuses from CTLA4Ig-treated dams were exposed to drug. On the basis of these data, CTLA4Ig was considered not teratogenic in mice, rats, or rabbits, supporting the administration of CTLA4Ig and belatacept to women of childbearing potential (WOCBP)
- In a fertility and early embryonic development study with CTLA4Ig in rats, there were no effects on reproductive function in male or female rats, and no effect on early embryonic development at doses up to 200 mg/kg administered i.v. every 3 days (human exposure multiple of 11-fold)
- In a pre- and post-natal development study with CTLA4Ig in rats, there were no effects on the F_0-generation dams at doses up to 200 mg/kg administered i.v. every 3 days (human exposure multiple of 11-fold), and no effects on the F_1-generation rats at $\leq$ 45 mg/kg (human exposure multiple of 3-fold). At 200 mg/kg, drug-related changes in the F_1-generation were limited to females, and consisted of an increase (9-fold) in the T-cell-dependent antibody response to keyhole limpet hemocyanin and moderate diffuse inflammation of the thyroid gland of 1 rat. These findings are considered to represent the lower threshold limit for effects of CTLA4Ig on immune parameters in the F_1-generation rats, as these changes were either limited to only 1 sex or 1 animal, and no other immune parameters were affected (splenic-lymphocyte and natural-killer cell phenotypes, serum Ig levels, and presence of antinuclear antibodies).

Clinical Pharmacology

A single-dose Phase 1 study with belatacept was performed in 40 healthy volunteers. Subjects received single i.v. infusions of either 0.1, 1, 5, 10, or 20 mg/kg belatacept. At each dose level, 6 subjects received active drug and 2 received placebo. Analysis of the safety data indicates that single i.v. doses were well tolerated. No deaths or serious adverse events (SAEs) were reported. All reported clinical AEs were of mild or moderate intensity. No histamine-like peri-infusional AEs were reported. No clinically significant changes in vital signs or laboratory parameters were observed. There was no evidence for the development of anti-belatacept antibodies. When the dose increased in a ratio of 1: 5: 10: 20, the geometric mean area under the concentration-time curve from time zero extrapolated to infinity values increased in a ratio of 1: 4.6: 9.9: 18.5; therefore, belatacept kinetics were found to be dose-proportional between 1 and 20 mg/kg. The half-life was adequately characterized between the 5 and 20 mg/kg dose levels, and ranged between 176 – 210 hours (7-9 days). Following a single i.v. infusion of

1-20 mg/kg, total body clearance (CLT) values ranged from 0.45-0.49 mL/h/kg. The steady-state volume of distribution (VSS) values were small, indicating that the drug (with high molecular weight) was confined mainly in the plasma and extracellular space. Both CLT and VSS appeared to be dose independent between the 1 and 20 mg/kg dose levels. Overall, the PK of belatacept appears to be linear following i.v. administration in healthy subjects.

Phase 2 Study in Rheumatoid Arthritis

Study IM103002 was a Phase 2 pilot study that assessed the efficacy, safety, and immunogenicity of multiple i.v. doses of belatacept, CTLA4Ig, and placebo in 214 subjects with RA. Eligible subjects had a diagnosis of RA for ≤ 7 years had failed at least 1 disease-modifying anti-rheumatic drug therapy, including etanercept, and had active disease (≥ 10 swollen joints, ≥ 12 tender joints, an erythrocyte sedimentation rate ≥ 28 mm/h, and morning stiffness ≥ 45 minutes). Overall, belatacept demonstrated dose-dependent efficacy in this subject population, as evidenced by American College of Rheumatology scores. With respect to safety, no deaths were reported during the treatment or follow-up period (through Day 169), and 12 subjects reported SAEs, although no SAEs were considered drug related by the investigators.

Phase 2 Study in Solid Organ Transplantation

Study Design

Study IM103100 was a 1-year, partially-blinded, randomized, active-controlled, multiple-dose, multicenter non-inferiority study in de novo renal transplant recipients. All subjects received basiliximab induction and background maintenance immunosuppression with MMF and corticosteroids. Subjects were randomized in a 1:1:1 ratio to treatment with belatacept (more intensive [MI] or less intensive [LI] regimens) or CsA (open-label dosed twice daily to achieve a specified trough serum concentration range). Belatacept was administered in a double-blind fashion, with the investigator and subject blinded to the identity of the belatacept dose regimen.

Belatacept subjects were dosed with 10 mg/kg on Days 1, 5, 15, 29, 43, 57, 71, 85, 113, 141, and 169 (MI regimen) or 10 mg/kg on Days 1, 15, 29, 57, and 85 (LI regimen). Subjects were reallocated on Days 85 (LI regimen) and 169 (MI regimen) to a 5 mg/kg dose of the drug every 4 or 8 weeks through Day 365.

