

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125288Orig1s000

MICROBIOLOGY REVIEW(S)

**MICROBIOLOGY/IMMUNOLOGY TEAM LEADER REVIEW
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

DATE: March 24, 2011

SUBMISSION: BLA # 125288 (SN-055)

REVIEWER: Shukal Bala, Ph.D.
Microbiology/Immunology Team Leader
Division of Special Pathogen and Transplant Products
Office of Antimicrobial Products

SUBJECT: Protein: Belatacept

(b) (4)



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(b) (4)



Indication: Prevention of organ rejection in adult patients receiving renal transplants

Applicant: Bristol-Myers Squibb

1. Introduction and Background

The subject of this Biological License Application (BLA) is Nulojix® (belatacept; LEA29Y) for the prevention of acute rejection in patients receiving a renal allograft. The applicant has proposed the dose of 10 mg/kg on days 1, 5, end of weeks 2, 4, 8, and 12 followed by a lower maintenance dose of 5 mg/kg every 4 weeks starting at week 16.

The key microbiological and immunologic studies were reviewed in the original BLA submission. The studies included (a) proof of concept studies supporting the mechanism by which belatacept exhibits its effect and activity *in vitro* and in animal models of transplantation, and (b) clinical microbiology/immunology studies (for details see Dr Aaron Ruland's Microbiology/Immunology review dated March 16, 2010 and Dr Shukal Bala's, Microbiology/Immunology Team Leader review dated March 18, 2010).

The applicant was issued a complete response (CR) letter on May 1, 2010. One of the deficiencies identified in the CR letter was the following:

“Regarding the indication of prophylaxis of organ rejection in adult patients receiving renal transplants, you have not provided sufficient long-term data to evaluate the long-term effect on the rate of post-transplant lymphoproliferative disorder (PTLD), renal effects, cardiovascular events, graft and patient survival.”

To address this deficiency, the applicant was requested to “provide the 36-month data from Phase 3 studies, particularly data on outcomes such as mortality, graft loss, glomerular filtration rate, PTLD and other serious adverse reactions.”

In this submission the applicant has responded to the deficiencies and submitted the following studies that are relevant to the review of microbiology and immunology:

- Nonclinical: a study report to evaluate whether belatacept crosses the blood brain barrier in non human primates and its effect on immune cells.
- Clinical: 36 month follow up data for 2 of the phase 3 clinical trials (IM103008 and IM103027).

Belatacept was derived from abatacept (Orencia®) which is approved for the treatment of moderate to severe rheumatoid arthritis. Abatacept is a homodimeric recombinant human fusion protein (human cytotoxic T-lymphocyte antigen-4, CTLA4-IgG1) constructed from a mutated extracellular domain of CTLA-4 and the Fc portion (hinge-CH2-CH3 domains) of a human IgG1 antibody. Belatacept was modified by mutation of 2 amino acids [lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y)] in the ligand binding region.

Belatacept, like abatacept, prevents co-stimulation of the T-cells, by CD80/CD86 on APC, by indirectly blocking signaling through CD28 receptor. Mutation of 2 residues [lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y)] in the ligand binding region allow belatacept to bind to human CD80 and CD86 with greater avidity than abatacept. Overall, the results show that belatacept is more active against human and nonhuman primate cells whereas abatacept is more active against rodent cells.

Binding of belatacept with CD80 and CD86 on the surface of antigen presenting cells blocks interaction with CD28 on the surface of T-cells. Blockage of CD80/CD86 and CD28 interaction inhibits T-cell proliferation and the production of the cytokines such as interleukin-2, interferon- γ , interleukin-4, and TNF- α . Belatacept does not suppress/block complement mediated or antibody dependent cellular cytotoxicity. Activated T lymphocytes are the predominant mediators of immunologic rejection.

This review summarizes the additional immunological findings from

- (1) a nonclinical study, and
- (2) 36 month follow-up data from two phase 3 clinical trials.

For details of these studies please see the Dr Aaron Ruland's Microbiology/Immunology review, dated March 23, 2011.

2. Review and Comments:

2.1. Nonclinical study

The nonclinical study was conducted to evaluate whether belatacept crosses the blood brain barrier in non human primates (cynomolgus monkeys) and its effect on infiltration of immune cells to the brain. The results of this non-clinical study are important because a higher incidence of PTLT was reported in Epstein Barr virus (EBV) negative renal transplant recipients in the belatacept arms, compared to cyclosporine, in the clinical trials submitted with the original submission; a higher incidence of PTLT in the central nervous system (CNS) was also observed among patients treated with belatacept compared to cyclosporin. The concern was whether belatacept could cross the blood-brain barrier and contribute to the disease process.

Study Design:

Cynomolgus monkeys were administered belatacept (10 or 50 mg/kg) or saline (n=4/treatment group) once weekly (days 1, 8, 15, 22 and 29) via intravenous infusion in a peripheral vein. Animals were monitored at regular intervals for body weight and other physical parameters at different time intervals. At day 30, animals were euthanized and brain tissue evaluated for cellular infiltration by immunofluorescence staining that included detecting the presence of cells expressing CD3 (T-lymphocytes), CD20 (subset of B-cells), CD68 (macrophage, dendritic cell, granulocyte), CD80 (B-cell, macrophage, dendritic cell), CD86 (antigen presenting cells, brain cells), CD209 (dendritic cells and macrophages), von Willebrand Factor (endothelial cells), and major histocompatibility complex (MHC) class-II antigens (HLA DP DQ DR; present on antigen presenting cells, astrocytes and glial cells) using anti-human antibodies. The source of the anti-human antibodies or the cross-reactivity with monkey tissues was not specified in the study report. Belatacept levels were also measured in the brain and serum.

Results:

Levels of Belatacept in CSF and Serum

The results show a dose-dependent increase in belatacept levels in the brain although the levels were lower in the CSF compared to serum on day 30, a day after administration of the last intravenous infusion of belatacept (10 or 50 mg/kg) once weekly for 4 weeks. Relative to serum levels, the levels of belatacept in CSF were <0.05 % of systemic exposures. The belatacept levels in the brain were deemed to be due to blood contamination of both the brain and the CSF and were

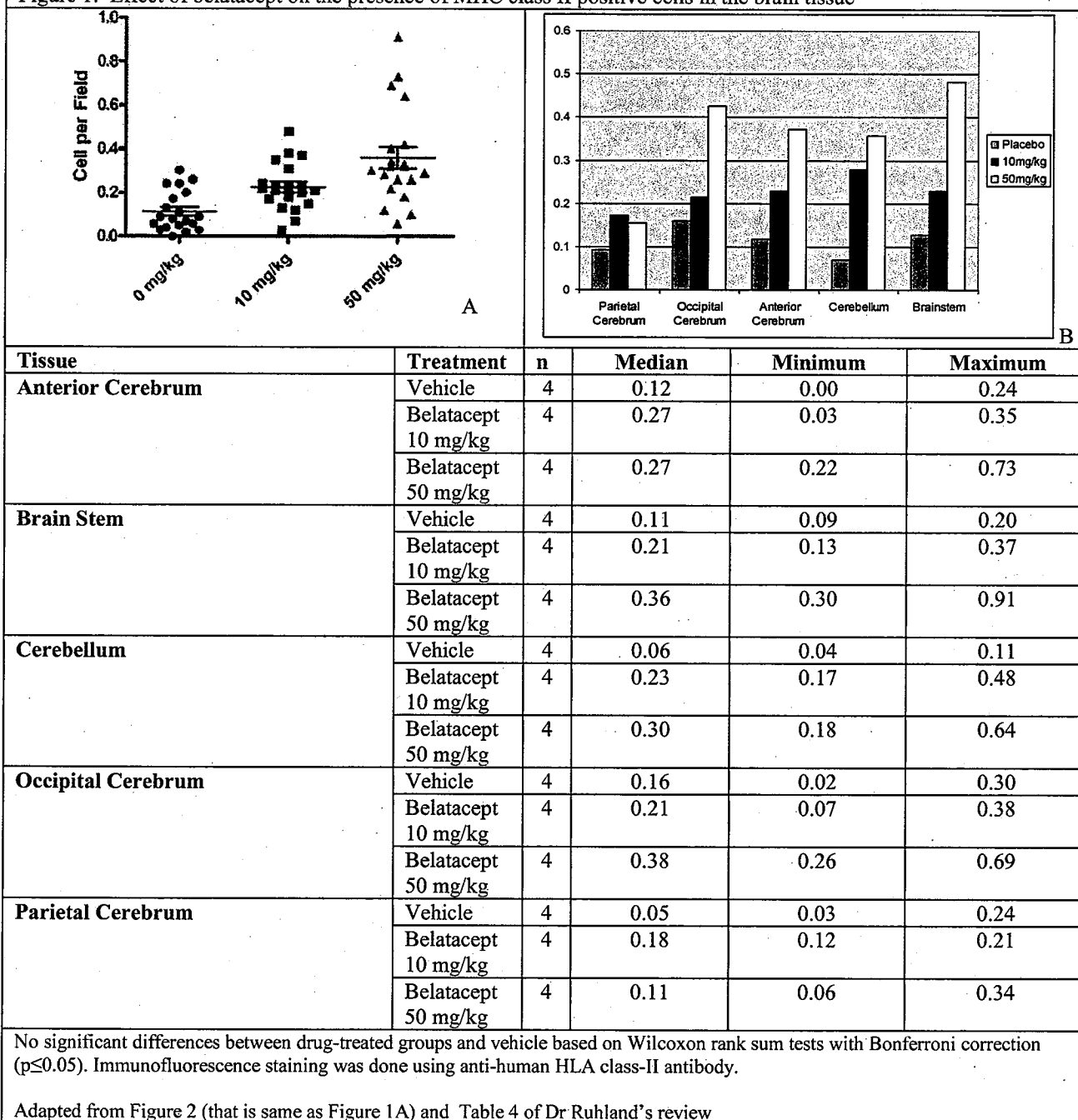
not indicative of belatacept crossing the blood-brain barrier; two expert opinions provided by the applicant supported this conclusion.

Cell Phenotyping

Immunofluorescent staining revealed no change in the number of cells expressing CD3, CD20, CD68, CD80, CD86, CD209, or von Willebrand Factor. Although the distribution of CD20+ cells (B-lymphocytes) was similar in the belatacept treated and vehicle groups this finding should be interpreted with caution since B-cell population is heterogeneous and all B-cells do not express CD20 (Cao *et al.*, 2008, *Am J Clin Pathol*, 129: 926).

The results show an increase in the number of MHC class-II (HLA- DP DQ DR) positive cells in the belatacept treated animals compared to vehicle group (Figure 1). Although the applicant states that the increase is likely not biologically significant and did not reach statistical significance, the observation was consistent across sections from different parts of the brain tissues examined and appeared in most cases to be dose-dependent (Figure 1). Although the difference in the number of cells expressing MHC class-II antigen between treated and control groups is about 2 to 3 fold, it is important to keep in mind that anti-human reagents were used for identification of these cell surface molecules and therefore, the possibility of lower affinity and avidity of the antibody against antigens within tissues from cynomolgus monkeys exists. Therefore, the possibility of even greater difference cannot be ruled out. It would have been useful to measure the intensity of staining in addition to enumerating the number of positive cells.

Figure 1: Effect of belatacept on the presence of MHC class II positive cells in the brain tissue



An increase in the presence of MHC class-II positive cells in the brain could be a reflection of cell activation; this observation may be related to the possibility of an increased incidence of PTLD presenting in the CNS of some patients treated with belatacept. This conclusion is based upon the fact that reactivated EBV is present in MHC class-II positive cells (B-cells) and even a single infected cell could clonally expand upon crossing the blood brain barrier and present as PTLD of the CNS in humans. Although EBV is not known to infect cynomolgus monkeys, a related primate virus, rhesus lymphocryptovirus (LCV) is known to produce an EBV-like disease in primates. Published study (Schmidtko *et al.*, 2002, *Transplantation*, 73: 1431) suggests LCV reactivation in a renal transplant model nonhuman in primates under immunosuppression over a 5-year period. The

LCV serostatus was not measured in cynomolgus monkeys treated with belatacept. Although the clinical relevance is not known the possibility of cell activation contributing to the PTLD cannot be ruled out.

It is an important scientific observation that supports one the component of the immune system being affected after treatment with belatacept. Therefore, it will be important to state in the "Mechanism of Action" subsection (12.1) or some other section of the labeling that treatment with belatacept increased the number of cells expressing major histocompatibility class-II antigens in the brain tissue in nonhuman primates.

2.2. Clinical Microbiology and Immunology: 36 month follow up data for 2 of the phase 3 clinical trials (IM103008 and IM103027).

PTLD and EBV status:

From the review of original BLA submission a higher incidence of PTLD was reported in patients in the belatacept containing regimen compared to the cyclosporin containing regimen (for details see Microbiology/Immunology review dated March 16, 2010 and Microbiology/Immunology Team Leader review dated March 18, 2010). Although the incidence is higher in EBV⁻ (seronegative) patients compared to EBV⁺ (seropositive) patients, EBV⁺ patients also developed PTLD including CNS PTLD. Therefore, it is important that EBV serostatus should be determined by using appropriate tests with high specificity that detect IgG antibodies against viral capsid antigen and Epstein Barr nuclear antigen. The possibility of reactivation of EBV does exist.

No additional PTLD cases were reported in this submission.

Appropriate statements have been incorporated in the "Highlight" and "Warnings and Precautions" (section 5.1) sections of the labeling.

Effect on antibody response (donor specific antibody)

The donor specific antibodies (DSAs) were measured by a single flow cytometric HLA antibody identification technique to differentiate class I from class II DSA (Flow Specific Beads) and single antigen beads were then used to determine the specificity of the donor specific anti-HLA antibodies. The data showed that there was a decrease in the number of patients developing DSA in the belatacept compared to cyclosporine arms at 12, 24, and 36 months. The review of 36 month follow up data supports the findings previously summarized for 12 and 24 months (for details see Microbiology/Immunology Team Leader review dated March 18, 2010), that less number of patients treated with belatacept developed *de novo* DSAs compared to cyclosporine. However, the results should be interpreted with caution as the number of patients that were positive in both belatacept and cyclosporin arms were very small and no correlation was established between decrease in DSA and graft survival or graft rejection.

3. The Labeling:

3.1. Current version of the labeling (agreed upon in communication dated March 21, 2011)

Section 12.1: Mechanism of Action

Belatacept, a selective costimulation blocker, binds to CD80 and CD86 on antigen-presenting cells thereby blocking CD28 mediated costimulation of T cells (lymphocytes). *In vitro*, belatacept inhibits T-lymphocyte proliferation and the production of the cytokines interleukin-2, interferon- γ , interleukin-4, and TNF- α . Activated T lymphocytes are the predominant mediators of immunologic rejection.

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3.2. Comments:

1. As discussed in section 2.1 of this review, the non human primate study shows an increased in the number of cells of MHC class-II (HLA- DP DQ DR) in the brain from cynomolgus monkeys treated with belatacept compared to vehicle. Although the clinical relevance of such finding is unknown this observation does reflect cell activation which is an important step in antigen presentation and development of immune responses just like its effect on T-lymphocyte proliferation and the production of the cytokines interleukin-2, interferon- γ , interleukin-4, and TNF- α . Therefore, it is recommended that the following statement should be added to the mechanism of action section (12.1) or some other section of the labeling:

In cynomolgus monkeys, belatacept increased the number of cells expressing major histocompatibility class-II antigens in the brain tissue. However, the clinical relevance of these findings is unknown.

On April 13, 2011, the applicant revised the text and proposed to add the following statement to the "Animal Toxicology and/or Pharmacology" section 13.2:

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The text proposed by the applicant does not appear to be factual and can be potentially misleading. Therefore, the following text was proposed for a more accurate representation of the data:

Following 5 doses (10 mg/kg or 50 mg/kg, once a week for 5 weeks) of systemic administration, belatacept was not detected in brain tissue of normal healthy

cynomolgus monkeys. The number of cells expressing major histocompatibility complex (MHC) class-II antigens (potential marker of immune cell activation) in the brain were increased in monkeys administered belatacept compared to vehicle control. However, distribution of some other cells expressing CD68, CD20, CD80, and CD86, typically expressed on MHC class II-positive cells, was not altered and there were no other histological changes in the brain. The clinical relevance of the findings is unknown.

2. Minor edits are recommended in the following statements for more accurate presentation of data and avoid off-label promotion:

[REDACTED] (b) (4)

3. It is recommended that the following statement should be deleted

[REDACTED]

[REDACTED] (b) (4)

[REDACTED] (b) (4)

The applicant may consider a prospective study with an endpoint which reports the development of DSA and its relationship with graft survival and graft loss.

In labeling received on May 3, 2011, the applicant agreed to all the changes discussed above for sections 12.1, 13.2, and 14.1.

3.3. FDA's proposed version of the labeling:

Section 12.1: Mechanism of Action

Belatacept, a selective T-cell (lymphocyte) costimulation blocker, binds to CD80 and CD86 on antigen-presenting cells thereby blocking CD28 mediated costimulation of T lymphocytes. *In vitro*, belatacept inhibits T lymphocyte proliferation and the production of the cytokines interleukin-2, interferon- γ , interleukin-4, and TNF- α . Activated T lymphocytes are the predominant mediators of immunologic rejection.

[REDACTED] (b) (4)

4. Recommendations

The BLA should be approved with respect to Microbiology/Immunology.

Shukal Bala
Shukal Bala, Ph.D.
Microbiology/Immunology Team Leader
DSPTP

Concurrence:

Division Director/Renata Albrecht, M.D.  Date 6/3/11

DSPTP

CC:

DSPTP/PM/GermainJ

MICROBIOLOGY/IMMUNOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

BLA #: 125288
(SN-055)

REVIEWER: Aaron M. Ruhland
CORRESPONDENCE DATE : 9/24/2010
CDER RECEIPT DATE : 9/24/2010
REVIEW ASSIGN DATE : 9/24/2010
REVIEW COMPLETE DATE: 3/23/2011

APPLICANT: Bristol-Myers Squibb
P.O. Box 4000
Princeton, NJ 08543

DRUG CATEGORY: Immunosuppressant

INDICATION: Prevention of organ rejection and preservation of a functioning allograft
in adult patients receiving renal transplants

DOSAGE FORM: Intravenous infusion

PRODUCT NAMES:

- a. **PROPRIETARY:** Nulojix®
- b. **NONPROPRIETARY:** Belatacept, LEA29Y, BMS-224818
- c. **CHEMICAL:** Not applicable

STRUCTURAL FORMULA:

Modified human CTLA4 (L104 to E, A29 to Y)-IgG1 fusion protein (hinge-CH2- CH3 domains)

Molecular weight: ~90 kDa
Molecular formula: Not applicable

SUPPORTING DOCUMENTS: IND 9418

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1. Executive Summary

The subject of this Biological License Application (BLA) is Nulojix[®] (belatacept) for the prevention of acute graft rejection in patients receiving a renal allograft. Belatacept was derived from abatacept, a human CTLA4-IgG1 fusion protein which binds to CD80/CD86 on antigen presenting cells (APCs). In this application, the applicant has submitted the results of two phase 3 trials, IM103008 and IM103027, with data through month 36. A full review of the data generated from these trials through month 12 and the preclinical studies supporting the activity of belatacept has been conducted (for details see Microbiology/Immunology review dated 03-16-2010 and Microbiology/Immunology Team leader review dated 03-18-2010). This review focuses on the development of donor specific antibody (DSA) in all patients in the intent to treat population (ITT) and also determines the development of DSA in patients after discontinuation of treatment due to either adverse event(s) or acute rejection. The results show that patients treated with belatacept, particularly those treated with the less intensive regimen (which is proposed for approval), developed less *de novo* DSA and for those patients who had DSA at baseline, less persistence of DSA compared to the cyclosporine A (CsA) treated arm. The data also indicate that patients treated with belatacept who test positive for DSA are not more likely to experience an episode of acute rejection than those who test negative for DSA. Additionally, patients treated with belatacept who test positive for DSA are not at more risk of undergoing acute rejection than patients treated with CsA who test positive for DSA. Additionally, there is no evidence that patients who discontinue belatacept develop DSA at a higher rate.

The 1-month study of belatacept in healthy non-human primates was conducted to determine the penetration and effect of belatacept on the blood:brain barrier (BBB) and brain immune cellularity. The objective was to determine whether the results could be related to an increase in the incidence of post transplant lymphoproliferative disorder (PTLD) presenting in the central nervous system (CNS) seen in the clinical trials in belatacept treated patients, particularly those who were EBV⁻ at the time of transplant. Results showed that little, if any, belatacept crossed the BBB, however a noticeable increase in the number of MHC class II⁺ cells was found in all regions of the brain examined in belatacept treated animals. A concomitant increase in CD20⁺ (B-cells) or CD68⁺ (macrophage, dendritic cells, granulocytes) was not seen. The applicant claims this increase in MHC II⁺ cells is insignificant though this observation should not be readily dismissed since even a slight increase in B-cell trafficking to the brain could theoretically manifest as PTLD since a single reactivated EBV⁺ B-cell clone could enter the brain, clonally expand, and manifest as PTLD of the CNS.

2. Introduction and Background

The subject of this Biological License Application (BLA) is Nulojix[®] (belatacept; LEA29Y) for the prevention of acute rejection in patients receiving a renal allograft. The original BLA application was previously reviewed (for details see Microbiology/Immunology review dated 03-16-2010 and Microbiology/Immunology Team Leader review dated 03-18-2010) and the applicant was issued a complete response (CR) letter requesting 36 month data on outcomes such as mortality, graft loss, glomerular filtration rate (GFR), PTLD and other serious adverse reactions. (b) (4)

(b) (4) Other bases for the CR letter concerned deficiencies in product quality and product quality microbiology. In this submission the applicant has responded to these deficiencies.

Patients treated with belatacept showed an increased risk for post-transplant lymphoproliferative disease (PTLD) and in particular PTLD presenting in the central nervous system (CNS). In order to understand the potential mechanism behind the CNS PTLD cases, additional investigative nonclinical studies in cynomolgus monkeys were requested to evaluate whether belatacept penetrates the brain or affects brain permeability. In this submission, the applicant has included a copy of the study report evaluating the potential of belatacept to cross blood-brain barrier.

Belatacept was derived from abatacept, a human CTLA4-IgG1 fusion protein which binds to CD80/CD86 on antigen presenting cells (APCs). Belatacept, like abatacept, was developed to prevent costimulation of T-cells by indirectly blocking signaling through its CD28 receptor. By binding CD80 and CD86 on the APC with the modified CTLA-4 domain of belatacept, it is thought that costimulation of the T-cells will be prevented by blocking the interaction of CD28 expressed on T-cells with the same ligands. The applicant has proposed the dose regimen of 10 mg/kg on days 1, 5, 14, 28, and month 2 and 3 after transplant, then 5 mg/kg every month starting at month 4.

3. Preclinical/Nonclinical Microbiology/Immunology Study

Nonclinical studies supporting mechanism of action and activity *in vitro* as well as *in vivo* were reviewed previously (for details see Microbiology/Immunology review dated 03-16-2010 and Microbiology/Immunology Team Leader review dated 03-16-2010).

The applicant has submitted a report of the study that evaluated the possibility of belatacept (BMS-224818) to cross the blood brain barrier in male monkeys and its influence on the expression of CD80, CD86 and trafficking of immune cells across the blood brain barrier. This was particularly important since in clinical trials increased incidence of PTLD was reported in Epstein Barr virus (EBV) negative renal transplant recipients in the belatacept arms. Cynomolgus monkeys were obtained from (b) (4) and administered belatacept (10 or 50 mg/kg) or saline (n=4/treatment group) once weekly (days 1, 8, 15, 22 and 29) via intravenous infusion in a peripheral vein. Notably, no other control arms using other immunosuppressants were used for comparison. The animals were sacrificed on day 30. Blood was obtained on day -5 before administration of belatacept. Blood samples were also collected before and after dosing on day 22 (at 3 minutes, 2, 6, 24, 72 and 120 hours), day 29 (before the last dose) and on day 30 (before necropsy). Blood was analyzed for plasma belatacept concentration, serum chemistry and coagulation and hematological parameters. Cerebrospinal fluid (CSF) was collected on day 30 prior to sacrifice and analyzed for belatacept and protein concentration, red blood cell count, and absolute total nucleated cell count. In addition to a standard organ workup for toxicokinetics studies, sections from different regions of the brain were obtained. At necropsy, brain sections were examined *in situ* then obtained and fixed in 10% neutral buffered formalin, another fixative or embedded in optimum cutting temperature compound (OCT) and frozen for histochemical and fluorescence staining. Sections were obtained from the anterior cerebrum, parietal cerebellum (with hippocampus), occipital cerebrum, cerebellum and

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brain stem at the level of the pons. Additional specimens [parietal cerebrum (with hippocampus), cerebellum, brain stem at the level of the pons] were obtained and remained unfixed and frozen for analysis of belatacept, IgG and albumin by ELISA. The ELISA for belatacept had been previously validated for use in determining belatacept concentrations in serum, though its use on CSF and homogenized protease digested brain slices has not been validated but the applicant reports that quality control data indicate the method was precise and accurate for these sample matrices. The manufacturer of ELISA assays used to determine IgG and albumin were Life Diagnostics Inc. and Bethyl Laboratories Inc., respectively. The applicant did not note the species specificity of the assays but states that the methods were tested and confirmed to be of use in detecting IgG and albumin from brain homogenates.

Microscopic pathology, standard histologic (hematoxylin and eosin stain) and immunologic evaluations were performed with the sections. Immunologic evaluations were performed on frozen sections of the brain, spleen, thymus and lymph nodes by immunofluorescent staining of OCT preserved sections with fluorochrome conjugated antibodies specific for immune cell epitopes and belatacept and analyzed by confocal microscopy. Spleen sections were used as a positive control to allow the conclusion of negative results for cell immunostaining. Thymus tissue was used as the positive control for anti-belatacept immunostaining. For immunohistochemical evaluations, the antibodies tested included anti-human MHC-II, anti-human CD209 (dendritic cell marker), anti-human CD68 (macrophage, dendritic cell, granulocyte), anti-human von Willebrand factor (endothelial cell), anti-human CD80 (B-cell, macrophage, dendritic cell), anti-human CD3 (T-cell), and anti-human CD20 (B-cell). The number of positive cells was calculated as a function of the area of the field and the number of positive cells per field. A single section was processed for each animal for each cell marker. For each section, the entire tissue area was evaluated for MHC II and CD209 which resulted in 202 to 1254 fields per tissue sample evaluated. For other cell markers, 25 congruent fields per sample were scored. For belatacept, 2 sections of each tissue were evaluated from each animal in their entirety.

There were no significant changes in serum chemistry, coagulation or hematological parameters. A dose proportional increase in serum levels of belatacept was observed at days 22 and 30 (Tables 1 and 2). Belatacept levels on Day 30, i.e., 24 hours after the last dose, were also similarly dose proportional (see Table 2 below).

Table 1. Day 22 pharmacokinetic parameters		
Parameter	Belatacept Dose	
	10 mg/kg/week	50 mg/kg/week
C _{3 minutes} (µg/mL) *	380	1,890
AUC _{0-168hours} (µg·hr/mL)	15,200	72,400
* C _{3 minutes} is the serum belatacept concentration at first postdose sampling time after IV administration on Day 22		

Low levels of belatacept were detected in the CSF by ELISA on day 30 and compared to day 30 belatacept levels (Table 2). Relative to serum levels, the levels of belatacept in CSF were <0.05 % of systemic exposures. The applicant states that these levels in CSF were likely due to contamination by belatacept in the blood at the time of collection and are consistent with the levels of hematologic cell contamination seen across all treatment groups including saline treated animals upon analysis (< 50 RBC/µL). However, two

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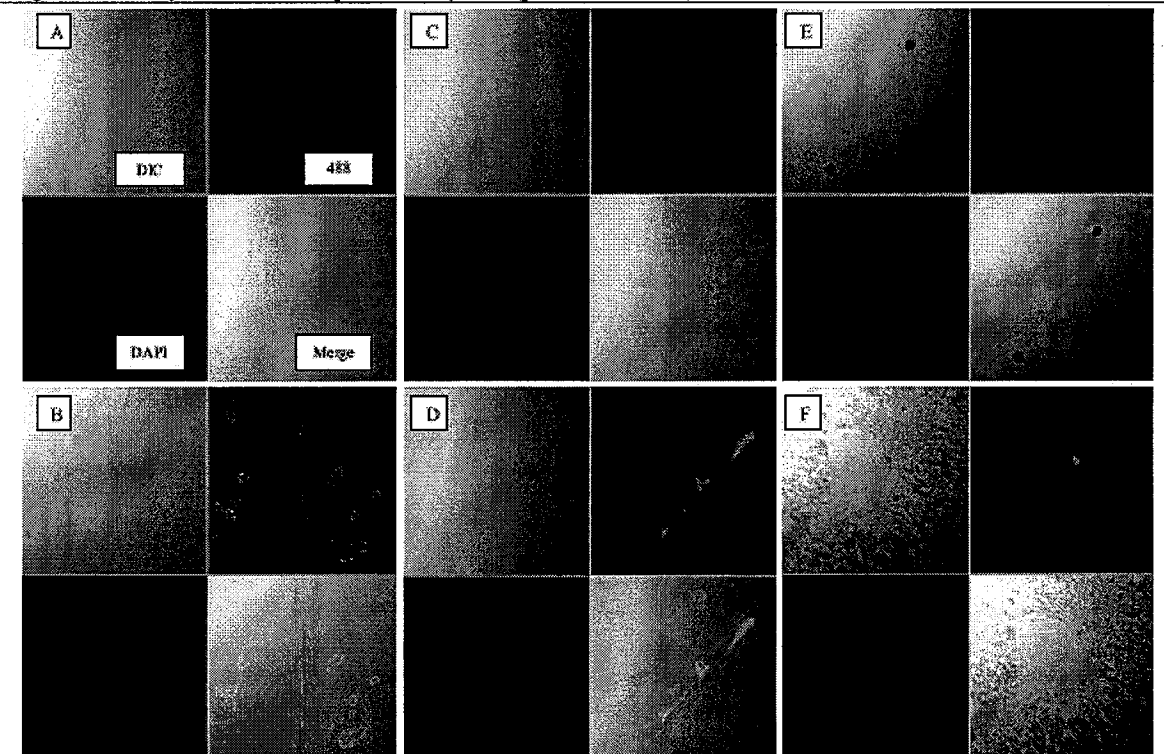
animals in the belatacept arms (animals 2004 and 3001) had 1068 and 316 RBC/ μ L, respectively; these animals did not have concomitant rise in nucleated white blood cells in the CSF; all animals had < 20 WBC/ μ L in CSF.

Low levels of belatacept were found in the brain tissue by ELISA as well (Table 2). These levels were < 0.07% of systemic exposures. Although the animals were perfused at sacrifice, these levels appear to be due to residual blood that remained within the microvasculature of the brain and are compatible with standard histological analysis of the brain which showed low levels of blood cells left within the microvasculature at the time of necropsy. Expert opinion provided by the sponsor supports this conclusion. Immunofluorescent staining showed the presence of belatacept within the brain microvasculature but not in the extravascular tissue within the brain (Figure 1).

Table 2. Mean belatacept concentrations of monkey CSF, brain and serum

Dose (mg/kg/week)	Time	Belatacept concentration (ng/mL or ng/g for tissue)			CSF/Serum ratio	Brain/Serum Ratio	CSF/Brain ration
		CSF	Brain (mean of 3 regions)	Serum			
10	Day 30	61.86	91.05	132940.11	0.000471	0.000669	0.914
50	Day 30	240.51	355.54	647229.28	0.000373	0.000552	0.772

Figure 1. Analysis of belatacept in the thymus (positive control) and brain tissue



Tissues were immunostained with an anti-belatacept antibody and imaged at 40 $\times$ magnification with DIC (upper left), AlexaFluor 488 conjugated anti-belatacept (upper right), and 4',6-diamidino-2-phenylindole (DAPI) nuclear staining (lower left); merge of the three channels is in the lower right image of each panel. Immunostaining of thymus tissue from (A) a vehicle-control and a (B) monkey treated with 50 mg/kg belatacept to demonstrate specificity of the anti-belatacept antibody. Cerebellum tissue from a (C) vehicle-control and a (D) 50 mg/kg/week belatacept and parietal cerebrum tissue from a (E) vehicle-control and a (F) a high dose monkey demonstrate belatacept presence in the microvasculature

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There were no differences noted in the ratio of albumin and IgG in the brain between belatacept and vehicle control animals (Table 3). For both vehicle control and monkeys treated with 50 mg/kg/week belatacept the range of brain to serum ratios was 0.03 to 0.16% and 0.02 to 0.03 for IgG and albumin, respectively. The ratios of belatacept in the brain compared to blood were consistent with those of IgG and albumin which do not normally cross the blood brain barrier.

Table 3. Immunoglobulin G (IgG) and albumin concentrations and ratios in the brain and serum					
Group		Immunoglobulin G Concentration Results			
		Brain (ng/mL)	Serum (ng/mL)	Ratio	Ratio %
Vehicle	Mean	13444.7	13323365.6	0.00108	0.108
	SD	3417.0	2878276.0	0.000462	0.0462
Belatacept 50mg/kg/week	Mean	4902.1	8384278.1	0.000630	0.0630
	SD	2530.1	1310051.5	0.000408	0.0407
		Albumin Concentration Results			
Vehicle	Mean	4538.1	23836883.7	0.000196	0.0196
	SD	260.3	4892805.6	0.0000381	0.00381
Belatacept 50mg/kg/week	Mean	4635.4	23036665.1	0.000204	0.0203
	SD	845.1	3440096.6	0.0000385	0.00386

Immunofluorescent staining revealed an increase in the number of MHC-II positive cells (Figure 2, Table 4). The applicant states that there are 15 data points graphed per treatment but it appears that 20 data points per treatment were included. The applicant states that each data point is from one of each of the 3 tissues evaluated from each monkey. However, tissues were taken from 5 locations: 3 in the cerebrum (parietal, occipital and anterior) and 1 each from the brain stem and cerebellum. It is unknown whether the applicant means that each data point is from one of the 5 tissues examined for each monkey as this would equate to 20 data points for 4 monkeys in each treatment group.

The applicant states that the increase is likely not biologically significant, and the increase in numbers did not reach statistical significance. The applicant states that the increase on average was a single cell per 4 fields examined (0.25 cells/field). The observation was consistent across all brain tissues examined and appeared in most cases to be dose dependent. This data suggests an increased trafficking of positive cells or increased expression of MHC class-II in the brain; the results were not statistically significant which may be due to small number of animals. This observation should not be completely dismissed and may be a clue in explaining the increased incidence of PTLD presenting in the CNS of some patients treated with belatacept. This conclusion is based upon the fact that reactivated EBV is present in MHC II positive cells (B-cells) and theoretically a single infected cell could clonally expand upon crossing the BBB and present as PTLD of the CNS in humans. CD20 staining did not show an increase in B-cells (Figure 2), however not all B-cells express CD20 (particularly plasma cells), nor do all B-cell myelomas present with CD20⁺ cells (Cao *et al*, 2008, *Am J Clin Pathol*, 129: 926). However, there is a lack of evidence of increased B-cells in the brain of belatacept treated animals. A primate virus, rhesus lymphocryptovirus (LCV) does produce an EBV-like disease in primates, however the serostatus of the monkeys was not reported and there were no indication of PTLD in this 30-day study. Published studies have been conducted which suggest LCV reactivation in a renal transplant model in primates under immunosuppression over a 5-year period (Schmidtke *et al.*, 2002, *Transplantation*, 73:

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1431). There were no T-cells or CD209⁺ cells found in the brain, nor was a statistically significant number of CD68⁺ cells (Figure 3).

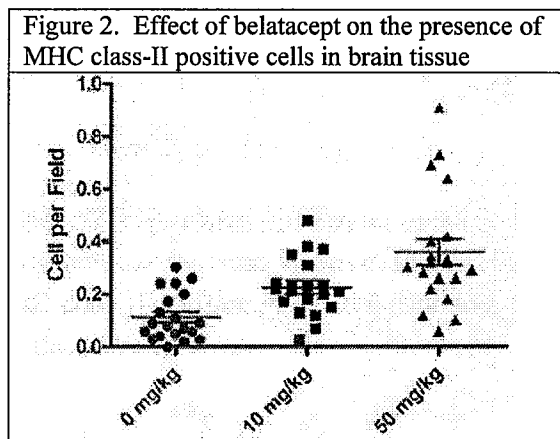
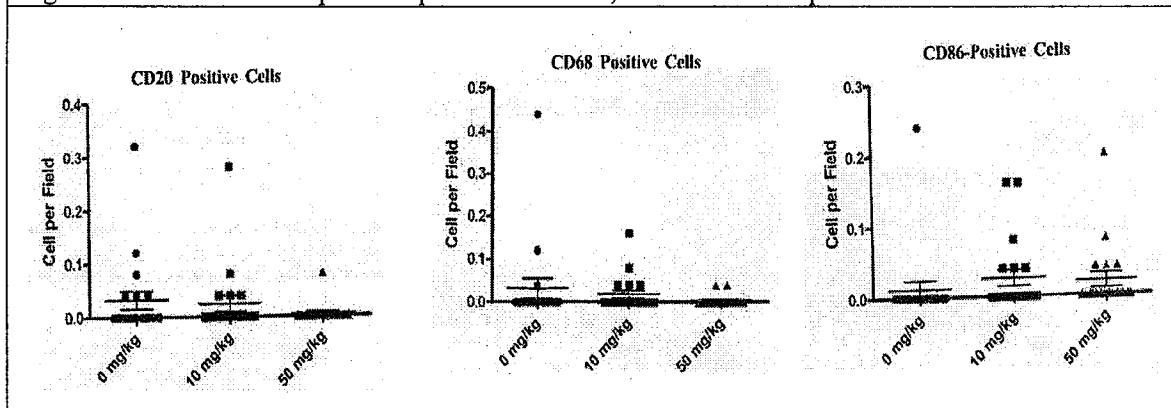


Table 4. Effect of belatacept on the presence of MHC class II positive cells in the brain tissue

Tissue	Treatment	n	Median	Minimum	Maximum
Anterior Cerebrum	Vehicle	4	0.12	0.00	0.24
	Belatacept 10 mg/kg	4	0.27	0.03	0.35
	Belatacept 50 mg/kg	4	0.27	0.22	0.73
Brain Stem	Vehicle	4	0.11	0.09	0.20
	Belatacept 10 mg/kg	4	0.21	0.13	0.37
	Belatacept 50 mg/kg	4	0.36	0.30	0.91
Cerebellum	Vehicle	4	0.06	0.04	0.11
	Belatacept 10 mg/kg	4	0.23	0.17	0.48
	Belatacept 50 mg/kg	4	0.30	0.18	0.64
Occipital Cerebrum	Vehicle	4	0.16	0.02	0.30
	Belatacept 10 mg/kg	4	0.21	0.07	0.38
	Belatacept 50 mg/kg	4	0.38	0.26	0.69
Parietal Cerebrum	Vehicle	4	0.05	0.03	0.24
	Belatacept 10 mg/kg	4	0.18	0.12	0.21
	Belatacept 50 mg/kg	4	0.11	0.06	0.34

No significant differences between drug-treated groups and vehicle based on Wilcoxon rank sum tests with Bonferroni correction ($p \leq 0.05$)

Figure 3. Effect of belatacept on the presence of CD20, CD68 and CD86 positive cells in the brain



4. Clinical Immunology

In the original BLA submission, the applicant presented data from 3 trials (IM103008, IM103027, and IM103100) that evaluated the presence of donor specific antibody (DSA) in patients who underwent renal transplant and were treated with belatacept more intensive (belatacept MI; group A), belatacept less intensive (belatacept LI; group B), and cyclosporine A (CsA; group C) based regimens. In this submission the sponsor has provided information after additional follow-up up to 36 months in 2 of the trials (IM103008 and IM103027)

Blood samples for antibody testing were obtained at baseline and at pre-specified times after transplant. A sample was also obtained at the time of any suspected rejection episode(s). The assays were performed blindly at the (b) (4)

. A specific algorithm was followed regarding the detection of DSAs. DSAs were determined by a single flow cytometric HLA antibody identification technique to differentiate class I from class II DSA (Flow Specific Beads) and single antigen beads were then used to determine the specificity of the antibodies and matched to donor HLA. The classification of positive or negative DSA for a patient sample was based on the shifts of the sample relative to the negative control markers. Any significant shift of the bead population to the right of the negative control marker was considered positive. A significant shift was considered as >50% of the bead population moving to the right of the negative control marker. If the population remains to the left of the negative control markers then it was considered negative. A Luminex-based assay was also used when samples were strongly positive by standard flow cytometry.

The proportion of subjects who had DSAs for either class I or class II DSA at baseline and through Month 36 was calculated for each treatment group by the applicant. It should be noted that this is a cumulative analysis, i.e., once a subject tests positive at a given timepoint, that subject remained positive and was included in the analysis for the later time points regardless of whether the DSA persisted in that patient. Antibody data was in AS.xpt dataset under the column heading ANLYPROC. Analysis was based upon class I donor antibody (coded as category 87 in the datasets) and class II donor antibody (coded as category 88 in the datasets) and included the specificity of the DSA detected. A reanalysis of the data through month 36 was conducted.

The analysis performed by the applicant does not specify the total number of patients evaluated during a given time frame or whether subjects who test positive at a given time have developed *de novo* DSA or whether the DSA were present from baseline. This review summarizes the information available based on an independent analysis. The analysis is based on the number of patients tested and the number who tested positive for DSA at different time points. For the purpose of this review, this analysis will be referred to as "period analysis" from here onward. Additionally, a breakdown of the data was performed to distinguish patients who had persistent DSA present from baseline and those who developed *de novo* DSA after receiving the allograft. The development of DSA was compared in those patients who were diagnosed with acute rejection and those who were not. Finally, an analysis of those patients who discontinued protocol defined treatment due to adverse events or acute rejection was conducted to determine the rate of development of DSA in these patients.

4.1 Trial IM103008

This was a phase 3, randomized, partially-blinded, active-controlled, parallel group, multi-center trial also known as the “Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT)”. The trial enrolled 738 patients, of which 666 were randomized and transplanted (ITT population). Subjects were randomized and transplanted with organs from standard criteria donors on day 1; the day 1 dose of the following drug regimens was given pre-operatively for the 36 month study:

- Belatacept MI regimen (n=219) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 3 (weeks 2, 4, 6, 8, 10, and 12) and then every 4 weeks through month 6 (weeks 16, 20, and 24). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Belatacept LI regimen (n=226) - intravenous belatacept (10 mg/kg) on days 1, 5, 14 and 28, then every 4 weeks through the end of month 3. At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Active control (n=221) – oral CsA given twice daily to achieve serum trough concentrations of 150 – 300 ng/mL during the first month and 100 – 250 ng/mL thereafter. If delayed graft function was diagnosed, patients may have received polyclonal anti-lymphocyte therapy until CsA could be initiated.

All subjects also received a background immunosuppressive regimen consisting of basiliximab induction, mycophenolate mofetil (MMF) and corticosteroid maintenance therapy.

Data which classified whether patients had class I or class II DSA were available for 671 pts in AS.xpt data set (i.e., 671 unique subject IDs;) for belatacept MI (n=220), belatacept LI (n=228) and CsA (n=223). It is of note that number of patients in this dataset is 671 whereas in the ITT population it was 666; the reasons for such discrepancy are unclear. This data differed somewhat from the 12 month data previously reviewed (for details see microbiology/immunology review dated 3-16-10) even at baseline where 38 pts were enrolled even though they were positive for DSA at screening with 12, 13, and 13 patients treated with belatacept MI, belatacept LI, and CsA, respectively. The reason for these differences was not identified. Since DSA testing occurred at different dates month 6 (range) and month 12 (range) in different patients, this analysis used day 185 as the cut-off for 6 months and day 395 for 12 month analysis. This allowed more of the patients to be examined and more closely replicated the applicant's data, though the applicant did not provide definitions on what ranges were used in their analysis. Note that the total number evaluated is the cumulative number of unique subjects evaluated from day 1 and does not mean that all patients had DSA evaluation in the period from month 6 to month 12. A period analysis was performed to determine this (see below). Overall, the conclusions drawn from this data are consistent with the 12-month data in that a fewer number of patients tested positive for DSA over 36 months in the belatacept treated groups than CsA (Table 5). By 36 months, similar numbers of patients in both belatacept arms (LI and MI) tested positive for DSA.

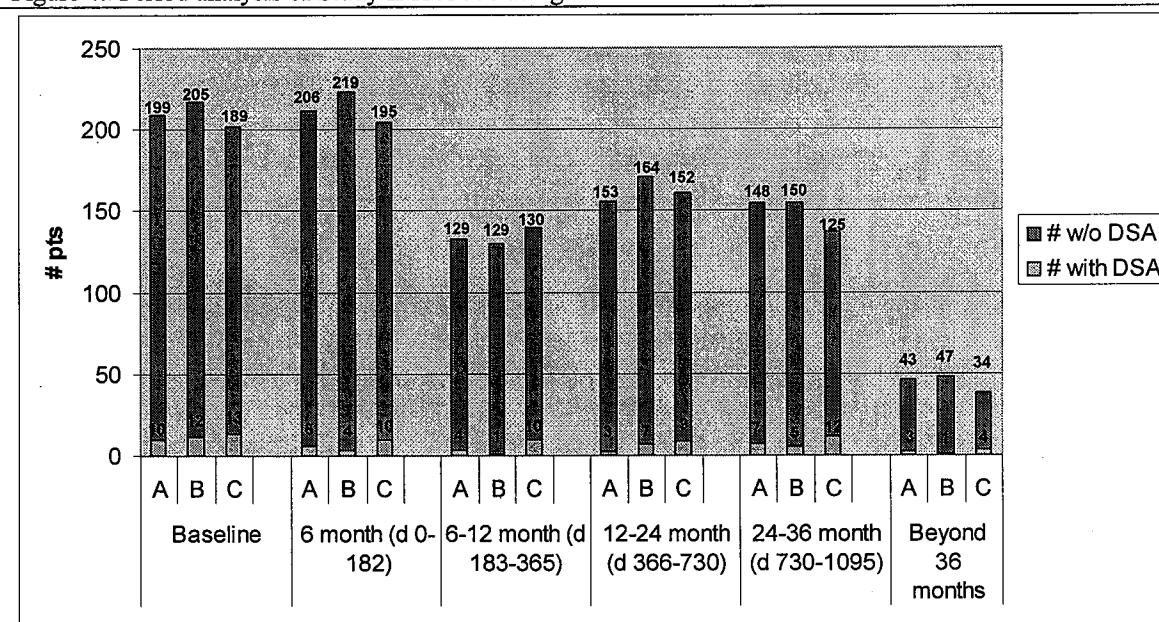
Table 5. Summary of subjects with anti-donor HLA antibodies through month 36 in ITT population in 103008

		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	219	228	217
	Anti-donor HLA positive (%)	12 (5.5%)	13 (5.7%)	13 (6.0%)
Month 6 (through day 185)	No. evaluated	213	223	206
	Anti-donor HLA positive (%)	6 (2.8%)	4 (1.8%)	10 (4.9%)
Month 12 (through day 395)	No. evaluated	216	224	212
	Anti-donor HLA positive (%)	8 (3.7%)	4 (1.8%)	15 (7.1%)
Month 24 (through day 760)	No. evaluated	217	224	212
	Anti-donor HLA positive (%)	10 (4.6%)	10 (4.5%)	19 (9.0%)
Month 36 (through day 1125)	No. evaluated	217	224	212
	Anti-donor HLA positive (%)	14 (6.5%)	13 (5.8%)	24 (11.3%)

N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.

A period analysis of the 36-month data was also conducted for review which was not performed by the applicant (Figure 4). The data show that at each time period examined, more patients in the CsA arm tested positive for DSA than in the belatacept arms. The number of patients examined during each time window was lower than those who were screened between start of treatment and 6 months, however the distribution of missing data appears equal across treatment groups.

Figure 4. Period analysis of Study IM103008 using 36 month data



A=Belatacept MI; B=Belatacept LI; C=CsA

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The data were analyzed further to separate patients with persistent DSA from those developing *de novo* DSA. Through month 12, more patients remained positive with persistent DSA from baseline in the CsA group (Table 6). However, the number of patients with persistent DSA were similar among all treatment groups by months 24 and 36. The data show that the biggest difference in the development of *de novo* antibodies occurs through month 12 when comparing belatacept to CsA (Table 7); more CsA treated patients did develop *de novo* DSA from month 12 to month 36 than belatacept treated patients. Overall, twice as many patients developed *de novo* DSA in the CsA arm compared to the belatacept LI and MI arms. A summary of this data is shown in Figure 5.

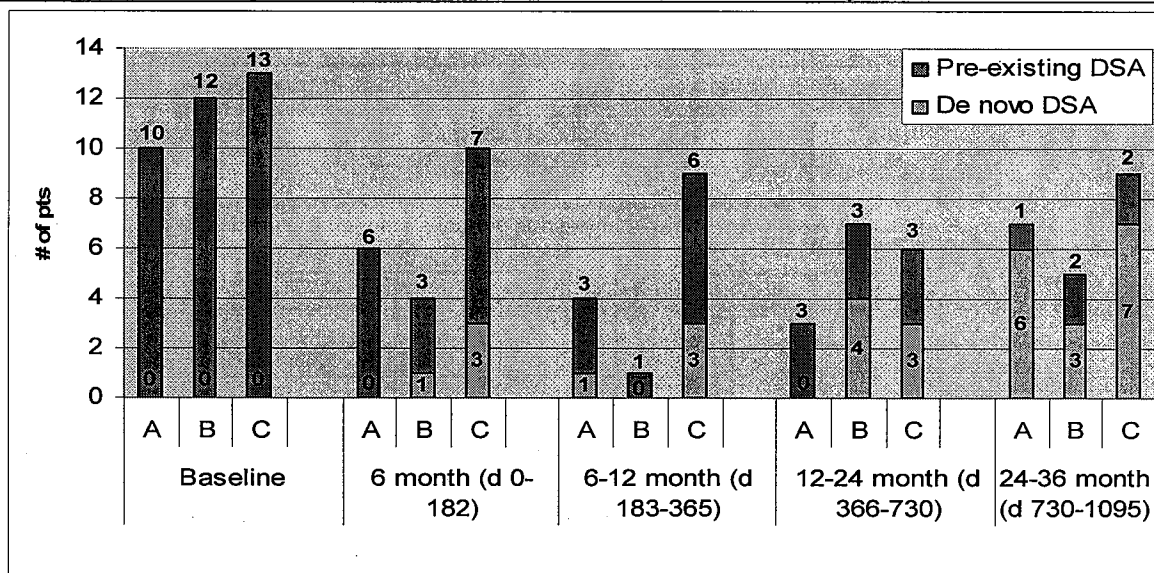
Table 6. Patients positive at baseline who tested positive for DSA after treatment started through month 36

Treatment	Baseline	Month 6	Month 12	Month 24	Month 36
Belatacept MI	10	6	3	3	1
Belatacept LI	12	3	1	3	2
CsA	13	7	6	3	2

Table 7. Patients negative at baseline with *de novo* DSA through month 36

Treatment	Month 6	Month 12	Month 24	Month 36	Total
Belatacept MI	0	1	0	6	7
Belatacept LI	1	0	4	3	8
CsA	3	3	3	7	16

Figure 5. Separation of persistent versus *de novo* DSA in Study IM103008 using 36-month data



A=Belatacept MI; B=Belatacept LI; C=CsA

Up to Month 36, acute rejection (AR) occurred in 24% (MI), 17% (LI), and 10% (CsA) of the patients, respectively, and most cases of AR occurred by Month 6 (Table 8). After

Month 24, no new cases of AR occurred in MI or LI while 1 new case occurred in the CsA group.

Table 8. Acute rejection rates in Study IM103008 using 36-month data

Acute Rejection (n, %) ^d			
<i>Month 12^a</i>	49 (22.4)	39 (17.3)	16 (7.2)
Difference from CsA (97.3% CI)	15.1 (7.9, 22.7) ^e	10.0 (3.3, 17.1)	---
<i>Month 24^a</i>	53 (24.2)	39 (17.3)	20 (9.0)
Difference from CsA (97.3% CI)	15.2 (7.5, 23.0)	8.2 (1.2, 15.4)	---
<i>Month 36 (from current CSR)</i>	53 (24.2)	39 (17.3)	21 (9.5)
Difference from CsA (97.3% CI)	14.7 (7.0, 22.6)	7.8 (0.6, 15.0)	---

^d Acute Rejection is defined as central biopsy proven rejection that was either (1) clinically suspected by protocol defined reasons or (2) clinically suspected by other reasons and treated.

The applicant presented a table which compared DSA status to the occurrence of acute rejection (Table 9). The data show that most patients who experienced acute rejection were negative for DSA throughout the trial although the data indicate that a higher percentage of patients treated with CsA that had an episode of AR developed DSA at some point in the trial. The data do not suggest that those patients treated with belatacept and testing positive for DSA are at a higher risk of acute rejection. No causal association should be drawn however between the presence of DSA and AR.

Table 9. Applicant reported development of anti-donor HLA antibodies by acute rejection status up to month 36. All randomized and transplanted subjects (ITT)

	No. subjects positive/no. subjects with/without AR assessed for antibodies					
	Belatacept MI (A)		Belatacept LI (B)		CsA (C)	
	AR	No AR	AR	No AR	AR	No AR
Baseline	2/52 (4%)	8/165 (5%)	2/39 (5%)	11/187 (6%)	0/20 (0%)	13/193 (7%)
Antibody positive by Month 6	2/52 (4%)	4/161 (3%)	0/39 (0%)	4/185 (2%)	1/19 (5%)	9/189 (5%)
Antibody positive by month 12	2/52 (4%)	4/163 (3%)	0/39 (0%)	4/185 (2%)	2/19 (10%)	12/191 (6%)
Antibody positive by month 24	2/52 (4%)	6/164 (4%)	2/39 (5%)	6/185 (3%)	3/20 (15%)	13/192 (7%)
Antibody positive by month 36	6/52 (12%)	8/165 (5%)	3/39 (8%)	9/185 (5%)	4/20 (20%)	20/192 (10%)

Source Data: Table S.5.10.2 and Table S.8.2B in Month 24 CSR

AR: acute rejection

This data was replicated by the reviewer. A subset of the adar.xpt dataset was created which only included those patients whose diagnosis was clinically suspected and biopsy proven rejection (category ARF 1). This yielded 114 total patients who were diagnosed with acute rejection following transplant (belatacept MI=53; belatacept LI=39; CsA=22). Of these 114 patients, 113 patients had the actual date of their acute rejection episodes recorded and also had DSA information reported after start of treatment (belatacept MI=52; belatacept LI=39; CsA=22). Each of these numbers was used as the denominator

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for the column labeled as AR for each treatment group. A cumulative analysis similar to the data of Table 5 (see above) was used to determine each patient's DSA status at each of the time points. Only those patients who experienced an acute rejection episode prior to day 1096 (36 months) were included. The conclusions are similar to those of the applicant (Table 10).

Table 10. Reviewer reported development of anti-donor HLA antibodies by acute rejection status up to month 24: All randomized and transplanted subjects (ITT)						
	No. subjects positive/no. subjects with/without AR assessed for antibodies					
	Belatacept MI (A)		Belatacept LI (B)		CsA (C)	
	AR	No AR	AR	No AR	AR	No AR
Baseline	2/52 (3.8%)	10/167(6.0%)	2/39 (5.1%)	11/189 (5.8%)	0/22 (0%)	13/195 (6.7%)
Antibody positive by Month 6 (day 182)	2/52(3.8%)	4/161 (2.5%)	0/39 (0%)	4/184 (2.2%)	1/22 (4.5%)	9/184 (4.9%)
Antibody positive by month 12 (day 395)	2/52 (3.8%)	6/164 (3.7%)	0/39 (0%)	4/185 (2.2%)	3/22 (13.6%)	12/190 (6.3%)
Antibody positive by month 24 (day 760)	4/52 (7.7%)	6/165 (3.6%)	2/39 (5.1%)	8/185 (4.4%)	4/22 (18.1%)	15/190 (7.9%)
Antibody positive by month 36 (day 1125)	6/52 (11.5%)	8/165 (4.8%)	3/39 (7.7%)	10/185 (5.4%)	5/22 (22.7%)	19/190 (10%)

An analysis was performed to determine whether patients who discontinued belatacept treatment due to an adverse event (AE) were more likely to develop DSA than patients who remained on belatacept. Patients were analyzed based on being entered as discontinued on study medication in AE.xpt dataset [parameter AEACN (Action Taken with Study Treatment) study drug discontinued was coded as category 5]. Sixty-nine total patients discontinued due to AE (belatacept MI = 18, belatacept LI = 16, CsA = 35) and these appear to include dose adjustments for the CsA arm. The replacement therapy following discontinuation of belatacept was not given. Of the 69 patients, 59 were tested for DSA at the time of and after the AE. Results showed that 3 belatacept MI and 2 CsA treated patients developed DSA only after discontinuation while no patients who discontinued the belatacept LI regimen due to an AE developed DSA after discontinuation (Table 13).

Table 11. Development of DSA in patients who discontinued study treatment due to adverse event		
Treatment Arm	Number of discontinuations due to AE who had DSA data	Number testing positive for DSA only after discontinuation
Belatacept MI	18	3
Belatacept LI	16	0
CsA	35	2

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An analysis was performed to determine whether patients who discontinued belatacept treatment due to an acute rejection episode were more likely to develop DSA than patients who remained on belatacept. Patients were analyzed based on being entered as discontinued on study medication in AR.xpt dataset [parameter ARACN (Action Taken with Study Treatment) category 5: study drug discontinued]. Eighty-two total patients discontinued due to AR (belatacept MI = 27, belatacept LI = 28, CsA = 27) and these appear to include dose adjustments for the CsA arm. The replacement therapy following discontinuation of belatacept was not given. Of the 82 patients, 74 were tested for DSA at the time of and after the AE (belatacept MI=24, belatacept LI=25, CsA=25). Results showed that 1 belatacept MI, 2 belatacept LI and 5 CsA treated patients developed DSA after discontinuation (Table 12). There does not appear to be a significant rise in the number of patients who develop DSA following discontinuation of belatacept due to acute rejection.

Table 12. Development of DSA in patients who discontinued study treatment due to acute rejection		
Treatment Arm	Number of discontinuations due to AR who had DSA data	Number testing positive for DSA only after discontinuation
Belatacept MI	24	1 (4.2%)
Belatacept LI	25	2 (8%)
CsA	25	5 (20%)

4.2 Trial IM103027

This was a phase 3, randomized, partially-blinded, active-controlled, parallel group, multi-center trial also known as the “Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial – EXTended Criteria Donors (BENEFIT-EXT). The trial enrolled 595 patients, of which 543 were randomized and transplanted (ITT population). Subjects were randomized and transplanted with kidneys from extended criteria donors on day 1; the day 1 dose of the following drug regimens was administered pre-operatively for the 36 month study:

- Belatacept MI regimen (n=184) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 3 (weeks 2, 4, 6, 8, 10, and 12) and then every 4 weeks through month 6 (weeks 16, 20, and 24). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Belatacept LI regimen (n=175) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 1 (weeks 2 and 4) and then every 4 weeks through month 3 (weeks 8 and 12). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Active control (n=184) – oral CsA given twice daily to achieve serum trough concentrations of 150 – 300 ng/mL during the first month and 100 – 250 ng/mL thereafter through the end of the trial. If delayed graft function was diagnosed, patients may have received polyclonal anti-lymphocyte therapy until CsA could be initiated.

All subjects also received a background immunosuppressive regimen consisting of basiliximab induction, mycophenolate mofetil (MMF) and corticosteroid maintenance

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therapy. The donor organ had to meet at least 1 of the following conditions to be considered extended criteria to be transplanted into patients participating in this trial:

- Donor age ≥ 60 years
- Donor age 50 – 59 years and 1 of the following: cerebrovascular accident (CVA) + hypertension + serum creatinine (SCr) > 1.5 mg/dL or CVA + hypertension or CVA + SCr > 1.5 mg/dL or hypertension + SCr > 1.5 mg/dL
- Anticipated cold ischemic time ≥ 24 hours
- Donor with cardiac death (non-heart beating donor)

Data which classified patients as having either class I or class II DSA was available for 557 patient in AS.xpt data set (i.e. 557 unique subject IDs) for belatacept MI (n=186), belatacept LI (n=181) and CsA (n=190) groups. The reason for the difference between the number of patients who were randomized to treatment and those identified as the ITT population was not identified. Overall, the conclusions drawn from this data are consistent with the Trial IM103008 data although a sharp decline in the number of patients who tested positive for DSA in the belatacept LI arm was not observed as in Trial IM103008, however fewer patients developed DSA compared to CsA (Table 13). This data also closely replicated that presented by the sponsor in the original BLA submission which presented data through 12 months.

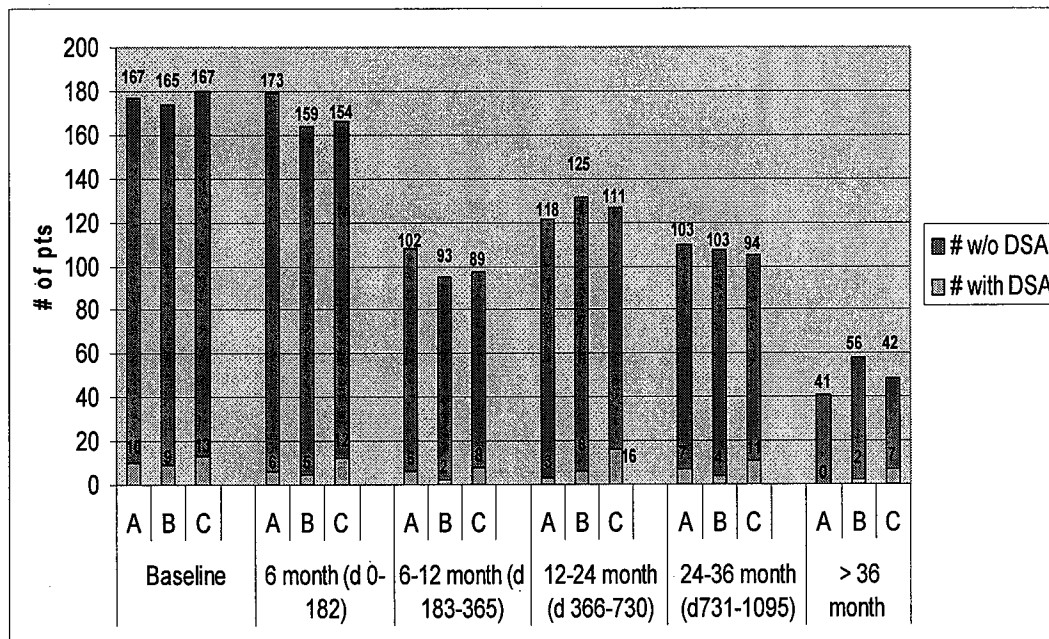
Table 13. Reviewer summary of subjects with anti-donor HLA antibodies in ITT population in Study 103027 using 36 month data

		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	177	174	180
	Anti-donor HLA positive (%)	10 (5.6%)	9 (5.2%)	13 (7.2%)
Month 6 (through day 185)	No. evaluated	179	165	166
	Anti-donor HLA positive (%)	6 (3.4%)	5 (3.0%)	12 (7.2%)
Month 12 (through day 395)	No. evaluated	179	171	174
	Anti-donor HLA positive (%)	9 (5.0%)	9 (5.3%)	16 (9.2%)
Month 24 (through day 760)	No. evaluated	179	171	175
	Anti-donor HLA positive (%)	12 (6.7%)	11 (6.4%)	23 (13.1%)
Month 36 (through day 1125)	No. evaluated	179	171	175
	Anti-donor HLA positive (%)	15 (8.4%)	11 (6.4%)	27 (15.4%)

N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.

A period analysis of the 36-month data was also conducted for review which was not performed by the applicant (Figure 6). This data indicates that many more patients treated with CsA developed DSA in each period examined compared to belatacept LI (group B). Unlike Study IM103008, it appears that patients who developed DSA did so fairly equally across all time periods examined.

Figure 6. Reviewer period analysis of subjects with anti-donor HLA antibodies in ITT population in Study 103027 using 36 month data



A=Belatacept MI; B=Belatacept LI; C=CsA

This data were further analyzed separate patients with persistent DSA and those developing *de novo* DSA. More patients remained positive with persistent DSA from baseline in the CsA group compared to belatacept LI (Table 14). This difference remained consistent through month 36. Similarly, the development of *de novo* antibodies was less in belatacept treated patients compared to CsA (Table 15). Overall, over three times as many patients developed *de novo* DSA in the CsA arm compared to the belatacept LI arm. A summary of this data is shown in Figure 7.

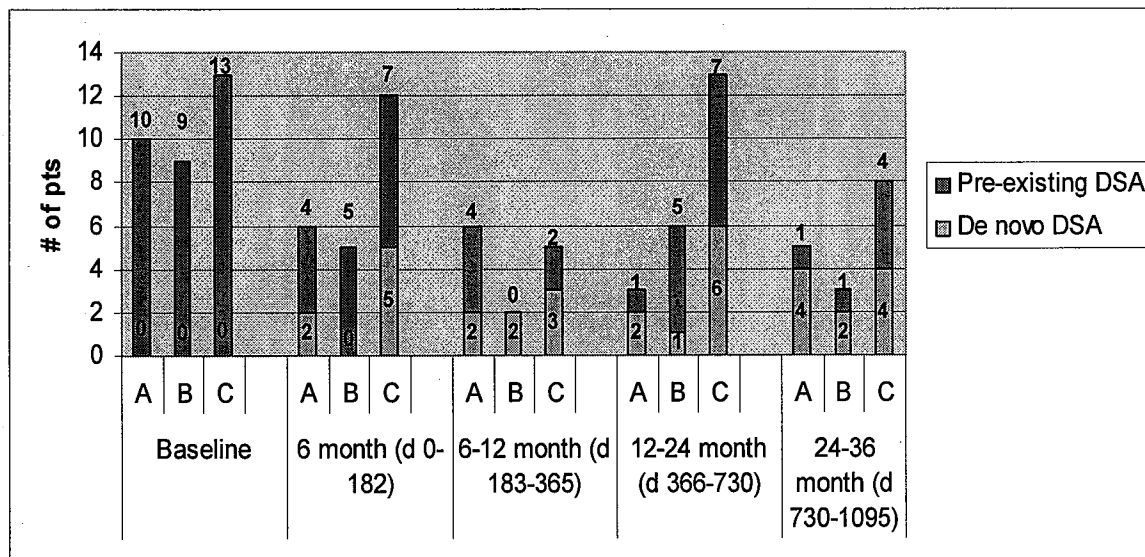
Table 14. Study IM103027: Patients positive at baseline who tested positive for DSA after treatment started through month 36

Treatment	Baseline	Month 6	Month 12	Month 24	Month 36
Belatacept MI	10	4	4	1	1
Belatacept LI	9	5	0	5	1
CsA	13	7	2	7	4

Table 15. Study IM103027: Patients negative at baseline with *de novo* DSA through month 36

Treatment	Month 6	Month 12	Month 24	Month 36	Total
Belatacept MI	2	2	2	4	10
Belatacept LI	0	2	1	2	5
CsA	5	3	6	4	18

Figure 7. Reviewer analysis of subjects with persistent versus *de novo* anti-donor HLA antibodies in ITT population in Study 103027 using 36 month data



A=Belatacept MI; B=Belatacept LI; C=CsA

The occurrence of AR was similar across all treatment groups and occurred in 18% (MI), 19% (LI), and 16% (CsA) of the patients. Most events of AR occurred by Month 6 and that after Month 36, there was no additional case of AR (Table 16). This data was replicated by the reviewer.

Table 16. Acute rejection rates in Study IM103027 using 36-month data

Acute Rejection (n, %) ^d				
Month 12 ^a	32 (17.4)	31 (17.7)	26 (14.1)	
Difference from CsA (97.3% CI)	3.3 (-5.2, 11.8)	3.6 (-5.0, 12.3)	-	
Month 24 ^a	32 (17.4)	32 (18.3)	28 (15.2)	
Difference from CsA (97.3% CI)	2.2 (-6.4, 10.8)	3.1 (-5.7, 12.0)	-	
Month 36	33 (17.9)	33 (18.9)	29 (15.8)	
Difference from CsA (97.3% CI)	2.2 (-6.6, 10.9)	3.1 (-5.8, 12.1)	-	

^a Month 24 CSR.

The applicant presented a table which compared DSA status to the occurrence of acute rejection (Table 17). The data show that for belatacept treated patients the presence of DSA was not as predictive in determining whether a patient had an episode of AR in comparison to the CsA treated patients. Patients in the belatacept arms who tested positive for DSA appeared to have less episodes of acute rejection than patients treated with CsA.

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Table 17. Applicant reported development of anti-donor HLA antibodies by acute rejection status up to month 24: All randomized and transplanted subjects (ITT) in Study 103027

	No. subjects positive/no. subjects with/without AR assessed for antibodies					
	Belatacept MI (A)		Belatacept LI (B)		CsA (C)	
	AR	No AR	AR	No AR	AR	No AR
Baseline	1/32 (3.1%)	9/141 (6.4%)	3/30 (10.0%)	6/137 (4.4%)	3/27 (11.1%)	10/147 (6.8%)
Antibody positive by Month 6	2/32 (6.3%)	4/147 (2.7%)	1/31 (3.2%)	4/134 (3.0%)	4/25 (16.0%)	8/141 (5.7%)
Antibody positive by month 12	2/32 (6.3%)	7/147 (4.8%)	1/32 (3.1%)	6/138 (4.3%)	5/27 (18.5%)	10/146 (6.8%)
Antibody positive by month 24	2/32 (6.3%)	7/147 (4.8%)	2/32 (6.3%)	7/139 (5.0%)	6/27 (18.5%)	15/148 (10.1%)
Antibody positive by month 36	3/32 (9.4%)	10/147 (6.8%)	2/32 (6.3%)	9/139 (6.5%)	7/27 (25.9%)	19/148 (12.8%)

Source Data: Table S.5.10.2 and Table S.8.2B in Month 24 CSR

This data was replicated by the reviewer. A total 95 patients were diagnosed with clinically suspected and biopsy proven acute rejection through month 36 following transplant (belatacept MI=33; belatacept LI=33; CsA=29). All of these patients had the actual date of their acute rejection episodes recorded and also had DSA information reported after start of treatment. Each of these numbers was used as the denominator for the column labeled as AR for each treatment group. A cumulative analysis similar to the data in Table 13 (see above) was used to determine each patient's DSA status at each of the time points. Only those patients who experienced an acute rejection episode prior to day 1096 (36 months) were included. The results show that a higher percentage of patients treated with CsA who underwent an episode of AR were DSA positive at some point in the trial (Table 18). No causal inferences between the presence of DSA and AR should be drawn, however. DSA positive patients treated with belatacept do not appear to be at any increased risk of AR compared to CsA patients. Though minor differences exist in the exact numbers reported by the sponsor and those calculated by the reviewer, these conclusions are in line with those of the sponsor.

Prevention of acute rejection

Table 18. Reviewer reported development of anti-donor HLA antibodies by acute rejection status up to month 24: All randomized and transplanted subjects (ITT) in Study 103027						
	No. subjects positive/no. subjects with/without AR assessed for antibodies					
	Belatacept MI (A)		Belatacept LI (B)		CsA (C)	
	AR	No AR	AR	No AR	AR	No AR
Baseline	1/33 (3.0%)	9/144 (6.3%)	3/33 (9.1%)	6/141 (4.3%)	4/29 (13.8%)	9/151 (6.0%)
Antibody positive by Month 6 (day 185)	2/33 (6.1%)	4/146 (2.7%)	1/33 (3.0%)	4/132 (3.0%)	4/29 (13.8%)	8/137 (5.8%)
Antibody positive by month 12 (day 395)	2/33 (6.1%)	7/146 (4.5%)	2/33 (6.1%)	7/138 (5.1%)	5/29 (17.2%)	11/145 (7.6%)
Antibody positive by month 24 (day 760)	3/33 (9.1%)	9/146 (6.2%)	2/33 (6.1%)	9/138 (6.5%)	6/29 (20.1%)	17/146 (11.6%)
Antibody positive by month 36 (day 1125)	4/33 (12.1%)	11/146 (7.5%)	2/33 (6.1%)	9/138 (6.5%)	7/29 (24.1%)	20/146 (13.7%)

An analysis was performed to determine whether patients who discontinued belatacept treatment due to an adverse event (AE) were more likely to develop DSA than patients who remained on belatacept. Patients were analyzed based on being entered as discontinued on study medication in AE.xpt dataset [parameter AEACN (Action Taken with Study Treatment) category 5: study drug discontinued]. A total of 120 patients discontinued due to AE (belatacept MI = 37, belatacept LI = 39, CsA = 44) and these appear to include dose adjustments for the CsA arm. The replacement therapy following discontinuation of belatacept was not specified. Results showed that 2 belatacept MI, 3 belatacept LI and 2 CsA treated patients developed DSA only after discontinuation (Table 19). It does not appear that more patients who discontinued belatacept LI developed DSA only after discontinuation compared to CsA.

Table 19. Development of DSA in patients who discontinued study treatment due to adverse event		
Treatment Arm	Number of discontinuations due to AE who had DSA data	Number testing positive for DSA only after discontinuation
Belatacept MI	37	2 (5.4%)
Belatacept LI	39	3 (7.7%)
CsA	44	2 (4.5%)

An analysis was performed to determine whether patients who discontinued belatacept treatment due to an acute rejection episode were more likely to develop DSA than patients who remained on belatacept. Patients were analyzed based on being entered as discontinued on study medication in AR.xpt dataset [parameter ARACN (Action Taken with Study Treatment) category 5: study drug discontinued]. A total of 73 patients discontinued due to AR (belatacept MI = 26, belatacept LI = 21, CsA = 26) and these appear to include dose adjustments for the CsA arm. The replacement therapy following discontinuation of belatacept was not specified. Of the 73 patients, 63 were tested for

Prevention of acute rejection

DSA at the time of and after the AE (belatacept MI=25, belatacept LI=17, CsA=21). Results showed that 1 belatacept MI, 1 belatacept LI and 2 CsA treated patients developed DSA only after discontinuation (Table 20). There does not appear to be a significant rise in the number of patients who develop DSA following discontinuation of belatacept due to acute rejection.

Table 20. Development of DSA in patients who discontinued study treatment due to acute rejection		
Treatment Arm	Number of discontinuations due to AR who had DSA data	Number testing positive for DSA after discontinuation
Belatacept MI	25	1 (4%)
Belatacept LI	17	1 (5.9%)
CsA	21	2 (9.5%)

5. Conclusions

The focus of this clinical microbiology/immunology review was the development of donor specific antibody (DSA) in all patients in the intent to treat population (ITT) and also to determine the development of DSA in patients after discontinuation of treatment due to either adverse event(s) or acute rejection. The results show that patients treated with belatacept, particularly those treated with the less intensive regimen which is proposed for approval, developed less *de novo* DSA and for those patients who had DSA at baseline, less persistence of DSA compared to the cyclosporine A (CsA) treated arm. The data also indicate that patients treated with belatacept who test positive for DSA are not at more risk of undergoing acute rejection than patients treated with CsA who test positive for DSA. Additionally, there is no strong evidence that patients who discontinue belatacept due to adverse events or acute rejection go on to develop DSA at a high rate.

The applicant has also submitted the final study report for a 1-month study of belatacept in healthy non-human primates. The purpose of this study was to determine the penetration and effect of belatacept on the blood:brain barrier (BBB) and brain immune cellularity. This study was performed to determine whether the results could be related to an increase in the incidence of PTLD presenting in the central nervous system (CNS) seen in the clinical trials in belatacept treated patients, particularly those who were EBV⁻ at the time of transplant. Results showed that little, if any, belatacept crossed the BBB, however a noticeable increase in the number of MHC class II⁺ cells was reported in all regions of the brain examined in belatacept treated animals. A concomitant increase in CD20⁺ (B-cells) or CD68⁺ (macrophage, dendritic cells, granulocytes) was not seen which could be due to presence of glial cells, astrocytes and other brain cells expressing class-II antigen. The applicant claims this increase in MHC II⁺ cells is insignificant though this observation should not be readily dismissed since even a slight increase in B-cell trafficking to the brain could theoretically manifest as PTLD since a single reactivated EBV⁺ B-cell clone could enter the brain, clonally expand, and manifest as PTLD of the CNS.

6. The label

(b) (4)

6.2 Comments

1. The statement (b) (4) should be changed to "In non human primate models of renal transplantation, belatacept monotherapy prolonged graft survival and decreased the production of anti-donor antibodies, compared to vehicle." This statement is more descriptive of the observations made in the non human primate studies of renal transplantation.
2. (b) (4)
The sponsor may consider a prospective study with an endpoint which reports the development of DSA and its relationship with clinical outcome.
3. A statement describing the observation of increased MHC class II cells in the brain of monkeys receiving belatacept should be put into the labeling. Such a statement might read as "In cynomolgus monkeys, belatacept increased the number of cells expressing major histocompatibility class-II antigens in the brain tissue. However, the clinical relevance of these findings is unknown."

6.3 FDA's version of the label

Additions are double underlined and deletions struck out.

12.1 Mechanism of Action

Belatacept, a selective T-cell (lymphocyte) costimulation blocker, binds to CD80 and CD86 on antigen-presenting cells thereby blocking CD28 mediated costimulation of T lymphocytes. *In vitro*, belatacept inhibits T lymphocyte proliferation and the production

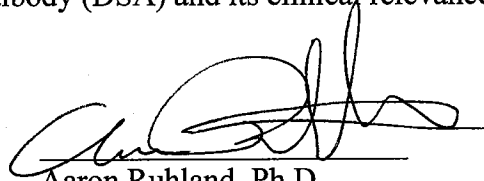
Prevention of acute rejection

of the cytokines interleukin-2, interferon- γ , interleukin-4, and TNF- α . Activated T lymphocytes are the predominant mediators of immunologic rejection.

(b) (4)

7. Recommendations:

1. The BLA is approvable from a microbiological/immunological standpoint pending an acceptable version of the labeling (for details see section 6.3).
2. The applicant should consider a prospective study with the goal of determining the incidence of donor specific antibody (DSA) and its clinical relevance.



Aaron Ruhland, Ph.D.
Microbiology/Immunology Reviewer
DSPTP

Concurrence:

Microbiology Team Leader/Dr Shukal Bala Shukal Bala Date 3/23/11
DSPTP

CC:

DSPTP/PM/GermainJ



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Center for Drug Evaluation and Research
WO Bldg 51
10903 New Hampshire Ave.
Silver Spring, MD 20993

Date: 3/2/2011
To: Administrative File, STN 125288/0/65
From: Bo Chi, Ph.D., CDER/OC/DMPQ/MAPCB/BMT
Endorsement: Patricia Hughes, Ph.D., Team Leader, CDER/OC/DMPQ/MAPCB/BMT
Subject: Review of a BLA amendment in response to a complete response letter
Applicant: Bristol-Myers Squibb Company
US License: 1713
Facility: Bristol-Myers Squibb Holdings Pharma, LTD., Manati, Puerto Rico
FEI: 2650089
Product: belatacept
Dosage: 250 mg/vial, lyophilized powder, intravenous
Indication: Renal transplant immunosuppression
PDUFA date: June 15, 2011

Recommendation: BLA 125288, as amended, is recommended for approval from a CMC sterility assurance and product quality microbiology perspective with the following post-market commitment:

Provide information and summary data on the container closure integrity test used to replace the sterility test in the stability program in a CBE-30 by June 30, 2012.

Review Summary

This is a resubmission to Biologics License Application (BLA) STN125288/0 for belatacept for the prophylaxis of organ rejection and preservation of a functioning allograft in adults receiving a renal transplant. This resubmission addresses the deficiencies listed in the May 1, 2010 complete response letter. This review assesses BMS' response to six CMC microbiology deficiency listed in the complete response letter.

(b) (4)

Conclusion

- I. The BLA 125288, as amended, is recommended for approval from sterility assurance and product quality microbiology perspective with the following post-market commitment:

Provide information and summary data on the container closure integrity test used to replace the sterility test in the stability program in a CBE-30 by June 30, 2012.

- II. Information and data in this submission not related to drug product sterility assurance was not evaluated and should be reviewed by a DTP reviewer.
- III. A follow-up inspection to a warning letter was conducted at the BMS, Manati, Puerto Rico facility starting on February 23, 2011. The compliance status of the drug product manufacturing facility is currently pending.

Cc: WO51: Chi
WO51: Hughes
WO22: Germain
HFD-328, eCTD Blue Files (STN 125288)

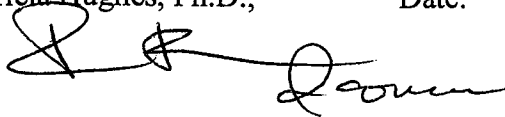
Archived File: S:\archive\BLA\125288\125288.0.65.rev.mem.BLA.3.2.2011.doc

SIGNATURES/DISTRIBUTION LIST

Primary BMT Reviewer: Bo Chi, Ph.D., *BC*
Concurring BMT Team Leader: Patricia Hughes, Ph.D.,

Date: 3/7/11

Date: 3/7/11

Handwritten signatures of Bo Chi and Patricia Hughes. The signature of Bo Chi is on the left, and the signature of Patricia Hughes is on the right, written in a cursive style.



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Center for Drug Evaluation and Research
WO Bldg 51
10903 New Hampshire Ave.
Silver Spring, MD 20993

Date: 3/15/2010
To: Administrative File, STN 125288/0
From: Bo Chi, Ph.D., CDER/OC/DMPQ/MAPCB/BMT *BC 3/23/10*
Endorsement: Patricia Hughes, Ph.D., Team Leader, CDER/OC/DMPQ/MAPCB/BMT *PH 3/23/10*
Subject: New Biologic License Application (BLA)
Applicant: Bristol-Myers Squibb Company
US License: 1713
Facility: Bristol-Myers Squibb Holdings Pharma, LTD., Manati, Puerto Rico
FEI: 2650089
Product: belatacept
Dosage: 250 mg/vial, lyophilized powder, intravenous
Indication: Renal transplant immunosuppression
PDUFA date: May 1, 2010

Recommendation: The drug product part of this application, as amended, is not recommended for approval from sterility assurance and product quality microbiology perspective.

Review Summary

BMS has submitted this Biologics License Application (BLA), STN 125288/0, requesting the approval of belatacept for the prophylaxis of organ rejection and preservation of a functioning allograft in adults receiving a renal transplant. Belatacept bulk drug substance is manufactured at the BMS facility located in East Syracuse, NY (BMS-SYR). Belatacept drug product is manufactured at the BMS facility in Manati, Puerto Rico (BMS-MAN). The application contains CMC information in an eCTD format.

(b) (4)



(b) (4)

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Reviewer comment: The information provided is adequate.

Satisfactory

(b) (4)

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cGMP Status:

The pre-license inspection of the drug product manufacture site of BMS at Manati, Puerto Rico was inspected in the week of 3/15/2010. The compliance status of the facility is pending the outcome of the recent inspection.

Conclusion

- I. The BLA is not recommended for approval from a sterility assurance and product quality microbiology perspective.
- II. Information and data in this submission not related to drug product sterility assurance was not evaluated and should be reviewed by a DTP reviewer.
- III. The pre-license inspection of the drug product manufacture site of BMS at Manati, Puerto Rico was inspected in the week of 3/15/2010. The compliance status of the facility is pending the outcome of the recent inspection.

Cc: WO51: Chi
WO51: Hughes
WO22: Germain
HFD-328, eCTD Blue Files (STN 125288)

Archived File: S:\archive\BLA\125288\125288.0.rev.mem.BLA.3.15.2010.doc

BLA STN125288/0 is not recommended for approval. The deficiencies are listed below:

1. The sensitivity (leak volume and the corresponding leak size) of the dye ingress test used to validate the integrity of the drug product container closure should be correlated to a microbial challenge test (using the same vacuum and pressure challenge conditions as the dye ingress test) and evaluated. Provide information and summary data of the microbial challenge test.
2. [REDACTED] (b) (4)
3. With regard to the validation study for shipping of belatacept from the BMS distribution center to consumers, please justify that the [REDACTED] (b) (4) temperature profile selected represent the worst case [REDACTED] (b) (4) temperature conditions. The temperature inside the truck during [REDACTED] (b) (4); the temperature outside the truck.
4. The updated drug product endotoxin specification [REDACTED] (b) (4) does not provide a safety margin over the specified limit for parental drugs (5 EU/kg) at the lowest belatacept infusion solution concentration (2 mg/mL). Please lower the endotoxin specification to include a safety margin. [REDACTED] (b) (4)
5. Please provide microbiology data to support the following labeling claim [REDACTED] (b) (4)
[REDACTED] In lieu of microbiology data, the product labeling should recommend that the constituted and diluted product may be stored for a maximum of 4 hours at 2° to 8°C or room temperature.
6. Provide information and summary data on the container closure integrity test developed in support of finished product on stability when available.



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Food and Drug Administration
Center for Drug Evaluation and Research
WO Bldg. 51
Silver Spring, MD 20993

Date: 22 March 2010

To: Administrative File, STN 125288/0
From: Michelle Y. Clark-Stuart, MGA, MT (ASCP), Reviewer, CDER/OC/DMPQ/MAPCB/BMT
Through: Patricia Hughes, Ph. D., Team Leader, CDER/OC/DMPQ/MAPCB/BMT
Subject: Amended New Biologic License Application (BLA) Review from a microbiology perspective and for acceptability of the East Syracuse manufacturing site (FEI=1317461)

MYC
3/24/10

08/29/2011

US License # 1713
Applicant Bristol-Myers Squibb
Product belatacept (Nulojix®)
Facility Bristol-Myers Squibb Co., 6000 Thompson Road, East Syracuse, NY, Reg. #1317461
Indication prophylaxis of organ rejection and preservation of functioning allograft in adult patients receiving renal transplant
Dosage: Lyophilized powder for injectable solution (250mg/vial)

Due date: 01 May 2010

Recommendation: The drug substance (DS) part of this BLA, as amended, is recommended for approval from a product quality/microbiology and CGMP perspective.