Evaluation Criteria

The primary efficacy variable was the incidence of clinically-suspected and biopsy-proven acute rejection (CSBPAR) at 6 months post-transplantation. CSBPAR was defined as an increase in serum creatinine (SCr) of at least 0.5 mg/dL compared to the baseline value in the absence of other factors known to adversely affect renal function that led the investigator to suspect acute rejection, which was then confirmed by centrally-assessed biopsy. Secondary efficacy variables were the incidence of all biopsy-proven acute rejections, including those without an increase in SCr of at least 0.5 mg/dL, as well as the composite endpoints of CSBPAR or presumed acute rejection and biopsy-proven acute rejection or presumed acute rejection at 6 months and 1 year,

and death and/or graft loss at 1 year. 'Presumed acute rejection' was defined as an elevation in SCr (at least 0.5 mg/dL compared to the baseline value in the absence of other factors known to adversely affect renal function) that led the investigator to suspect and treat the subject for acute rejection without a biopsy to confirm the diagnosis, or despite a biopsy that did not confirm acute rejection. All biopsies were assessed in a blinded fashion by a central pathologist. The primary cause of graft loss and death was also adjudicated.

The safety evaluation included AEs (including infections), vital signs, physical examinations, electrocardiograms (ECGs), and laboratory parameters (hematology, biochemistry, and urinalysis). Topics of special interest were renal function (glomerular filtration rate [GFR], as determined by iohexol clearance, SCr, and calculated creatinine clearance or GFR at 1, 6, and 12 months), blood pressure (BP) parameters (systolic pressure [SBP] and diastolic blood pressure [DBP], presence of hypertension), fasting serum cholesterol and triglycerides (TGs), and the presence of post-transplant diabetes mellitus (PTDM).

Efficacy Results

The efficacy results for Study IM103100 study are presented in the following sections. Overall, the mean duration of exposure was comparable across all 3 treatment groups. Specifically, mean duration of exposure was 300, 308, and 294 days in the belatacept MI and LI groups and the CsA group, respectively.

Acute Rejection

The primary endpoint, CSBPAR at 6 months, occurred infrequently in all treatment groups (Table 1.3.4.3A). The incidence rate was slightly lower in the belatacept groups than in the CsA group. The criteria for non-inferiority to CsA were easily satisfied for both belatacept groups; however, the number of events was too small to support any further conclusions regarding the relative efficacy of the 3 regimens. The distribution of events by severity (as indicated by histological grade) was similar across the 3 treatment groups. Identical results were observed at 12 months.

The secondary endpoint of biopsy-proven acute rejection occurred 2 to 4 times more frequently than the primary endpoint of CSBPAR, indicating that most biopsy-proven acute rejection episodes were subclinical (ie, not associated with an increase in SCr $\geq$ 0.5 mg/dL). These episodes of subclinical rejection were observed on biopsies taken to satisfy the protocol requirements, according to local practice, or for other reasons than an increase in SCr $\geq$ 0.5 mg/dL.

Biopsy-proven acute rejection occurred most frequently in the belatacept LI group. As the rate of CSBPAR was comparable across treatment groups, the difference in the rate of biopsy-proven acute rejection was due to an increase in the number of subclinical rejection episodes in the belatacept LI arm. Reallocation to 8-week treatment was associated with an increased frequency of subclinical rejection.

Overall, the histological severity grade of acute rejection episodes appeared to be similar across the 3 treatment groups. While Grade IIB rejection, as assessed by Banff 97 criteria, occurred more frequently in the belatacept groups, the number of such events

was small, and the confidence intervals (CIs) around the incidence rates broadly overlapped (belatacept MI: 1.0.%-12.5%; belatacept LI: 1.1%-13%; and CsA: 0%-6.5%).

Table 1.3.4.3A: Acute Rejection - Study IM103100

Banff Grade for Acute Rejections	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
CSBPAR (ITT Analysis) by Month 6 (Primary Endpoint)[a]			
Mild Acute (IA)	2 (2.7%)	0	1 (1.4%)
Mild Acute (IB)	0	0	1 (1.4%)
Moderate Acute (IIA)	2 (2.7%)	3 (4.2%)	2 (2.7%)
Moderate Acute (IIB)	1 (1.4%)	1 (1.4%)	2 (2.7%)
Total	5 (6.8%)	4 (5.6%)	6 (8.2%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	-1.5 (-10.0, 7.0)	-2.6 (-10.9, 5.7)	---
BPAR (ITT Analysis) by Month 6			
Mild Acute (IA)	2 (2.7%)	3 (4.2%)	3 (4.1%)
Mild Acute (IB)	0	1 (1.4%)	1 (1.4%)
Moderate Acute (IIA)	6 (8.1%)	8 (11.3%)	7 (9.6%)
Moderate Acute (IIB)	3 (4.1%)	5 (7.0%)	2 (2.7%)
Total	11 (14.9%)	17 (23.9%)	13 (17.8%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	-2.9 (-14.9, 9.0)	6.1 (-7.1, 19.4)	---
BPAR (ITT Analysis) by Month 12			
Mild Acute (IA)	3 (4.1%)	4 (5.6%)	3 (4.1%)
Mild Acute (IB)	1 (1.4%)	4 (5.6%)	1 (1.4%)
Moderate Acute (IIA)	5 (6.8%)	8 (11.3%)	7 (9.6%)
Moderate Acute (IIB)	5 (6.8%)	5 (7.0%)	2 (2.7%)
Total	14 (18.9%)	21 (29.6%)	13 (17.8%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	1.1 (-11.4, 13.6)	11.8 (-2.0, 25.5)	---

[a] The results for CSBPAR were the same for all treatment groups at Month 12.

BPAR = biopsy-proven acute rejection, CI = confidence interval, CSBPAR = clinically-suspected and biopsy-proven acute rejection, and ITT = intent to treat.

Recurrent Acute Rejection

Recurrent acute rejections are summarized in Table 1.3.4.3B. Overall, the average number of rejection episodes per subject (~1.2) was similar among the 3 treatment groups.

Table 1.3.4.3B: Frequency of Biopsy-proven Acute Rejections - Study IM103100 (Intent-to-Treat Analysis)

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
No. of Subjects with Acute Rejection	14	21	13
1 Episode	11/14 (78.6%)	18/21 (85.7%)	12/13 (92.3%)
2 Episodes	2/14 (14.3%)	3/21 (14.3%)	0
3 Episodes	1/14 (7.1%)	0	1/13 (7.7%)

Chronic Allograft Nephropathy (CAN)

Biopsy specimens were also examined for CAN by an independent blinded central histopathologist using Banff 97 working classification of kidney transplant pathology.[i] By Month 12, CAN was approximately 30%-50%, in relative terms, less common with belatacept than with CsA (Table 1.3.4.3C).

Table 1.3.4.3C: Biopsy-proven Chronic Allograft Nephropathy - Study IM103100

Biopsy-proven Chronic Allograft Nephropathy	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Month 3	3 (4.1%)	2 (2.8%)	9 (12.3%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	-8.3 (-17.1, 0.5)	-9.5 (-18.0, -1.0)	---
Month 6	6 (8.1%)	3 (4.2%)	11 (15.1%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	-7.0 (-17.3, 3.3)	-10.8 (-20.3, -1.4)	---
Month 12	15 (20.3%)	11 (15.5%)	22 (30.1%)
Difference in Event Rates (LEA29Y - CsA) with Asymptotic 95% CI (%)	-9.9 (-23.8, 4.1)	-14.6 (-28.1, -1.2)	---

Note: Biopsy-proven chronic allograft nephropathy was assessed by the central pathologist; Day 1
baseline biopsies were included. In an analysis restricted to subjects with at least 1 post-baseline
biopsy, the rates of biopsy-proven chronic allograft nephropathy at 12 months were 29%, 20%,
and 44% for the LEA29Y MI, LEA29Y LI, and CsA groups, respectively.
CI = confidence interval.

Subject and Graft Survival

Death and/or graft loss occurred infrequently in all treatment groups, and was least frequently reported in the belatacept LI group (Table 1.3.4.3D). Most graft losses occurred for technical, rather than immunological reasons.

Five deaths (4 in the CsA group and 1 in the belatacept MI group) appear in Table 1.3.4.3D, which was analyzed according to the intent-to-treat (ITT) principle. Two of these deaths – both in the CsA group – occurred on therapy or within 56 days of the last dose of study therapy. Accordingly, these deaths also are counted under the prespecified safety conventions, and appear in the safety summaries in Section 1.3.4.4.

Three other deaths qualify under the ITT principle, but not under the safety conventions because they either never received study drug or the death was an event subsequent to the discontinuation of study drug + 56 days. These deaths appear in Table 1.3.4.3D, but not in the safety summaries in Section 1.1.4.4. One death in the CsA group, and 1 in the

belatacept MI group, occurred > 56 days after the last dose of study therapy. One death in the CsA group occurred in a subject who was randomized, but never treated.

Table 1.3.4.3D: Subject Death and Graft Loss by Month 12 - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Deaths by Month 12 (ITT Analysis)[a]			
Total No. of Deaths	1 (1.4%)	0	4 (5.5%)
Reasons:			
Cardiac	0	0	2 (2.7%)
Infection / Sepsis	1 (1.4%)	0	0
Pulmonary Embolism	0	0	1 (1.4%)
Other - Unknown	0	0	1 (1.4%)
Graft Loss by Month 12 (ITT Analysis)			
Total No. of Graft Losses	3 (4.1%)	1 (1.4%)	3 (4.1%)
Reasons:			
Renal Vein or Renal Artery Thrombosis	1 (1.4%)	1 (1.4%)	2 (2.7%)
Other - Infarction (Etiology Unknown, Possibly Ongoing Rejection)	1 (1.4%)	0	0
Other - PTLD as Treatment of PTLD	1 (1.4%)	0	0
Other - Combination - Persistent DGF; Acute Rejection; Infection	0	0	1 (1.4%)

Error! Bookmark not defined. Includes all deaths reported up to 12 months post-randomization, by treatment group assigned.