Review Summary:

Belatacept is a genetically engineered fusion protein that selectively blocks full activation (IL-2 production) of T-lymphocytes by binding specifically to B7-1 and B7-2 on the Antigen Presenting Cells (APC), and inhibiting the co-stimulatory pathway. It is a second-generation higher avidity mutant of abatacept (Orencia® STN 125118). The secreted protein is manufactured in the Chinese Hamster Ovary (CHO) cell line. This review covers drug substance produced (b) (4), used in the manufacture of drug product used in the Phase III clinical trials. The application contains information to support commercial production of belatacept drug substance at Bristol-Myers Squibb (BMS), located in East Syracuse, NY. This BLA was submitted in the eCTD format. This review covers the evaluation of the drug substance aspects of the submission from a microbial control and microbiology product quality perspective. The following amendments to the quality section of the submission were reviewed in addition to the original

application: eCTD sequence number 0002 dated 7/31/09 (clarification of addresses listed for the (b) (4) facilities), 0003 dated 8/6/09 (notification that Module 2.3.2.S.2.3 - Common Technical Document Quality Overall Summary was incomplete as originally sent, this is a resubmission of replacement corrected Module 2.3.2.S.2.3), 0005 dated 9/4/09 (high level description identifying the differences and similarities of abatacept and belatacept with respect to the CMC manufacturing process), 0007 dated 9/18/09 (response to FDA facsimile requesting additional microbiology, CMC, and clinical information), 0022 dated 1/6/10 (updated 3.2.S.7 Stability), 0023 dated 1/8/10 (updated 3.2.S.2.5, .6, and S.4.3 sections -shipping validation, (b) (4) hold time, and bioburden assay qualification), 0028 dated 2/2/10 (updated 3.2.A.1 for (b) (4), 0031 dated 2/1/10 (drug product response to questions), and 0037 dated 2/23/10 (updated 3.2.S.6 per OBP request), 0043 dated 3/17/10 (endotoxin qualification reports). All items were adequately addressed during the review period.

(b) (4)

Satisfactory

(b) (4)

Conclusion

- I. The BLA and amendments for the new belatacept drug substance manufactured at the Bristol-Myers Squibb East Syracuse, NY manufacturing site was evaluated for microbial control strategy, and contamination/cross-contamination controls related to the Drug Substance (Section 3.2.S) and Facilities and Equipment (Section 3.2.A.1). The drug substance part of this BLA, as amended, is recommended for approval from a product quality/microbiology and CGMP perspective.
- II. All other sections of the BLA not related to microbial control and microbial product quality attributes should be reviewed by the OBP/DTP reviewer.
- III. No inspectional follow-up items for drug substance.

cc: WO51: Hughes
WO51: Clark-Stuart
WO22: Germain
HFD-328, e-CTD Files (STN 125288)

Archived File: S:\archive\BLA\125288\125288.0.rev.mem.BLA.03-22-10.doc

MICROBIOLOGY/IMMUNOLOGY TEAM LEADER REVIEW
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

DATE: March 18, 2010

SUBMISSION: BLA # 125288 (Original)

REVIEWER: Shukal Bala, Ph.D.
Microbiology Team Leader
Division of Special Pathogen and Transplant Products
Office of Antimicrobial Products

SUBJECT: Protein: Belatacept

(b) (4)

(b) (4)

(b) (4)

Indication: Prevention of organ rejection in adult patients receiving renal
transplants

Applicant: Bristol-Myers Squibb

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1. Executive Summary:

Belatacept, like abatacept, prevents co-stimulation of the T-cells, by CD80/CD86 on APC, by indirectly blocking signaling through CD28 receptor. Mutation of 2 residues [lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y)] in the ligand binding region allow belatacept to bind to human CD80 and CD86 with greater avidity than abatacept. Overall, the results show that belatacept is more active against human and nonhuman primate cells whereas abatacept is more active against rodent cells.

Binding of belatacept with CD80 and CD86 on the surface of APC blocks interaction with CD28 on the surface of T-cells. Blockage of CD80/CD86 and CD28 interaction inhibits T-cell proliferation and the production of the cytokines such as interleukin-2, interferon- γ , interleukin-4, and TNF- α . Belatacept does not suppress/block complement mediated or antibody dependent cellular cytotoxicity. Activated T lymphocytes are the predominant mediators of immunologic rejection.

Preclinical studies also suggest an effect on interaction of CD80/CD86 (on the surface of APC) and CD152 (CTLA-4; on the surface of activated T-cells) as measured by IDO activity; however, such an effect was not shown in the small number of patients tested.

In primate models of renal transplantation, belatacept prolonged kidney graft survival and inhibited the production of anti-donor antibodies. Such an effect appears to be reversible. The applicant states that there was no effect on the different T-cell subsets. Belatacept in combination with sirolimus and basiliximab extended pancreatic islet graft survival. Effect on immune responses *in vivo* was also measured in normal mice, rats, rabbits and monkeys. The results show that belatacept was effective in suppressing antibody response to sRBC and L6 antigens in monkeys and KLH in rodents. Here again, the results show abatacept to be more active in rodents and belatacept in primates. The effect of long term treatment with abatacept on T-cell subsets was also measured in rats. Overall, the results show an increase in the absolute number of T-cells, CD4 and CD8 cells in the peripheral blood and a decrease in regulatory T-cells. Also, inflammation was reported in adult rats and signs of autoimmunity such as thyroiditis and insulinitis in juvenile rats. Such changes appear to be reversible.

In clinical studies, fewer belatacept-treated patients had detectable antibodies to donor-specific human leukocyte antigens (HLA) compared to CsA-treated patients, although acute rejection episodes were reported in similar or greater number of patients. Production of antibodies to donor-specific HLA antigens is associated with antibody-mediated rejection and poor graft outcomes. The results of flow cytometric analysis in blood samples from a small number of patients enrolled in the phase 2 and 3 clinical trial show a transient decrease in regulatory T-cells (CD25+, FOXP3+) in patients treated with either belatacept+ basiliximab or CsA+basiliximab.

In general, transplant recipients are on intense immunosuppressive regimen and are more susceptible to development of opportunistic infections and tumors. A higher incidence of PTLD was reported in patients in the belatacept containing regimen compared to the CsA containing regimen. Although the incidence is higher in EBV⁻ (seronegative) patients compared to EBV⁺ (seropositive) patients, EBV⁺ patients also developed PTLD including CNS PTLD. Therefore, it is important that EBV serostatus should be determined by using appropriate tests with high specificity that detect IgG antibodies against VCA and

EBNA. The possibility of reactivation of EBV does exist. Reactivation is generally measured by presence of IgM antibodies against VCA antigen (Figure A) or detection of virus by methods such as PCR. There are no FDA cleared PCR methods for detection of virus. However, tests for detection of IgM antibodies against VCA antigens are FDA cleared. If the presence of IgM antibodies is demonstrated in a patient it is recommended that patients should not be administered belatacept.

Figure A: Antibody titers to EBV antigens distinguish between primary and resolved infection¹

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Interpretive criteria for determining primary and past EBV infection

VCA IgG	VCA IgM	EBNA IgG	EA IgG	Interpretation
-	-	-	-	No infection, past or present
-	+	-	-	Early phase of primary infection
+	+	-	-/+	Acute phase of primary infection
+	+	+	+/-	Late phase of primary infection/reactivation
+	-	+	-/+	Past infection

Adapted from Figure 46 and Table 26 of Dr. Ruhland's review.

Conclusions

The BLA is approvable pending an acceptable version of the labeling. It is recommended that labeling should specify the increased risk of developing PTLTD in

- EBV negative patients, and
- Patients with IgM antibodies against VCA but **no** IgG antibodies against VCA and EBNA.

It is also recommended that certain standards be maintained for the testing of EBV serostatus. The EBV tests to be used should be highly specific for IgG antibodies against VCA and EBNA antigens. These specifications should be made in appropriate sections of the labeling so that the message goes to the prescribing physicians and the clinical microbiology laboratories responsible for EBV testing.

¹Gulley, ML and Tang, W. (2008) Laboratory Assays for Epstein-Barr Virus-Related Disease. *J Mol Diagn.*, 10: 279-292

2. Introduction and Background

The subject of this Biological License Application (BLA) is Nulojix® (belatacept; LEA29Y) for the prevention of acute rejection in patients receiving a renal allograft. The has proposed the dose of 10 mg/kg on days 1, 5, 14, 28, months 2 and 3 followed by a lower dose of 5 mg/kg every month starting at month 4.

Belatacept was derived from abatacept (Orencia®) which is approved for the treatment of moderate to severe rheumatoid arthritis. Abatacept is a homodimeric recombinant human fusion protein (human cytotoxic T-lymphocyte antigen-4, CTLA4-IgG1) constructed from a mutated extracellular domain of CTLA-4 and the Fc portion (hinge-CH2-CH3 domains) of a human IgG1 antibody. Belatacept was modified by mutation of 2 amino acids [lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y)] in the ligand binding region.

In Phase 2 and 3 trials, the mean belatacept C_{min} achieved in renal transplant patients was

- 20 µg/mL at month 1 post-transplant.
- > 7 µg/mL at months 2 to 4 post-transplant.
- > 4 µg/mL from months 6 post-transplant and onwards.

The half life of belatacept is about 8 to 10 days. No metabolites were identified in any of the studies (for details see clinical pharmacology review).

This review summarizes the microbiologic findings from (a) proof of concept studies supporting the mechanism by which belatacept exhibits its effect and activity *in vitro* and in animal models of transplantation, and (b) clinical microbiology/immunology studies (for details see microbiology/immunology review by Dr Aaron Ruhland, dated 3/16/10).

2.1. Immunobiology of Transplantation

Transplantation is a process of transferring grafts (organs, tissues, or cells) from one individual to another. Graft rejection is a potential complication of transplantation in which lymphocytes play an important role. Rejection may be mediated by a variety of cell types, which recognize the graft as non-self or foreign and attack it. The process may occur within several different time frames. Hyperacute rejection relies on pre-formed antibodies that activate complement and occurs within a week. Acute rejection employs either antibodies that develop in response to the graft and activate complement (vascular rejection) or is mediated more directly by cells (cellular rejection) including cytotoxic T-cells and macrophages. Chronic rejection is considered multifactorial and represents a culmination of both immunologic and nonimmunologic damage to graft. Since antibody production (against T-cell dependent antigens) and cell-mediated immunity both depend on functional T cells, a diminution or obliteration of T cell responsiveness has frequently been the goal of drug development for this indication. Steroids, cyclosporine A (CsA), tacrolimus, monoclonal antibodies, and combinations of these agents have proven to be effective in suppressing cellular (broadly speaking, T cell-dependent) and antibody (T and B cell-dependent) -mediated rejection mechanisms.

For optimal T-cell activation to occur, it is essential that (1) alloantigens be recognized by T-cell receptors, (2) T-cells are costimulated with antigen presenting cells (APC) such as macrophages/dendritic cells/B-cells, and (3) T-cells proliferate and acquire effector

functions (Figure 1). Several soluble mediators derived from macrophages, lymphocytes, and other nonlymphoid cells play an important role in the regulation of immune responses and graft rejection. Interleukin (IL) -2 and IL-4, produced by activated T-cells (CD4 and CD8 cells), play an important role in the clonal expansion of T-cells. Some growth factors produced by non T-cells such as IL-7 (produced by stromal cells and important for T-cell maturation, proliferation and differentiation) and IL-15 (produced by activated macrophages, muscle cells, keratinocytes, renal epithelial cells and endothelial cells) are shown to exhibit IL-2 like activities which includes providing support for induction of T- and NK- cell proliferation, cytotoxic T cell function and production of interferon (IFN)- γ and tumor necrosis factor (TNF)- α .

T-cell activation is dependent on a network of positive and negative regulatory factors that modulate survival, proliferation and differentiation of T lymphocytes (Figure 1). The receptors important for T-cell activation and regulation include:

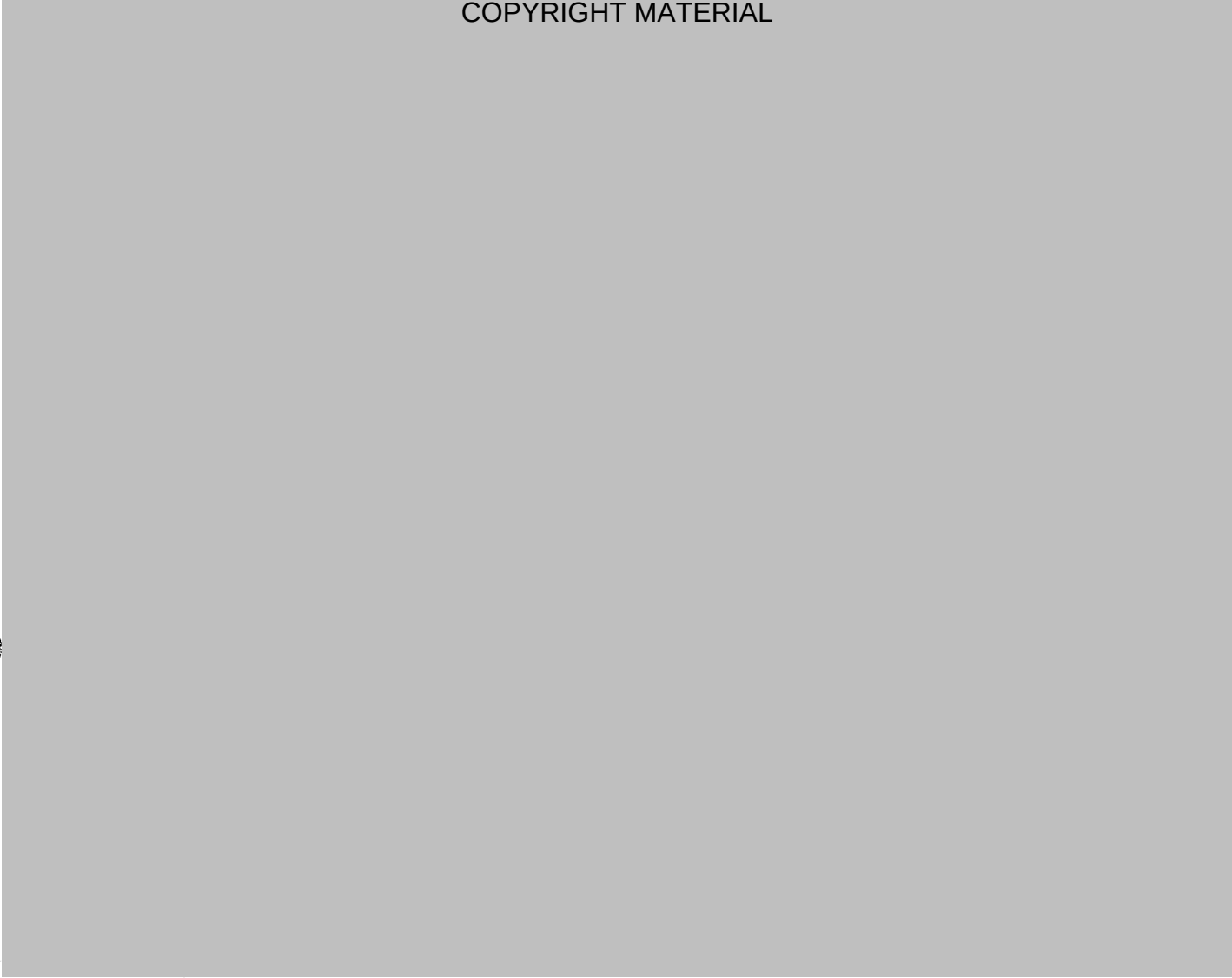
- T-cell receptor (TCR): Engagement of TCR by a specific major histocompatibility complex (MHC) forms a TCR-MHC complex that initiates signal transduction after recognition of specific antigen.
- Ligation of co-stimulatory molecules:
 - **CD28:** CD28 is expressed on both resting and activated T-cells and binds CD80 and CD86 (B7-2) on the surface of APC. CD28 signals are important for cytokine (IL-2, IL-4, IL-5, IL-13, interferon- γ , GM-CSF, and IL-8) and chemokine (e.g., CCL5 that interacts with CCR1 CCR3, and CCR5 receptors) production that may promote type-2 T-helper response and T-cell survival by promoting BCL-X that belongs to BCL-2 family. TCR binding in the absence of CD28 ligation leads to apoptosis or anergy/tolerance.
 - **CD152:** CD152 (also called as CTLA-4), is expressed on activated T-cells and is considered a T-cell inhibitory or regulatory receptor. CD152 binds CD80 and CD86 with higher affinity than CD28. Studies also suggest that interaction of CD80 and CD152 may mediate a stronger signal than CD152 and CD86. Interaction of CD152 with CD80/86 expressed on the APC also initiates reverse signaling in the APC that induces expression of indoleamine-pyrrole 2, 3-dioxygenase (IDO). IDO catalyzes the degradation of tryptophan, an essential amino-acid to N-formyl kynurenine. Depletion of tryptophan may inhibit the growth of microbes and proliferation of T-cells. Studies suggest that in models of transplantation, IDO can down-regulate the T-cell alloresponse *in vitro* and promote tolerance in murine models of pancreatic islet transplantation and allogeneic T-cell transfer². However, the physiologic role of IDO in transplantation has not been determined.

CD28 and CD152 have opposing effects in regulating immune responses. CD28 decreases and CD152 increases the threshold for T-cell activation. It is possible that belatacept could compete with CD152 for interaction with CD80/CD86 and potentially inhibit IDO expression in APC *in vivo*.

² Hainz U, Jürgens B, Heitger A. (2007) The role of indoleamine 2,3-dioxygenase in transplantation. *Transpl Int.* 20:118-127.

Figure 1: Diagrammatic representation of interaction of antigen presenting cells with T-cells and sites of action of available immunosuppressants that inhibit the three-signal model of T-cell activation and proliferation³

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3. Proof of Concept Studies

3.1. Mechanism of Action

Belatacept binds to CD80 and CD86 molecules present on the surface of antigen presenting cells such as macrophages, monocytes and B-cells and prevents co-stimulation of T-cells (Figure 2). The avidity of belatacept is higher compared to abatacept against both human CD80 and CD86 molecules; whereas abatacept exhibited a higher avidity, compared to belatacept, against murine CD80 and CD86 molecules. In one experiment, both belatacept and abatacept showed similar avidity against the murine CD86 molecule. Belatacept and abatacept showed similar binding to CD80 expressed on transfected

³ Kahan, BD. (2003) Individuality: the barrier to optimal immunosuppression. *Nature Reviews Immunology* 3: 831-838.

Chinese hamster ovary cells (CHO) whereas belatacept bound with more avidity to CD86 on the surface of CHO cells.

Figure 2: Diagrammatic representation of T-cell fate under different conditions of TCR engagement⁴

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3.2. Activity *In Vitro*

3.2.1. Effect on T-cell proliferation

Belatacept, like abatacept, suppresses human T-cell proliferation (both CD4 and CD8 cells) induced by antigen presenting cells. Belatacept is more active in inhibiting human T-cell proliferation compared to murine cells whereas murine cells are more sensitive to abatacept than belatacept. Studies also show that belatacept is more effective in blocking proliferation when CD4 cells are incubated with CHO cells expressing CD80 molecule (IC₅₀ 100 ng/mL) compared to those expressing CD86 molecule (IC₅₀ 250 ng/mL). Belatacept IC₅₀ values are lower than abatacept suggesting belatacept to be a more active blocker. It is unclear whether the number of CD80 and CD86 molecules expressed on the surface of CHO cells was same or different or such a differential effect reflects differential avidity of belatacept to CD80 and CD86 molecules.

The activity of belatacept appears to be reversible; the human T-cells + irradiated B-cells (PM-LCL) cultured in the presence of belatacept remained unresponsive in a secondary culture but not in the absence of belatacept.

3.2.2. Effect on cytokine production

Belatacept decreased the production of IL-2, IL-4, IFN- γ and TNF- α in mixed lymphocyte reaction cultures of human peripheral blood T-cells and irradiated human B-cell line cells (PM-LCL). Such an effect was concentration dependent with the IC₅₀ values of 0.029 μ g/mL, 0.20 μ g/mL, 0.060 μ g/mL to 0.20 μ g/mL, and 0.16 μ g/mL, respectively. Belatacept IC₅₀ values required for CD86 occupancy appear to correlate with function as measured by IFN- γ production in an allogeneic reaction of human T-cells and

⁴ Alegre, M-L, Frauwirth, KA, and Thompson, CB. (2001) T-cell regulation by CD20 and CTLA-4. *Nature Reviews Immunology* 1: 220-228.

monocyte derived dendritic cells. Overall, the results suggest that belatacept is more effective than abatacept in inhibiting cytokine production in response to alloantigen.

3.2.3. *Effect on complement and antibody mediated cellular cytotoxicity*

Belatacept does not mediate human complement-dependent cellular cytotoxicity and antibody dependent cellular toxicity; this could be due to the fact that the hinge region of the Fc portion (IgG1) of belatacept is mutated.

3.2.4. *Effect on receptor occupancy*

Receptor occupancy by belatacept was evaluated by a competitive assay using FUN-1 antibody and some commercially available anti-CD86 antibodies (IT2.2, HA5.2B7, BU63, (b) (4)) that compete for CD86 receptor expressed on monocytes. The results showed HA5.2B7 to be less effective in displacing CD86 bound belatacept compared to other antibodies. *In vitro* studies show that saturation of the receptors by belatacept occurred at a concentration of approximately 0.1 µg/mL and 1 µg/mL for CD80 and CD86, respectively. Studies suggest that the saturation level of CD86 on the APC correlates with the activity of belatacept in blocking T-cell response to stimulation in a mixed lymphocyte reaction.

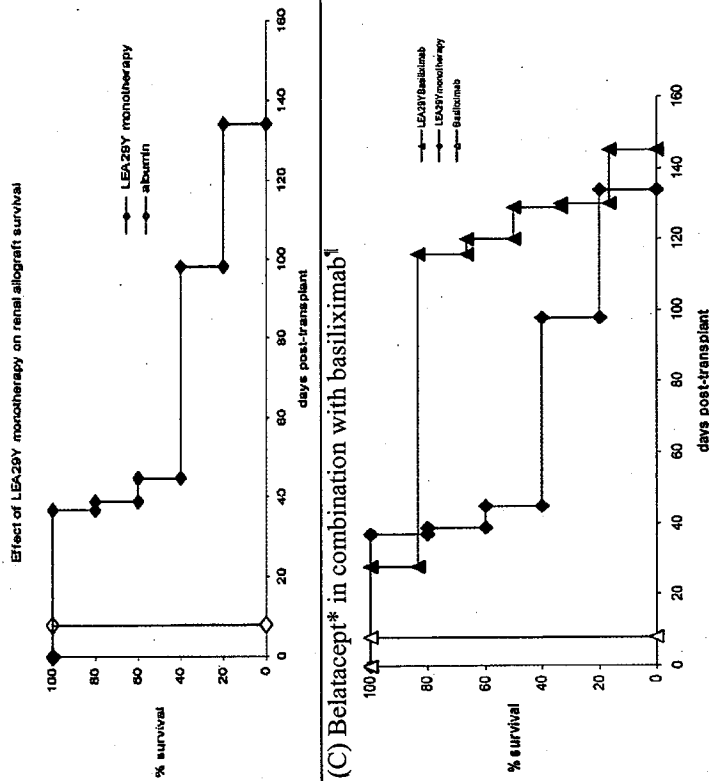
3.3. *Activity In Vivo*

3.3.1. *Kidney transplant model*

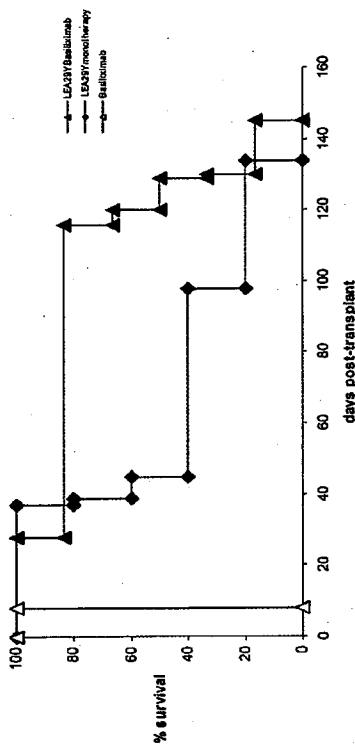
The activity of belatacept alone or as part of an immunosuppressive regimen was measured in a primate orthotopic transplant model. The rhesus monkeys underwent bilateral nephrectomy with the donor kidney anastomosed to the recipient vena cava (renal vein) and distal aorta (renal artery). The monkeys were treated with belatacept alone or in combination with mycophenolate mofetil (MMF) and steroids or basiliximab. The animals were followed at regular intervals; the kidney graft survival was measured by serum creatinine, BUN and serum chemistries. Kidney allograft histology was conducted at the time of necropsy. Other parameters monitored include serum levels of belatacept and anti-belatacept antibodies, anti-donor antibodies, PBMC subset analysis and renal allograft histology of formalin fixed sections. The results showed that belatacept alone or in combination with MMF + steroids or basiliximab extended the survival of kidney allografts (Figure 3).

No detectable anti-donor antibody responses were reported in monkeys treated with albumin (control group) or those treated with basiliximab as the monkeys (2 per group) died by 8 days post-transplantation. None of the monkeys treated with belatacept alone or in combination with MMF+steroids or basiliximab developed antibodies until day 100 i.e., 30 days after the last dose. About 40 % of the monkeys developed antibodies between days 100 and 130. Similarly in monkeys treated with belatacept + basiliximab about 40% of the monkeys developed anti-donor antibodies. In monkeys treated with MMF or steroids significant anti-donor antibodies were detected in about 80% of the monkeys by day 25. Overall the results suggest that belatacept is effective in prolonging graft survival and inhibiting the development of anti-donor antibodies.

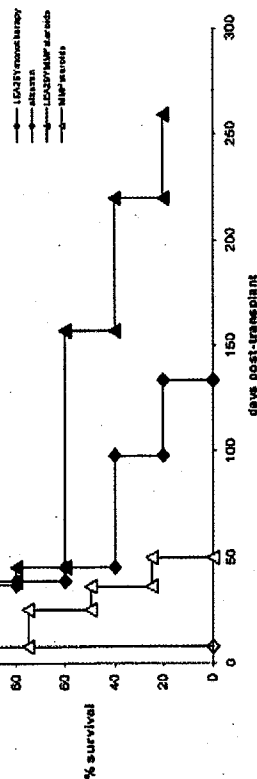
Figure 3: Effect on graft survival (A, B, and C) and anti-donor antibodies (D).
(A) Belatacept* alone



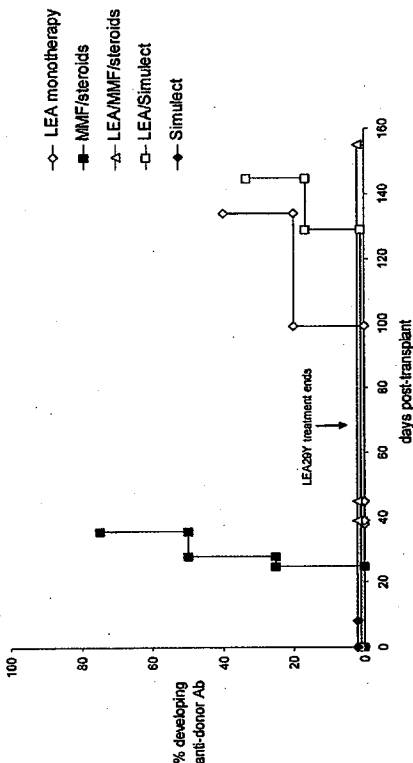
(C) Belatacept* in combination with basiliximab[†]



(B) Belatacept* in combination with MMF** and steroids***



(D) Anti-donor antibodies



LEA=belatacept

*Belatacept: administered intraoperatively 10 mg/kg, followed by 15 mg/kg on day 2 and then 20 mg/kg on days 14, 28, 42, 56 and 70 post-surgery.

**MMF: 15 mg/kg, bid administered intramuscularly days 1 to 14 followed by once daily at the same dose through 6 months post-transplant.

***Solumedrol: 12 mg administered intravenously intraoperatively, and then as a tapered intramuscular injection on post-operative day(s): 1 (20mg), 2 (16 mg), 3 (12 mg), 4 (8 mg), 5 (4 mg), 6 – 14 (3 mg), and 15 – 182 (1 mg).

[†]Basiliximab: 0.3 mg/kg, intravenously intraoperatively and on day 4.

Experimental Group	Survival (days)
Human Albumin (n=2)	8, 8
Belatacept (n=6)	37, 39, 45, 98, 134
MMF/Steroids (n=4)	8, 25, 36, 50
Belatacept/MMF/Steroids (n=5)	39, 45, 157, 220, >260
Basiliximab (n=2)	7, 8
Belatacept/Basiliximab (n=6)	28, 116, 120, 129, 130, 145

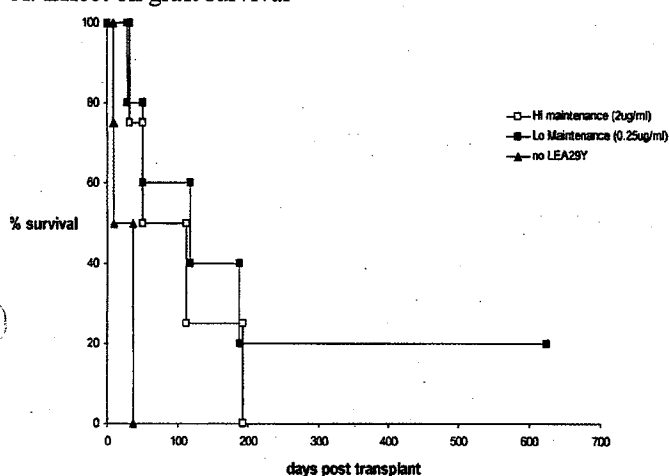
Adapted from Table 6 and Figures 28, 30, 31, and 33 of Dr Ruhland's review

The applicant states that there was no effect of treatment on absolute lymphocyte cell count and its subsets (CD3, CD4, CD8 and CD20). However, the results were not available for review.

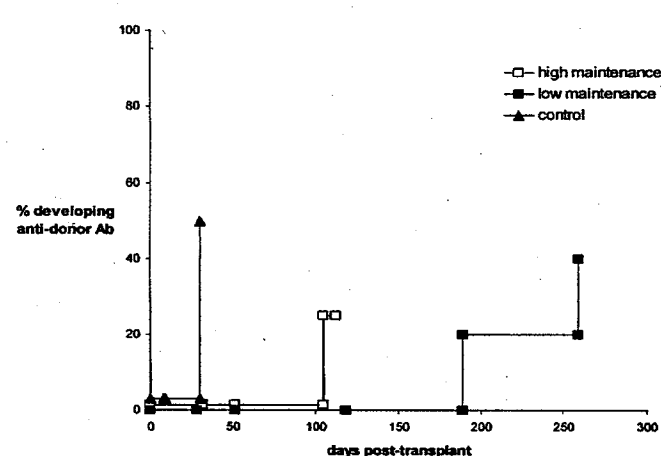
In another experiment, the effect of 2 different (low and high) maintenance regimens of belatacept in combination with MMF, steroids and basiliximab was measured. The experimental design was same as described above. The results in Figure 4 show that low and high maintenance regimens are similar in improving graft survival (except for one animal in the low maintenance group that showed survival >625 das) compared to the control group. Anti-donor antibodies did develop in 20% and 40% of the monkeys during the maintenance phase.

Figure 4: Effect of low and high maintenance on graft survival and anti-donor antibodies

A: Effect on graft survival



B: Effect on anti-donor antibodies



Experimental Group	Survival (days)
Belatacept (high maintenance) (n=4)	32, 51, 112, 198
Belatacept (low maintenance) (n=5)	28, 51, 118, 189, >625
Basiliximab/MMF/Steroids (n=4)	8, 9, 36, 36

Control group: A combination of basiliximab, MMF and steroids.

High maintenance group: Same background immunosuppression + belatacept (10 mg/kg on day 0, 15 mg/kg on day 4, 20 mg/kg on days 14, 30, 49, 68 and 91, and then 20 mg/kg every 23 days until day 344. These doses were given to target different trough concentrations through day 365 (day 0 – 30 = 20 µg/mL; day 30 – 90 = 7µg/mL; day 90 – 365 = 2µg/mL).

Low maintenance group: Same background regimen + belatacept (same regimen as above) through day 90, except the long term maintenance (days 90 – 365) had a target trough of 0.25 µg/mL so NHP were given 20 mg/kg every 33 days.

Adapted from Table 7 and Figures 34 and 36 of Dr. Ruhland's review

3.3.2. Pancreatic transplant model

The activity of belatacept + sirolimus + basiliximab as part of an immunosuppressive regimen was measured in a primate pancreatic transplant model. The rhesus monkeys underwent pancreatectomy and a week later were intra-hepatically implanted with allogeneic pancreatic cells by injecting into the colic vein. Allograft function was measured daily by glucose tolerance test (insulin and c-peptide production). The results in Figure 5 show that sirolimus + basiliximab were not effective in prolonging graft survival whereas belatacept + sirolimus + basiliximab prolonged graft survival.

Shortened graft survival in the control group (sirolimus + basiliximab) was associated with early (day 11) production of IFN- γ (measured by ELISpot assay). In the group treated with belatacept + sirolimus + basiliximab, IFN- γ production was suppressed until day 185; IFN- γ production was restored about a month after discontinuation of therapy thereby indicating reversal of the effect of belatacept on T-cells.

The anti-donor antibodies were measured by flow cytometry and the results in Figure 5 show no change in mean channel fluorescence in the regimen containing belatacept compared to the control group thereby suggesting suppression of antibody response. However, the timing of the assay for antibody measurement was not specified.

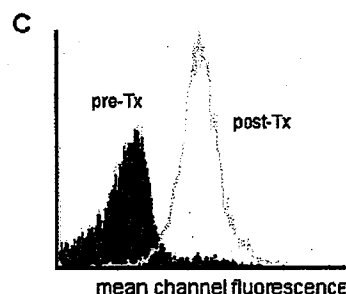
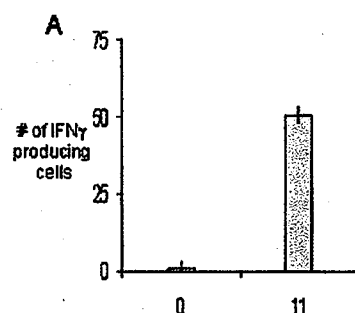
Figure 5: Survival of islet pancreatic cells

Animal ID	IEQ/kg	Survival (insulin independence)	Treatment	MHC mismatches	
				Class I	Class II
RKf-7	22,250	204	Belatacept/Rapa/ α IL-2R	2	ND
RUF-7	17,087	190	Belatacept/Rapa/ α IL-2R	ND	3
RRe-7	20,266	216	Belatacept/Rapa/ α IL-2R	2	6
RWt-6	16,033	56	Belatacept/Rapa/ α IL-2R	2	3
RMv-6	8,201	> 200	Belatacept/Rapa/ α IL-2R	1	3
RQz-6	12,980	7	Rapa/ α IL-2R	2	5
RIb-7	10,903	7	Rapa/ α IL-2R	1	4

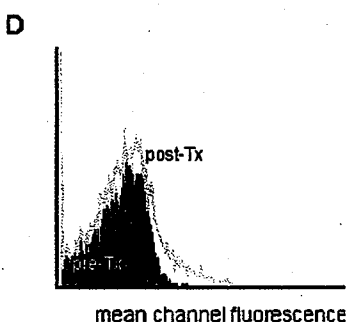
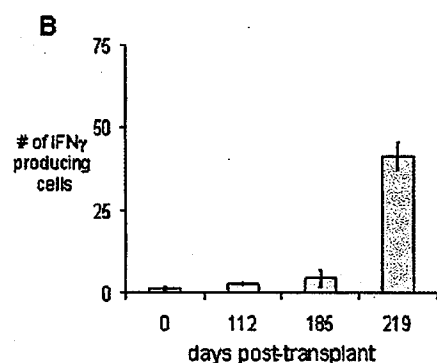
ND= no difference in the alleles which were typed; IEQ=islet equivalents implanted; rapa = Sirolimus; α IL-2R = basiliximab.

A and B: Allogeneic IFN- γ

C and D: Development of anti-donor antibodies



A and C: Sirolimus* + basiliximab**



B and D: Belatacept*** + sirolimus + basiliximab

*Sirolimus: Sirolimus was given orally (1.25 mg/kg) through day 50 and then as a 1 mg/kg dose through day 100. The dose was further tapered until terminated on day 121.

** Basiliximab: 0.3 mg/kg administered intravenously intra-operatively and on day 4.

***Belatacept: 10 mg/kg intra-operatively followed by 15 mg/kg on days 4, 20 mg/kg on day 14 and then every two weeks until day 154.

Adapted from Table 8 and Figure 37 of Dr. Ruhland's review

3.3.3. Effect of belatacept on immune response

■ Effect on antibody response

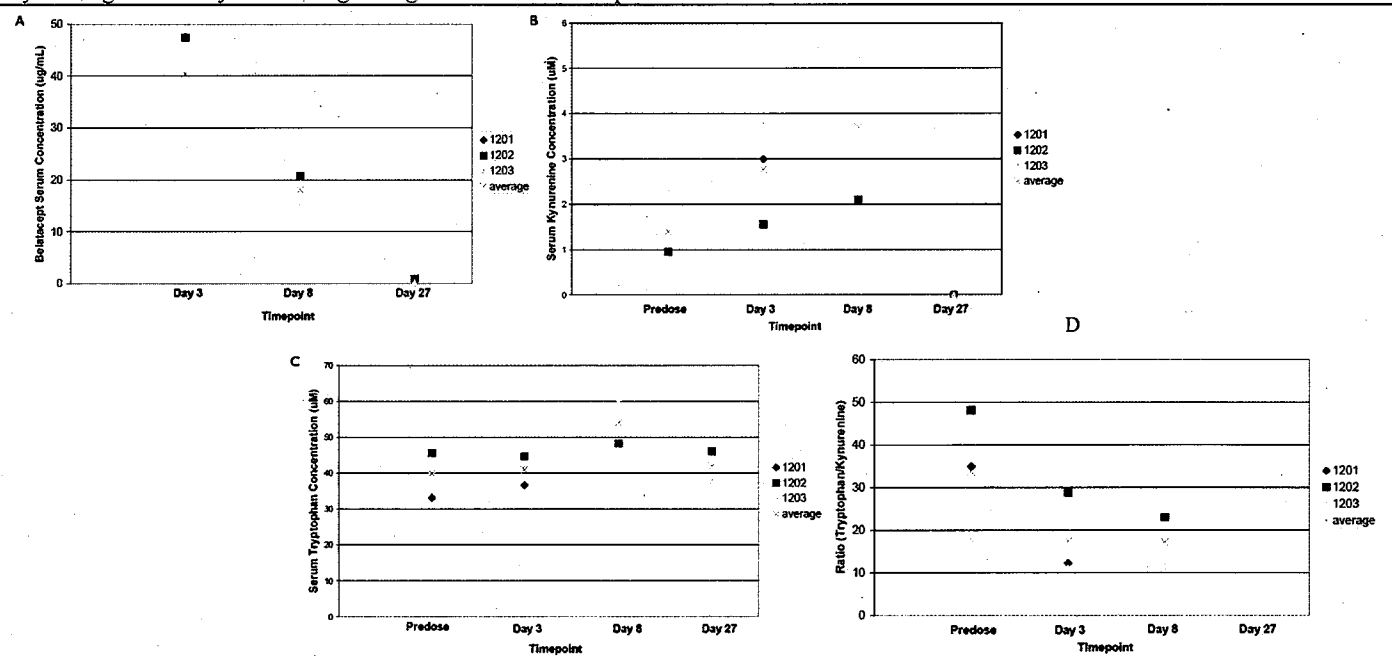
The effect of belatacept on antibody response to antigens such as keyhole limpet hemocyanin (KLH) was measured in mice, rats, and rabbits; in monkeys, the antibody responses to sheep erythrocytes (sRBC) and murine L6 antigen were measured. The studies in mice, rats and rabbits show suppression of anti-KLH antibody response. Such an effect may be dose-dependent as in one experiment an increase in anti-KLH antibody titer was reported at a low dose of 0.03 mg/kg. In primates, suppression of antibody response to sRBC and murine L6 antigen was reported. Overall, the studies show that belatacept is more active in primates and abatacept is more active in mice, rats and rabbits.

As mentioned above (see Figure 3D and 4B), belatacept suppresses anti-donor antibodies. However, such an effect appears to be reversible.

■ Effect on IDO activity

The effect of a single dose of belatacept (10 mg/kg, i.v.) on indoleamine 2,3 dioxygenase (IDO) activity in the APC was examined in 3 cynomolgus monkeys by examining the IDO dependent catalysis of the conversion of tryptophan to kynurenine; decreases in the T/K ratio suggests stimulation of IDO activity. The results showed no change in tryptophan levels and an increase in the serum kynurenine levels leading to a decrease in T/K ratio in the serum of the primates (Figure 6). The relevance of alterations in kynurenine levels or T/K ratio in blood to the site of injury or graft survival is unclear.