DGF = delayed graft function, ITT = intent to treat, and PTLD = post-transplant lymphoproliferative disorder.

Adverse Events

Overall Adverse Events

The overall incidence of AEs is summarized in Table 1.3.4.4A.

Table 1.3.4.4A: Overall Incidence of Adverse Events Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

	No. (%) of Subjects		
	LEA29Y MI (N=74)	**LEA29Y LI (N=71)**	**CsA (N=71)**
Adverse Events	73 (98.6)	69 (97.2)	68 (95.8)
Discontinued Due to Adverse Events	13 (17.6)	15 (21.1)	14 (19.7)
Related Adverse Events	43 (58.1)	40 (56.3)	50 (70.4)
Serious Adverse Events	50 (67.6)	52 (73.2)	41 (57.7)
Related Serious Adverse Events	20 (27.0)	23 (32.4)	21 (29.6)
Deaths[a]	0	0	2 (2.8)

[a] Includes all deaths up to 56 days after last dose of study therapy, by therapy received.

The rate of AEs, including AEs resulting in discontinuation, was similar across the 3 treatment groups. The rate of SAEs was somewhat higher for both belatacept treatment groups than for the CsA treatment group. As described below, this difference is due to an increased number of reports of AEs of transplant rejection, not subsequently confirmed as transplant rejection, in the belatacept treatment groups.

The incidence of AEs, by Medical Dictionary for Drug Regulatory Activities (MedDRA) system organ class and preferred term, is summarized in Table 1.3.4.4B.

Table 1.3.4.4B: Most Frequent Adverse Events (At Least 10% in Any Group) Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

MedDRA System Organ Class Preferred Term	No. (%) of Subjects		
	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=71)
Subjects with Any Adverse Events	73 (98.6)	69 (97.2)	68 (95.8)
Blood & Lymphatic System Disorders	29 (39.2)	28 (39.4)	40 (56.3)
Leukopenia	14 (18.9)	12 (16.9)	21 (29.6)
Anemia	13 (17.6)	12 (16.9)	21 (29.6)
Cardiac Disorders	10 (13.5)	10 (14.1)	10 (14.1)
Endocrine Disorders	4 (5.4)	8 (11.3)	9 (12.7)
Gastrointestinal Disorders	45 (60.8)	45 (63.4)	42 (59.2)
Nausea	19 (25.7)	18 (25.4)	16 (22.5)
Diarrhea	17 (23.0)	18 (25.4)	17 (23.9)
Constipation	16 (21.6)	22 (31.0)	20 (28.2)
Vomiting	11 (14.9)	14 (19.7)	11 (15.5)
General Disorders & Administration Site Conds.	43 (58.1)	40 (56.3)	42 (59.2)
Edema Peripheral	23 (31.1)	20 (28.2)	21 (29.6)
Pyrexia	15 (20.3)	19 (26.8)	15 (21.1)
Pain	7 (9.5)	6 (8.5)	9 (12.7)
Fatigue	6 (8.1)	6 (8.5)	9 (12.7)
Edema	6 (8.1)	7 (9.9)	11 (15.5)
Immune System Disorders	22 (28.7)	29 (40.8)	16 (22.5)
Transplant Rejection	19 (25.7)	23 (32.4)	11 (15.5)
Infections & Infestations	54 (73.0)	52 (73.2)	53 (74.6)
Urinary Tract Infection	17 (23.0)	17 (23.9)	22 (31.0)
Cytomegalovirus Infection	11 (14.9)	10 (14.1)	13 (18.3)
Nasopharyngitis	9 (12.2)	10 (14.1)	11 (15.5)
Injury, Poisoning & Procedural Complications	44 (59.5)	45 (63.4)	45 (63.4)

Table 1.3.4.4B: Most Frequent Adverse Events (At Least 10% in Any Group) Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

MedDRA System Organ Class Preferred Term	No. (%) of Subjects		
	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=71)
Incision Site Complication	17 (23.0)	16 (22.5)	13 (18.3)
Post Procedural Pain	14 (18.9)	17 (23.9)	15 (21.1)
Graft Dysfunction	9 (12.2)	10 (14.1)	10 (14.1)

Table 1.3.4.4B: Most Frequent Adverse Events (At Least 10% in Any Group) Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

MedDRA System Organ Class Preferred Term	No. (%) of Subjects		
	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=71)
Investigations	26 (35.1)	22 (31.0)	29 (40.8)
Blood Creatinine Increased	13 (17.6)	10 (14.1)	13 (18.3)
Metabolism & Nutrition Disorders	36 (48.6)	35 (49.3)	42 (59.2)
Hypophosphatemia	14 (18.9)	24 (33.8)	15 (21.1)
Hyperlipidemia	9 (12.2)	8 (11.3)	6 (8.5)
Hypercholesterolemia	6 (8.1)	4 (5.6)	9 (12.7)
Hypokalemia	5 (6.8)	5 (7.0)	9 (12.7)
Musculoskeletal & Connective Tissue Disorders	26 (35.1)	20 (28.2)	20 (28.2)
Arthralgia	8 (10.8)	6 (8.5)	4 (5.6)
Back Pain	8 (10.8)	3 (4.2)	6 (8.5)
Nervous System Disorders	26 (35.1)	20 (28.2)	26 (36.6)
Headache	13 (17.6)	10 (14.1)	8 (11.3)
Tremor	8 (10.8)	10 (14.1)	14 (19.7)
Psychiatric Disorders	18 (24.3)	27 (38.0)	20 (28.2)
Insomnia	12 (16.2)	19 (26.8)	17 (23.9)
Renal & Urinary Disorders	28 (37.8)	27 (38.0)	25 (35.2)
Reproductive System & Breast Disorders	7 (9.5)	12 (16.9)	7 (9.9)
Respiratory, Thoracic & Mediastinal Disorders	23 (31.1)	24 (33.8)	29 (40.8)
Cough	7 (9.5)	8 (11.3)	11 (15.5)
Dyspnea	5 (6.8)	6 (8.5)	9 (12.7)
Skin & Subcutaneous Tissue Disorders	26 (35.1)	18 (25.4)	18 (25.4)
Vascular Disorders	27 (36.5)	29 (40.8)	29 (40.8)
Hypertension	16 (21.6)	17 (23.9)	22 (31.0)

Note: The number of adverse events for transplant rejections includes investigator-reported transplant rejections, often obtained at the time of biopsy, irrespective of central blinded histological evaluation and/or local

evaluation. All cases of centrally-confirmed clinically-suspected and biopsy-proven acute rejection and biopsy-proven acute rejection are reported in Table 1.3.4.3.A.

MedDRA = Medical Dictionary of Drug Regulatory Activities.

Transplant rejection was reported more commonly with both doses of belatacept than with CsA. Subsequent evaluation revealed that these reports reflected episodes of suspected acute rejection later disproven by central biopsy, as well as episodes that resolved spontaneously without treatment. All reported AEs of transplant rejection that were subsequently confirmed by biopsy have been counted in the efficacy summaries provided above. AEs commonly observed during CsA treatment, such as anemia, leukopenia, hirsutism, tremor, hypomagnesemia, and hypertension, were reported less frequently with belatacept than with CsA in this study. Infectious complications occurred with comparable frequency. Pulmonary edema and proteinuria were reported more frequently with belatacept than with CsA. The significance of these events requires further evaluation.

Serious Adverse Events

The most frequent SAEs are summarized by MedDRA system organ class and preferred term in Table 1.3.4.4C. As noted above, SAEs were reported somewhat more frequently in the belatacept treatment groups than in the CsA group. This difference is accounted for by an increased frequency of reporting acute rejection as an AE in the belatacept groups. Subsequent evaluation revealed that these reports reflected episodes of suspected rejection later disproven by central biopsy, as well as episodes that resolved spontaneously without treatment. All reported AEs of transplant rejection that were subsequently confirmed by biopsy have been counted in the efficacy summaries provided above in Section 1.1.4.3.

Three subjects treated with the belatacept MI regimen developed PTLD. One case occurred on treatment and the others occurred 2 months and > 1 year after discontinuation of the study drug. The subject that developed PTLD on treatment was Epstein-Barr virus (EBV) negative and received an EBV positive allograft. This subject was diagnosed with PTLD 9 months after transplantation from a biopsy of a lesion near the basal ganglia, and belatacept was discontinued. The subject died 5 months later from *Pneumocystis carinii* pneumonia and recurrent *Cytomegalovirus* (CMV) infection while receiving dexamethasone and sirolimus. A second subject was diagnosed with PTLD 4 months after transplantation and 2 months after discontinuation of belatacept with initiation of tacrolimus. The diagnosis was based upon a renal allograft biopsy performed for suspected acute rejection. The tumor tissue and urine tested positive for EBV, and retrospective analysis of stored sera from the recipient tested negative for EBV. This subject underwent a transplant nephrectomy. A final subject received 4 doses of belatacept before discontinuation for a Grade IIB rejection, which was treated with a 10-day course of OKT3®. PTLD was diagnosed from an excisional biopsy of an anterior cervical lymph node 12 months after discontinuation of study drug. Additional information on these cases is provided in the IB.

One subject treated with the belatacept MI regimen developed breast cancer after 12 months of treatment. In retrospect, the baseline mammogram for this subject was abnormal. No subjects treated with the belatacept LI regimen developed malignancies.