Figure 6: Serum concentrations of (A) belatacept, (B) kynurenine and (C) tryptophan, and (D) tryptophan/kynurenine ratio in cynomolgus monkeys following a single dose of belatacept



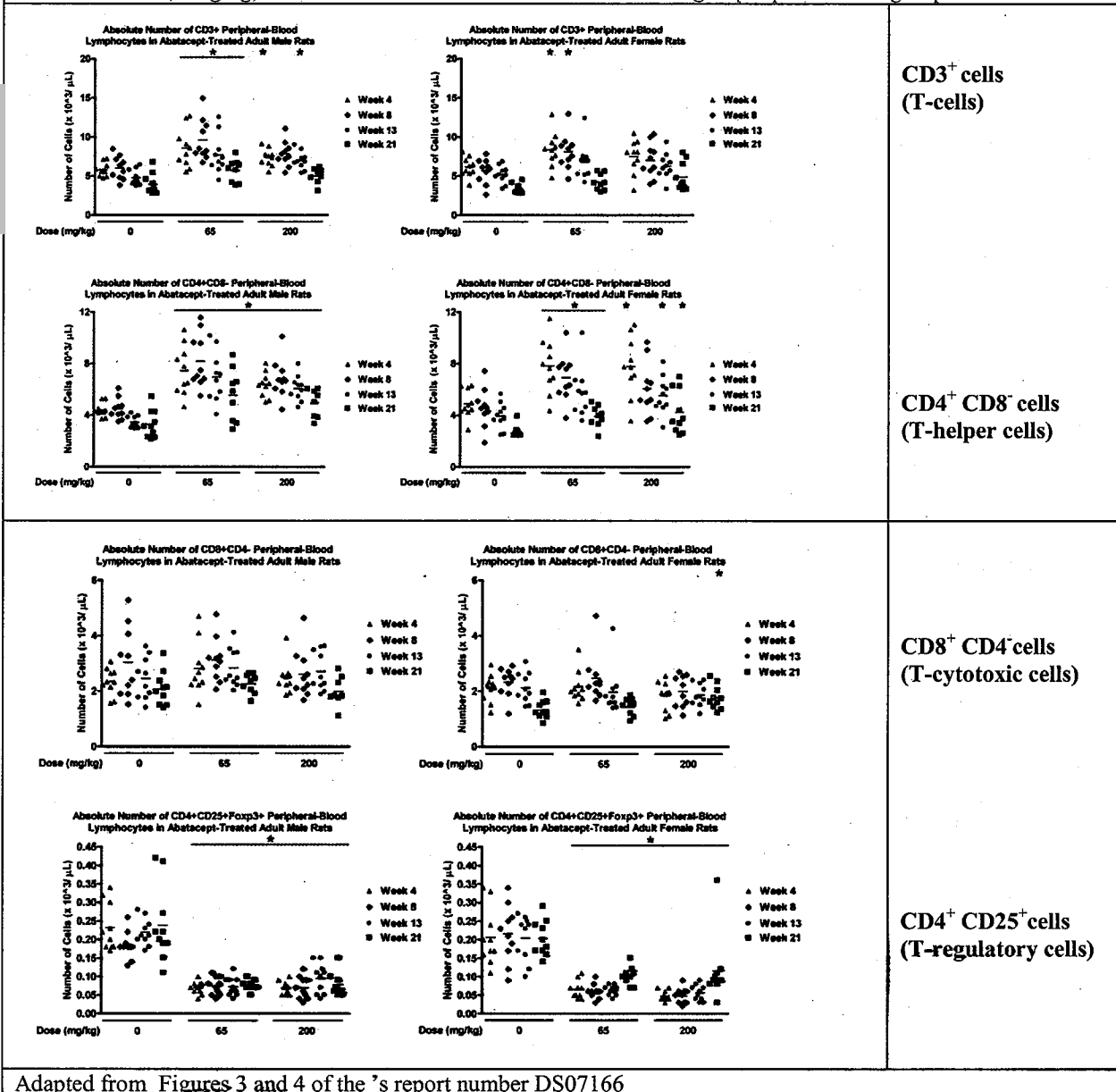
Adapted from Figures 44 and 45 of Dr Ruhland's review

■ *Effect on T-cell subsets*

In an animal model of transplantation, the applicant stated that there was no effect of belatacept on lymphocyte count or its subsets. The results were not available for review.

Administration of abatacept in adult rats, administered at 4 to 10 fold higher doses, increased absolute number of T-cells, CD4 and CD8 cells in the peripheral blood (Figure 7). This was associated with a decrease in the number of regulatory T-cells and inflammation of thyroid and pancreatic islets. The results also show a trend towards recovery of different T-cell subsets after discontinuation of treatment. Similar observations, of slightly higher magnitude, were made in juvenile rats (for details see Dr Ruhland's review). The effect of high doses of belatacept was not examined.

Figure 7: Absolute number of peripheral T-cytotoxic and T-regulatory cells at weeks 4, 8, and 13 (during treatment) and 21 (follow up) in male and female rats. An * indicates statistically significantly different from vehicle-control (0 mg/kg). The horizontal line in the data for each dose group represents the group mean.



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▪ *Effect on host resistance*

In host resistance models of *Mycobacterium tuberculosis*, *Pneumocystis jiroveci* and murine cytomegalovirus (MCMV) in mice abatacept had no effect on susceptibility to infection under the experimental conditions tested. Belatacept was not tested.

In the *M. tuberculosis* murine model, abatacept at a dose of 0.5 mg/kg had no effect on IFN- γ production.

The results from the limited studies should be interpreted with caution and do not rule out the possibility of emergence of infections in transplant recipients.

4. Clinical Microbiology/Immunology

The results of the three clinical trials (phase 3: IM103008 and IM103027; phase 2: IM103100) show that belatacept prevents acute rejection similar to comparator (CsA) and also decreased incidence of antibody mediated rejection (for details see Medical Officer's review). Some of the issues related to (1) the development of PTLT and Epstein Barr virus (EBV) serostatus, and (2) effect on immune cells and function are discussed below:

4.1. PTLT and EBV serostatus

4.1.1. PTLT

Post-transplant lymphoproliferative disorder (PTLT) is described as an abnormal proliferation of lymphocytes following transplant and includes B-cell neoplasia and hyperplasia. Most cases of PTLT occur as the result of host immune failure to control progressive infection with Epstein Barr virus (EBV), a consequence of the impaired host T-cell immune responses that normally control the proliferation of EBV-infected B-cells. PTLT typically presents as a tumor consisting of B-cells (CD20+) which also test positive for EBV. Patients who test serologically negative for EBV antibodies prior to transplant and the initiation of immunosuppression are considered to be at a higher risk for PTLT. However PTLT can occur in both EBV⁺ and EBV⁻ recipients.

4.1.2. Clinical trial findings

In the 3 clinical trials, the EBV serostatus was determined for 95% (1357 of the 1425) of the patients prior to enrollment in the clinical trial. PTLT developed in 16 patients of which 14 were in the belatacept arm and 2 in the CsA arm (Table 1). Of the 14 patients treated with belatacept who developed PTLT, 7 were EBV negative (of 96 total EBV negative patients on belatacept) and 6 were EBV positive (of 805 total EBV positive patients on belatacept) and 1 was of unknown status. EBV serostatus prior to transplant was unknown in 2 of the patients that developed PTLT (one in the Belatacept arm and 1 in the CsA arm). Of the 14 patients in the belatacept arm, 8 developed CNS PTLT. None of the 2 patients in the CsA arm developed CNS PTLT.

Based on the clinical trial results there was no association between EBV serostatus and development of CNS PTLT.

Table 1. Tests used to determine EBV status in the 15 patients who developed PTLD.

Clinical Trial - Patient ID	Treatment Arm	EBV				Manufacturer	Specificity	Sensitivity
		Donor Status	Recipient Status	Test Used	Assay Type			
103008 - 20019	CsA	+	-	VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103008 - 20060	Belatacept - LI	+	+	Captiva EBV VCA IgG	ELISA	Trinity Biotech	100%	95.5%
103008 - 20256	Belatacept - LI	+	+	VCA IgM EBNA IgG	ELISA	Zeus Scientific	89.9 - 97.5% 98 - 100%	70.8-98.9% 90-97.3%
103008 - 20039	Belatacept - MI	+	-	VCA IgG	ELISA	Wampole Laboratories	98%	98%
103008 - 20734	Belatacept - MI	+	+	LIAISON EBV IgM, LIAISON VCA IgG, LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103008 - 20548	Belatacept - MI	-	-	VCA IgG, VCA IgM	ELISA	Demeditec Diagnostics	100% 97.4%	98% 97.2%
103027 - 10029	Belatacept - LI	Unknown	-	EBNA-1 IgG	ELISA	Diamedix		
103027 - 10093	Belatacept - LI	Unknown	+	ETI-VCA-IgG, ETI-EBV-IgM reverse, ETI-EA-IgG, ETI-EBNA-IgG	ELISA	DiaSorin, Inc.	97%, 86%, 95%, 99%	98% 96% 95% 92%
103027 - 10593	Belatacept - LI	+	-	EBV-VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103027	Belatacept - LI	Unknown	+	VCA-EBV IgG/IgM	IFA	In-house	100%	100%
103027 - 10185	Belatacept - MI	+	+	LIAISON EBV IgM LIAISON VCA IgG LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103027 - 10447	Belatacept - MI	Unknown	-	Unknown	Unknown	Unknown	Unknown	Unknown
103100 - 00025	CsA	Unknown	Unknown	EBNA-1 IgG	ELISA	Diamedix	Unknown	Unknown
103100 - 00006	Belatacept - MI	Unknown	-	Unknown	Unknown	Unknown	Unknown	Unknown
103100 - 00031	Belatacept - MI	Unknown	Unknown	EBNA-1 IgG	ELISA	Diamedix	Unknown	Unknown
103100 - 00004	Belatacept - MI	Unknown	-	VCA IgG, EBNA-1 IgG	ELISA	Medac Diagnostika	97.9%, 98%	96.3%, 100%

Adapted from Table 32 of Dr Ruhland's review. ☐ represents patients that developed CNS PTLD

(Although EBV serostatus was unknown for some patients however, the test was specified. The results in Table represent information provided by the)

One of the limitations of EBV testing of samples from patients enrolled in the clinical trial is the variety of EBV serological tests employed by the different study centers involved in the clinical trials. Overall, 108 different assays were combined into 81 different panels and used in the clinical trials at 95 different laboratories. A majority of the testing was done at the site laboratories. Ninety-seven of the assays were noted as “FDA cleared” in the dataset provided by the sponsor. The sponsor provided product brochures for 31 of the assays and also provided the specificity and sensitivity of many of the assays as well. The performance of the assays was variable with some assays being less specific than others. The specificity of the IgG antibody based EBV tests in the 14 PTLD patients with known EBV status was $\geq 96\%$ (for details see Dr Ruhland’s review).

4.1.3. EBV testing

Several serological tests are available as an aid in determining whether a person has a current, past or reactivation of EBV infection. In general, EBV panels are interpreted as shown in Figure 8.

Figure 8: Antibody titers to EBV antigens distinguish between primary and resolved infection ⁵				
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Interpretive criteria for determining primary and past EBV infection				
VCA IgG	VCA IgM	EBNA IgG	EA IgG	Interpretation
-	-	-	-	No infection, past or present
-	+	-	-	Early phase of primary infection
+	+	-	-/+	Acute phase of primary infection
+	+	+	+/-	Late phase of primary infection/reactivation
+	-	+	-/+	Past infection
Adapted from Figure 46 and Table 26 of Dr. Ruhland’s review.				

To determine a current or active infection, a typical panel includes testing for IgM antibodies to EBV viral capsid antigen (VCA) or use the Paul-Bunnell heterophile antibody test. To determine past infection and convalescence, several assays are available and cleared by the FDA that test for IgG antibodies to VCA, EBV nuclear antigen (EBNA) and EBV early antigen (EA). IgG antibodies to VCA and EBNA persist for life in most people. IgG antibodies to EA may also be present, but

⁵Gulley, ML and Tang, W. (2008) Laboratory Assays for Epstein-Barr Virus-Related Disease. *J Mol Diagn.*, 10: 279-292.

this titer tends to decrease to an undetectable level within months of clearing the primary infection. Reappearance or rising titers of antibody to EA is indicative of reactivation of EBV if noted in the presence of clinical signs of disease. False-positive antibody titer results may occur on the basis of cross-reactivity with several viruses (e.g. CMV, rubella, and adenovirus) and some parasites such as *Toxoplasma gondii*.

Since the risk of developing PTLD is life-threatening and irreversible, it is recommended that EBV testing should be based on a panel of tests with high specificity that can detect IgG antibodies against VCA and EBNA and IgM antibodies against VCA. An EBV⁻ patient should not be treated with belatacept. Also, belatacept treatment should be avoided in patients with **no** IgG antibodies against VCA and EBNA but is IgM⁺ against VCA.

4.1.4. CDRH consultation

The Division consulted with the Center for Devices and Radiological Health (CDRH), Office of In Vitro Devices, regarding the appropriateness of the EBV assays. The CDRH microbiology reviewers, Mr. Peter Summers and Mr. Li Li, agreed with the Division's assessment of and recommended that a panel of tests which measure IgG antibodies against VCA and EBNA be included for testing (for details see Attachment-1). Although there is a warning in the EBV test brochures that specify that "the performance of the EBV antibody test has not been established in immunocompromised patients" it is possible that immunocompromised patients may not be able to induce immune response and therefore will be EBV⁻. EBV⁻ subjects should not be treated with belatacept.

4.2. Effect on Immune Response

4.2.1. Effect on lymphocyte subsets

The results of flow cytometric analysis in samples from a small number of patients enrolled in the phase 2 and 3 clinical trial show a transient decrease in regulatory T-cells (CD25⁺, FOXP3⁺) in patients treated with either belatacept+basiliximab or CsA+basiliximab.

Figure 9: Short term effects of belatacept (bel) and basiliximab (bas) treatment on regulatory T-cells⁶

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Short-term effects of belatacept and basiliximab treatment of FOXP3⁺ Tregs. Human PBMCs were cell surface stained using a combination of anti-CD4, anti-CD25 and anti-CD127 mAbs. Once fixed, the cells were stained additionally with anti-FOXP3 mAb. (A) This panel represents the changes of FOXP3⁺ Tregs in the whole cohort of patients before treatment and at different time points post-treatment in either the Belatacept or CNI arms. The cells are gated on CD4⁺ and percentage of CD25⁺ cells were analyzed. Data are representative of 10–22 independent individuals from either Belatacept group or CNI group, shown as Mean ± SD with both competing (black bar) and noncompeting (grey bar) anti-CD25 mAbs. (B) This panel represents the ratio of CD25⁺FOXP3⁺ versus CD25⁺FOXP3⁺ with competing anti-CD25 mAb. Each solid black triangle represents an independent individual, the solid bar represents average percentage among the 10–22 individuals. (C) This panel represents the quantitative changes in CD4⁺FOXP3⁺ cells before treatment and at different time points post-treatment in both the belatacept group and CNI groups. Each solid black diamond represents an individual, the solid bar represents the average percentage among the individuals examined. (D) This panel represents the percentage of CD4⁺CD127^{lo/-} cells in the whole cohort of patients before treatment and at different time points post-treatment. Each solid black diamond represents an independent individual, the solid bar represents the average percentage among the individuals from both belatacept and CNI groups.

Adapted from Figure 49 of Dr Ruhland's review

4.2.2. Effect on lymphocyte proliferation

Effect on lymphoproliferation was measured using an Immunknow[®] assay (Cylex Inc, Columbia, MD). The Immunknow[®] assay measures the synthesis of ATP. CD4 T-cells were isolated from a group of patients treated with belatacept and stimulated with a nonspecific mitogen, phytohemagglutinin, *in vitro*. No significant changes over time in ATP levels following belatacept administration were observed for any of the subjects. The mean ATP levels in the 8- and 4-week

⁶ Bluestone *et al.*, 2008. The effect of costimulatory and interleukin 2 receptor blockade on regulatory T-cells in renal transplantation. *Am J Transplant*, 8:2086-2096.

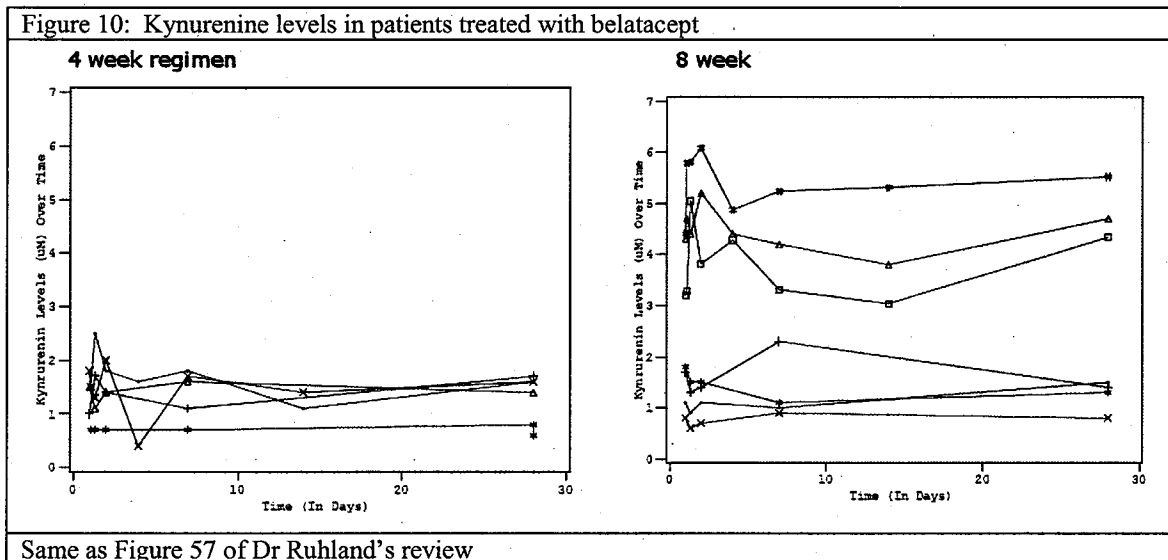
treatment groups were 282.3 and 333.0 ng/mL which is close to value proposed by the of ~280 ng/mL for normal ATP levels in transplant recipients. It appears that the baseline measurements prior to transplant and treatment were not made. There was no correlation between the alteration in ATP levels and belatacept concentrations or clinical response.

4.3.3. Effect on antibody response

A lower incidence of anti-donor HLA antibodies was reported in patients in the belatacept regimen compared to the comparator arm. There was no correlation between the presence of anti-donor HLA antibodies and antibody mediated rejection.

4.2.4. Effect on IDO activity

The effect of belatacept treatment on IDO activity was measured in a small number of patients in study IM103100. Blood samples were collected before and at different time points after belatacept dosing to determine tryptophan (the substrate of IDO) and kynurenine (a metabolite of tryptophan produced by IDO) levels. There was a wide variability in the kynurenine levels and 3 patients had high kynurenine levels at all time points than the remaining 4 patients; no consistent rise in kynurenine was observed following belatacept treatment (Figure 10). The applicant states that there was no change in tryptophan levels. Overall the results suggest no monitorable effect on IDO activity.

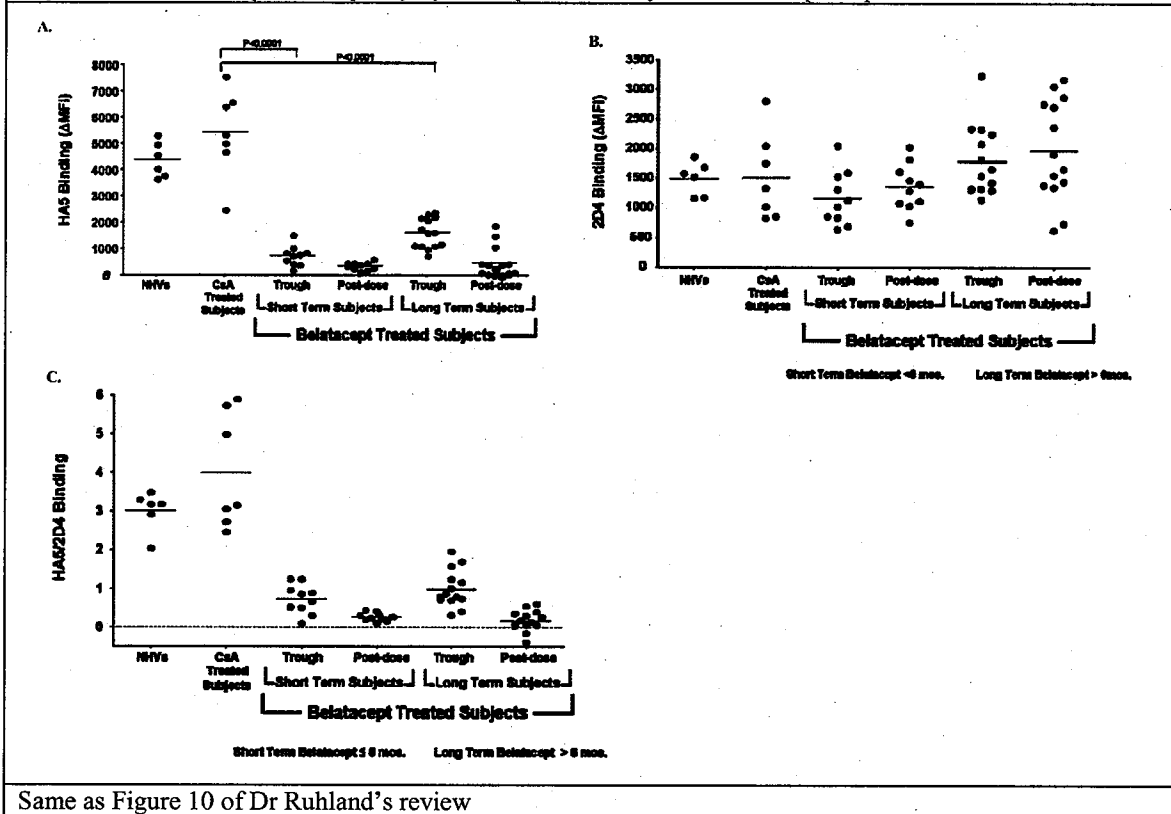


Effect on Receptor Occupancy

Receptor occupancy by belatacept was evaluated by a competitive assay using HA5.2B7 antibody that competes for CD86 receptor expressed on monocytes (based on preliminary experiments this antibody was shown to be less effective in displacing CD86 bound belatacept compared to other antibodies; for details see section 2.2). Whole blood samples were collected from patients in clinical trial IM103100 (called long term treatment; >1 year treatment with 5 mg/kg belatacept every 4 or 8 weeks) and IM103027 and IM103008 (called short term treatment; 10 mg/kg belatacept for 2 – 6 months). However, the actual time of collection of samples was not specified. The results in Figure 11 show a higher CD86 occupancy in short term patients than the longer term patients just before the next dose. The receptor occupancy was similar post dose in both short term and long term treatment groups.

BEST
POSSIBLE
COPY

Figure 11: CD86 receptor occupancy by a competition assay in renal transplant patients



5. Recommendations

The BLA is approvable pending an acceptable version of the labeling. It is recommended that labeling should specify the increased risk of developing PTLT in

- EBV negative patients, and
- Patients with IgM antibodies against VCA but **no** IgG antibodies against VCA and EBNA.

It is also recommended that certain standards be maintained for the testing of EBV serostatus. The EBV tests to be used should be highly specific for IgG antibodies against VCA and EBNA antigens. These specifications should be made in appropriate sections of the labeling so that the message goes to the prescribing physicians and the clinical microbiology laboratories responsible for EBV testing. For changes to microbiology sections of the package insert please see Dr. Ruhland's review.

Shukal Bala

Shukal Bala, Ph.D.
Microbiology/Immunology Team Leader
DSPTP

Concurrence:

Division Director/Renata Albrecht, M.D.

DSPTP

CC:

DSPTP/PM/GermainJ

Date 6/3/11

Attachment – 1

- 1. Consult Review:** Mr. Peter Summers, M.S., and Page 23
Mr. Li Li, M.S.
Microbiologist
Division of In Vitro Diagnostic Device Evaluation & Safety
Center for Devices and Radiological Health (CDRH)

This includes correspondence by email from Mr Peter Summers. The CDRH's response is italicized.

- 2. Background information prepared for consultation from CDRH** Page 25

From: Summers, Peter L
Sent: Tuesday, January 19, 2010 12:36 PM
To: Bala, Shukal
Cc: Summers, Peter L
Subject: RE: Emailing: K991459.pdf

We would like to discuss the following points:

If the Division does decide to state that only patients who are serologically positive for EBV antibodies prior to transplant be treated with belatacept, then will it be appropriate to state the following in the labeling:

- 1) A panel of tests which measure IgG antibodies against VCA and EBNA be included for testing.

We agree that evidence of VCA and EBNA IgG antibodies in the patient demonstrates that the individual has been infected with EBV. However, there probably is a warning in the labeling that states that the performance of the EBV antibody test has not been established in immunocompromized patients.

- 2) Is there any other information which should be considered or included in the labeling?

No comment without additional information.

- 3) Will any other details need to be included in the Black Box or Section 5.1?

No comment without additional information.

Hope this helps

Peter

From: Bala, Shukal
Sent: Tuesday, January 19, 2010 12:18 PM
To: Summers, Peter L
Subject: FW: Emailing: K991459.pdf

From: Bala, Shukal
Sent: Tuesday, December 29, 2009 1:30 PM
To: Summers, Peter L
Cc: Scherf, Uwe; Ruhland, Aaron; Archdeacon, Patrick; Meyer, Joette M; Albrecht, Renata
Subject: RE: Emailing: K991459.pdf

Hi Peter,

As discussed with you about a week ago, our Team has prepared some background information for you. I will block some time, in calendar for middle of January, for our discussion. Please let me know who else to invite.

Meanwhile. please let me know if you need additional information.

Thanks,
Shukal

<< File: Belatacept - CDRH.doc >>

From: Scherf, Uwe
Sent: Tuesday, October 13, 2009 2:11 PM

Nulojix® (Belatacept)

Bristol Myers Squibb

To: Bala, Shukal; Summers, Peter L
Subject: RE: Emailing: K991459.pdf

Peter,

thanks for taking care of this, Shukal, feel free to work with Peter on any additional issues.

Best,

Uwe

From: Bala, Shukal
Sent: Tuesday, October 13, 2009 1:18 PM
To: Summers, Peter L
Cc: Scherf, Uwe
Subject: RE: Emailing: K991459.pdf

Thanks Peter, this is helpful. If any other question comes up, I shall let you know.

Thanks,
Shukal

From: Summers, Peter L
Sent: Tuesday, October 13, 2009 9:16 AM
To: Bala, Shukal; Scherf, Uwe
Cc: Summers, Peter L
Subject: Emailing: K991459.pdf

<< File: K991459.pdf >> more cleared Diasorin EBV assays

2. Background information prepared for consultation from CDRH

APPEARS THIS WAY
ON ORIGINAL

PTLD:

Post-transplant lymphoproliferative disorder (PTLD) is described as an abnormal proliferation of lymphocytes following transplant and includes B-cell neoplasia and hyperplasia. The disease is thought to occur as a result of the immunosuppression used for prevention of acute rejection in transplant recipients. Treatment typically involves discontinuing or decreasing immunosuppressant drugs while chemotherapy, radiotherapy and surgery may be curative for localized lesions. Most cases of PTLD occur as the result of host immune failure to control progressive infection with Epstein Barr virus (EBV), a consequence of the impaired host T-cell immune responses that normally control the proliferation of EBV-infected B-cells. PTLD typically presents as a tumor consisting of B-cells (CD20+) which also test positive for EBV. Patients who test serologically negative for EBV antibodies prior to transplant and the initiation of immunosuppression are considered to be at a higher risk for PTLD. However PTLD can occur in both EBV+ and EBV-recipients.

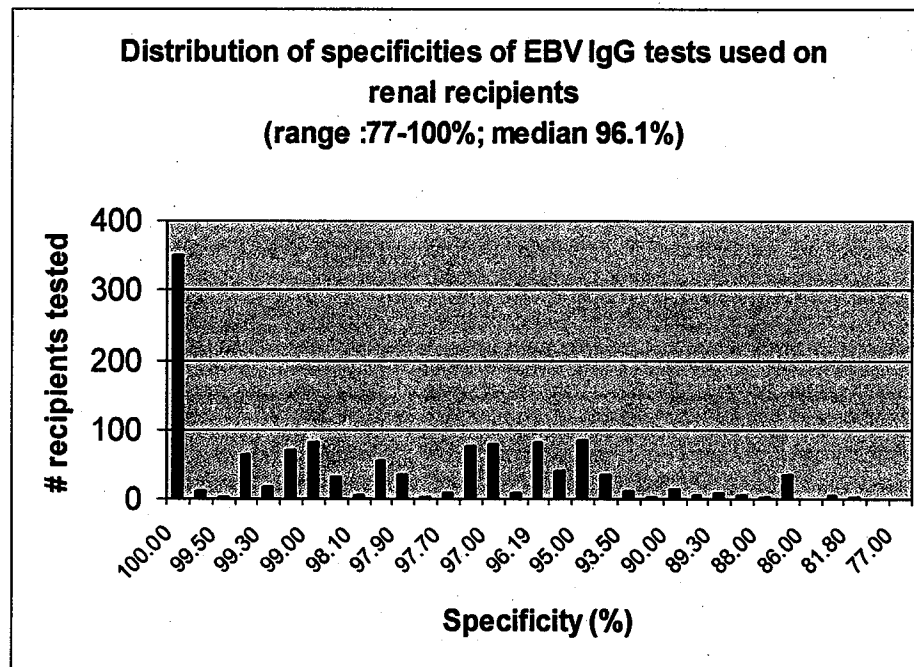
EBV tests:

Several serological tests are available as aid in determining whether a person has a current, past or reactivation of EBV infection. In general, EBV panels are interpreted as shown in Table 1. To determine a current, active infection, typical panels test for IgM antibodies to EBV viral capsid antigen (VCA) or use the Paul-Bunnell heterophile antibody test. On the other hand, there are multiple IgG antibodies which can be measured in order to determine past infection and convalescence. Assays are available which test for IgG antibodies to VCA, EBV nuclear antigen (EBNA) and EBV early antigen (EA). IgG antibodies to VCA and EBNA persist for life in most people. IgG antibodies to EA may also be present, but this titer tends to decrease to an undetectable level within months of clearing the primary infection. Reappearance or rising titers of antibody to EA is indicative of reactivation of EBV if noted in the presence of clinical signs of disease. False-positive VCA antibody titer results may occur on the basis of cross-reactivity with other herpes viruses, e.g. CMV. False positive results may also occur due to polyclonal activation of B-cells by CMV, *Toxoplasma gondii*, rubella, adenovirus or an unknown agent.

Table 1: Interpretive criteria for determining primary and past EBV infection				
VCA IgG	VCA IgM	EBNA IgG	EA IgG	Interpretation
-	-	-	-	No infection, past or present
-	+	-	-	Early phase of primary infection
+	+	-	-/+	Acute phase of primary infection
+	+	+	+/-	Late phase of primary infection/reactivation
+	-	+	-/+	Past infection

In the clinical trials of belatacept, EBV serological testing was performed on a majority of the patients enrolled (Table 2). Many different EBV serological tests were employed by the different study centers involved in the clinical trials. Overall, 108 different assays were combined into 81 different panels and used in the clinical trials at 95 different laboratories. A majority of these were site laboratories. Ninety-seven of the assays were noted as "FDA cleared" in the dataset provided by the sponsor. The sponsor provided product brochures for 31 of the assays and also provided the specificity and sensitivity of many of the assays as well. The performance of the assays was variable with some assays being less specific than others (range 77 – 100%; Figure 1).

Figure 1.



In the clinical trials, 1357 recipients were tested for EBV serostatus and 14 developed PTLD. Two additional patients also developed PTLD but their EBV status prior to transplant is unknown. Of the 14 patients treated with belatacept who developed PTLD, 7 were EBV negative (of 96 total EBV negative patients on belatacept) and 6 were EBV positive (of 805 total EBV positive patients on belatacept; Table 2) and 1 was of unknown status. For details of the clinical trial see attachment 1.

Table 2: Recipient EBV status prior to transplant and development of PTLD						
Treatment	EBV negative recipient		EBV positive recipient		Unknown Recipient Status	
	Total	Developed PTLD (% of total)	Total	Developed PTLD (% of total)	Total	Developed PTLD (% of total)
Belatacept MI	45	5 (11.1%)	404	2 (0.49%)	28	1 (3.6%)
Belatacept LI	51	2 (3.9%)	401	4* (1.0%)	20	0 (0%)
Cyclosporine A	57	1 (1.8%)	399	0 (0%)	20	1 (5%)

N.B. *Tabulated with up-to-date data as of 12-2009. MI = More intensive regimen; LI = Less intensive regimen

Table 3 summarizes the EBV serological tests used on the 16 patients who developed PTLD. The specificity of the IgG antibody based EBV tests in the 14 patients with known EBV status was $\geq 96\%$.

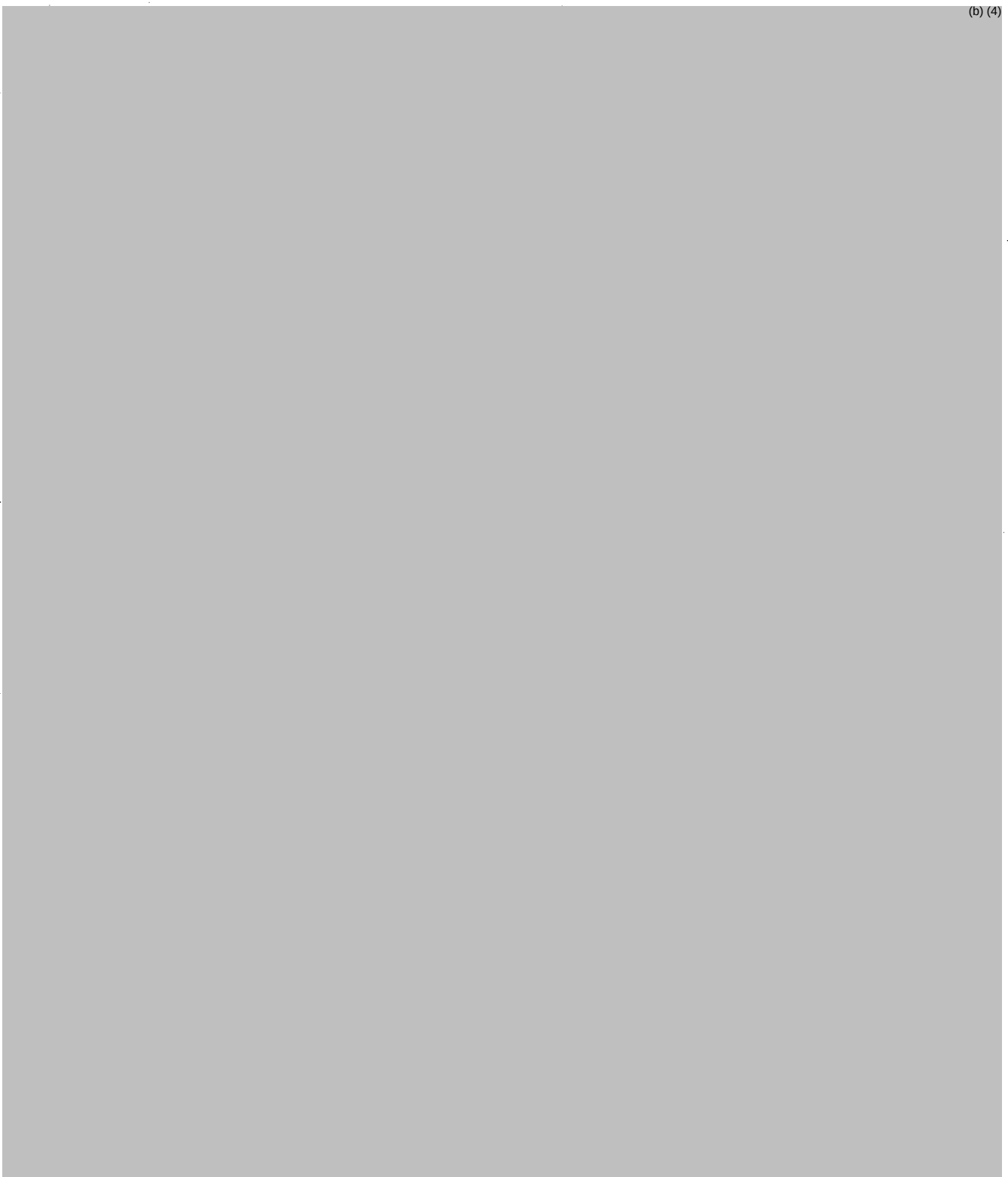
Table 3: Tests used to determine EBV status in the 15 patients who developed PTLD. (unk = unknown or not provided by)

Patient ID	Treatment Arm	Donor EBV status	Recipient EBV status	EBV test used	Assay Type	Manufacturer	Specificity	Sensitivity
103008 - 20019	CsA	+	-	VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103008 - 20060	Belatacept - LI	+	+	Captiva EBV VCA IgG	ELISA	Trinity Biotech	100%	95.5%
103008 - 20256	Belatacept - LI	+	+	VCA IgM, EBNA IgG	ELISA	Zeus Scientific	89.9-97.5%, 98-100%	70.8-98.9% 90-97.3%
103008 - 20039	Belatacept - MI	+	-	VCA IgG	ELISA	Wampole Laboratories	98%	98%
103008 - 20734	Belatacept - MI	+	+	LIAISON EBV IgM, LIAISON VCA IgG, LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103008 - 20548	Belatacept - MI	-	-	VCA IgG, VCA IgM	ELISA	Demedtec Diagnostics	100% 97.4%	98% 97.2%
103027 - 10029	Belatacept - LI	unknown	-	EBNA-1 IgG	ELISA	Diamedix	unknown	unknown
103027 - 10093	Belatacept - LI	unknown	+	ETI-VCA-IgG, ETI-EBV-IgM reverse, ETI-EA-IgG, ETI-EBNA-IgG	ELISA	DiaSorin, Inc.	97%, 86%, 95%, 99%	98% 96% 95% 92%
103027 - 10593	Belatacept - LI	+	-	EBV-VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103027 - 10044	Belatacept - LI	unknown	+	VCA-EBV IgG/IgM	Indirect fluorescent antibody	"in house method"	100%	100%
103027 - 10185	Belatacept - MI	+	+	LIAISON EBV IgM, LIAISON VCA IgG, LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103027 - 10447	Belatacept - MI	unknown	-	unknown	unknown	unknown	unknown	unknown
103100 - 00025	CsA	unknown	unknown	EBNA-1 IgG	ELISA	Diamedix	unknown	unknown
103100 - 00006	Belatacept - MI	unknown	-	unknown	unknown	unknown	unknown	unknown
103100 - 00031	Belatacept - MI	unknown	unknown	EBNA-1 IgG	ELISA	Diamedix	unknown	unknown
103100 - 00004	Belatacept - MI	unknown	-	VCA IgG, EBNA-1 IgG	ELISA	Medac Diagnostika	97.9%, 98%	96.3%, 100%

Shaded cells represent information unavailable

The sponsor is proposing to include a Black Box in the labeling with the following text:

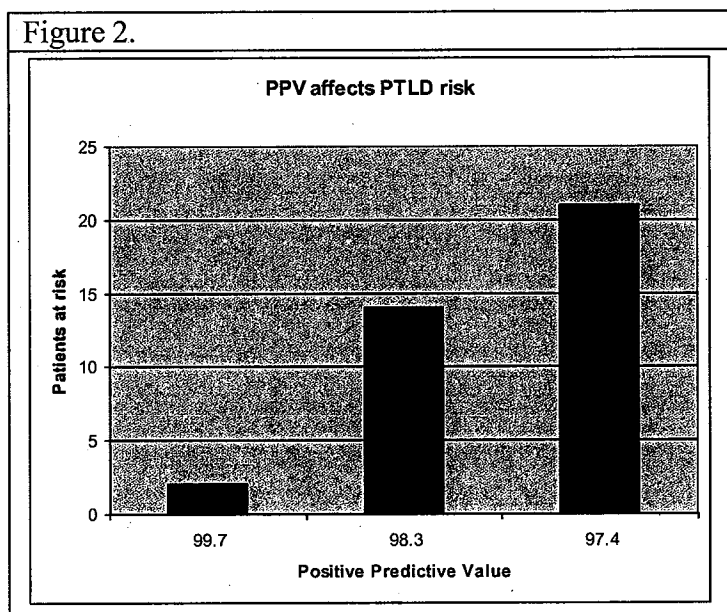
(b) (4)



The recommends that only patients who test as serologically positive for EBV antibodies prior to transplant be treated with belatacept. If this recommendation is made, it appears that the

performance of the assay used will be important. In particular, the specificity and PPV of the assay will be critical factors to consider.

As an example using the trials of belatacept which enrolled 805 EBV “positive” patients, more patients would have been put at risk of being a false positive if the specificity and hence PPV were lower (Figure 2). The PPV values of 99.7, 98.3 and 97.4 were calculated assuming 90% prevalence of EBV (+) serology and specificities of 98, 85 and 77 %, respectively. These false positive recipients, if administered belatacept, may have been at increased risk for EBV related PTLD since they are “true” negatives.



Less than 100% specificity allows the possibility of false positive results when screening EBV serology. A panel which yields positive IgG antibody results against different antigens (e.g. VCA, EBNA) may be more specific than the positive result of a single IgG antibody test for determining past infection against a single antigen. Patients who are negative for all antibodies are considered to have never been infected with EBV. For a patient to be considered as having a past infection, the patient should test positive for IgG antibodies against VCA and EBNA.

We would like to discuss the following points:

If the Division does decide to state that only patients who are serologically positive for EBV antibodies prior to transplant be treated with belatacept, then will it be appropriate to state the following in the labeling:

- 1) A panel of tests which measure IgG antibodies against VCA and EBNA be included for testing.
- 2) Is there any other information which should be considered or included in the labeling?
- 3) Will any other details need to be included in the Black Box or Section 5.1?

Appendix 1: Details on the Clinical Trial

In the clinical trials of belatacept, a higher incidence of PTLTD was found in patients in the belatacept arms when compared to the cyclosporine A (CsA) control arm (1.4% versus 0.42%). Table 1 lists a summary of PTLTD cases across clinical trials IM103008, IM103027, and IM103100. Sixteen cases of PTLTD were reported from these trials: IM103008 (n=6), IM103027 (n=6) and IM103100 (n=4). Fourteen of the PTLTD cases were in belatacept treated patients, and 2 cases were in CsA treated patients. Of the fourteen PTLTD cases occurring in the belatacept arms, 8 occurred in patients receiving the more intensive (MI) regimen and 6 occurred in patients receiving the less intensive (LI) regimen. Please note that this represents an incidence of PTLTD in 14/949 (1.5 %) and 2/476 (0.42 %) of the patients treated with belatacept (both regimens) and CsA, respectively.