Two subjects treated with CsA developed malignancies – squamous cell carcinoma of the skin and thyroid cancer – while a third subject developed a parathyroid nodule not yet confirmed to be malignant.

Table 1.3.4.4C: Most Frequent (At Least 5% in Any Group) Serious Adverse Events Through Day 56 After Double-blind Period (Randomized, Transplanted and Treated Population) - Study IM103100

MedDRA System Organ Class Preferred Term	No. (%) of Subjects		
	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=71)
Subjects with Any Serious Adverse Events	50 (67.6)	52 (73.2)	41 (57.7)
Blood & Lymphatic System Disorders	2 (2.7)	3 (4.2)	4 (5.6)
Gastrointestinal Disorders	7 (9.5)	7 (9.9)	5 (7.0)
General Disorders & Administration Site Conds.	5 (6.8)	8 (11.3)	7 (9.9)
Pyrexia	4 (5.4)	8 (11.3)	6 (8.5)
Immune System Disorders	20 (27.0)	23 (32.4)	13 (18.3)
Transplant Rejection	18 (24.3)	20 (28.2)	9 (12.7)
Infections & Infestations	17 (23.0)	12 (16.9)	18 (25.4)
Cytomegalovirus	5 (6.8)	4 (5.6)	7 (9.9)
Pyelonephritis	4 (5.4)	1 (1.4)	2 (2.8)
Urinary Tract Infection	2 (2.7)	0	4 (5.6)
Injury, Poisoning & Procedural Complications	8 (10.8)	6 (8.5)	9 (12.7)
Investigations	8 (10.8)	2 (2.8)	4 (5.6)
Blood Creatinine Increased	8 (10.8)	2 (2.8)	4 (5.6)
Metabolism & Nutrition Disorders	1 (1.4)	2 (2.8)	4 (5.6)
Renal & Urinary Disorders	9 (12.2)	11 (15.5)	9 (12.7)
Respiratory, Thoracic & Mediastinal Disorders	6 (8.1)	3 (4.2)	4 (5.6)
Vascular Disorders	3 (4.1)	5 (7.0)	8 (11.3)

MedDRA = Medical Dictionary of Drug Regulatory Activities.

Topics of Special Interest

Renal Function

Renal function was assessed by measurement of iohexol using a plasma disappearance method. As iohexol is a true glomerular filtration marker, measurement of iohexol clearance may be regarded as a 'gold standard' technique. Because iohexol clearance measures are relatively difficult to perform, renal function was also assessed by measurement of SCr and application of several SCr-based formulas for prediction of renal function. Unlike iohexol, creatinine is not a true glomerular filtration marker, and is relatively insensitive to changes in renal function in subjects with GFRs > 50-60 mL/min.

Iohexol clearance was greater in the belatacept treatment groups than in the CsA group at all time points (Table 1.3.4.5A). Data collection was not complete, however, and it is difficult to predict the impact of missing data on the observed results. SCr and creatinine-based estimates of renal function, which were obtained more consistently than iohexol clearance, also generally favored belatacept, although to a lesser degree.

Table 1.3.4.5A: Renal Function - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Mean (SD) Measured Serum Creatinine (mg/dL) (ITT Analysis)			
Month 1	n=68 1.3 (0.46)	n=69 1.3 (0.52)	n=65 1.6 (1.02)
Month 6	n=62 1.2 (0.41)	n=66 1.2 (0.33)	n=54 1.4 (0.69)
Month 12	n=60 1.2 (0.46)	n=59 1.2 (0.55)	n=50 1.4 (1.29)
Mean (SD) Iohexol Clearance Rate (mL/min/1.73 m^2) (ITT Analysis)			
Month 1	n=53 59.7 (17.34)	n=51 60.2 (14.45)	n=48 54.0 (19.33)
Month 6	n=41 62.2 (25.60)	n=41 64.5 (19.48)	n=31 56.0 (19.54)
Month 12	n=32 66.3 (20.71)	n=37 62.1 (15.90)	n=27 53.5 (16.43)
Mean (SD) Calculated GFR (mL/min/1.73 m^2) Using Levey Formula (ITT Analysis)			
Month 1	n=68 65.3 (21.43)	n=69 65.6(20.61)	n=65 56.9 (20.92)
Month 6	n=62 70.1 (21.09)	n=65 69.9 (20.04)	n=54 63.3 (26.496)
Month 12	n=60 72.4 (22.53)	n=59 73.2 (22.54)	n=50 68.0 (28.06)

GFR = glomerular filtration rate, ITT = intent to treat, and SD = standard deviation.

Renal Function Following Biopsy-proven Acute Rejection

In general, based on the last available SCr values, renal function following biopsy-proven acute rejection (including clinically-suspected and subclinical events) was similar among subjects in the 3 treatment groups (belatacept MI: 1.9 mg/dL; belatacept LI: 1.6 mg/dL; CsA: 2.3 mg/dL).

Mean SCr values for subjects who had recurrent acute rejections are summarized in Table 1.3.4.5B. Subjects with recurrent acute rejection had similar or better most recent mean SCr at last follow-up in the belatacept treatment groups as compared to the CsA treatment group.

Table 1.3.4.5B: Most Recent Mean Serum Creatinine Values Post Acute Rejection - Study IM103100 (Intent-to-Treat Analysis)

Mean Serum Creatinine (mg/dL)	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
1 Episode of Acute Rejection	1.7	1.6	2.3
> 1 Episode of Acute Rejection	2.5	1.6	2.7
Non-Grade IIB Acute Rejection	1.7	1.6	2.3
Grade IIB Acute Rejection	2.2	1.5	2.2

Mean SCr values for subjects with more severe acute rejections (moderate - Grade IIB) are also summarized in Table 1.3.4.5B. Grade IIB rejections were observed in 5 subjects in both belatacept groups and 2 subjects in the CsA group. The mean SCr values at last follow-up were comparable between the belatacept MI and CsA groups, but lower in the belatacept LI group.

Cardiovascular and Metabolic Effects

Mean SBP was 3-4 mm Hg higher with CsA than with belatacept by Month 12, despite a somewhat higher rate of antihypertensive medication use in the CsA group (Table 1.3.4.5C). Total cholesterol was slightly lower with belatacept than with CsA, as were both the non-high-density lipoprotein (non-HDL) and HDL fractions (Table 1.3.4.5D). Lipid-lowering medications were used more frequently with CsA (53%) than with belatacept (32%-36%). HbA1c and the incidence of PTDM were slightly lower with belatacept than with CsA (Table 1.3.4.5E).

Table 1.3.4.5C: Blood Pressure - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Mean (SD) Diastolic Blood Pressure (mm Hg) (ITT Analysis)			
Month 1	n=58 81 (11.0)	n=63 79 (11.3)	n=57 82 (9.9)
Month 6	n=49 79 (9.4)	n=50 77 (10.2)	n=41 81 (8.2)
Month 12	n=52 78 (9.2)	n=47 75 (11.7)	n=39 78 (7.6)
Mean (SD) Systolic Blood Pressure (mm Hg) (ITT Analysis)			
Month 1	n=58 135 (15.6)	n=63 136 (17.0)	n=57 135 (14.4)
Month 6	n=49	n=50	n=41

Table 1.3.4.5C: Blood Pressure - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
	130 (16.3)	129 (16.3)	136 (17.1)
Month 12	n=52 130 (17.3)	n=47 129 (15.7)	n=39 133 (18.7)
Incidence of Hypertension (ITT Analysis)			
Months 0 - 1	72 (97.3%)	68 (95.8%)	71 (97.3%)
Months 1 - 3	n=73 65 (89.0%)	n=70 58 (82.9%)	n=72 66 (91.7%)
Months 3 - 6	n=73 65 (89.0%)	n=70 57 (81.4%)	n=71 66 (93.0%)
Months 6 - 9	n=72 64 (88.9%)	n=69 57 (82.6%)	n=67 61 (91.0%)
Months 9 - 12	n=72 63 (87.5%)	n=69 58 (84.1%)	n=64 59 (92.2%)
Antihypertensive Medication Use at 12 months (ITT Analysis)			
Subjects on Antihypertensive Medications	63 (87.5%)	57 (82.6%)	59 (92.2%)
No. of Classes			
1	17 (27.0%)	20 (35.1%)	9 (15.3%)
2	20 (31.8%)	22 (38.6%)	24 (40.7%)
3	16 (25.4%)	10 (17.5%)	18 (30.5%)
≥ 4	10 (15.9%)	5 (8.8%)	8 (13.6%)

Note: Hypertension was defined as diastolic blood pressure ≥ 90 mm Hg and/or systolic blood pressure ≥ 140 mm Hg or use of any antihypertensive medication.

ITT = intent to treat and SD = standard deviation.

Table 1.3.4.5D: Lipids - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Mean (SD) Total Cholesterol (mg/dL) (ITT Analysis)			
Month 1	n=69 222 (64.7)	n=69 210 (45.6)	n=65 239 (53.7)
Month 6	n=63 204 (40.4)	n=65 202 (47.7)	n=54 224 (54.8)
Month 12	n=60 198 (41.4)	n=58 201 (40.0)	n=50 212 (44.2)
Mean (SD) non-HDL Cholesterol (mg/dL) (ITT Analysis)			
Month 1	n=68 159 (62.7)	n=68 142 (40.2)	n=64 169 (51.5)
Month 6	n=62 150 (39.4)	n=64 143 (42.1)	n=51 165 (55.1)
Month 12	n=59 145 (36.7)	n=56 144 (35.8)	n=48 151 (43.4)
Mean (SD) HDL Cholesterol (mg/dL) (ITT Analysis)			
Month 1	n=68 64 (19.4)	n=68 68 (21.7)	n=64 70 (21.6)
Month 6	n=62 54 (14.8)	n=65 56 (19.0)	n=52 62 (20.1)
Month 12	n=60 53 (15.7)	n=57 56 (13.5)	n=48 59 (18.5)
Subjects on Lipid-lowering Medications (ITT Analysis)			
Months 0 - 1	21 (28.4%)	10 (14.1%)	23 (31.5%)
Months 1 - 3	n=73 16 (21.9%)	n=70 15 (21.4%)	n=72 26 (36.1%)
Months 3 - 6	n=73 22 (30.1%)	n=70 20 (28.6%)	n=71 30 (42.3%)
Months 6 - 9	n=72 24 (33.3%)	n=69 22 (31.9%)	n=67 31 (46.3%)
Months 9 - 12	n=72 26 (36.1%)	n=69 22 (31.9%)	n=64 34 (53.1%)