Table 1. Incidence of PTLTD among all patients enrolled in clinical trials			
Clinical Trial	Treatment Arm	Total Enrolled	Patients with PTLTD (%)
IM103008	Belatacept MI	219	3 (1.4%)
	Belatacept LI	226	2 (0.9%)
	Cyclosporine A	221	1 (0.5%)
IM103027	Belatacept MI	184	2 (1.1%)
	Belatacept LI	175	4 (2.3%)
	Cyclosporine A	184	0 (0%)
IM103100	Belatacept MI	74	3 (4%)
	Belatacept LI	71	0 (0%)
	Cyclosporine A	71	1 (1.4%)
Total		1425	15 (1%)

Of the fourteen PTLTD cases in belatacept arm, 7 cases occurred in EBV negative recipients, 6 cases occurred in EBV positive recipients and 1 case was of unknown recipient EBV status. A total of 96 recipients treated with belatacept were EBV negative prior to transplant. It appears that EBV negative recipients receiving belatacept were at the highest risk of developing PTLTD (7/96 or 7.3% of EBV negative patients on belatacept compared to 1/57 or 2.3% for CsA treated patients). A comparison between belatacept and CsA can not be made for EBV positive patients who developed PTLTD because no EBV positive recipients developed PTLTD in the CsA arms. It is assumed that PTLTD occurs in EBV positive recipients receiving CsA at some rate greater than 0%, however it appears that these trials did not enroll enough patients in the CsA arms to determine this rate. As it appears now, EBV positive recipients receiving belatacept are also at risk of developing PTLTD (6/805 or 0.75% of EBV positive patients on belatacept compared to 0/399 or 0% for CsA treated patients). It should be noted that one recipient in the CsA arm and one patient in a belatacept arm were of unknown EBV status. In the case of CsA, this patient represents one of the two recipients who developed PTLTD

Table 2. Recipient EBV status prior to transplant and development of PTLD						
Treatment	EBV negative recipient			EBV positive recipient		
	Total	Developed PTLD	% of total	Total	Developed PTLD	% of total
Belatacept MI	45	5	11.1%	404	2	0.49%
Belatacept LI	51	2	3.9%	401	4	1.0%
Cyclosporine A	57	1	1.7%	399	0	0%

MICROBIOLOGY/IMMUNOLOGY REVIEW
DIVISION OF SPECIAL PATHOGEN AND TRANSPLANT PRODUCTS

BLA #: 125288
(SN-000)

REVIEWER: Aaron M. Ruhland
CORRESPONDENCE DATE : 6/30/2009
CDER RECEIPT DATE : 7/1/2009
REVIEW ASSIGN DATE : 7/8/2009
REVIEW COMPLETE DATE: 3/16/2010

SPONSOR: Bristol-Myers Squibb
P.O. Box 4000
Princeton, NJ 08543

DRUG CATEGORY: Immunosuppressant

INDICATION: Prevention of organ rejection and preservation of a functioning allograft in adult patients receiving renal transplants

DOSAGE FORM: Intravenous infusion

PRODUCT NAMES:

- a. **PROPRIETARY:** Nulojix®
- b. **NONPROPRIETARY:** Belatacept, LEA29Y, BMS-224818
- c. **CHEMICAL:** Not applicable

STRUCTURAL FORMULA:

Modified human CTLA4 (L104 to E, A29 to Y)-IgG1 fusion protein (hinge-CH2-CH3 domains)

Molecular weight: ~90 kDa
Molecular formula: Not applicable

SUPPORTING DOCUMENTS: BB-IND 9418

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1. Executive Summary

The subject of this Biological License Application (BLA) is Nulojix® (belatacept; LEA29Y) for the prevention of acute graft rejection in patients receiving a renal allograft. Belatacept was derived from abatacept, a human CTLA4-IgG1 fusion protein which binds to CD80/CD86 on antigen presenting cells (APCs). Belatacept has been modified by mutation of residues lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y) in the ligand binding region. Preclinical studies indicate that these mutations allow belatacept to bind to human CD80 and CD86 with greater avidity than abatacept. It is thought that the binding of belatacept to CD80/CD86 on APCs prevents the interaction of these costimulatory molecules with CD28 expressed on the surface of T-cells. This interaction would prevent the requisite signal 2, or costimulation, required for activation of antigen specific, naïve resting T-cells. *In vitro*, belatacept prevented proliferation of human T-cells in an allogeneic mixed lymphocyte reaction (MLR) at a lower inhibitory concentration than abatacept. In similar reactions, belatacept prevented the production of IL-2, IL-4, TNF- α , and IFN- γ . While preclinical studies suggested an effect of belatacept on the APC (IDO assays; see below), similar results were not found in the human trials at the doses tested.

In a primate model, belatacept alone or as part of an immunosuppressive regimen extended the survival of renal allografts. A combination of belatacept and sirolimus following basiliximab induction also extended pancreatic islet graft survival. The observation that patients had a lower incidence of antibody mediated rejection in clinical trials of belatacept is supported by extensive preclinical experiments showing belatacept prevents a humoral response (i.e. antibody production) to different antigens. In preclinical studies in rats treated with abatacept, the regulatory T-cell subset was diminished by as much as 70%. These findings were often accompanied by signs of autoimmune disease which typically progressed following washout. In primate studies of belatacept, similar results were not found. Moreover, in patients on long term treatment with belatacept and MPA following basiliximab induction, regulatory T-cells were not decreased and no decrease in suppressor function was noted. An increase in Foxp3⁺ T-cells in renal biopsy sections taken during episodes of acute rejection was also reported. Similar results were found in another analysis of belatacept treated patients. Immunophenotyping of blood lymphocytes revealed a decrease in CD4⁺ T-cells relative to CD8⁺ T-cells while Foxp3⁺ regulatory T-cells remained stable. A correlation of belatacept and autoimmunity in human patients has not been substantiated by clinical observations.

In clinical trials, recipients treated with belatacept had a lower incidence of testing positive for anti-donor HLA antibodies. No correlation between patients with and without episode(s) of acute rejection and anti-HLA antibodies was found. A higher incidence of post-transplant lymphoproliferative disorder (PTLD) was found in patients treated with belatacept compared to the control arm. Recipients who were serologically negative for Epstein Barr virus (EBV) antibody were the most susceptible to PTLD though it also appears that the incidence of PTLD in EBV positive patients was higher than the control arm. The applicant recommends that only patients who test as

Nulojix[®] (Belatacept)Bristol Myers Squibb

serologically positive for EBV be treated with belatacept. Many different tests were employed across the clinical trial sites and laboratories to determine EBV status. The performance of these assays was variable with some assays being less specific than others (range 77 – 100%). Less than 100% specificity allows the possibility of false positive results when screening EBV serology. In practice, false positive recipients, if administered belatacept, may be at increased risk for EBV related PTLT. It is recommended that the labeling for belatacept specifically state that practitioners and clinical laboratory technicians employ a panel of the most specific assays possible when screening recipients for EBV status to ensure a high positive predictive value. Panels which employ a panel of antibodies that determine the stage of patient EBV infection based on the presence or absence of antibodies of different isotypes and antigen specificities may allow for a more specific result and this aspect should be specified in the labeling.

Belatacept is approvable from a microbiological/immunological standpoint pending an acceptable version of the labeling.

2. Introduction and Background

The subject of this Biological License Application (BLA) is Nulojix[®] (belatacept; LEA29Y) for the prevention of acute rejection in patients receiving a renal allograft. The applicant has proposed the dose regimen of 10 mg/kg on days 1, 5, 14, 28, and month 2 and 3, then 5mg/kg every month starting at month 4.

Belatacept is a homodimeric recombinant fusion protein constructed from a mutated extracellular domain of human cytotoxic T-lymphocyte antigen-4 (CTLA-4) and the Fc portion (hinge-CH2-CH3 domains) of a human IgG1 antibody. Belatacept was derived from abatacept (Orencia[®]), a human CTLA4-IgG1 fusion protein which binds to CD80/CD86 expressed on antigen presenting cells (APCs). Belatacept has been modified by mutation of 2 amino acids [lysine 104 (L104) to glutamic acid (E) and alanine 29 (A29) to tyrosine (Y)] in the ligand binding region of abatacept. These mutations resulted in increased avidity of belatacept for its targets, CD80 and CD86. Belatacept, like abatacept, was developed to prevent costimulation of the T-cells by indirectly blocking signaling through its CD28 receptor. By binding CD80 and CD86 on the APC with the modified CTLA-4 domain of belatacept, it is thought that costimulation of the T-cells will be prevented by blocking the interaction of CD28 expressed on T-cells with the same ligands.

Abatacept is currently FDA approved for treatment of adult rheumatoid arthritis and juvenile idiopathic arthritis. Abatacept is administered every 2 weeks for up to 4 weeks followed by every 4 weeks and the dose depends on body weight (500 mg to 1000 mg). The package insert also specifies that abatacept should not be administered with TNF-antagonists.

Biology of Transplantation

T-cells are the predominant immune cell type involved in mediating acute rejection of solid organ allografts. Current immunosuppressive regimens administered following solid organ transplant are typically based upon calcineurin inhibitors (CNIs) such as tacrolimus or cyclosporine A (CsA) and noncalcineurin inhibitors such as sirolimus and mycophenolate. While the use of CNIs has significantly reduced incidence of acute rejection and lengthened graft and patient survival, the CNIs are not without deleterious side effects. Due to their mechanism of action, CNIs also affect non-target cells and long term use contributes to chronic allograft rejection, nephrotoxicity, cardiovascular pathology and metabolic disorders. It is thought that an agent which is more specifically targeted to blocking T-cell activation may continue to prevent acute rejection while decreasing the long term side effects associated with CNIs.

Calcineurin is a widely expressed protein phosphatase which, in T-cells, plays a major role in their activation through signals initiated by the T-cell receptor (TCR). While the use of CNIs has significantly reduced incidence of acute rejection and lengthened graft and patient survival, the CNIs are not without deleterious side effects. Due to their mechanism of action, CNIs also affect non-target cells and long term use contributes to chronic allograft rejection, nephrotoxicity, cardiovascular pathology and metabolic disorders. It is thought that a compound which is more specifically targeted to preventing T-cell activation may continue to prevent acute rejection while decreasing the long term side effects associated with CNIs.

Naïve, resting T-cells typically require 2 signals from APC for activation. Signal 1 is mediated by the TCR while signal 2 is based upon a second costimulatory signal. CD28, a disulfide-linked homodimeric transmembrane receptor, is a critical costimulatory receptor for T-cell activation. Both CD28 and a related molecule, CTLA-4 (cytotoxic T-lymphocyte antigen-4, CD152), bind the same ligands: CD80 (B7.1) and CD86 (B7.2). CD80/86 are predominantly expressed on APC though liver sinusoidal endothelial cells may also be induced to express CD80/86. CD28 is expressed on both resting and activated cells, whereas CTLA-4 is only detectable on activated cells.

CD28 and CTLA-4 are thought to mediate opposing effects on the T-cell. CD28 delivers an activating costimulatory signal. The CD28 signal in conjunction with TCR signaling potentiates T-cell survival, function, expression of IL-2, the IL-2 receptor and other cytokines. Signals delivered to the T-cell through CD28 ligation activate phospholipase C, p21^{ras}, phosphoinositol-3-kinase (PI3K), sphingomyelinase/ceramide, and 5-lipoxygenase. These signals regulate the activation of several transcription factors involved in expression of IL-2 and its receptor. CD28·TCR signaling results in I κ B- α phosphorylation, which causes its degradation. This releases NF- κ B and allows its translocation into the nucleus. Also, the mitogen-activated protein kinases extracellular signal-regulated kinase (ERK) and jun NH2-terminal kinase (JNK) are activated on signaling and induce c-Fos and c-Jun transcription and the subsequent formation of the AP-1 transcription factor. Other cytokines and molecules influenced by CD28 signaling include Bcl-x_L, IL-4, IL-5, IL-13, interferon- γ , GM-CSF, IL-8 and RANTES (i.e., regulated on activation, normal T-cell expressed and secreted). Engagement and signaling through the TCR in naïve T-cells in the absence of CD28 costimulation may lead to

antigen specific tolerance (hyporesponsiveness), anergy or cell death. CD28 signaling may also be important in thymic T-cell selection; some evidence suggests the engagement of CD28 in conjunction with TCR activation leads to deletion of self reactive T-cells. This has implications in the prevention of autoimmunity.

CTLA-4 is expressed transiently after T-cell activation and is more avid than CD28 for CD80 and CD86. In contrast to CD28, CTLA-4 delivers a signal which abrogates T-cell activation. Studies suggest that CTLA-4 ligation activates cellular phosphatases and inhibits signaling through the TCR (Guntermann and Alexander, 2002, *J Immunol.*, 168:4420). This mechanism allows a counter-regulatory downregulation and cessation of the T-cell response following stimulation. Mice which are genetically deficient for CTLA-4 expression do not survive and succumb to uncontrollable autoimmune disease.

CTLA-4 has ~100-fold higher avidity than CD28 for CD80/86. The difference in avidity may be due in part to evidence which suggests that the CTLA-4 homodimer has two binding sites for CD80 or CD86 whereas the CD28 homodimer is now thought to only contain a single ligand binding site (Collins *et al.*, 2002, *Immunity*, 17: 201).

Experimental evidence also suggests that CD80 has two binding sites in contrast to a single receptor binding site on CD86. These data imply that the interaction of CD80 and CTLA-4 may mediate a stronger signal than CTLA-4 and CD86. Therefore, due to less opposition for binding of CTLA-4 to CD86 because of the stronger CTLA-4:CD80 interactions, the CD28:CD86 interaction may be more stimulating to the T-cell despite CD86 having weaker affinity than CD80 for CD28 (Sansom *et al.*, 2003, *Trends Immunol.*, 24: 313). This data, combined with other observations that CD86 knockout mice are more immunocompromised than CD80 knockout mice, leads to the conclusion that the CD86:CD28 interaction is the more important stimulatory signal on the T-cell. However, this may also be due to CD86 expression being generally more abundant than that of CD80. These two mechanisms make it difficult to discern between functional differences that are due to differences in expression patterns and those that are due to differences in structures. Indeed, another study has shown that the actual signal propagated by CD28:CD80 is more stimulatory in transfected CHO cells (Manzotti *et al.*, 2006, *Eur J Immunol*, 36: 1413).

Interaction of CTLA-4 with CD80/86 expressed on the APC also initiates reverse signaling in the APC. CTLA-4 expressed on the T-cell has been shown to increase indoleamine 2,3 dioxygenase (IDO) activity in the APC (Mellor and Munn, 2004, *Nature Immunol.*, 4:762). IDO catalyzes the degradation of the essential amino acid tryptophan to N-formylkynurenine. IDO is the first and rate-limiting enzyme of tryptophan catabolism through the kynurenine pathway. Through the depletion of tryptophan, it is thought that the growth of microbes and proliferation of T-cells is hampered by IDO activity. In models of transplantation, data suggest that IDO can down-regulate the T-cell alloresponse *in vitro* and promote tolerance in murine models of pancreatic islet transplantation and allogeneic T-cell transfer (Hainz *et al.*, 2007, *Transpl Int.*, 20:118). Belatacept could potentially induce IDO expression in APC *in vivo*. However, the physiologic role of IDO in human organ transplantation has not been determined.

Nulojix® (Belatacept)

Bristol Myers Squibb

Pharmacokinetic parameters were measured after single and multiple doses of belatacept. The results in Table 1 show an increase in AUC with an increase in dose in renal transplant recipients. In Phase 2 and 3 trials, the mean belatacept C_{min} achieved in renal transplant patients were $> 20 \mu\text{g/mL}$ at Month 1 post-transplant and $> 7 \mu\text{g/mL}$ at Months 2 to 4 post-transplant and $> 4 \mu\text{g/mL}$ from Months 6 post-transplant and onwards. The half life of belatacept is about 8 to 10 days. No metabolites were identified in any of the studies (for details see clinical pharmacology review).

Table 1. Pharmacokinetic parameters (Mean [range]) in healthy subjects and renal transplant patients after 5 and 10 mg/kg intravenous infusions

Pharmacokinetic parameter	Healthy subjects (after 10 mg/kg single dose; n=15)	Renal transplant patients (after 5 mg/kg multiple doses; n=14)	Renal transplant patients (after 10mg/kg multiple doses; n=10)
Peak concentration (C_{max}) [$\mu\text{g/mL}$]	300 (190 – 492)	139 (80 – 176)	247 (161 – 340)
AUC* [$\mu\text{g}\cdot\text{h/mL}$]	26398 (18964 – 40684)	14090 (7906 – 20510)	22252 (13575 – 42144)
Terminal half-life ($t_{1/2}$) [days]	9.8 (6.4 – 15.6)	8.2 (3.1 – 11.9)	9.8 (6.1 – 15.1)
Systemic clearance	0.39 (0.25 – 0.53)	0.51 (0.33 – 0.75)	0.49 (0.23 – 0.70)
Volume of distribution (V_{ss}) [L/kg]	0.09 (0.25 – 0.53) [†]	0.12 (0.09 – 0.17)	0.11(0.067 – 0.17)

* AUC=AUC (INF) after single dose and AUC (TAU) after multiple dose, where TAU=4 weeks

† The range for this parameter appears to be a editorial error in the draft labeling

3. Preclinical/Nonclinical Microbiology

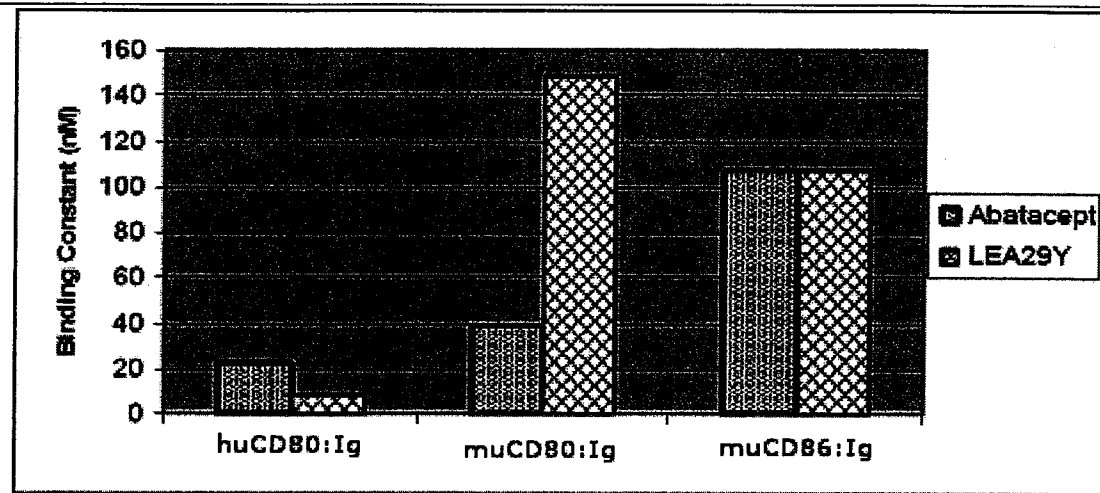
3.1 Mechanism of action

The development and characterization of belatacept (LEA29Y) has been described (Larsen *et al.*, 2005, *Am J Transplant*, 5:443). cDNA codon based mutagenesis of abatacept (CTLA-4-Ig) and expression in COS cells yielded a molecule with increased avidity for CD80/CD86 compared to abatacept (Table 2). The binding of belatacept and abatacept to immunoglobulin fusion proteins of human B7 molecules was analyzed using surface plasmon resonance detection with a ^{(b) (4)} instrument. The software used for evaluation allowed on-rate, off-rate and dissociation constant (K_D) to be calculated. The results revealed that abatacept had an approximate 2x greater affinity for human CD80 than CD86 (K_d of 13.9 nM versus 6.5 nM for CD86 and CD80 respectively). Belatacept bound with approximately 4-fold increased avidity for CD86 and 2-fold increased avidity for CD80 when compared to abatacept. The on-rates for abatacept and belatacept binding to CD80-Ig and CD86-Ig were similar, however, the off-rates differed. These data indicate that the increase in avidity (lower K_d) of belatacept is predominantly due to slower off-rates when comparing it to abatacept. Compared to abatacept, belatacept had ~2x slower off-rate from CD80-Ig and ~4x slower off-rate from CD86-Ig. Therefore the mutations differentially increased the avidity of belatacept for each ligand and by doing so actually equalized the affinity of belatacept for both ligands [K_d of 3.21 nM (289 $\mu\text{g/mL}$) and 3.66 nM (329 $\mu\text{g/mL}$) for CD86 and CD80, respectively].

Table 2. Summary of equilibrium and kinetic constants for belatacept (belatacept) compared to CTLA4Ig (abatacept)				
Analyte	Ligand	On-rate k_{on} (1/Ms)	Off-rate k_{off} (1/s)	Dissociation Constant (K_D) (nM)
Abatacept	huCD80Ig	3.44 ± 0.29	2.21 ± 0.18	6.51 ± 1.08
Belatacept	huCD80Ig	2.96 ± 0.20	1.08 ± 0.05	3.66 ± 0.41
Abatacept	huCD86Ig	5.95 ± 0.57	8.16 ± 0.52	13.9 ± 2.27
Belatacept	huCD86Ig	6.42 ± 0.40	2.06 ± 0.03	3.21 ± 0.23

Another study compared the binding properties of belatacept and abatacept to human and murine CD80/CD86 molecules (Study 930023829). The binding of belatacept and abatacept to immunoglobulin fusion proteins of human and murine CD80/86 molecules was performed using surface plasmon resonance detection with a (b) (4) instrument. No further details of the experiment were given. For human CD80 abatacept had a higher K_D (i.e. a higher rate of dissociation) than belatacept due to a higher off-rate (Table 3). For murine CD80, abatacept had a lower K_D associated with its lower off-rate (note that lower K_D means higher affinity). The binding of abatacept and belatacept to murine CD86 was similar (Figure 1). Therefore it can be stated that belatacept has a higher affinity for human CD80 than abatacept and abatacept has a higher affinity for murine CD80 than belatacept. Though the absolute values for affinity constants do not match the previous experiment, the conclusions are the same. These differences may account for the differences in activities observed when comparing the two molecules *in vitro* and *in vivo* and may depend on the cell origin or animal used (see experiments below).

Figure 1. (b) (4) analysis of the dissociation constants of abatacept and belatacept to human and murine B7 molecules

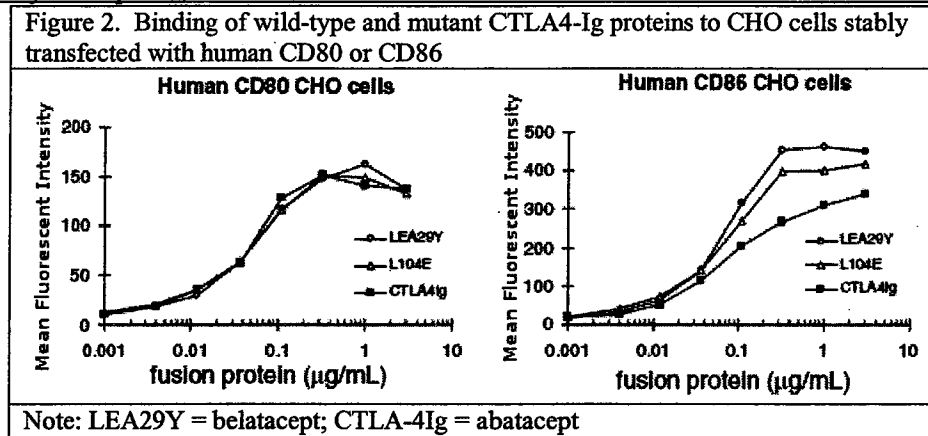


Note: LEA29Y=belatacept

Table 3. Binding kinetics of abatacept and belatacept

Analyte	Ligand	On-rate k_{on} (1/Ms)	Off-rate k_{off} (1/s)	Dissociation Constant (K_D) (nM)
Abatacept	huB7-1 Ig	3.2×10^5	6.6×10^{-3}	22
Abatacept	muB7-1 Ig	2.1×10^5	8.5×10^{-3}	39
Abatacept	muB7-2 Ig	1.4×10^5	1.4×10^{-2}	107
Belatacept	huB7-1 Ig	3.3×10^5	2.6×10^{-3}	8
Belatacept	muB7-1 Ig	2.1×10^5	3.1×10^{-2}	148
Belatacept	muB7-2 Ig	1.8×10^5	1.9×10^{-2}	107

The binding of belatacept to human CD80 or CD86 expressed on transfected Chinese hamster ovary cells (CHO) was examined and compared to abatacept and another CTLA-4 fusion protein L104E. Transfected CHO cells were incubated with each antibody and the extent of binding was determined by flow cytometry. In this model, no difference in the mean fluorescent intensity of CD80 binding was found between belatacept and abatacept at any concentration (Figure 2). For CD86, belatacept bound more avidly than abatacept as demonstrated by increased fluorescent intensity at sub-saturating concentrations.



Receptor Occupancy Assay

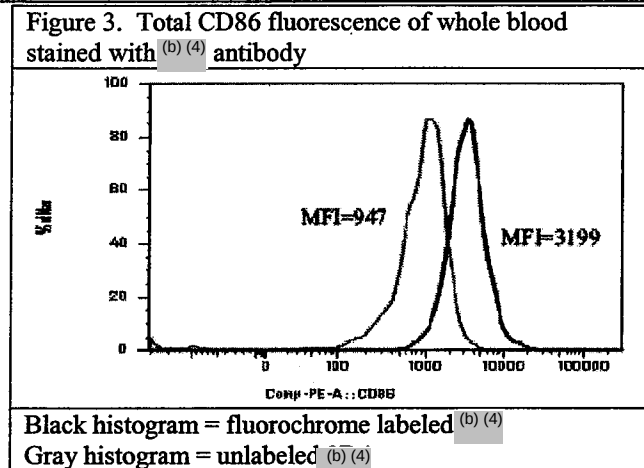
The sponsor developed an assay to measure CD86 receptor occupancy by belatacept on monocytes present in human whole blood or isolated peripheral blood mononuclear cells [PBMC; (Report # 930023833)]. The sponsor proposes to use the results of this assay to measure CD86 receptor occupancy in clinical samples. The assay is a competition assay based on the binding of another antibody FUN-1 (BD Biosciences, San Jose, CA) which competes with belatacept for CD86 expressed on monocytes. The assay did not measure CD80 receptor occupancy.

PBMCs were isolated from heparinized whole blood by centrifugation through lymphocyte separation medium (CellGro, Manassas, VA). First, cells (1×10^6) were incubated for 15 minutes at room temperature with various concentrations (0.1 – 100 µg) of belatacept in FACS buffer. After blocking Fc receptors with nonspecific IgG, monocytes were labeled with FITC conjugated anti-CD14. The cells were then incubated with a titration of FUN-1-PE (0.0625 – 2 µg). Cells were analyzed on a Becton Dickinson FACSCalibur flow cytometer and gated for monocytes based on their forward scatter/side scatter profiles and FITC (i.e. CD14) staining intensity. This population was plotted on a histogram of PE intensity (i.e. FUN-1 binding). For whole blood, which included analysis of clinical samples, a citrate-dextrose-A anticoagulant was used, some incubation times differed and erythrocytes were lysed prior to analysis.

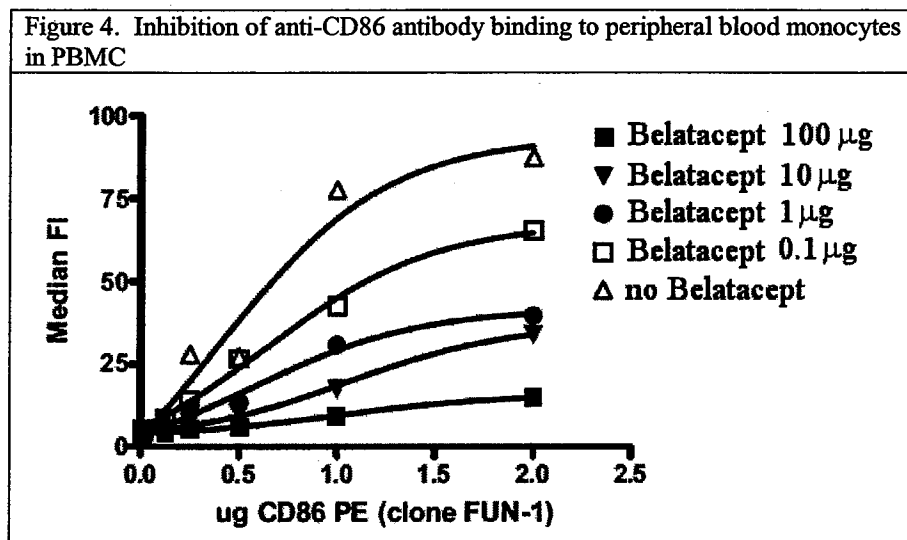
Specific binding was calculated with the following formula:

$$MFI_T - MFI_{NS} = \Delta MFI$$

MFI_T was total mean fluorescence, and MFI_{NS} was nonspecific median fluorescence of cells incubated with unlabeled anti-CD86 and ΔMFI was the relative measurement of specific binding. Figure 3 depicts a typical result of whole blood analysis with another anti-CD86 antibody, ^{(b) (4)}(BMS proprietary antibody). The ΔMFI for this sample would be 2252 arbitrary units.

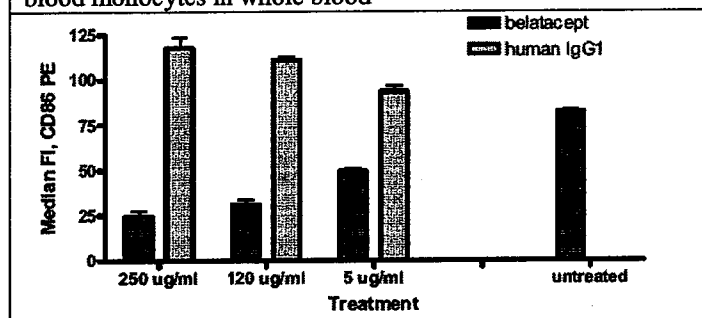


In competition assays, PE labeled FUN-1 binding was inhibited by increasing concentrations of belatacept. In the absence of belatacept, FUN-1 staining intensity saturated at concentrations of $\sim 2 \mu\text{g}$ when analyzing isolated PBMC (Figure 4). The applicant notes that when $100 \mu\text{g}$ of belatacept was added to the reaction, FUN-1 binding decreased by ~ 9 -fold and indicated near complete saturation of CD86 by belatacept at this concentration.



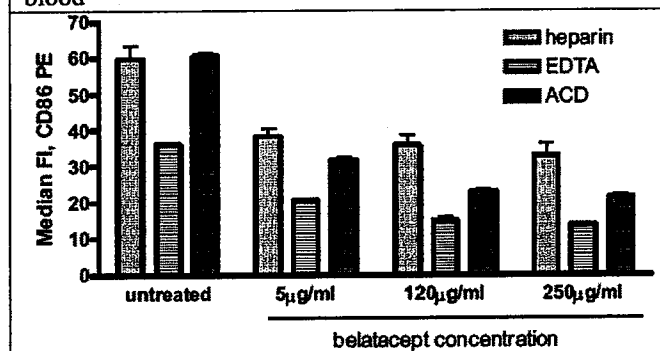
The assay was then optimized for staining whole blood to allow the assay to be more readily applied to clinical samples. In whole blood, belatacept also inhibited the binding of FUN-1 in a dose dependent manner (Figure 5). The concentration of FUN-1 used in this experiment was not specified.

Figure 5. Belatacept inhibits anti-CD86 binding to peripheral blood monocytes in whole blood



The effect of the anticoagulant used with whole blood was tested. Blood was drawn into syringes containing acid-citrate dextrose-A (ACD-A), heparin or ethylene diamine tetra-acetic acid (EDTA) and then incubated with 5 – 250 µg of belatacept for 1 hour. The results show that for untreated cells, EDTA inhibited some FUN-1 binding while heparin and ACD-A allowed similar levels of staining (Figure 6). When belatacept was added to the reaction, maximum inhibition of FUN-1 binding by belatacept was achieved with ACD-A than heparin, therefore it was chosen in further optimizing experiments.

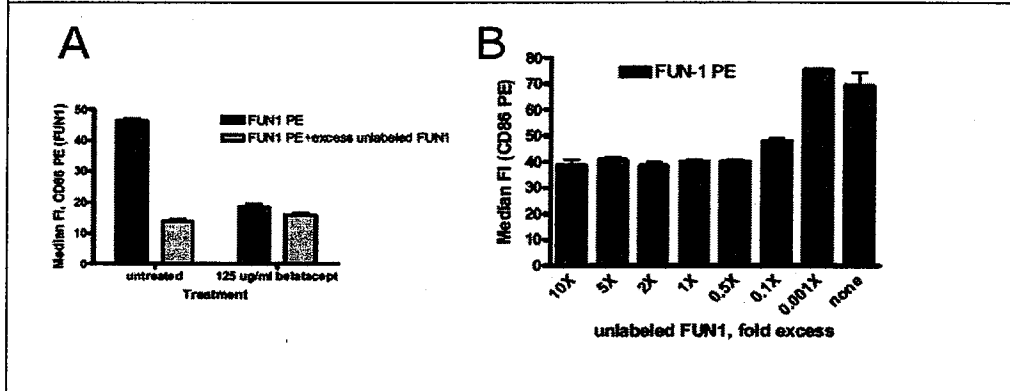
Figure 6. Effect of anticoagulant on belatacept-mediated inhibition of anti-CD86 binding to monocytes in whole blood



Since 100% inhibition of FUN-1 binding was not achieved with the highest concentrations of belatacept tested, an experiment was designed to determine the level of background fluorescence FUN-1-PE was contributing due to nonspecific binding. Unlabeled FUN-1 was added (10x excess) before incubation with PE-labeled FUN-1. The results showed that excess unlabeled FUN-1 was able to reduce the MFI to < 20 units (Figure 7A). A similar effect was seen even in the presence of 0.5x excess unlabeled FUN-1 (Figure 7B). This observation indicates that unlabeled FUN-1 binds with higher affinity than the PE-labeled antibody. The level of fluorescence that remains detected is considered background and is similar to the level achieved with 125 µg of belatacept in the presence or absence of unlabeled FUN-1. Therefore, this remainder of

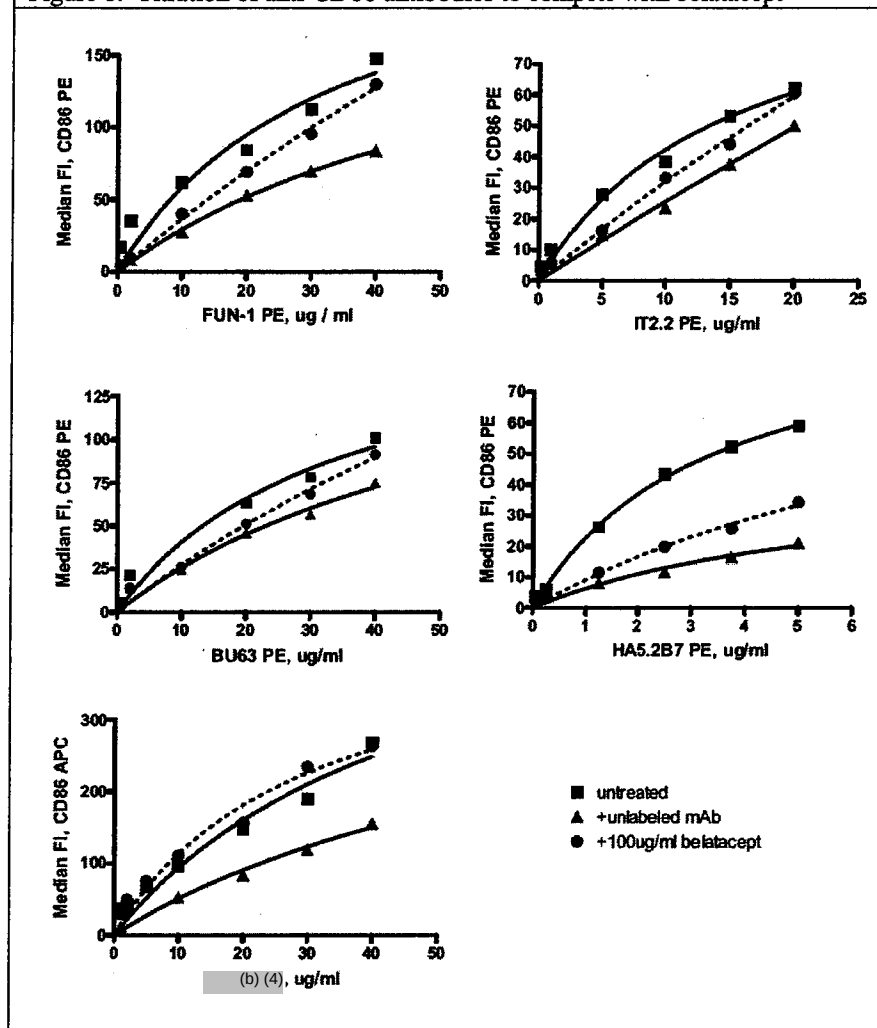
fluorescence in the presence of belatacept is considered background and it appears belatacept is completely saturating CD86 on the monocyte at these concentrations.

Figure 7. Determination of, and compensation for, background fluorescence associated with anti-CD86 antibodies



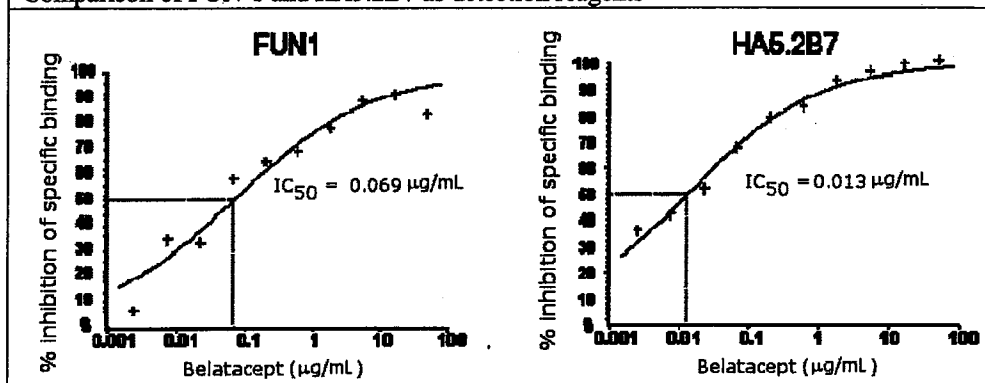
FUN-1 binding was compared to other commercially available anti-CD86 antibodies to determine which optimally competed with belatacept for binding to CD86 in whole blood. Criteria for selection of the most appropriate reagent included magnitude of signal, highest specific binding (e.g. total binding minus non-specific binding), and the lowest potential for the antibodies to displace belatacept. Following an initial incubation with 100 μ g belatacept (15 minutes/room temperature), PE-labeled IT2.2 (Bd Biosciences, San Jose, CA), HA5.2B7 (Immunotech, Quebec, Canada), BU63 [Caltag (Invitrogen), Carlsbad, CA] and (b) (4) were added to cells at various concentrations and incubated for 30 minutes on ice to determine antibody displacement of belatacept. The results showed that at the highest concentrations tested, FUN-1, IT2.2 and BU63 appeared to completely displace belatacept (Figure 8). No inhibition of binding by belatacept was observed at these concentrations. At lower concentrations, a loss in maximum MFI was seen indicating incomplete displacement of belatacept by each antibody. HA5.2B7 displaced belatacept by only 60% at the highest concentration tested (5 μ g/mL), though the applicant notes concern regarding its low staining intensity. Further experiments were conducted with FUN-1 and HA5.2B7.

Figure 8. Titration of anti-CD86 antibodies to compete with belatacept



The sensitivity of the CD86 receptor competition assay was evaluated with FUN-1 and HA5.2B7. The IC_{50} value of each antibody was compared in whole blood samples. Various concentrations of each antibody were screened against 6 donor samples for their ability to inhibit belatacept binding to CD86. Similar results were obtained across samples. A typical curve is shown in Figure 9. The mean IC_{50} value across samples was $0.85 \mu\text{g/mL}$ for FUN-1 and $0.06 \mu\text{g/mL}$ for HA5.2B7. The applicant states that the lower IC_{50} for HA5.2B7 may indicate its low ability to displace CD86-bound belatacept as noted above. HA5.2B7 was chosen for use in measuring CD86 saturation of clinical samples because of its lesser belatacept displacement, great assay sensitivity, and better correlation to inhibitory activity.

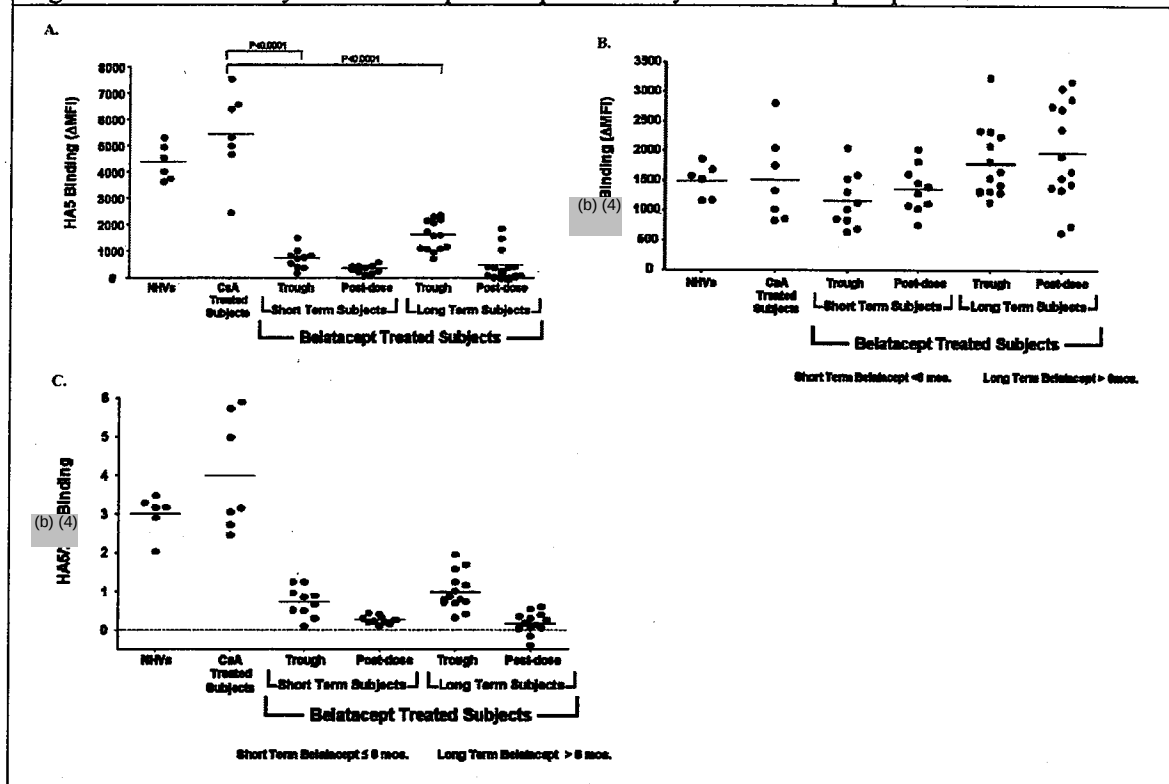
Figure 9. *In vitro* whole blood CD86 receptor competition assay sensitivity to belatacept: Comparison of FUN-1 and HA5.2B7 as detection reagents



Testing of whole blood by receptor occupancy assay

Whole blood from renal transplant patients in the clinical trials were tested by the receptor occupancy assay. For samples from study IM103100, the patients were considered long term (>1 year post transplant), and receiving 5 mg/kg belatacept every 4 or 8 weeks. For studies IM103027 and IM103008, subjects were considered short term and had been receiving 10 mg/kg belatacept for 2 – 6 months. The exact time points at which the blood samples were collected after transplantation or after initiation of the first dose were not specified. Blood was obtained prior to and after the belatacept dose was given and assayed with HA5.2B7. Levels of free CD86 on the surface of monocytes before and after belatacept infusion were compared to patients receiving CsA. Cells were also stained with (b) (4) to assess total CD86 levels before and after infusion. The results show that postdose in both short term and long term subjects, CD86 was almost completely saturated as shown by a lack of HA5 binding (Figure 10A). Short term patients had higher CD86 occupancy by belatacept at trough, i.e., just before the next dose, than the longer term patients (Figure 10 A and B). This difference may be reflective of higher mean CD86 expression by long term subjects in general as shown by (b) (4) staining (Figure 10B). Indeed the difference between short and long term occupancy at trough becomes more similar if HA5 staining is normalized to (b) (4) staining (Figure 10C). Extrapolation estimated that patients receiving 10 mg/kg belatacept had ~82% of CD86 receptors saturated at trough and ~94% saturated following the dose. Patients receiving 5 mg/kg belatacept had ~75% and ~96% CD86 receptor saturation at trough and following belatacept administration. Although the applicant states the assay appears useful for clinical purposes, a correlation of receptor occupancy to pharmacokinetic parameters or clinical outcome such as episodes of acute rejection was not attempted by the applicant.