ITT = intent to treat and SD = standard deviation.

Table 1.3.4.5E: Diabetes - Study IM103100

	LEA29Y MI (N=74)	LEA29Y LI (N=71)	CsA (N=73)
Mean (SD) HbA1c (%) (ITT Analysis)			
Month 1	n=67 5.7 (0.83)	n=69 5.7 (0.78)	n=67 5.9 (0.95)
Month 6	n=61 5.9 (0.95)	n=58 5.9 (0.84)	n=53 6.1 (1.09)
Month 12	n=60 5.8 (0.83)	n=57 5.8 (0.67)	n=52 6.2 (0.84)
New-onset PTDM / Treated for Hyperglycemia (ITT Analysis)			
Total No. Subjects Without Pre-transplant Diabetes	67	65	60
Month 1	0	0	1 (1.7%)
Month 6	0	0	2 (3.3%)
Month 12	2 (3.0%)	0	3 (5.0%)

Note: PTDM was defined as the need for treatment of hyperglycemia with either an oral agent or insulin for a total of ≥ 4 weeks in a subject not known to be diabetic prior to transplant.

HbA1c = hemoglobin A1c, ITT = intent to treat, PTDM = post-transplant diabetes mellitus, and SD = standard deviation.

Immunogenicity

No immunogenicity was observed in Study IM103100.

Exploratory Analysis in an Extended Criteria Donor Population or in Subjects with Impairment of Renal Function Post-transplant

ECD criteria were not prospectively defined in Study IM103100. An analysis of subjects who received an allograft with possible impaired renal function at the time of transplantation was explored in a retrospective fashion. Criteria for this analysis were donor age ≥ 60 years and CIT ≥ 24 hours. In addition, subjects who experienced either DGF (defined as dialysis treatment within first week of transplantation) or slow graft function (defined as SCr at Day 5 ≥ 3 mg/dL without dialysis) were also analyzed. Tables 1.3.4.6A and 1.3.4.6B show key efficacy and safety data, retrospectively, from these respective analyses.

Table 1.3.4.6A: Evaluation of Subjects from Study IM103100 with Donor Age ≥ 60 Years or Cold-ischemic Time ≥ 24 Hours

	LEA29Y MI (N=17)	LEA29Y LI (N=16)	CsA (N=10)
Subject Death or Graft Loss by Month 12, n (%)	0	1 (6.3%)	0
Acute Rejection (Clinically-suspected) by Month 6, n (%)	1 (5.9%)	0	0
Acute Rejection (Clinically-suspected and Subclinical) by Month 6, n (%)	1 (5.9%)	3 (18.8%)	1 (10.0%)
Chronic Allograft Nephropathy by Month 12, n (%)	4 (23.5%)	2 (12.5%)	5 (50.0%)
Median Glomerular Filtration Rate by Month 12 (mL/min/1.73 m^2)	(n=11) 61.5	(n=10) 57.4	(n=4) 48.0
Malignancies, n	0	0	0

Table 1.3.4.6B: Evaluation of Subjects from Study IM103100 with Slow or Delayed Graft Function

	LEA29Y MI (N=32)	LEA29Y LI (N=32)	CsA (N=30)
Subject Death or Graft Loss at Month 12, n (%)	2 (6.3%)	0	4 (13.3%)
Acute Rejection (Clinically-suspected) at Month 6, n (%)	5 (15.6%)	2 (6.3%)	4 (13.3%)
Acute Rejection (Clinically-suspected and Subclinical) at Month 6, n (%)	7 (21.9%)	8 (25.0%)	9 (30.0%)
Chronic Allograft Nephropathy at Month 12, n (%)	8 (25.0%)	5 (15.6%)	12 (40.0%)
Median Glomerular Filtration Rate at Month 12 (mL/min/1.73 m^2)	(n=11) 56.0	(n=20) 56.4	(n=10) 48.0
Malignancies, n	1	0	1

In these analyses, belatacept demonstrated results consistent with the results in the overall study population. Of note, there were favorable trends with respect to GFR and CAN with comparable acute rejection rates between the belatacept and CsA treatment groups.