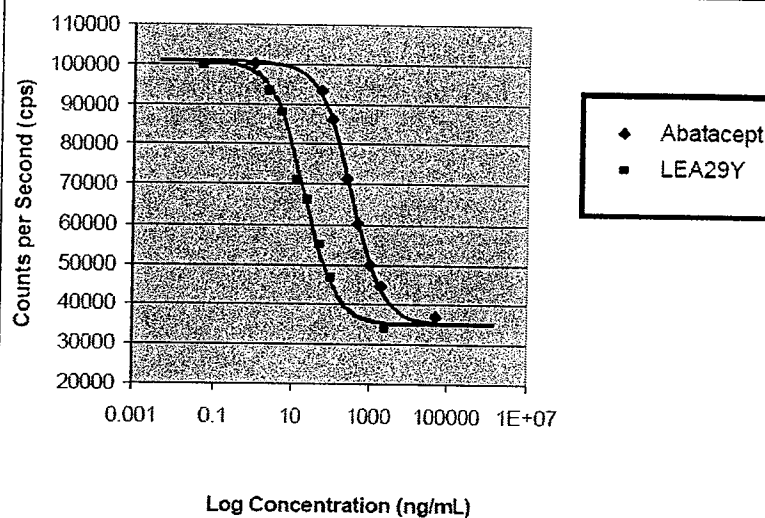
Figure 10. Clinical utility of CD86 receptor competition assay in renal transplant patients



3.2 Activity *in vitro*

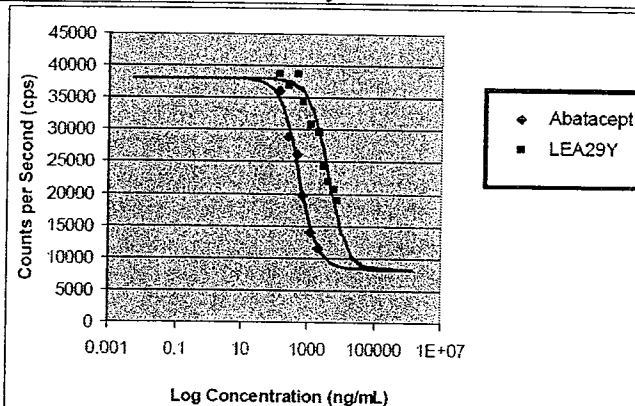
An experiment compared the effect of belatacept and abatacept on stimulation of a human T-cell line transfected with a plasmid constructed with the luciferase gene under the control of the IL-2 promoter (Study 930023829). Jurkat T-cells were incubated with abatacept or belatacept with simultaneous stimulation with anti-CD3 antibody and co-culture with the CD80/CD86 expressing cell line, Daudi B-cells. No other details of the experiment were given. The results showed that belatacept (LEA29Y) was ~16x more potent against human cells than abatacept (Figure 11). The EC₅₀ for belatacept and abatacept were 20 and 310 ng/mL respectively.

Figure 11. Comparison of belatacept and abatacept in a human T-cell costimulation assay



Another experiment compared the effect of belatacept to abatacept on proliferation of stimulated murine splenic cells. The cells were stimulated with anti-murine CD3 and the extent of proliferation was measured by incorporation of ^3H -thymidine. The results showed that abatacept was ~7x more potent than belatacept (Figure 12). The EC_{50} values obtained for belatacept and abatacept were 3,500 and 480 ng/mL, respectively.

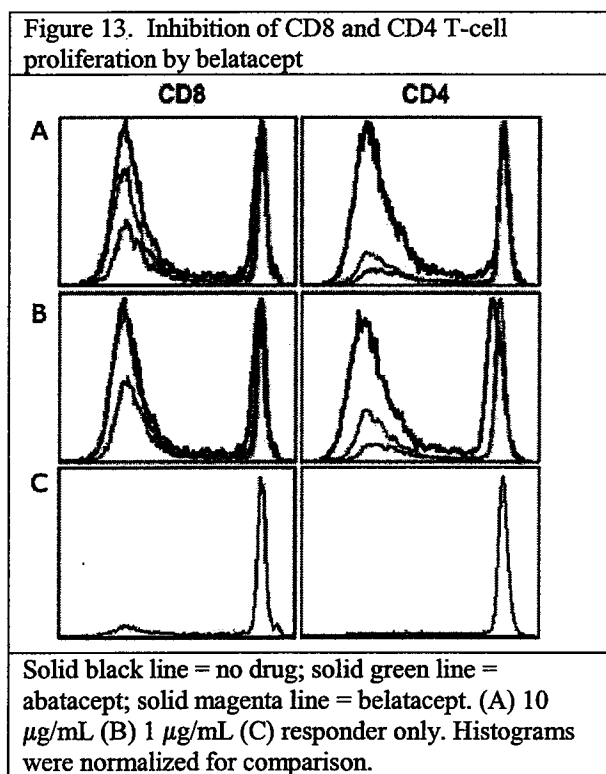
Figure 12. Comparison of belatacept and abatacept in a murine T-cell stimulation assay



The results of the human T-cell stimulation assay were compared to those obtained with murine splenic T-cells in a proliferation assay. By comparing the EC_{50} values and setting the activity of abatacept to 100%, the relative activity of belatacept can be calculated for human and murine cells. As the data above shows, abatacept is more active than belatacept against murine cells and belatacept is more active against human cells (Table 4).

Table 4. Relative activity of belatacept in human and murine cell based <i>in vitro</i> bioassays		
	Human Cell Assay (%)	Murine Cell Assay (%)
Abatacept	100	100
Belatacept	1600	14

The ability of belatacept to inhibit CD8⁺ or CD4⁺ T-cell proliferation was determined (Larsen *et al.*, 2005, *Am J Transplant*, 5:443). Human CD8⁺ or CD4⁺ T-cells were purified from PBMC using magnetic bead separation and labeled with carboxyfluorescein succinimidyl ester (CFSE). CFSE is proportionally diluted into all subsequent daughter cells upon each successive cell division and can be quantified with flow cytometry. CD4 or CD8 T-cells were incubated alone (Figure 13-C) or in combination with mature allogeneic dendritic cells as stimulators and various concentrations of belatacept or abatacept. The cell combinations were cultured for 5 days, harvested and stained with either anti-CD4 or anti-CD8 and analyzed by flow cytometry. The results show that both CD4 and CD8 T-cells underwent proliferation when stimulated with activated allogeneic dendritic cells (heavy black line, Figures 13-A and B). When cultures contained either belatacept or abatacept proliferation was diminished. T-cells cultured in the absence of allogeneic stimulation (i.e. responder cells only) failed to proliferate (Figure 13-C). Belatacept was more active than abatacept and almost completely inhibited CD4⁺ proliferation. Abatacept had little effect on CD8⁺ proliferation, while belatacept significantly inhibited the proliferation of CD8⁺ T cells; however, a residual subpopulation remained resistant to blockade with belatacept (Figures 13-A and B).



The applicant has submitted the results of experiments which compared the *in vitro* activity of belatacept to abatacept by measuring the ability of T-cells to respond to stimulation in the presence of either fusion protein (Study 930009365 v2.0). In the first experiment, isolated CD4⁺ T-cells from human peripheral blood were incubated with CHO cells expressing either CD80 or CD86. The T-cell to CHO cell ratio was 10:1 (9.0×10^4 and 9.0×10^3 cell/well, respectively). Phorbol-myristate acetate (PMA; 1ng/mL) and various concentrations of belatacept and abatacept were then added. After 3 days of incubation, 1 μ Ci of [³H]-thymidine was added to each well and the cells were incubated for an additional 7 hours. The results show that belatacept inhibited human CD4⁺ T-cell proliferation more so than abatacept. For CD80 costimulation, the EC₅₀ for belatacept was 100 ng/mL and for abatacept 500 ng/mL (Figure 14). For CD86 costimulation, the EC₅₀ values were 250 ng/mL and 320 ng/mL for belatacept and abatacept, respectively (Figure 15). The data suggest that belatacept is more efficient at blocking CD80 costimulation than CD86 costimulation. However, no data were provided which compared the level of CD80 and CD86 expression on the CHO cells. Therefore no firm conclusion can be made whether this data indicates increased avidity of belatacept for CD80, differences in expression of CD80 and CD86, or if the data represent a differential biological effect such as CD86 mediating a stronger, more proliferative signal than CD80 in naïve CD4 T-cells and thus requiring a higher level of blockade than CD80 to achieve the same biological effect. Though results have often been conflicting, some evidence suggests that CD86 ligation mediates a stronger proliferative signal in the CD4 T-cell (Lang *et al.*, 2002, *J Immunol*, 168:3786). These results further confirm that belatacept is more active than abatacept in blocking human T-cell costimulation.

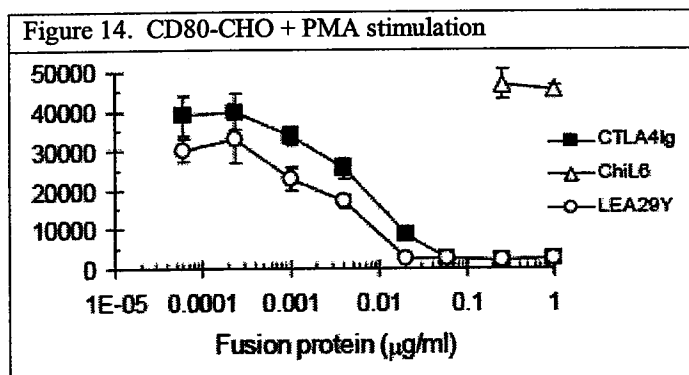
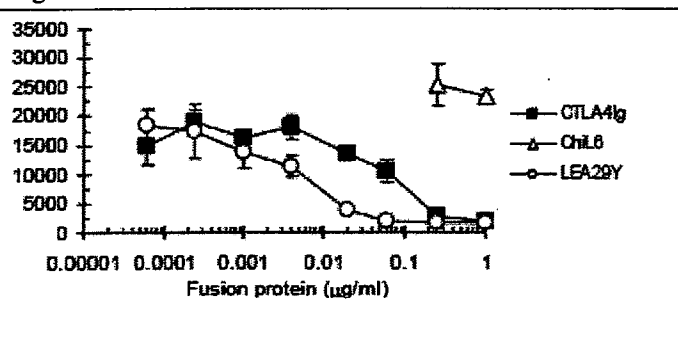
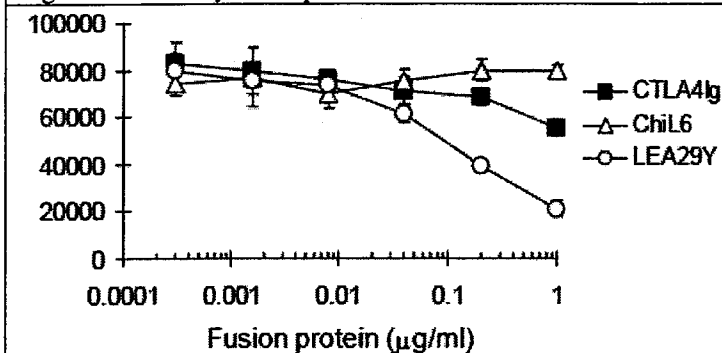


Figure 15. CD86-CHO + PMA stimulation

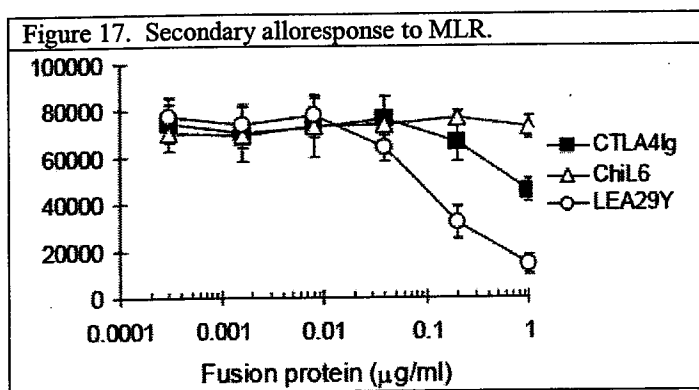


Another experiment examined the effect of belatacept on T-cell proliferation in primary and secondary allogeneic mixed lymphocyte reactions (MLRs). An irradiated human B-cell line (PM-LCL; 8×10^3 /well) was used as the antigen presenting cell line and was incubated with CD4⁺ T-cells (3×10^4 /well) isolated from human blood. PM-LCL cells are an EBV transformed cell line that equally express CD80 and CD86. Various concentrations of abatacept or belatacept were added to each well and the plates were incubated for 6 days at which time 1 µCi of [³H]-thymidine was added to each well and incubated for an additional 7 hours. The results showed that belatacept was more effective in reducing the allogeneic response than abatacept (Figure 16). The EC₅₀ values were 0.18 µg/mL and > 1.0 µg/mL for belatacept and abatacept, respectively.

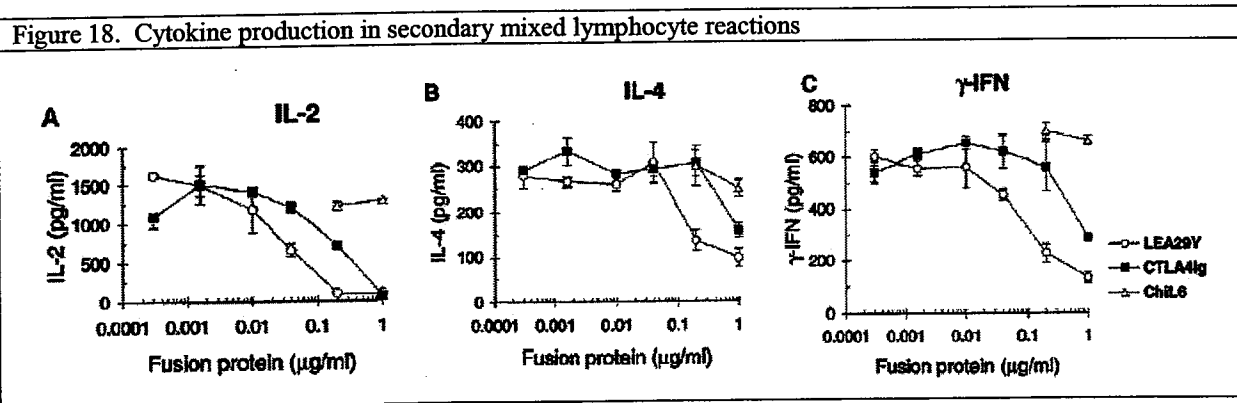
Figure 16. Primary alloresponse to MLR



A secondary response was measured by purifying seven-day primary allo-stimulated T-cells with lymphocyte separation medium (ICN, Aurora, OH). The cells were rested for 24 hours before the addition of fresh LCL-PM cells in the presence of various concentrations of belatacept and abatacept. After 3 days, the cells were pulsed and harvested as described for the primary reaction. The results are similar to the primary MLR and show that belatacept was more effective than abatacept in preventing this reaction (Figure 17). The EC₅₀ values were 0.20 µg/mL and > 1.0 µg/mL for belatacept and abatacept, respectively. These results suggest that the T-cells from the primary MLR are unresponsive in the secondary MLR when belatacept or abatacept is present. The results do not indicate that the T-cells became anergic to the alloantigens presented during the primary MLR since they remain reactive in the secondary MLR similar to control (ChIL6) at low concentrations (i.e. ≤ 0.01 µg/mL).



Supernatants from the primary and secondary MLR reactions were assayed for cytokine production. Supernatants were collected 24 hours after the addition of the allogeneic stimulator cells. An enzyme linked immunosorbent assay (ELISA) was performed to determine concentrations of IL-2, IL-4 and IFN- γ in the supernatants. The applicant states that only IL-2 was produced in the primary MLR and belatacept was ~6x more active than abatacept in preventing IL-2 production. The EC₅₀ values for this effect were 0.04 µg/mL and 0.25 µg/mL for belatacept and abatacept, respectively. Belatacept was more active than abatacept in preventing production of IL-2, IL-4 and IFN- γ after secondary stimulation (Figure 18). The belatacept EC₅₀ values for inhibition of IL-2, IL-4 and IFN- γ secretion were 0.03 µg/mL, 0.20 µg/mL, and 0.20 µg/mL, respectively; abatacept EC₅₀ values were 1.0 µg/mL, 0.14 and 1.0 µg/mL, respectively. Overall, the data suggest that the concentration of belatacept required for inhibiting stimulation and cytokine production in response to alloantigen was lesser than for abatacept.

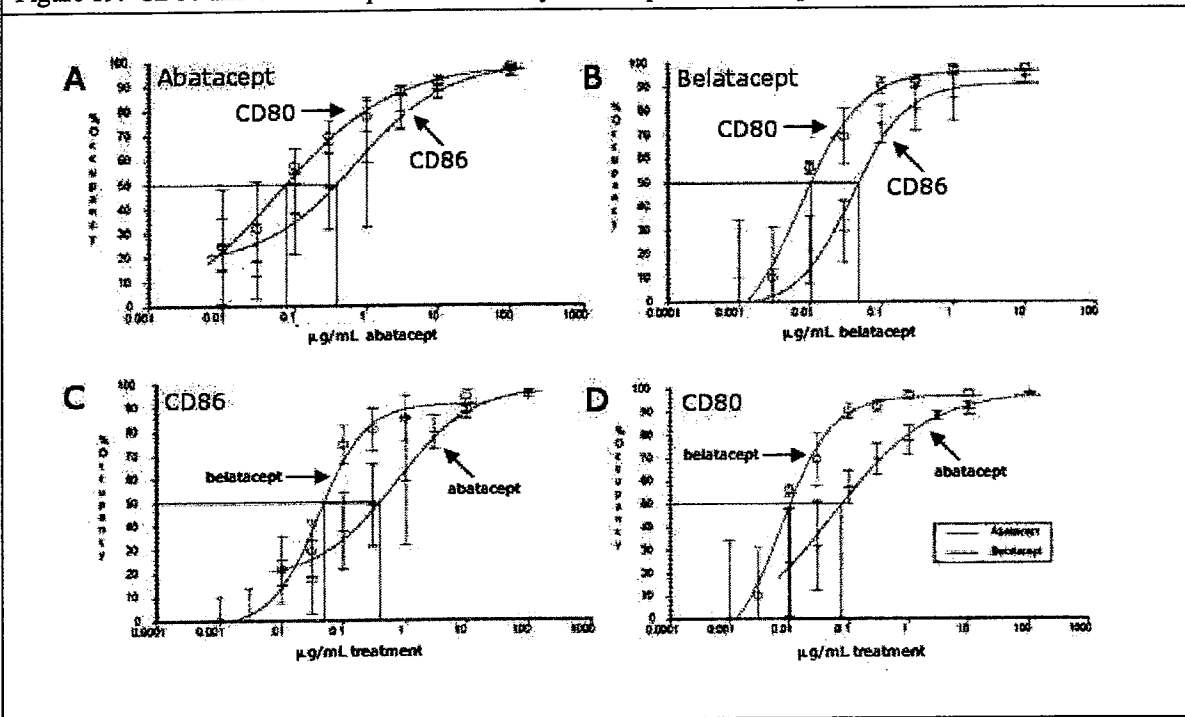


A study examined the extent of CD80/86 receptor saturation and its effect on inhibition of an MLR (Study 930028781). To determine the extent of receptor saturation by different concentrations of belatacept and abatacept, a receptor competition assay was used with cultured dendritic cells. T-cells were isolated by sheep red blood cell (SRBC) rosetting of human PBMC. Briefly, T-cells which rosette SRBC were separated from rosette negative cells by removing the non-rosetting layer at the top of the SRBC layer. The SRBC were then lysed and the resulting free T-cells were washed and resuspended in

cell culture medium (RPMI-10% fetal bovine serum). To purify monocyte derived dendritic cells, the SRBC-rosette negative fraction was washed and plated onto tissue culture plastic. After allowing the progenitor cells to adhere, the non-adherent cells were removed by decanting. Granulocyte-macrophage colony stimulating factor ($1 \mu\text{g/mL}$) and IL-4 (500 ng/mL) was added to the wells and replaced every 3 days for monocyte derived dendritic cells to differentiate. On the 6th day, LPS ($6 \mu\text{g}$) was added to each well. The dendritic cells were then used on day 7.

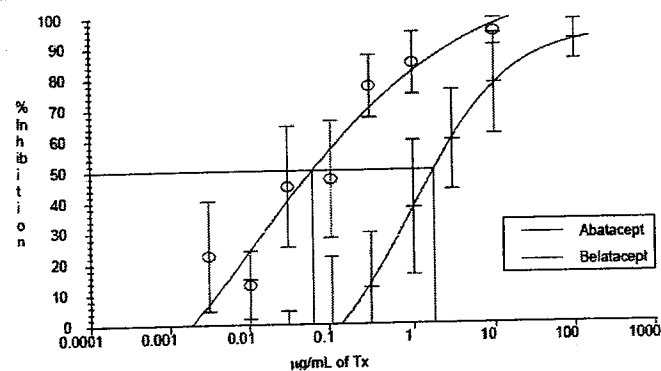
The concentration of belatacept or abatacept required to saturate CD80 or CD86 was then determined. For the CD86 receptor assay, the dendritic cells were incubated with murine IgG to block Fc receptors. To positively identify dendritic cells, the cells were incubated with anti-human HLA-DR antibody. A competition assay employing various concentrations of abatacept or belatacept against labeled anti-CD80 or anti-CD86 antibody was utilized. The results showed that the concentration of abatacept and belatacept required for 50% receptor saturation of CD86 was ~5x higher than for CD80 (belatacept $\text{EC}_{50} = 0.048 \mu\text{g/mL}$ for CD86 versus $0.010 \mu\text{g/mL}$ for CD80 and abatacept $\text{EC}_{50} = 0.387 \mu\text{g/mL}$ for CD86 and $0.076 \mu\text{g/mL}$ for CD80) (Figures 19-A and B). Additionally, the belatacept EC_{50} for each receptor was ~7-8x lower than the abatacept EC_{50} (Figures 19-C and D). These results mirror the above observations regarding the EC_{50} of belatacept for CD80 versus CD86 and may reflect the differential levels of CD80 and CD86 expression on the dendritic cell. This may be particularly evident in this model since LPS is known to strongly induce the expression of CD86 on dendritic cells (Saito *et al.*, 2006, *J Clin Exp Hematopathol*, 46: 31). Saturation of the receptors by belatacept occurred at approximately $0.1 \mu\text{g/mL}$ and $1 \mu\text{g/mL}$ for CD80 and CD86, respectively. While this data may be useful for estimating peripheral concentrations of belatacept needed to saturate activated dendritic cells, similar results obtained with peripheral blood monocytes may have been more informative to relate blood concentrations of belatacept to receptor occupancy.

Figure 19. CD80 and CD86 receptor saturation by belatacept and abatacept



An allogeneic reaction was set up as follows: The purified T-cells (responders; 1×10^6) from one human volunteer were cultured with the monocyte derived dendritic cells (stimulators; 1×10^5) from another volunteer in the presence of various concentrations of abatacept or belatacept. The cells were incubated for 72 hours and supernatants were analyzed for cytokine content using a multiplex Beadlyte Human Multi-cytokine Flex kit for IFN- γ , IL-2 and TNF- α (Upstate Biologicals, New York). Belatacept almost completely inhibited production of IFN- γ (Figure 20). An approximate 30-fold difference was noticed in IC_{50} values when comparing abatacept to belatacept for inhibition of IFN- γ production ($IC_{50} = 1.73 \mu\text{g/mL}$ and $0.060 \mu\text{g/mL}$ for abatacept and belatacept, respectively).

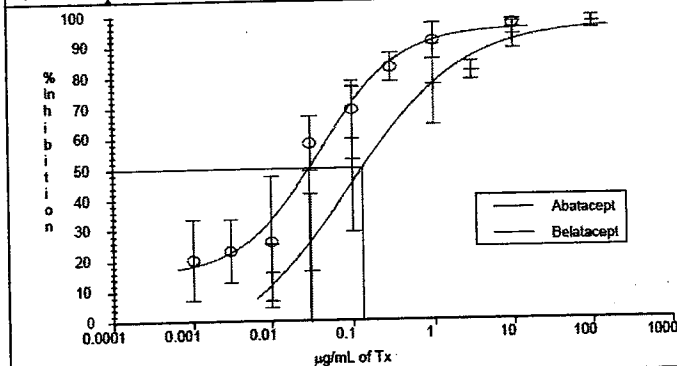
Figure 20. Inhibition of IFN- γ by abatacept and belatacept



IC₅₀ values: Abatacept = 1.73 $\mu\text{g/mL}$, belatacept = 0.060 $\mu\text{g/mL}$,
 IFN- γ Concentration range 7.65 – 4275 pg/mL

A similar trend was observed for IL-2 production (Figure 21). A 4.5-fold difference in the IC₅₀ values for inhibition of IL-2 production was noted (IC₅₀ = 0.133 $\mu\text{g/mL}$ and 0.029 $\mu\text{g/mL}$ for abatacept and belatacept, respectively).

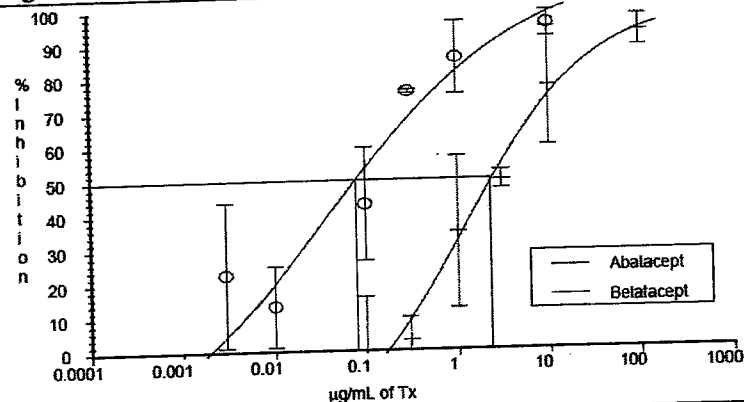
Figure 21. Inhibition of IL-2 production by abatacept and belatacept



IC₅₀ values: Abatacept = 0.133 $\mu\text{g/mL}$, belatacept = 0.029 $\mu\text{g/mL}$; IL-2 concentration range 8.91 – 576 pg/mL

An approximate 21-fold difference in IC₅₀ values was observed for TNF- α production (Figure 22; IC₅₀=3.44 $\mu\text{g/mL}$ and 0.16 $\mu\text{g/mL}$ for abatacept and belatacept, respectively).

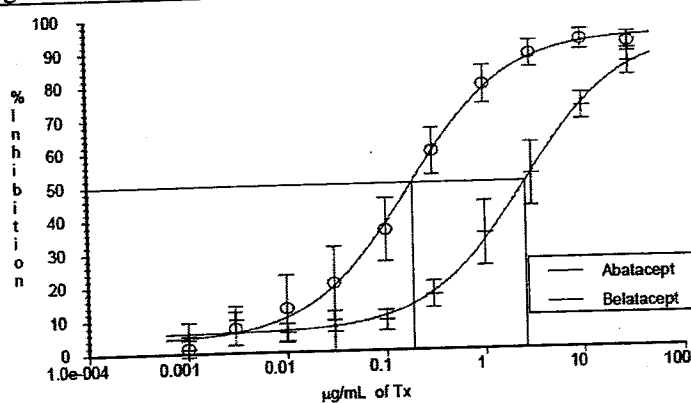
Figure 22. Inhibition of TNF- α production by abatacept and belatacept



IC₅₀ values: Abatacept = 3.44 μ g/mL, belatacept = 0.16 μ g/mL; TNF α concentration range : 4.53 – 303 pg/mL

The applicant also measured T-cell proliferation by ³H-thymidine incorporation (Figure 23). No other details on methodology for this experiment were included in the study report. The results show an ~14-fold difference in the IC₅₀ values with belatacept being more active at inhibiting proliferation in this reaction (IC₅₀ = 2.62 μ g/mL and 0.185 μ g/mL for abatacept and belatacept, respectively).

Figure 23. Inhibition of proliferation by abatacept and belatacept

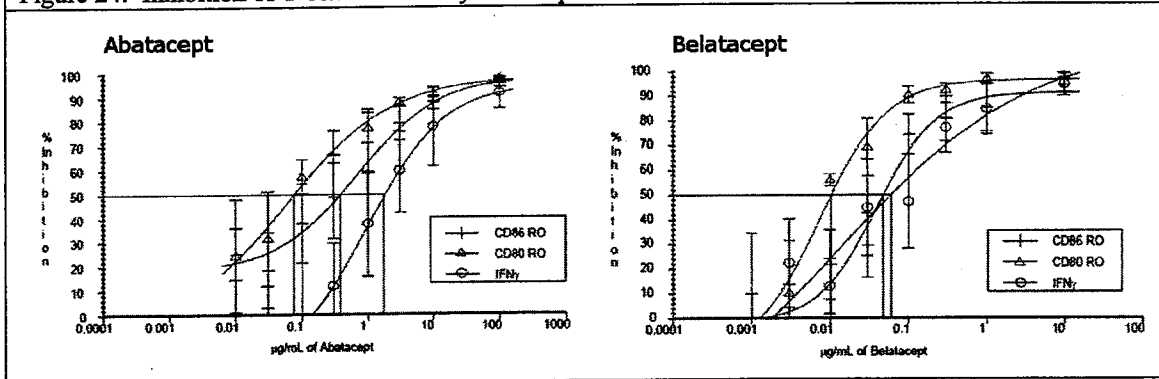


This experiment was repeated with different stimulator:responder pairings and similar results were obtained for each experiment (Table 5).

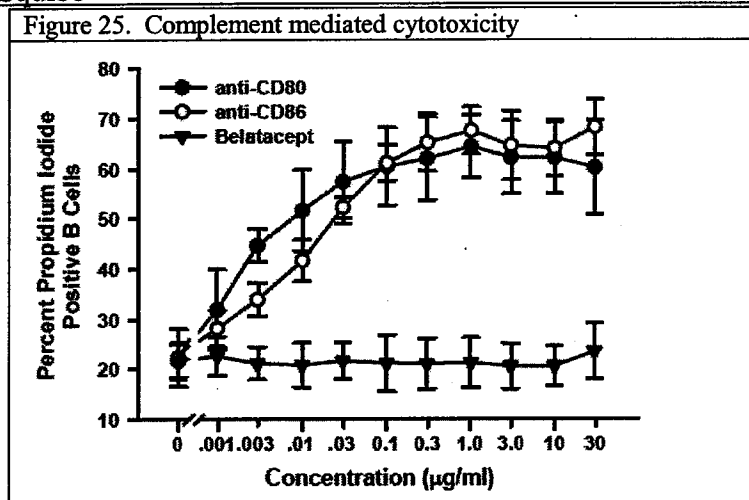
Table 5. Summary of mean IC ₅₀ values				
Parameter	Belatacept		Abatacept	
	Mean (n)	Standard Deviation	Mean (n)	Standard Deviation
Proliferation	0.072 (19)	0.08	1.045 (19)	0.906
IL-2	0.021 (3)	0.006	0.342 (3)	0.328
TNF- α	0.036 (3)	0.021	0.672 (3)	0.439
IFN- γ	0.050 (3)	0.022	0.846 (3)	0.49

Comparisons between the IC_{50} values for CD80 and CD86 receptor saturation were correlated with the IC_{50} values for inhibition of IFN- γ . It was found that IC_{50} value for CD86 more directly correlated with the IC_{50} value for inhibition of IFN- γ production than the IC_{50} for CD80 saturation (Figure 24). These results suggest that a concentration of belatacept or abatacept which achieves CD86 saturation is required to inhibit T-cell cytokine production and proliferation. At this concentration, CD80 is almost completely saturated. It appears that either both receptors are stimulating a T-cell response in this allo-reaction or that inhibition of the reaction is more dependent on more fully saturating CD86.

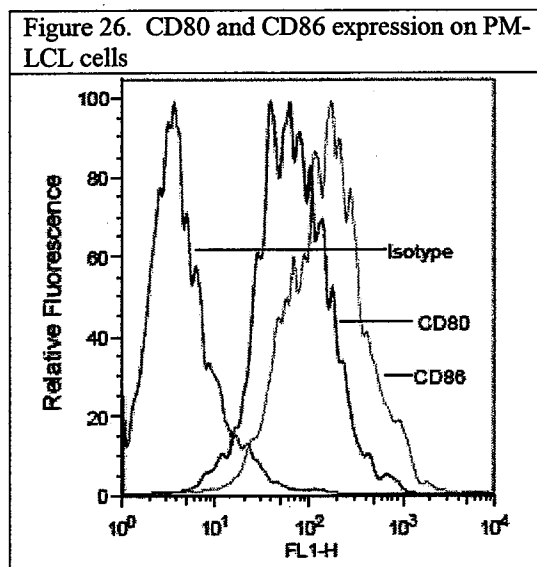
Figure 24. Inhibition of T-cell function by abatacept



Another study conducted by the applicant suggested that belatacept does not mediate human complement-dependent cellular cytotoxicity (Study 930015933). Since the hinge region of the Fc portion (IgG1) of belatacept was mutated, this assay was conducted to confirm that belatacept does not fix complement. PM-LCL B-cells (1×10^6) were incubated at 4°C in RPMI medium containing 10% fetal calf serum alone or with various concentrations of belatacept or CD80/86 antibodies known to fix complement [555681 (IgM) or 555662 (IgG2b); BD Pharmingen]. After 1 hour, rabbit complement (CedarLane Labs, Ontario, Canada) was added and the cells were incubated at 37°C for 2 hours. Propidium iodide was added to detect dead cells and the cell preparations were assayed by flow cytometry. The percentage of cells which stain positive for propidium iodide indicates the extent of complement mediated cell lysis. The results suggest that while the control antibodies for CD80 and CD86 demonstrate a concentration dependent increase in complement mediated lysis, belatacept does not mediate a similar effect (Figure 25).

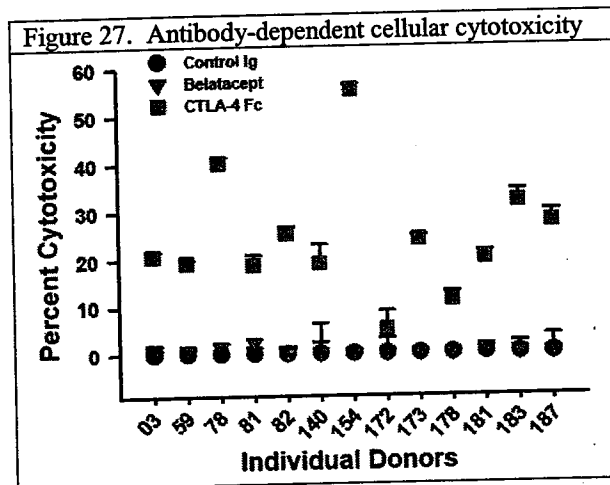


A study was undertaken to determine whether belatacept mediates antibody dependent cellular cytotoxicity (ADCC) by human peripheral blood mononuclear cells (PBMC; Study 930015715). Fc receptors present on peripheral NK cells, neutrophils and macrophages have the potential to mediate the antibody dependent killing of cells that have reacted with belatacept. The ability of the control antibodies used in this experiment to bind to PM-LCL B-cells was first confirmed by flow cytometry (Figure 26). The controls included for testing include an isotype control and antibodies directed against CD80 and CD86 (BD Biosciences, San Jose, CA).



LCL-PM target cells were labeled with sodium chromate ($\text{Na}_2^{51}\text{CrO}_4$; 100µCi per 10⁶ cells). The Fc receptor expressing effector PBMCs were isolated from 13 healthy volunteers by Ficoll gradient. The optimized ratio of cells (100:1; effector:target) were combined with 30 µg/mL of belatacept, CTLA-4 Fc (positive control), or ChiL6 (negative control). CTLA-4 Fc was constructed by fusing CTLA-4 with a non-mutated IgG1 Fc tail (Biosource, Camarillo, CA) and ChiL6 is an isotype matched chimeric

protein immunoglobulin. The cell preparations were then incubated for 6 hours at 37°C. The supernatants were then analyzed on a gamma counter to determine the extent of cell lysis according to the following formula: Cell lysis = (Experimental release – spontaneous release/maximal release – spontaneous release). Spontaneous release was determined with a cell preparation incubated without antibody and maximal release was calculated by addition of 1% Triton-X-100 to one of the cell preparations. The results showed that belatacept, similar to isotype control, does not mediate ADCC of target cells by PBMCs obtained from 13 different human volunteers (Figure 27). The CTLA-4 Fc positive control with no mutations in the Fc portion caused varying degrees of lysis of the target cells.



3.3 Activity *in vivo*

NHP Renal Transplant Model

The effect of belatacept on renal allograft acceptance in rhesus macaques was determined (Pearson and Larsen, 2002, Study 930008605 v2.0). The study was divided into 2 phases. In the first phase, the effect of belatacept monotherapy, or concomitant administration with either mycophenolate mofetil (MMF, Cellcept) or basiliximab (Simulect) on renal allograft survival was examined. All recipients were bred from a different colony than the donor and all monkeys were CMV positive. The monkeys underwent bilateral nephrectomy with the donor kidney anastomosed to the recipient vena cava (renal vein) and distal aorta (renal artery). Following surgery, the monkeys were randomized into five treatment groups (groups 2- 6):

1. Group 1 (n=2): The sponsor states that historical albumin treated controls served as the placebo group. It is unclear what the term "historical" control specifies. The five renal allograft recipients were treated with human serum alone in previous experiments and underwent simultaneous bilateral nephrectomy. Albumin (complete details of the vehicle were not specified) was administered

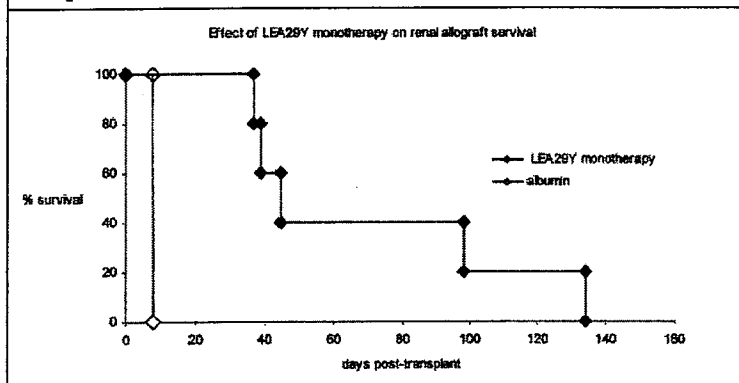
- intraoperatively and on days 2, 4, and 7 following surgery by intravenous injection.
2. Group 2 animals (n=6) were intravenously treated with belatacept alone intraoperatively (10 mg/kg), on day 2 (15 mg/kg) and then 20 mg/kg on days 14, 28, 42, 56 and 70 post-surgery. This dose was chosen based on prior pharmacokinetic studies to target a serum trough concentration of 20 µg/mL.
 3. Group 3 animals (n=4) were treated with MMF and solumedrol (methylprednisolone). MMF (15 mg/kg, bid) was administered intramuscularly at the time of transplant daily through day 14. Afterward, MMF was given once daily at the same dose through 6 months post-transplant. Solumedrol was given intravenously intraoperatively (12 mg), and then as a tapered intramuscular injection on post-operative day(s): 1 (20mg), 2 (16 mg), 3 (12 mg), 4 (8 mg), 5 (4 mg), 6 – 14 (3 mg), and 15 – 182 (1 mg).
 4. Group 4 animals (n=5) were given belatacept, MMF and solumedrol using the same respective drug regimens described for the other groups.
 5. Group 5 animals (n=2) received basiliximab given intravenously intraoperatively and on day 4 (0.3 mg/kg).
 6. Group 6 animals (n=6) were administered belatacept and basiliximab according to the same dosing regimens described above.

The macaques were monitored for allograft function (serum creatinine, BUN and serum chemistries) on post-op days 2, 7, and then weekly. Survival was also monitored and defined as the development of renal failure sufficient to require dialysis in a clinical setting (BUN>200 mg/dL, or hyperkalemia > 7.0, bicarbonate < 12 combined with rising serum creatinine). These animals were euthanized at the time of allograft failure and underwent autopsy. Results of the autopsy were not reported except for the results of renal allograft histology. Other parameters which were monitored included serum levels of belatacept and anti-belatacept antibodies, anti-donor antibodies, PBMC subset analysis and protocol directed biopsy and examination of renal allograft histology of formalin fixed sections.

The results showed that belatacept alone significantly prolonged survival over albumin treated historical controls (p=0.008, Table 6, Figure 28). However, between day 20 and 42 post-transplant, all monkeys in the belatacept group (group 2) showed signs of renal dysfunction (Figure 29). Protocol biopsies obtained at sacrifice or on day 42 were indicative of acute rejection as they showed significant lymphocytic infiltrates and tubulitis. The applicant notes that two of the monkeys experienced a "spontaneous improvement" in renal function and had prolonged survival to days 98 and 134. The data indicate activity of belatacept in prolonging allograft survival.

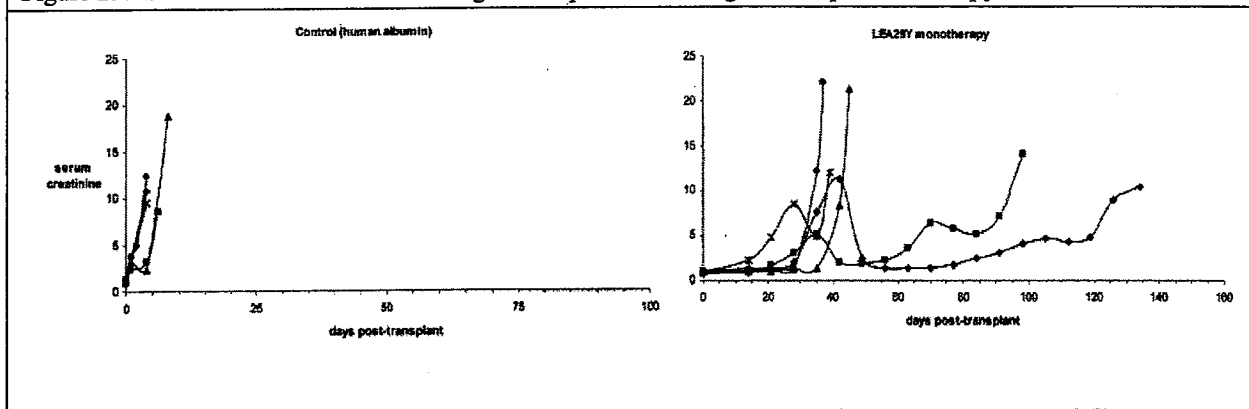
Table 6. Phase 1 survival summary		
Group Number	Experimental Group	Survival (days)
1	Human Albumin	8, 8
2	Belatacept Monotherapy	37, 39, 45, 98, 134
3	MMF/Steroids	8, 25, 36, 50
4	Belatacept/MMF/Steroids	39, 45, 157, 220, >260
5	Basiliximab monotherapy	7, 8
6	Belatacept/Basiliximab	28, 116, 120, 129, 130, 145

Figure 28. Kaplan-Meier survival plot of belatacept monotherapy compared to albumin treated controls



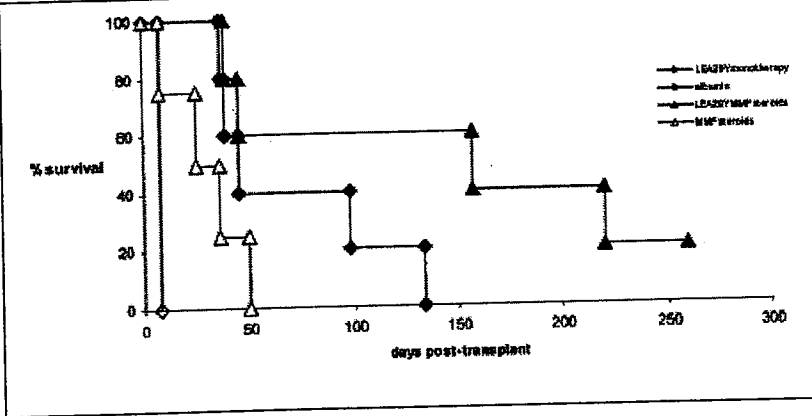
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Figure 29. Serum creatinine values of allograft recipients receiving belatacept monotherapy



The addition of belatacept to a regimen of MMF and steroids significantly improved graft survival. Group 3 (MMF/steroids) showed a median survival of 30 days whereas Group 4 (belatacept/MMF/steroids) had median survival of 157 days (range: 39 - >260 days) (Table 6, Figure 30). The combination of belatacept, MMF and steroids was also superior to belatacept monotherapy (Group 2).

Figure 30. Kaplan-Meier survival plot of belatacept in combination with MMF and steroids



Belatacept also enhanced survival of animals treated with basiliximab. While basiliximab monotherapy was not effective in enhancing graft survival over untreated controls, the combination of belatacept and basiliximab prolonged allograft survival over either agent alone (Table 6, Figure 31). Most allografts functioned normally until approximately day 100, at which time serum creatinine levels began to rise and acute rejection was confirmed by histology (Figure 32).

Figure 31. Effect of belatacept and basiliximab on renal allograft survival

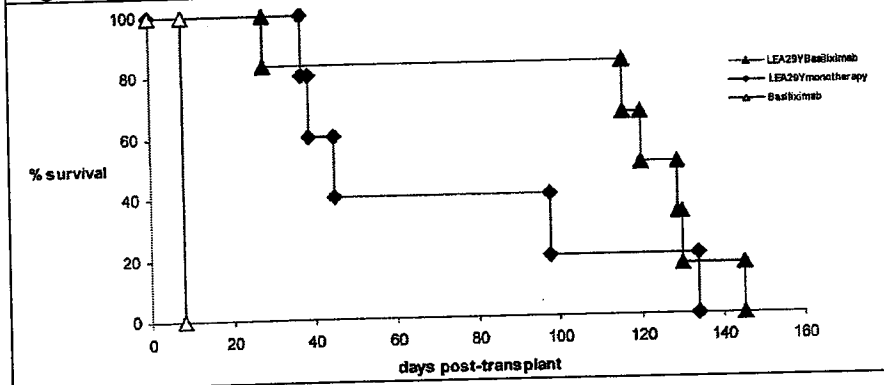
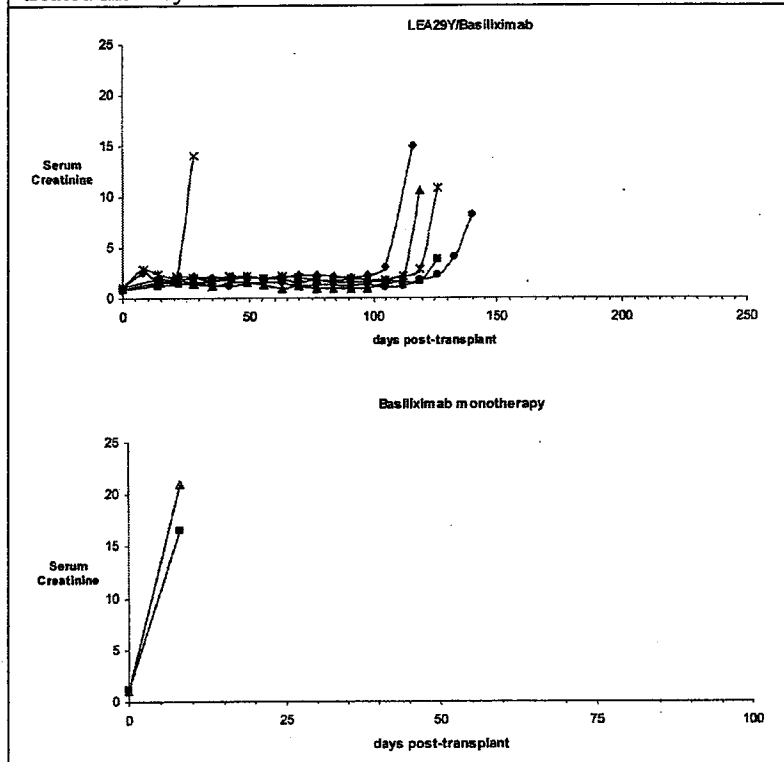
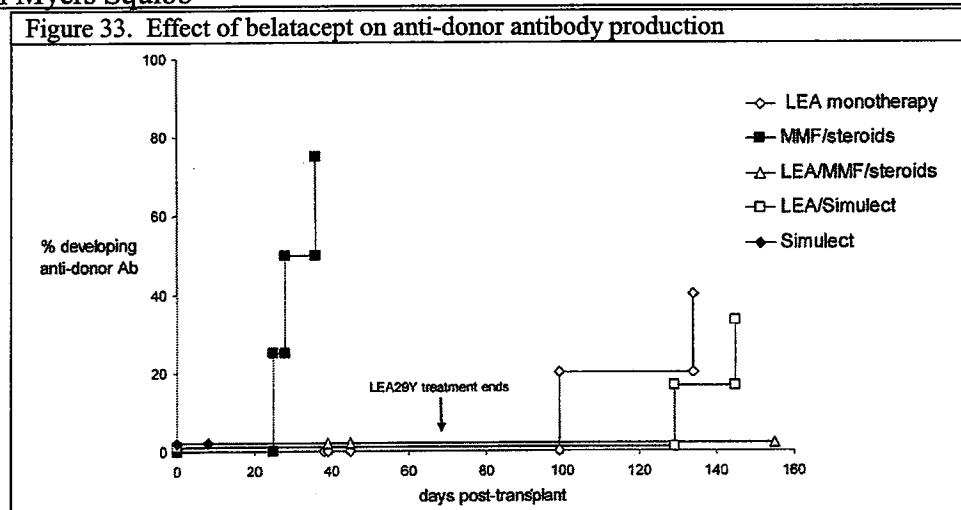


Figure 32. Serum creatinine levels in basiliximab and/or belatacept treated monkeys



Since survival was limited for Group 1 (albumin) and Group 5 (basiliximab monotherapy), no detectable anti-donor antibody responses were found in these monkeys. When survival was extended by administration of MMF and steroids, the monkeys showed significant development of anti-donor antibodies at the time of rejection. Notably, no monkeys developed anti-donor antibodies while being treated with any regimen containing belatacept. Belatacept dosing was discontinued on day 70 in all treatment groups; in monkeys that survived > 100 days, anti-donor antibodies were detected (Figure 33). Overall, this experiment suggests that belatacept has an additive effect when combined with other immunosuppressive agents and that belatacept is effective in preventing anti-donor antibody production during the first 70 days of therapy. A comparator arm which included a calcineurin inhibitor alone or in combination with MMF/steroids or basiliximab was not included in this study and would have been informative. The applicant additionally notes that no differences in peripheral blood lymphocyte populations were noted following belatacept treatment. Absolute lymphocyte count and cells positive for CD3, CD4, CD8 and CD20 were measured. However, the results were not available for review.



The second phase of the study explored the effect of different dosing regimens of belatacept in combination with MMF, steroids and basiliximab. All surgical techniques and assessments were the same as described for the previous experiment. The rhesus macaques were divided into 3 treatment groups. The controls (n=4) received a combination of basiliximab, MMF and steroids. A second group, the “high maintenance group”, received the same background immunosuppression in addition to belatacept given as 10 mg/kg on day 0, 15 mg/kg on day 4, 20 mg/kg on days 14, 30, 49, 68 and 91, and then 20 mg/kg every 23 days until day 344. These doses were given to target different trough concentrations through day 365 (day 0 – 30 = 20µg/mL; day 30 – 90 = 7µg/mL; day 90 – 365 = 2µg/mL). The “low maintenance group” received the same background regimen in addition to the same regimen of belatacept through day 90, however the long term maintenance (days 90 – 365) had a target trough of 0.25 µg/mL so NHP were given 20 mg/kg every 33 days. An arm which included a calcineurin inhibitor in combination with the background regimen was not included.

The results of the experiment show that while belatacept prolonged the survival of allograft recipients over the standard regimen, neither the high or low maintenance treatment significantly enhanced survival over the other, though one monkey in the low maintenance group had indefinite survival (Table 7, Figure 34). Examination of serum creatinine levels shows that the allograft functions normally until the time of rejection at which point a sharp rise is noted (Figure 35). The differences in the scale of the x-axis should be noted for each graph within Figure 35.

Table 7. Phase 2 survival summary	
Experimental Group	Survival (days)
Belatacept (high maintenance)	32, 51, 112, 198
Belatacept (low maintenance)	28, 51, 118, 189, >625
Basiliximab/MMF/Steroids	8, 9, 36, 36

Figure 34. High versus low maintenance belatacept effect on survival

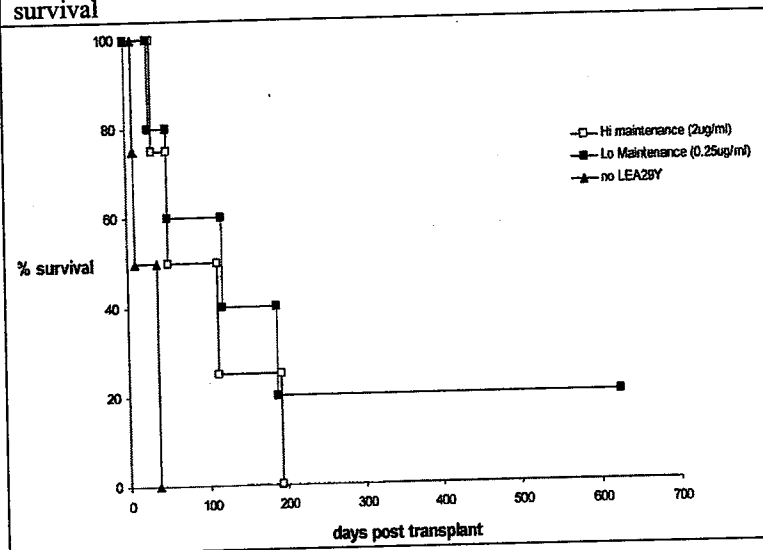
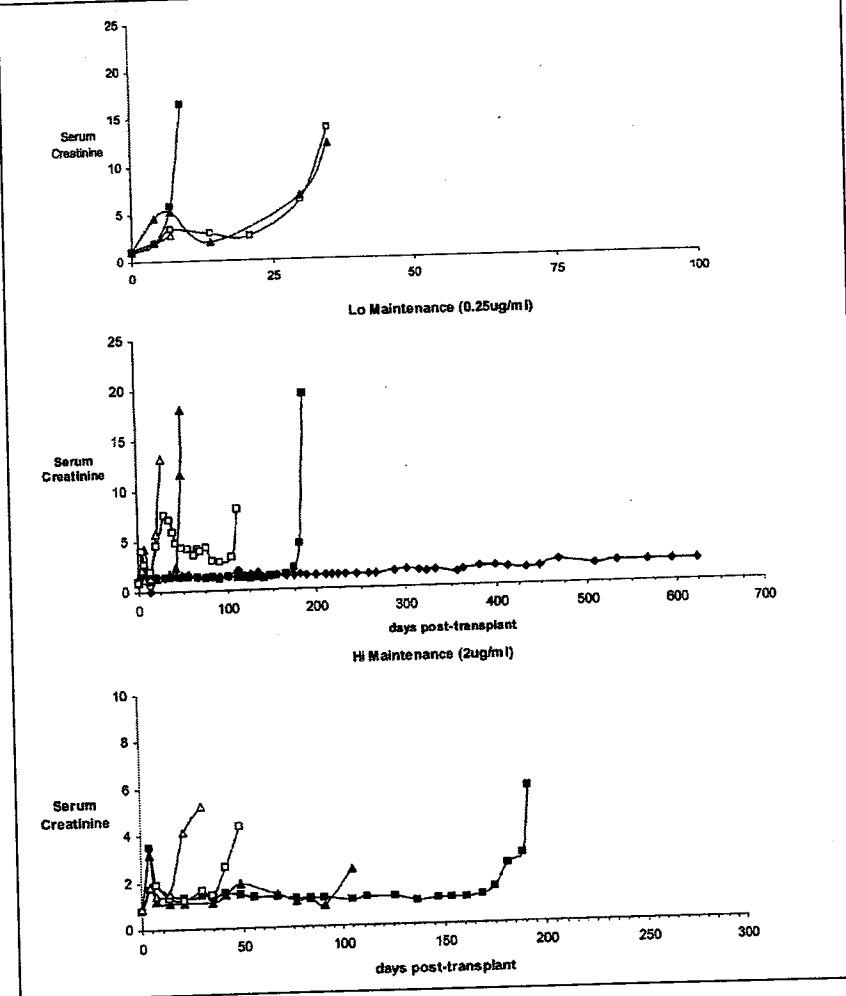
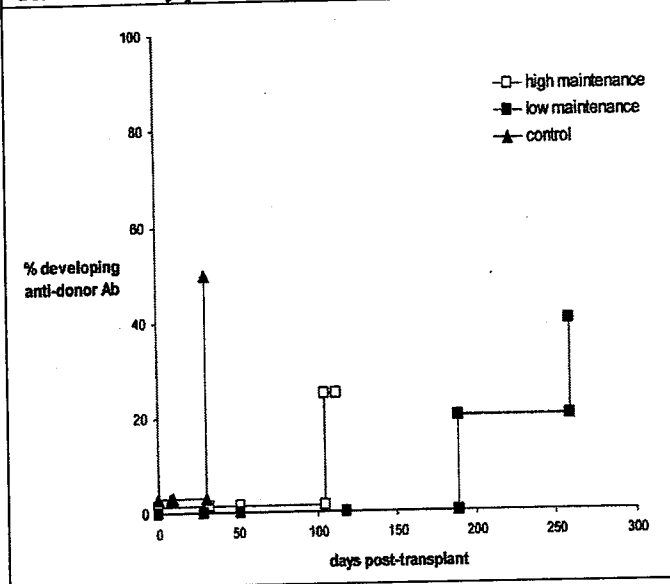


Figure 35. Serum creatinine levels in high and low maintenance belatacept treatments



Anti-donor antibodies did not develop in any monkeys receiving belatacept until after day 100 (Figure 36). By day 100, over 20% of the monkeys in the high maintenance group had developed antibodies. It is unknown if the sole monkey which survived beyond day 112 in this group was assayed again. The two monkeys in the low maintenance group which survived beyond day 100 developed anti-donor antibodies. Therefore these data suggest that development of anti-donor antibodies can occur during belatacept treatment albeit at a delayed onset compared to other treatment regimens.

Figure 36. Effect of different dosing regimens on anti-donor antibody production



Pancreatic Islet Cell Transplant

The effect of belatacept on the survival of donor pancreatic islet cells was examined (Pearson and Larsen, 2002, Study 930008605 v2.0). Diabetes was induced in rhesus macaques by pancreatectomy and confirmed with an intravenous glucose tolerance test before surgery. The MHC haplotype of the donor/recipient pairs was also determined (8 class I and 12 class II alleles). The investigators sought to maximize MHC mismatch between donor and recipient. At least one week following pancreatectomy, purified allogeneic pancreatic islet cells were intrahepatically implanted by injection into the left colic vein. After transplant, macaques were divided into two treatment groups:

1. Group 1: belatacept/sirolimus/basiliximab. Belatacept was administered intra-operatively (10 mg/kg) and then on days 4 (15 mg/kg), 14 and then every two weeks until day 154 (20 mg/kg). Basiliximab (0.3 mg/kg) was administered intravenously intra-operatively and on day 4. Sirolimus was given orally (1.25 mg/kg) through day 50 and then as a 1 mg/kg dose through day 100. The dose was further tapered until terminated on day 121.
2. Group 2: Sirolimus/basiliximab. The regimen followed the same dosing schedule as described for Group 1 without belatacept treatment.

After implantation, allograft function was monitored by daily measurement of fasting plasma glucose and monthly intravenous glucose tolerance tests measuring insulin and c-peptide production. Blood glucose was also recorded after lunch each day. Failure of the allograft was defined as development of hyperglycemia (two consecutive fasting glucose concentrations ≥ 125 mg/dL on subsequent days). The development of anti-donor antibodies was also monitored. PBMC alloreactivity was determined using peripheral

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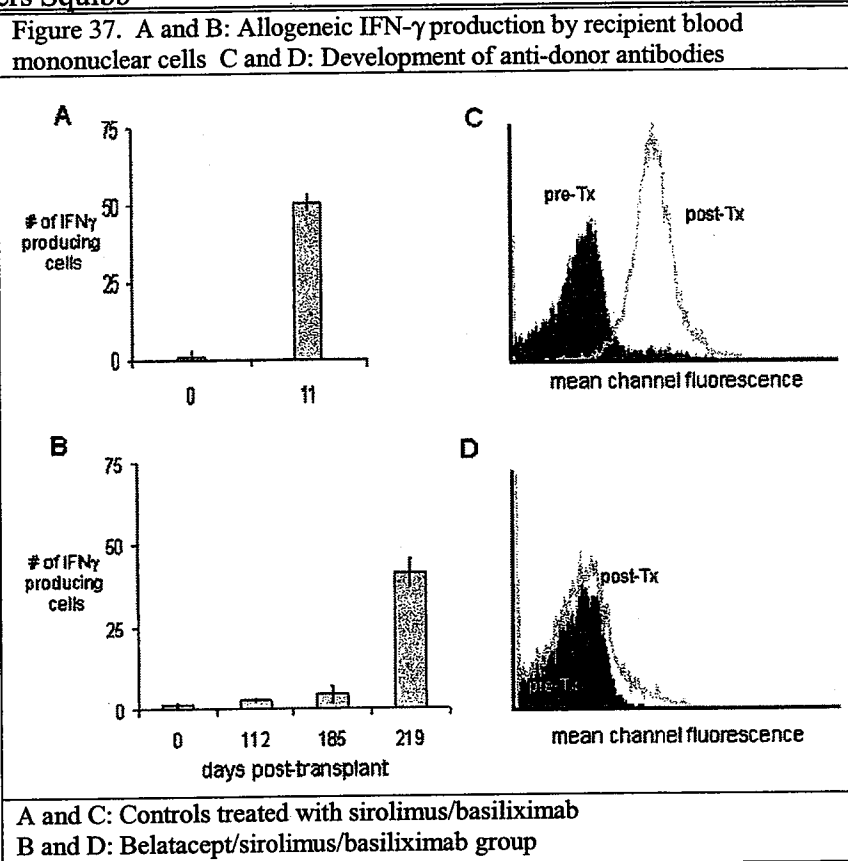
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blood obtained from donor and recipient macaques. Alloreactivity was defined as IFN- γ production as detected with an ELISpot assay. For this assay, an equal number of irradiated donor leukocytes (stimulators) and recipient leukocytes (responders) were plated in wells coated with a mouse anti-human IFN- γ antibody (Mabtech, Sweden). Plates were incubated for 14-16 hours, at which time an anti-IFN- γ biotinylated antibody was added. Plates were washed, developed and spots detected. Each spot is representative of a single IFN- γ secreting cell. The frequency was calculated by dividing the number of spots by the total number of responder cell plated.

The results showed that belatacept lengthens the survival (median 200 days) of islet allografts in rhesus macaques compared to monkeys treated with sirolimus and basiliximab (rapa/ α IL-2R) that survived for 7 days (Table 8). The degree of MHC mismatch between donor and recipient was similar between treatment groups. Glucose tolerance assays confirmed function of the graft and four of the five monkeys in the belatacept containing regimen remained rejection free throughout treatment even though sirolimus was discontinued by day 121. After immunosuppressive treatment ceased at day 150, a gradual hyperglycemia emerged and was treated with exogenous insulin.

Table 8. Survival of islet pancreatic cells					
Animal ID	IEQ/kg	Survival (insulin independence)	Treatment	MHC mismatches	
				Class I	Class II
RKf-7	22,250	204	Belatacept/Rapa/ α IL-2R	2	ND
RUF-7	17,087	190	Belatacept/Rapa/ α IL-2R	ND	3
RRe-7	20,266	216	Belatacept/Rapa/ α IL-2R	2	6
RWt-6	16,033	56	Belatacept/Rapa/ α IL-2R	2	3
RMv-6	8,201	> 200	Belatacept/Rapa/ α IL-2R	1	3
RQz-6	12,980	7	Rapa/ α IL-2R	2	5
RIb-7	10,903	7	Rapa/ α IL-2R	1	4
ND= no difference in the alleles which were typed; IEQ=islet equivalents implanted; rapa = Sirolimus; α IL-2R = basiliximab.					

The ability of recipient PBMC to respond to donor antigen was assayed by the ELISpot assay (Figure 37-A and B). The results show that the frequency of donor reactive PBMC quickly rises in the control animals which coincides with the shortened graft survival time in this group. Conversely, monkeys in the belatacept containing regimen had very few reactive PBMC until after therapy ceased. The development of anti-donor antibodies is also prevented by belatacept administration compared to control (Figure 37-D and C, respectively). The timing of the assay for antibody development was not specified.



Keyhole Limpet Hemocyanin antibody response

A study was conducted to compare belatacept and abatacept in a murine primary immune response model (Study numbers 092804KLH, 102704KLH, and 020805KLH).

The effect of abatacept and belatacept on the antibody response to keyhole limpet hemocyanin (KLH) was examined. BALB/c mice were injected intraperitoneally with KLH (250 μ g) on Day 0. On day 0, 7, and 14, the mice were also injected subcutaneously with various amounts of a control IgG, belatacept or abatacept (n=10 per group). Another group received no antibody treatment (n=5). On days 7, 14 and 21, blood was collected for ELISA analysis of antibodies against KLH and to determine levels of belatacept or abatacept. Antibody titer was defined as the inverse of the dilution of serum for which the optical density (OD) is equal to 5 times the OD of the background. The data are expressed as the ratio of the anti-KLH titer of the tested serum to the pooled serum titer of control IgG treated BALB/c mice immunized with KLH. The results showed that administration of 16 mg/kg abatacept inhibited the antibody response to KLH earlier than belatacept (Table 9). By 3 weeks, both groups demonstrated equal inhibition of antibody response. The maximal inhibition for abatacept was achieved after 2 doses (day 14), whereas maximum inhibition was not evident until 3 doses of belatacept were administered (day 21). This data infers that fewer doses of abatacept may be necessary to achieve maximum results and may coincide with data which show that abatacept binds to murine CD80 and CD86 more avidly than belatacept.

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Table 9. Study 092804KLH: Comparison of antibody response to KLH			
% inhibition of KLH-specific IgG in serum			
	Day 7	Day 14	Day 21
Abatacept (16 mg/kg)	68%	78%	76%
Belatacept (16 mg/kg)	47%	60%	74%
N.B. inhibition calculated by comparing % response to control IgG-treated group at same dose concentration			

An experiment examined antibody production against KLH in the presence of various concentrations of belatacept and abatacept. Experimental design was similar to that described above. It is evident that belatacept and abatacept given at the highest dose (and hence the highest serum drug levels at day 7) showed the most significant inhibition of antibody production and the lowest dose of each drug showed the least inhibition (Table 10). However, the early time points do not show a dose response. When comparing abatacept doses of 10 and 3 mg/kg and belatacept doses of 30 and 10 mg/kg, it seems the data does not follow a dose response. The applicant notes that plasma levels for abatacept when given at 10 mg/kg were too low to quantify on day 7. The lower limit of detection of the assay was not specified. The applicant offers the explanation that the mice may not have received the day 0 dose. This does not seem likely as these mice also had a decreased antibody response on day 7 which probably signals that these mice did get a dose of the study molecule on day 0 since no other doses were given in the interim. The lower inhibition rates for this treatment group may suggest that the dose given was less than 10 mg/kg. Given this discrepancy for both treatment groups, it is difficult to conclude a linear dose response. The final measurement taken on day 21 does show a more linear dose response for belatacept. The development of antibodies to abatacept and belatacept were also reported by the applicant for this study. The titer of anti-abatacept and anti-belatacept antibodies increased with decreasing dose with very little or no antibody evident at the highest doses of belatacept and abatacept. The development of neutralizing antibodies against the lower doses of abatacept and belatacept could cause a decrease in activity and hence be responsible for the observed effect. The applicant notes that belatacept was more immunogenic and thus may be responsible for the increased activity of abatacept in this model.

Table 10. Study 102704KLH: Dose dependent inhibition of antibody production to KLH				
% inhibition of KLH specific IgG in serum and serum drug levels				
Dose (mg/kg)	% inhibition			Serum Drug Levels (ng/mL on Day 7)
	Day 7	Day 14	Day 21	Mean $\pm$ SD
Abatacept				
30	61%	91%	95%	26109 $\pm$ 12733
10	47%	60%	70%	< LLQ *
3	51%	86%	85%	515 $\pm$ 567
1	55%	56%	55%	< LLQ
Belatacept				
100	74%	97%	97%	ND
30	53%	87%	94%	21014 $\pm$ 13287
10	77%	86%	85%	5148 $\pm$ 4089
3	-32%	1%	13%	< LLQ
N.B. inhibition calculated by comparing % response to control IgG-treated group at same dose concentration, except for abatacept 1g/kg, which is compared to 3 mg/kg IgG-treated group				
* Serum dose levels were significantly lower than anticipated indicating that mice may have not received day 0 dose of 10 mg/kg				
LLQ = below quantitative levels; ND = not done; SD = standard deviation				

Another experiment examined dose response. Antibodies against KLH were only measured on day 14 which was the day of “peak immune response in this model” (Table 11). The results showed a more consistent dose response (Figure 38). Abatacept was more active in this model with maximum inhibition achieved at a dose of 3 mg/kg whereas the maximum activity of belatacept was evident at the 30 mg/kg. The applicant notes that the decrease in serum levels for belatacept treated mice compared to abatacept treated mice at similar doses may be responsible for the decrease in activity seen with belatacept. The applicant notes that belatacept was more immunogenic and thus may be responsible for the increased activity of abatacept in this model. The difference could also be due to increased clearance of belatacept compared to abatacept.

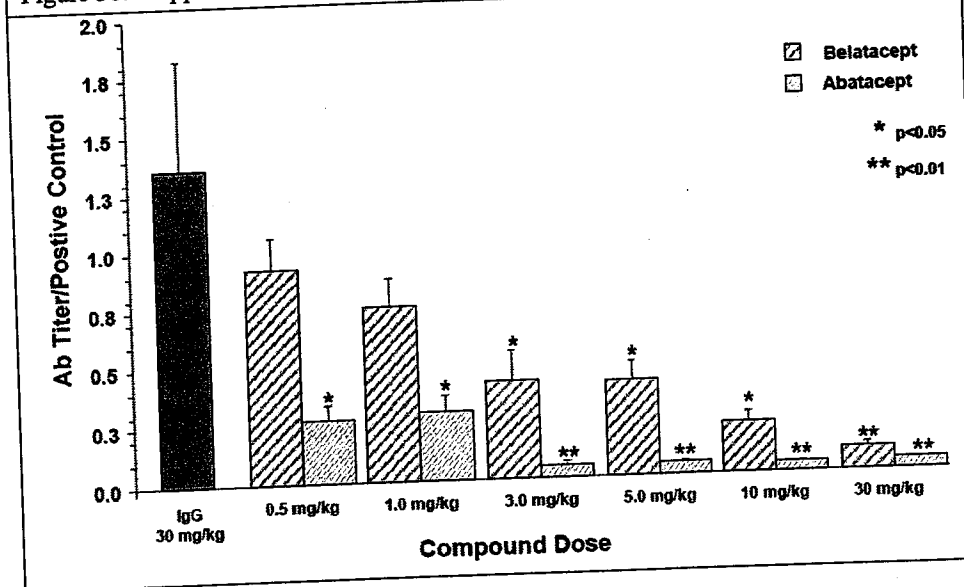
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Table 11. Study 020805KLH: Repeat dose response of abatacept and belatacept in inhibiting antibody response to KLH

Dose (mg/kg)	% inhibition (day 14)	Serum drug levels [(ng/mL on day 7), mean $\pm$ SD]	Serum drug levels [(ng/mL on day 14), mean $\pm$ SD]	Serum drug levels [(ng/mL on day 21), mean $\pm$ SD]
Abatacept				
30	97%	76437 $\pm$ 6749	112494 $\pm$ 11598	131356 $\pm$ 10808
10	97%	28651 $\pm$ 2905	39242 $\pm$ 2873	47565 $\pm$ 7913
5	96%	13782 $\pm$ 1819	16058 $\pm$ 6773	14055 $\pm$ 5304
3	96%	8000 $\pm$ 1031	6540 $\pm$ 2675	2076 $\pm$ 3204
1	78%	12 $\pm$ 19	< LLQ	< LLQ
0.5	80%	< LLQ	< LLQ	< LLQ
Belatacept				
30	93%	29045 $\pm$ 3920	9231 $\pm$ 11463	3401 $\pm$ 5295
10	84%	6846 $\pm$ 3080	47 $\pm$ 54	3 $\pm$ 0.4
5	70%	749 $\pm$ 973	< LLQ	< LLQ
3	69%	3 $\pm$ 3	< LLQ	< LLQ
1	45%	< LLQ	< LLQ	< LLQ
0.5	32%	< LLQ	< LLQ	< LLQ

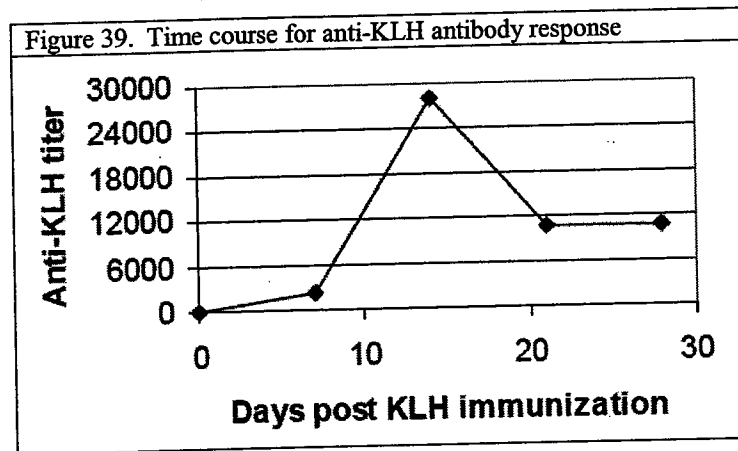
N.B. % inhibition calculated by comparing % response to control IgG 30 mg/kg group
 < LLQ = below quantitative levels and SD = standard deviation

Figure 38. Suppression of day 14 primary immune response to KLH

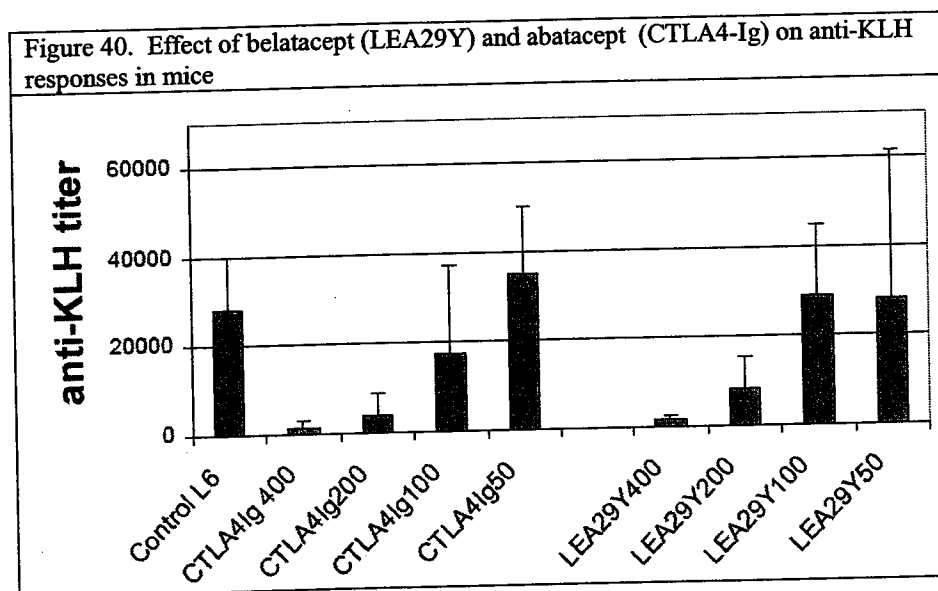


The applicant has provided a summary of another *in vivo* study in which antibody production to KLH was determined in mice treated with a single dose of belatacept or abatacept (Report 930008556). Belatacept and abatacept were tested at doses of 50, 100, 200 and 400 μ g, which the applicant states are approximately equivalent to doses of 2.5, 5.0, 10 and 20 mg/kg, respectively. Mice (n=5/treatment, except belatacept 400 μ g dose which had n=2) were immunized with KLH intraperitoneally on the same day as belatacept or abatacept treatment. Blood samples were then taken on days 7, 14, 21, and

28 to determine anti-KLH antibody production. Figure 39 shows that the anti-KLH response in control mice peaked at day 14.



The day 14 titers of belatacept (LEA29Y) and abatacept (CTLA4-Ig) treated mice were then compared (Figure 40). The results showed a trend with an inverse relationship between anti-KLH titers and treatment concentration. Abatacept was ~2x more active in mice with decreases in KLH antibody evident with the 100 µg dose whereas the effect of belatacept was evident beginning with the 200 µg dose.



Exploratory Study in Rats #1 KLH response

A study compared the bioactivity of belatacept to abatacept in rats (Study DS04256). Female Sprague-Dawley rats (n=10 per group) were administered saline, abatacept or belatacept (20 mg/kg) on days 1 and 8. On day 1, the rats were also immunized subcutaneously with KLH (1 mg) within 30 minutes prior to dosing with abatacept or belatacept. Serum was obtained from the rats on days 1 (before dosing and immunization), 8, 15, and 22. The serum was assayed for anti-KLH antibody by ELISA.

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KLH-specific antibodies were captured by coating microtiter plates with KLH and detected with alkaline phosphatase-conjugated goat anti-rat IgG and IgM antibodies. A serum dilution was considered positive if its post-dose titer was at least 9-fold greater than its baseline response (i.e. before dosing and immunization). The titer was reported as the inverse of the lowest dilution which yielded an absorbance that was 5-times the background absorbance measured for the microtiter plate. Pharmacokinetics, toxicological parameters and the development of anti-abatacept and anti-belatacept antibodies were also measured. The results showed that the endpoint titers of belatacept and abatacept treated rats were significantly lower than untreated controls. At all time points, suppression of anti-KLH antibody response was > 99% for abatacept and belatacept treated rats. While the mean anti-KLH antibody titers of control rats were 7,933, 39,086 and 78,042 for days 8, 15 and 22, respectively, the mean antibody titers for rats treated with abatacept were 29, 19, and 19 and for belatacept treated rats were 8, 15 and 22. Individual anti-KLH antibody titers were reported for each rat.

Exploratory Study in Rats #2 KLH response

Another study determined an abatacept or belatacept dose response in inhibiting the formation of anti-KLH antibodies in rats (Study DS04284). The animals, materials and methods for this study are the same as for Study DS04256 except various doses of abatacept and belatacept were given on day 1 and day 8. There were 9 total groups (n=10/ group). Serum was obtained from the rats on days 0 (before dosing and immunization), 8, 15, and 22. At doses of 1, 3, and 10 mg/kg, both molecules suppressed T-cell dependent development of anti-KLH antibody by $\geq 98\%$ at all time points assayed (Table 12). Abatacept at a dose of 0.3 mg/kg suppressed anti-KLH antibody by $\geq 98\%$ at all time points whereas belatacept at the same dose suppressed anti-KLH antibody production by 87, 65, and 49% on days 8, 15, and 22 respectively. This data further supports the observation that abatacept is more active in rodents. The individual anti-KLH antibody titers were reported for each rat and were related to the C_{trough} for each dose group. Since inhibition was > 98% for all treatment groups at all time points except belatacept (0.3 mg/kg), no definitive exposure/response curve could be generated. Trough concentrations for belatacept (0.3 mg/kg) were the lowest of any of the treatment groups.

Table 12. Belatacept and abatacept dose response of rats to development of KLH-specific antibodies *					
Compound	Dose (mg/kg)	% inhibition			AUC _(0-168 hours) (µg/mL x hour)
		Day 8	Day 15	Day 22	
Abatacept	0.3	99	99	98	231
	1	99	100	100	815
	3	99	100	100	2820
	10	100	100	100	7990
Belatacept	0.3	87	65	49	144
	1	99	100	100	545
	3	99	100	100	1450
	10	98	99	99	4640
* Expressed as percent suppression of T-cell-dependent antibody response to KLH relative to vehicle-control group					

Exploratory Study in Rats #3 KLH response

Another study examined dose dependence of inhibition of anti-KLH responses (Study DS05055). This study followed the same methodology of Study DS04256 and DS04284 except the responses of doses < 0.3 mg/kg were explored. Results were similar to the former studies except for the doses < 0.3 mg/kg (Table 13). Interestingly, belatacept at doses of 0.03 and 0.1 mg/kg and abatacept at the dose of 0.03 mg/kg actually enhanced anti-KLH antibody development at some time points.

Table 13. Belatacept and abatacept dose response of rats to development of KLH-specific antibodies *				
Compound	Dose (mg/kg)	% inhibition		
		Day 8	Day 15	Day 22
Abatacept	0.03	-120	-92	-64
	0.1	59	85	81
	0.3	90	99	100
	1	96	100	100
Belatacept	0.03	-269	-9	15
	0.1	-57	62	45
	0.3	10	59	60
	1	89	97	96
* Expressed as percent suppression of T-cell-dependent antibody response to KLH relative to vehicle-control group				

A study explored the effect of a two-week intermittent dose regimen of belatacept in female New Zealand White rabbits (Study DS04252). Belatacept or abatacept (10 mg/kg) was intravenously administered to female rabbits (n = 6/group) on days 1 and 8. A control group received saline. All animals were subcutaneously immunized with KLH (1 mg) thirty minutes prior to receiving treatment on day 1. Blood was drawn on day 8, 15 and 22 and anti-KLH antibody was measured in serum by ELISA as described above using goat anti-rabbit IgG for detection. Pharmacokinetic analysis showed that exposure to belatacept was 4-fold less than abatacept even though similar doses were administered. The endpoint titer was defined as described above. The results also showed that the

development of anti-belatacept or anti-abatacept antibodies correlated with serum levels of belatacept and abatacept. The results showed that both belatacept and abatacept were effective in blocking anti-KLH antibody production (Table 14). As observed in other rodent species, abatacept appeared to be more active in rabbits than belatacept. Interestingly, belatacept treated rabbits developed some anti-KLH antibody later in the observation period (day 22).

Table 14. Anti-KLH antibody production in rabbits treated with belatacept or abatacept				
		Treatment group:		
		Control	Belatacept	Abatacept
Day 8	Mean	114044	757	199
	Std. Dev.	218621	464	280
	% suppression	0	99.3	99.8
Day 15	Mean	410786	14833	953
	Std. Dev.	246104	16981	1156
	% suppression	0	96.4	99.8
Day 22	Mean	417497	139871	3325
	Std. Dev.	185180	187305	4613
	% suppression	0	66.5	99.2

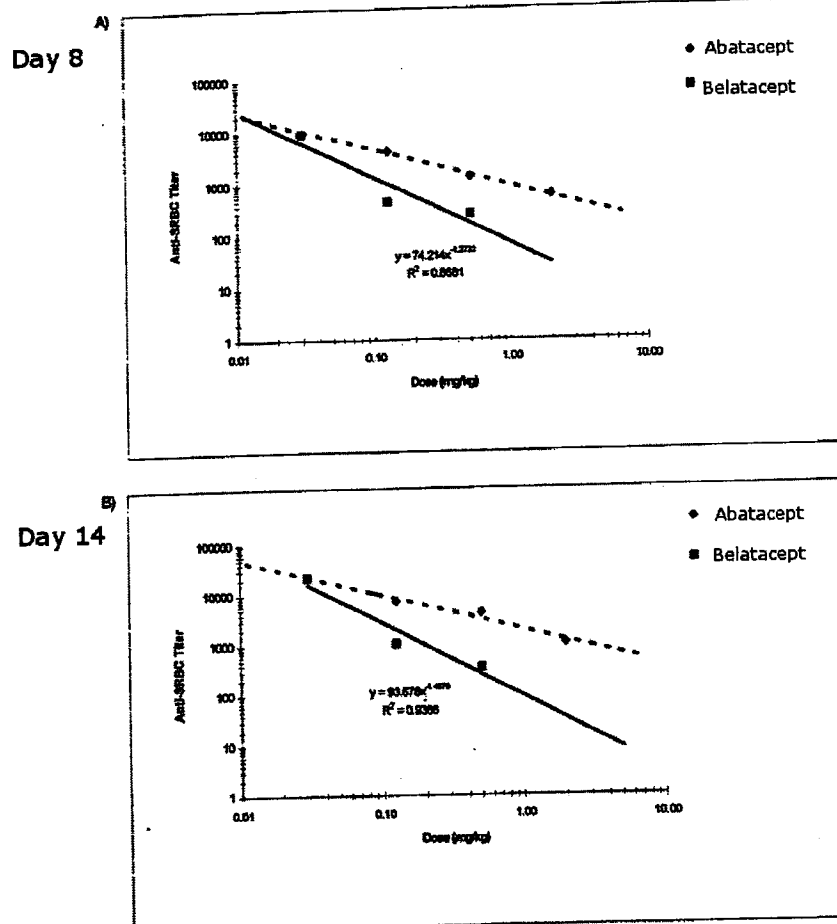
Another study examined the dose dependence of inhibition of the anti-KLH antibody response (Study DS04287). The methodology was the same as described for the above experiment in rabbits and 6 rabbits were used per dose group. Rabbits received 1, 3, or 10 mg/kg abatacept or 3, 10 or 20 mg/kg belatacept. The resulting data while having a high standard deviation suggest that abatacept and belatacept have similar effect at the lowest dose (3 mg/kg), but at higher doses, abatacept reduces the titer of anti-KLH antibody more than belatacept (~99-100% versus ~90-95% for abatacept and belatacept, respectively (Table 15).

Table 15. Anti-KLH antibody production in rabbits treated with belatacept or abatacept								
		Treatment group:						
		Control	Belatacept (mg/kg)			Abatacept (mg/kg)		
			3	10	20	1	3	10
Day 8	Mean	216810	9392	9955	1382	9061	490	625
	Std. Dev.	148234	8114	22327	1484	11968	201	636
	% suppression	0	96	95	99	96	100	100
Day 15	Mean	1429387	153899	75203	31028	92892	9330	5199
	Std. Dev.	837999	126252	153332	48740	106874	11526	6933
	% suppression	0	89	95	98	94	99	100
Day 22	Mean	2052037	389463	223751	167548	416938	29254	6247
	Std. Dev.	1238911	237069	284787	231202	366016	48970	7221
	% suppression	0	81	89	92	80	99	100

Another study examined the biological effect of a single dose of belatacept or abatacept in cynomolgus monkeys (Study No. 97607). Prior to administration of the test molecules, the monkeys were immunized with murine L6 antigen (5mg/kg, i.v.) and sheep erythrocytes (SRBC; 1.7 mL/kg of a 10% suspension). The monkeys [n=4 (2 male and 2 female)/dose group] were then intravenously injected with either belatacept (0.03, 0.125, or 0.5 mg/kg) or abatacept (0.125, 0.5, or 2.0 mg/kg). Serum samples were

obtained prior to dosing and on days 8, 14, 21, 28, and 42. Antibody titers to the SRBC and L6 were then determined using specific Immulon ELISA kits per manufacturer's instructions (Dynax, Chantilly, VA). The results were expressed as the end point titer. The end point titer was defined as the lowest antibody titer which yielded an absorbance equal to at least twice the mean background absorbance. The results showed that both molecules exhibited a dose dependent inhibition of the primary immune response to SRBC (Figure 41). Belatacept was 2- to 3-fold more effective than abatacept when defined by the dose that caused a 50% reduction in the peak anti-SRBC response (ID₅₀: 0.065 mg/kg vs. 0.031 mg/kg on day 8, 0.188 mg/kg vs. 0.057 mg/kg on day 14) and 6-11 fold more effective than CTLA4-Ig for the dose that caused a 90% reduction (ID₉₀: 0.664 mg/kg vs 0.108 mg/kg on day 8, 1.91 mg/kg vs. 0.17 mg/kg on day 14).

Figure 41. Anti-SRBC antibody titers versus dose of belatacept and abatacept treated monkeys



The applicant notes that an antibody response to L6 was late to develop, even in untreated monkeys, and results regarding a dose response were difficult to extrapolate (Table 16). In untreated monkeys, the anti-L6 antibody titer peaked at day 21 and day 28. No suppression of the anti-L6 titer was observed on day 21 in monkeys dosed with

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either 0.03 or 0.125 mg/kg belatacept. However, the monkeys which received the highest dose of belatacept did suppress anti-L6 antibody production more than the same dose of abatacept.

Table 16. Anti-murine L6 antibody response							
Treatment	Dose (mg/kg)	Anti-murine L6 total Ig Antibody response					
		Day					
		1	8	14	21	28	42
Vehicle	0	118	468	65610	149558	149558	113640
Abatacept	0.125	270	468	4209	65610	65610	86348
	0.5	68	270	1403	49853	86348	113640
	2.0	270	205	355	1846	5539	21370
Belatacept	0.03	118	355	65610	196830	149558	149558
	0.125	615	1066	12627	149558	113640	149558
	0.5	270	270	468	7290	49853	28783

The applicant extrapolated ID₅₀ and ID₉₀ to compare belatacept to abatacept. ID₅₀ and ID₉₀ are defined as the doses which would cause a reduction equal to 50 and 90% of the peak titer in the absence of treatment. When comparing the extrapolated ID₅₀ of the peak reduction in anti-SRBC response, belatacept was found to be approximately 2-3 fold more active than abatacept (Table 17). When this analysis was extended to determine the ID₉₀, belatacept was determined to be 6-11 fold more active than abatacept. The applicant does not rule out increased clearance of abatacept as a contributing factor to its lower activity in this model.

Table 17. Comparative immunosuppression by belatacept and abatacept						
Compound	ID ₅₀ (mg/kg)			ID ₉₀ (mg/kg)		
	SRBC		Murine L6	SRBC		Murine L6
	Day 8	Day 14	Day 21	Day 8	Day 14	Day 21
Abatacept	0.065	0.188	0.167	0.664	1.91	0.583
Belatacept	0.031	0.057	0.106	0.108	0.17	0.421

Effect on immune cell subsets

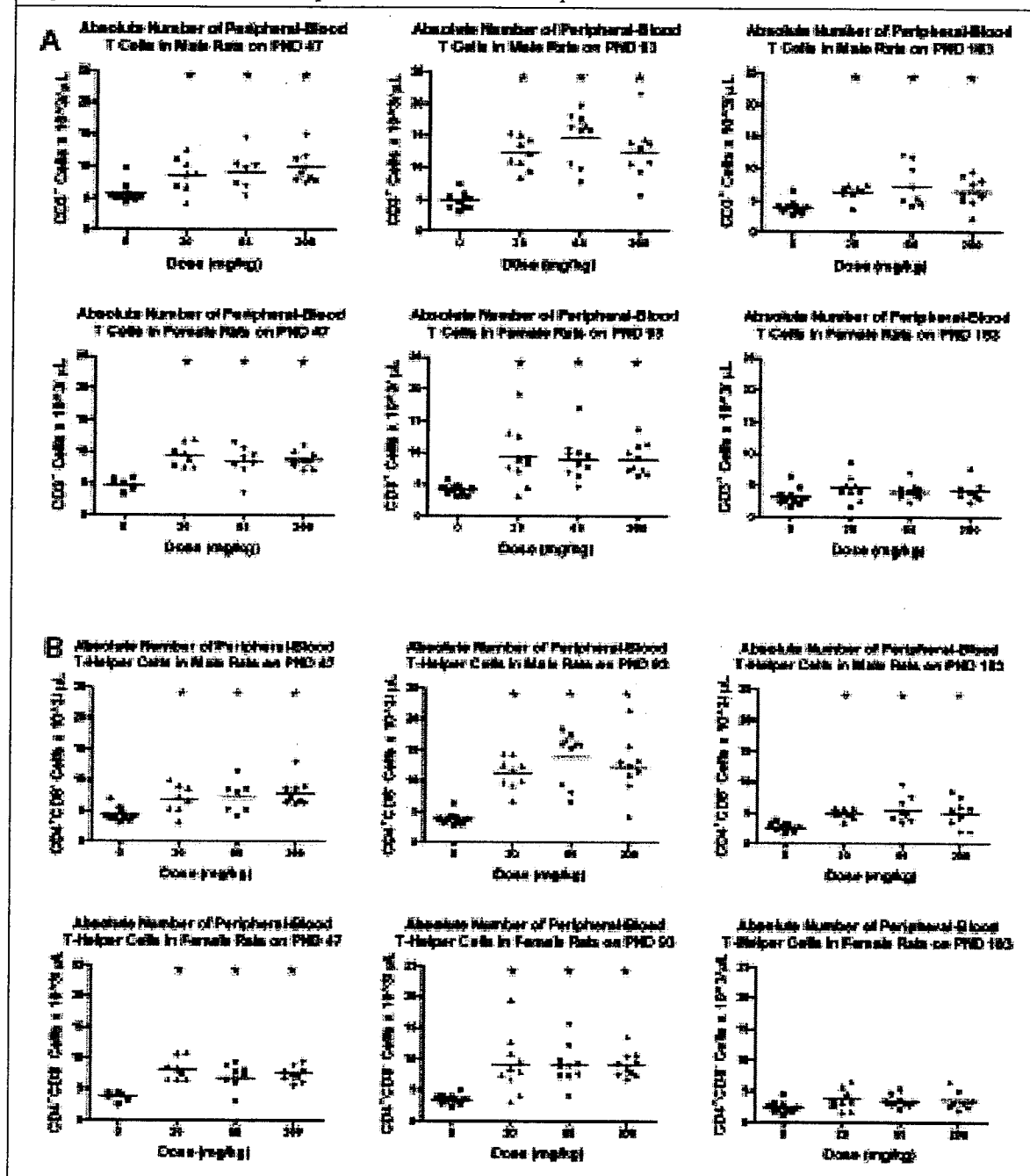
The effect of abatacept on immune cell subsets was measured in a thirteen week study in juvenile rats (Study DN07013). Abatacept (20, 65, 200 mg/kg) was administered thrice weekly on postnatal days (PND) 4 – 94. Results were compared to a separate group administered vehicle (0.9% saline). A subset of the rats were designated for analysis of total serum IgG, T-cell dependent antibody production to KLH (described above) and peripheral blood lymphocyte typing. Blood was obtained at sacrifice and on PND 47, 93 and 183 for phenotyping CD4⁺ CD8⁺ (T-helper cells), CD8⁺ CD4⁺ (cytotoxic T-cells), CD4⁺ C25⁺ Foxp3⁺ (regulatory T-cells), CD45RA⁺ (B-cells) and CD3⁺ CD161⁺ cells (NK cells). The rats were sacrificed on the last day of dosing or following a 3-month postdose period.

It is important to note that the abatacept doses exceed those required for activity in both man and rodent. In the above described studies examining antibody response to KLH in mice during abatacept exposure, the dosing regimens in this study exceed the dose (16

mg/kg/week) required to inhibit antibody by ~4 to 40-fold. The high dose of 200 mg/kg was anticipated to produce systemic exposures to abatacept that were as much as 11 times greater than in human adults at a single dose of 10 mg/kg once monthly.

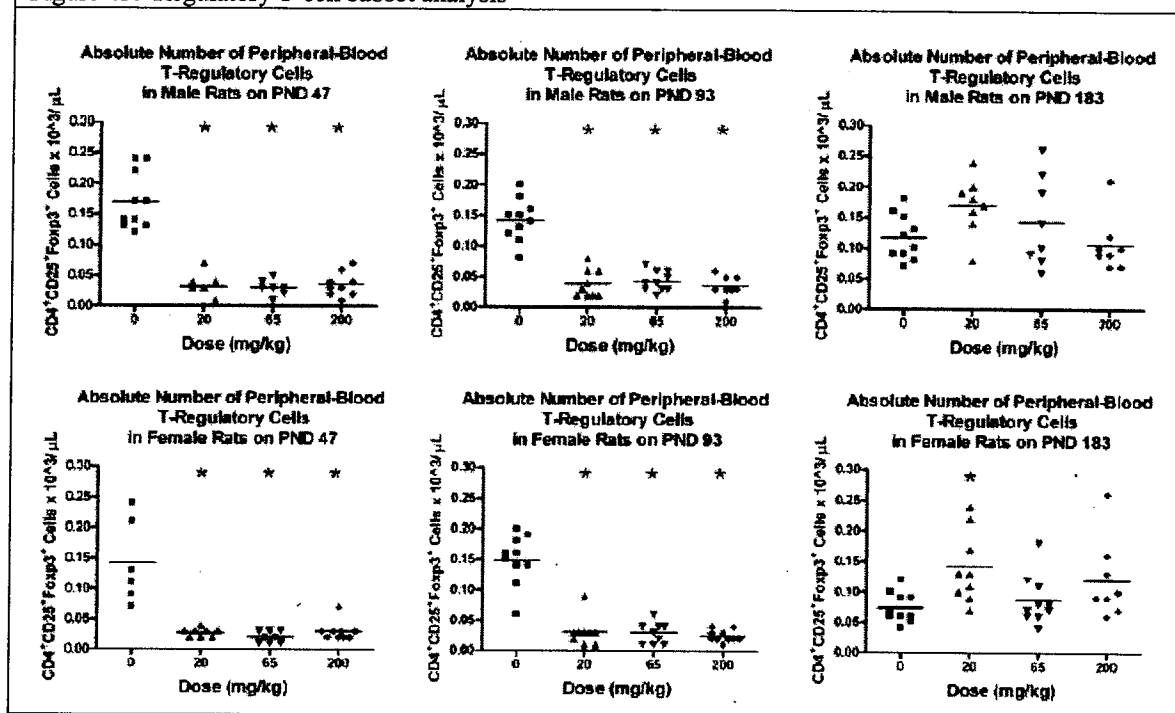
In lymphoid organs, increases in thymic and spleen weight were noted. Overall the absolute number of T-cells was increased in belatacept treated rats. This difference was mainly defined by an increase in CD4⁺ T-cells in belatacept treated rats (Figure 42). These effects began during the late-juvenile phase (PNDs 42 to 56), and generally progressed to the end of the dosing period (PND 93). Absolute numbers of peripheral-blood T cells and T-helper cells were increased 153 to 310% and 161 to 360% of control, respectively. Following the 3-month postdose period, total T-cell and T-helper cell numbers fell to control levels in females but remained elevated in males suggesting that the effect on these lymphocyte subsets was partially (males) to fully (females) reversible by this timepoint.

Figure 42. Absolute and T-helper cell counts in belatacept treated rats



Even though the absolute the number of CD4⁺ cells increased, the absolute number of T-regulatory cells decreased (Figure 43). T-regulatory cells were decreased 14 to 29% of control at the 20 mg/kg dose on PNDs 47 and 93. Regulatory T-cell numbers were similarly decreased in both sexes and on PND 93. These cells began to recover by day 183.

Figure 43. Regulatory T-cell subset analysis



An overall summary of the immunological changes is depicted in Table 18. Abatacept also progressively altered serum IgG concentration and T-cell-dependent antibody responses to keyhole limpet hemocyanin (KLH) at all doses.

Table 18. Summary of drug related changes in clinical immunology

Parameter		PND	Males			Females		
			20 mg/kg	65 mg/kg	200 mg/kg	20 mg/kg	65 mg/kg	200 mg/kg
Total T-cell (CD3 ⁺)		47	1.61x*	1.66x*	1.86x*	2.24x*	1.90x*	2.03x*
		93	2.95x*	3.60x*	3.23x*	2.64x*	2.60x*	2.65x*
		183	1.86x*	2.07x*	1.89x*	1.56x	1.39x	1.49x
T-Helper (CD4 ⁺ CD8 ⁻)		47	1.53x*	1.61x*	1.74x*	1.97x*	1.74x*	1.80x*
		93	2.60x*	3.10x*	2.66x*	2.31x*	2.17x*	2.20x*
		183	1.65x	1.89x*	1.67x*	1.47x	1.27x	1.33x
T-Regulatory (CD4 ⁺ CD25 ⁺ Foxp3 ⁺)		47	0.18x	0.18x*	0.24x	0.21x*	0.14x*	0.21x*
		93	0.29x	0.29x*	0.29x	0.20x*	0.20x*	0.20x*
		183	1.42x	1.17x	0.92x	2.00x*	1.29x	1.71x
Serum IgG		42	0.44x*	0.29x*	0.16x*	0.35x*	0.23x*	0.14x*
		93	0.08x*	0.07x*	0.04x*	0.09x*	0.05x*	0.03x*
		183	0.41x*	0.21x*	0.27x*	0.36x*	0.28x*	0.42x*
KLH Specific Antibody	IgM	48	0.15x	0.14x	0.15x	0.16x*	0.10x*	0.15x*
		176	2.01x	0.45x	0.42x	0.59x	0.89x	0.67x
	IgG	56	0.03x	0.03x	0.04x	0.02x	0.02x	0.02x
		184	0.76x	0.15x	0.03x*	1.52x	0.50x	0.10x
"x" = Treated group mean ÷ control group mean								
Statistical significance (= p ≤0.01).								

Pathological findings were not noted in the thymus but in the spleen expanded T-cell areas were noted within the marginal zone and as prominent periarteriolar lymphoid sheaths (PALS) in the spleens of abatacept treated animals (Table 19). In mesenteric and mandibular lymph nodes, expanded paracortical zones and decreased B-cells in germinal centers were seen in abatacept treated animals. Immunofluorescent analysis revealed expanded T-cell areas. In nonlymphoid organs, lymphocytic infiltration of the thyroid (thyroiditis) and pancreatic islet cells (insulinitis) at all doses was seen in some animals (overall incidence at the end of dosing of 24 and 9% for thyroiditis and insulinitis respectively). This autoimmune pathology increased throughout the recovery period. These findings suggest an autoimmune component and may be linked to the decreases in regulatory T-cells or a perturbation in thymic selection of self reactive T-cells seen in these animals at these doses. Withdrawal of immunosuppressant may exacerbate such immune cell mediated pathology.

Table 19. Summary incidence of drug-related histopathologic findings in rats

Histopathologic Finding		PND	Males				Females			
			Vehicle	20 mg/kg	65 mg/kg	200 mg/kg	Vehicle	20 mg/kg	65 mg/kg	200 mg/kg
Mesenteric Lymph Node	Paracortical Zone expansion	93	-	5	3	3	-	7	3	8
		183	-	-	-	-	-	-	-	-
	Germinal Centers decreased	93	-	8	10	9	-	10	10	10
		183	-	0	5	3	-	-	-	-
Mandibular Lymph Node	Paracortical Zone expansion	93	.*	3	5	3	-	7	4	6
		183	-	-	-	-	-	-	-	.*
	Germinal Centers decreased	93	.*	9	9	8	-	9	10	7*
		183	-	1	3	8	1	-	1	.*
Spleen	PALS prominent	93	-	9	10	9	-	10	10	10
		183	-	8	9	9	-	-	-	-
	Marginal Zone expansion	93	-	8	10	9	-	8	7	10
		183	-	-	-	1	-	-	-	-
Thyroid gland	Thyroiditis	93	-	2	3	2	-	2	1	4
		183	-	7	7	5	-	8	8	6
Pancreas	Islet inflammation	93	-	2	2	-	-	-	-	1
		183	-	3	6	4	-	-	2	1

PALS = perarteriolar lymphoid sheath
n= 10 rats evaluated/sex/group except where indicated (* = 7 rats evaluated)
“-“ = 0

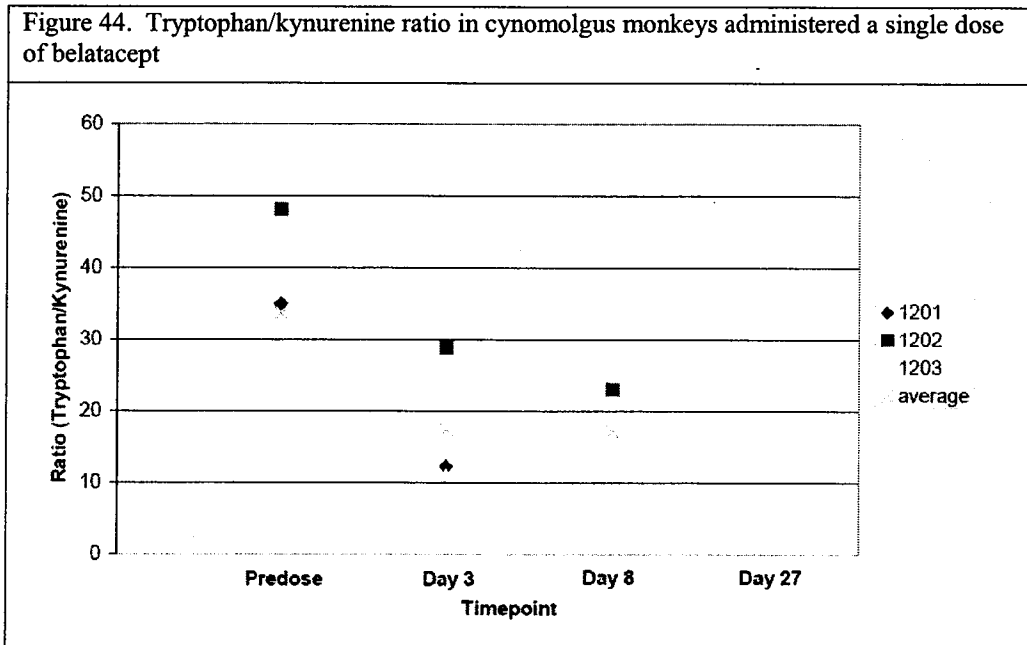
Similar studies have been performed in adult and juvenile rats with similar results regarding changes in peripheral immunophenotypes (Studies DS07166, DS07165).

Induction of indoleamine 2,3 dioxygenase

A study examined the bioactivity of belatacept after a single dose was administered to cynomolgus monkeys (Study DS06045). As stated above, belatacept binds to the APC and may prevent T-cell activation through blocking CD80/CD86 and CD28 and CTLA-4 interactions on the APC and T-cell respectively. It is unknown whether the immunosuppressive effect of belatacept could be due in part to the interaction of belatacept with CD80/CD86 on the APC similar to the interaction of CTLA-4 with CD80/CD86. As mentioned above in section 2, IDO catalyses the conversion of tryptophan to kynurenine. Studies have demonstrated the ability of IDO to inhibit the proliferation of intracellular pathogens and tumor cells by consumption of the essential amino acid tryptophan. Other studies have suggested that IDO also acts as a natural regulator and suppressor of T-cell responses. In this study, the activity of belatacept against antigen presenting cells (APCs) was determined by examining the activity of IDO. The activity of IDO can be indirectly extrapolated from the measurement of serum tryptophan (T) and kynurenine (K). Theoretically, stimulation of IDO activity should decrease tryptophan concentrations while kynurenine concentrations increase. Therefore, decreases in the T/K ratio may be indicative of stimulation of IDO activity.

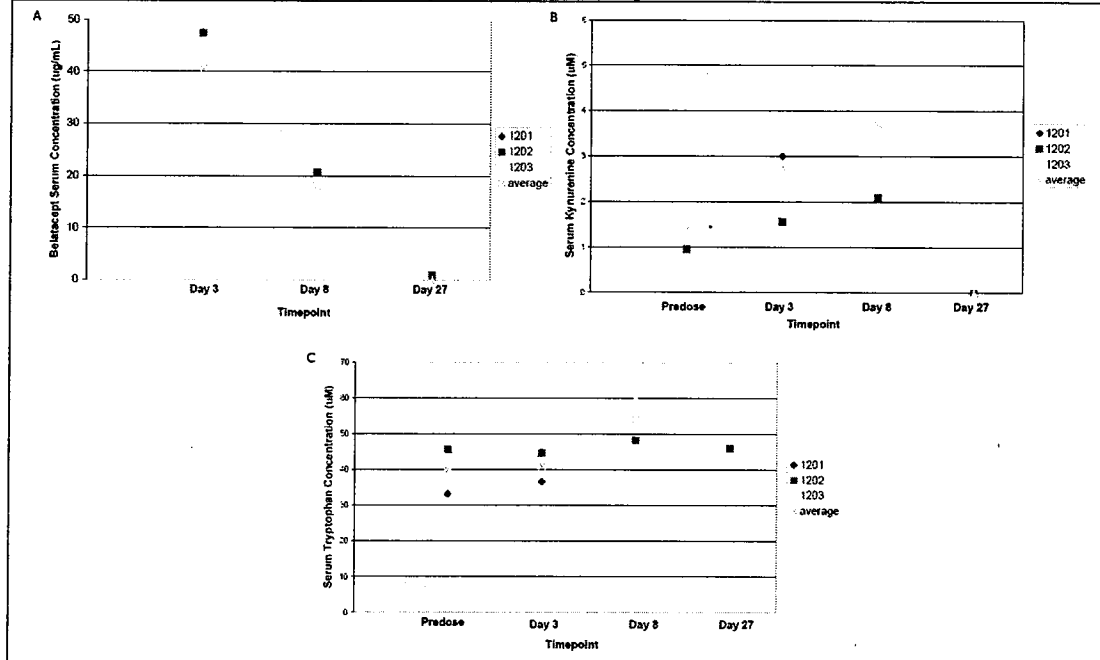
Three female cynomolgus monkeys received belatacept (10 mg/kg, i.v.) on day 0. Blood samples were collected pre-dose and on days 3, 8 and 27. Serum tryptophan and kynurenine were measured from blood samples using high performance liquid chromatography (HPLC). Serum concentrations of belatacept were measured by ELISA.

It is noted that one of the monkeys (#1201) died on day 5 due to unrelated reasons (injury). The results showed a decrease in the T/K ratio in the serum of the primates (Figure 44).



Measurements showed that kynurenine levels increased by day 3 following a single dose of belatacept and continued to increase through day 8. As belatacept was cleared from the monkey by day 27, serum kynurenine fell to undetectable levels (Figure 45-A and B). No effect on serum tryptophan levels was noted (Figure 45-C). The applicant contends that changes in serum tryptophan may not be relevant in this setting and the activity of belatacept to stimulate IDO in lymphoid tissues would decrease local concentrations of tryptophan while mediating an increase in serum kynurenine as it diffused into circulation. This remains possible, and more research regarding measurement of kynurenine levels as a biomarker of belatacept activity should be conducted.

Figure 45. Serum concentrations of (A) belatacept, (B) kynurenine and (C) tryptophan in cynomolgus monkeys following a single dose of belatacept



Effect on susceptibility to infection

An experiment was conducted to determine if abatacept affects outcome in a mouse model of chronic tuberculosis (Study DS04081). Abatacept was evaluated in a murine model of chronic tuberculosis. C57BL/6 mice were intranasally challenged with an aerosol of 15 cfu of *M. tuberculosis*. A chronic infection developed over 4 months at which time the mice were subcutaneously injected weekly with 0.5 mg/ mouse abatacept, anti-TNF- α antibody [MP6-XT22 (0.5 mg/mouse)] or phosphate buffered saline. Mice were then evaluated for survival, body weight, general health, bacterial load, histological assessment of the lung and spleen and IFN- γ production in the lung and lymph nodes by a cell based ELISpot assay measuring IFN- γ from T-cells in response to mycobacterial antigens. A cohort of each treatment group was sacrificed following 1, 2, 3, 4, 6, 8 and 16 weeks (n=6/group) of treatment. The results showed that all of the mice treated with abatacept survived through the observation period. These mice showed no difference in body weight, bacterial burden or IFN- γ production. In contrast, mice treated with anti-TNF- α antibody died before week 9 and were of decreased weight, increased bacterial burden and showed less IFN- γ production. This experiment was not performed with belatacept.

An experiment was conducted to determine if abatacept alters host resistance to *Pneumocystis jiroveci* and murine cytomegalovirus (MCMV) in a mouse model of skin grafting (Study 910044345). The study sought to determine if:

- abatacept compromised the host's immune response to primary infection with MCMV or PC

- prior abatacept therapy compromises the host's ability to respond to primary infection
- PC or MCMV infection promotes graft rejection through activation of the immune system in abatacept treated skin transplant-tolerant animals.

The results showed that administering abatacept (200µg, twice weekly for 2 weeks) to BDF1 mice in the MCMV model and abatacept (200µg, twice weekly for 3 weeks) in the PC model did not significantly alter clearance of infection in intact mice. An elevation of the viral titer was noted on day 4 and 21 in the MCMV infected mice treated with abatacept but no difference in clearance time was reported. In another model, mice were treated with abatacept at the time of skin graft (once daily for 7 days) and then infected with PC or MCMV once the skin graft was well established (100 days post grafting). No significant differences were noted other than an increased viral titer in MCMV infected mice on days 10 and 21 following infection, which did not alter final outcome. Neither MCMV nor PC infection caused rejection of the established skin graft. These results suggest that abatacept treatment prior to or at the time of infection does not immunosuppress the mouse to an extent that alters its ability to resist infection.

The effect of belatacept in any of these models was not explored.

4. Clinical Microbiology

The results of three pivotal clinical trials were submitted by the applicant in support of this BLA (IM103008, IM103027, IM103100). The results of the trials have indicated that belatacept prevents acute rejection similar to comparator (CsA) though not all margins were met when statistically analyzing these endpoints. Belatacept also decreased incidence of antibody mediated rejection and may not have the same extent of deleterious effects on long term renal function as current CNI based regimens such as decreased glomerular filtration rate due to nephrotoxicity (for details see Medical Officer's review).

4.1 Study IM103008

This was a phase 3, randomized, partially-blinded, active-controlled, parallel group study also known as the "Belatacept Evaluation of Nephroprotection and Efficacy as First-line Immunosuppression Trial (BENEFIT)". The study was conducted at 104 sites. The primary efficacy objectives of the trial were:

- Composite of subject and graft survival by 12 months
- Composite of measured glomerular filtration rate (GFR) < 60 mL/min/1.73 m² at month 12 or a decrease in measured GFR ≥ 10 mL/min/1.73 m² from month 3 to month 12
- Incidence of acute rejection

Secondary efficacy objectives were:

- Measured GFR at 12 months
- Biopsy-proven chronic allograft nephropathy (CAN) at 12 months

The trial enrolled 738 patients, of which 666 were randomized and transplanted (ITT population). Subjects were randomized and transplanted with organs from standard criteria donors on day 1; the day 1 dose of the following drug regimens was given pre-operatively for the 36 month study:

- Belatacept MI regimen (n=219) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 3 (weeks 2, 4, 6, 8, 10, and 12) and then every 4 weeks through month 6 (weeks 16, 20, and 24). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Belatacept LI regimen (n=226) - intravenous belatacept (10 mg/kg) on days 1, 5, 14 and 28, then every 4 weeks through the end of month 3. At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Active control (n=221) – oral CsA given twice daily to achieve serum trough concentrations of 150 – 300 ng/mL during the first month and 100 – 250 ng/mL thereafter. If delayed graft function was diagnosed, patients may have received polyclonal anti-lymphocyte therapy until CsA could be initiated.

All subjects also received a background immunosuppressive regimen consisting of basiliximab induction, mycophenolate mofetil (MMF) and corticosteroid maintenance therapy.

The pertinent results of the trial as reported by the applicant at month 12 are as follows (Table 20):

Table 20. Primary and secondary endpoint based results for BENEFIT trial IM103008			
Endpoint:	Belatacept MI	Belatacept LI	CsA
Subject and graft survival (n, %)	209 (95.4%)	218 (96.5%)	205 (92.8%)
Composite endpoint for measured patients with GFR < 60 or decrease ≥ 10 mL/min/1.73 m ² from Month 3 to 12 (n, %)	115 (55.0%)	116 (54.2%)	166 (77.9%)
Measured GFR (mL/min/1.73 m ²) (mean, standard deviation)	65.0 (30.0)	63.4 (27.7)	50.4 (18.7)
Acute rejection (n, %)	48 (21.9%)	39 (17.3%)	16 (7.2%)
Prevalence of CAN (n, %)	40 (18.3%)	54 (23.9%)	71 (32.4%)

The overall conclusions as summarized by the applicant:

- Patient and graft survival were comparable and met the pre-specified 10% non-inferiority (NI) margin

- Belatacept (MI and LI) resulted in improved renal function at 12 months as measured by GFR
- Belatacept (MI and LI) resulted in increased acute rejection at month 12. The LI regimen met the 20% NI margin whereas the incidence of acute rejection in the MI regimen did not meet the NI margin

Epstein Barr virus (EBV) serological status for donors and recipients was determined prior to randomization. Post transplant lymphoproliferative disease (PTLD) developed in some patients following transplant. Table 21 summarizes this data. There were 3 cases of PTLD in EBV (+) recipients among the 577 enrolled and all occurred in belatacept treated patients (incidence: 3/393 or 0.7% for belatacept treated and 0/184 or 0% for CsA treated patients). One-third of the cases in EBV (+) recipients treated with belatacept resulted in central nervous system (CNS) involvement. Among the 89 EBV (-) recipients, 3 cases of PTLD were reported (incidence 2/52 or 3.8% for belatacept treated and 1/37 or 2.7% for CsA treated patients). One-half of the cases in EBV (-) recipients treated with belatacept resulted in CNS involvement.

Table 21. EBV status and PTLD results for IM103008								
Treatment Arm	Donor EBV Status		Recipient EBV Status		(+) Recipient PTLD		(-) Recipient PTLD	
	Positive	Negative	Positive	Negative	No. total cases	No. w/ CNS involvement	No. total cases	No. w/ CNS involvement
Belatacept LI*	163	26	199	27	2	0	0	0
Belatacept MI*	140	40	194	25	1	1	2	1
CsA*	155	31	184	37	0	0	1	0
Total	458	97	577	89	3	1	3	1
* There were 37, 39, and 35 donor EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively.								

4.1.2 EBV assays used in Study IM103008

Of the 666 sample pairs (donor/recipient), 655 were not processed by a central lab. The site laboratory is not listed for 52 subjects and the other samples were tested at 82 different laboratories. A total of 71 different testing panels were used on the patients at these locales. The test used for 147 patients was not listed. Appendix 1-A lists the different panels, manufacturers and the sensitivity and specificity of each assay or panel as they were reported by the applicant.

4.2 Study IM103027

This was a phase 3, randomized, partially-blinded, active-controlled, parallel group study also known as the “Belatacept Evaluation of Nephroprotection and Efficacy as First-line

Nulojix® (Belatacept)

Bristol Myers Squibb

Immunosuppression Trial – EXTended Criteria Donors (BENEFIT-EXT)”. The study was conducted at 79 sites. The primary efficacy objectives of the trial were:

- Composite of subject and graft survival by 12 months
- Composite of measured glomerular filtration rate (GFR) < 60 mL/min/1.73 m² at month 12 or a decrease in measured GFR $\geq$ 10 mL/min/1.73 m² from month 3 to month 12

Secondary efficacy objectives were:

- Measured GFR at 12 months
- Biopsy-proven chronic allograft nephropathy (CAN) at 12 months
- Incidence of acute rejection

The trial enrolled 595 patients, of which 543 were randomized and transplanted (ITT population). Subjects were randomized and transplanted with kidneys from extended criteria donors on day 1; the day 1 dose of the following drug regimens was given pre-operatively for the 36 month study:

- Belatacept MI regimen (n=184) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 3 (weeks 2, 4, 6, 8, 10, and 12) and then every 4 weeks through month 6 (weeks 16, 20, and 24). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Belatacept LI regimen (n=175) - intravenous belatacept (10 mg/kg) on days 1 and 5, and then every 2 weeks through month 1 (weeks 2 and 4) and then every 4 weeks through month 3 (weeks 8 and 12). At this point, belatacept (5 mg/kg) was given every 4 weeks through completion of the trial.
- Active control (n=184) – oral CsA given twice daily to achieve serum trough concentrations of 150 – 300 ng/mL during the first month and 100 – 250 ng/mL thereafter. If delayed graft function was diagnosed, patients may have received polyclonal anti-lymphocyte therapy until CsA could be initiated.

All subjects also received a background immunosuppressive regimen consisting of basiliximab induction, mycophenolate mofetil (MMF) and corticosteroid maintenance therapy. The donor organ had to meet at least 1 of the following conditions to be considered extended criteria to be transplanted into patients participating in this trial:

- Donor age $\geq$ 60 years
- Donor age 50 – 59 years and 1 of the following: cerebrovascular accident (CVA) + hypertension + serum creatinine (SCr) > 1.5 mg/dL or CVA + hypertension or CVA + SCr > 1.5 mg/dL or hypertension + SCr > 1.5 mg/dL
- Anticipated cold ischemic time $\geq$ 24 hours
- Donor with cardiac death (non-heart beating donor)

4.2.1 Study Results

The pertinent results of the trial as reported by the applicant are as follows (Table 22):

Table 22. Primary and secondary endpoint based results for BENEFIT trial IM103027			
Endpoint:	Belatacept MI	Belatacept LI	CsA
Subject and graft survival (n, %)	158 (85.9%)	154 (88.0%)	156 (84.8%)
Composite endpoint for measured patients with GFR < 60 or decrease ≥ 10 mL/min/1.73 m ² from Month 3 to 12 (n, %)	124 (70.5%)	129 (76.3%)	151 (84.8%)
Measured GFR (mL/min/1.73 m ²) (mean, standard deviation)	52.1 (21.9)	49.5 (25.4)	45.2 (21.1)
Acute rejection (n, %)	32 (17.4%)	31 (17.7%)	26 (14.1%)
Prevalence of CAN (n, %)	82 (44.8%)	80 (46.0%)	95 (51.6%)

The overall conclusions as summarized by the applicant:

- Patient and graft survival were comparable and met the pre-specified 10% non-inferiority (NI) margin.
- Belatacept (MI) resulted in improved renal function at 12 months as measured by GFR; for belatacept (LI), the results favored the LI regimen over the CsA based regimen though they were not statistically significant.
- Belatacept (MI and LI) resulted in an increase of observed acute rejection at month 12. However, both of the belatacept regimens met the 20% NI margin.

EBV status for donors and recipients was determined prior to randomization. There were 91, 90, and 105 donor EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively. PTLTD was developed in 6 patients in the belatacept arms (Table 23). There were 3 cases of PTLTD among the 325 EBV (+) recipients receiving belatacept (incidence: 0.6% for belatacept treated and 0% for CsA treated patients). All of the PTLTD cases in EBV (+) recipients treated with belatacept resulted in central nervous system (CNS) involvement. Among the 33 EBV (-) recipients, 3 cases of PTLTD were reported (incidence: 9.1% for belatacept treated and 0% for CsA treated patients). Two of the PTLTD cases in EBV (-) recipients treated with belatacept resulted in CNS involvement.

Table 23. EBV status and PTLD results for IM103027

Treatment Arm	Donor EBV Status		Recipient EBV Status		(EBV+) Recipient PTLD		(EBV-) Recipient PTLD	
	Positive	Negative	Positive	Negative	No. total cases	No. w/ CNS involvement	No. total cases	No. w/ CNS involvement
Belatacept LI*	71	13	156	19	2 [†]	2	2	1
Belatacept MI*	81	13	169	14	1	1	1	1
CsA*	69	10	168	16	0	0	0	0
Total	221	36	493	49	3	3	3	2

* There were 91, 90, and 105 donor EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively. One recipient status in the belatacept MI group was unknown.
[†] One additional case of PTLD is added which was reported by the applicant in December of 2009 (See IND 9418, SDN481).

4.2.2 EBV assays used in Study IM103027

Of the 543 sample pairs (donor/recipient), 499 were not processed by a central lab. The site laboratory is not listed for 50 subjects and the other patient samples were tested at 63 different laboratories. A total of 55 different testing panels were used on the patients at these locales. The test used for 105 patients was not listed. Appendix 1-B lists the different panels, manufacturers and the sensitivity and specificity of each assay or panel as they were reported by the applicant. For more information regarding the performance of the EBV assays and the development of PTLD, see section 4.4.

4.3 Study IM103100

This was a phase 2, open-label, randomized, controlled study. The study was conducted at 41 sites. The primary objective of the trial was to assess the efficacy as measured by the incidence of clinically-suspected and biopsy proven acute rejection at 6 months. Secondary efficacy objectives included graft survival and function as well as safety parameters.

4.3.1 Study Results

The trial enrolled 216 patients who were randomized and transplanted (ITT population). Subjects were randomized and transplanted on day 1; the day 1 dose of the following drug regimens was given pre-operatively for the 36 month study:

- Belatacept MI regimen (n=74) - same as IM103008
- Belatacept LI regimen (n=71) - intravenous belatacept (10 mg/kg) on days 1, 15, 29, 57 and 85 and then every 4 or 8 weeks to achieve a serum trough of 2 or 0.25 µg/mL through completion of the trial. It is important to note that this regimen

lacks the day 5 dose given for the LI regimen in the phase 3 trials described above.

- Active control (n=71) – oral CsA given twice daily same as IM103008.

All subjects also received a background immunosuppressive regimen as in IM103008. The pertinent results of the trial as reported by the applicant are as follows (Table 24):

Table 24. Primary and secondary endpoint based results for trial IM103100			
Endpoint:	Belatacept MI	Belatacept LI	CsA
Death or graft loss (up to 12 months)	4 (5.4%)	1 (1.4%)	6 (8.2%)
Acute rejection (through 6 months) (n, %)	5 (6.8%)	4 (5.6%)	6 (8.2%)
Acute rejection (through 12 months) (n, %)	14 (18.9%)	21 (29.6%)	13 (17.8%)
Prevalence of CAN (n, %)	15 (28.8%)	11 (20.4%)	20 (44.4%)

The overall conclusions as summarized by the applicant are:

- Patient and graft survival of belatacept treated patients were comparable to those treated with CsA
- Renal function at 12 months as measured by GFR appeared better in the belatacept (MI and LI) arms compared to CsA
- Numerically lower rates of CAN in belatacept treated patients

Epstein Barr virus (EBV) status for donors and recipients was determined prior to randomization. There were 42, 44, and 41 donor EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively. There were 20, 27, and 20 recipient EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively. It appears that the EBV serological status of many recipients was unknown; this contrasts with the phase 3 studies. Post transplant lymphoproliferative disease developed in some patients (Table 25). There were no cases of PTLN in EBV (+) recipients among the 134 enrolled. Among the 15 EBV (-) recipients, 2 cases of PTLN were reported and both occurred in the belatacept MI regimen (incidence 2/11 or 18.2% for belatacept treated patients and 0/4 or 0% for CsA treated patients). Both of the cases in EBV (-) recipients treated with belatacept resulted in CNS involvement. There were also 2 cases of PTLN reported among patients for whom EBV status was unknown, 1 case occurred in a patients in the belatacept MI arm and the other in a CsA treated patient. Neither case involved the CNS.

Table 25. EBV status and PTLT results for IM103100

Treatment Arm	Donor EBV Status		Recipient EBV Status		(EBV +) Recipient PTLT		(EBV -) Recipient PTLT		Unknown Recipient PTLT	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Belatacept LI*	25	4	46	5	0	0	0	0	0	0
Belatacept MI*	21	9	41	6	0	0	2	2	1	0
CsA*	27	3	47	4	0	0	0	0	1	0
Total	73	16	134	15	0	0	2	2	2	0

* There were 42, 44, and 41 donor EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively. There were 20, 27, and 20 recipient EBV status results that were unknown for belatacept LI, belatacept MI or CsA treated patients, respectively.

4.3.2 EBV assays used in Study IM103100

None of the sample pairs (donor/recipient) were processed by a central lab. The site laboratory is not listed for 38 subjects and the other samples were tested at 17 different laboratories. A total of 15 different testing panels were used on the patients at these locals. The test used for 38 patients was not listed. Appendix 1-C lists the different panels, manufacturers and the sensitivity and specificity of each assay or panel as they were reported by the applicant. It also appears that at least 12 patients only had antibodies of the IgM isotype assayed. It is also noted that some patients had a single IgG antibody test while others had more than one. For more information regarding the performance of the EBV assays and the development of PTLT, see section 4.4.

4.4 Overall summary of EBV analysis results

4.4.1 Overview of assays to determine donor/recipient EBV status

The most important parameter for determining recipient EBV susceptibility is based on the presence of antibodies which indicate past infection. Effective diagnosis of EBV disease can be accomplished by testing for antibodies to several EBV-associated antigens. These antibodies include IgM and IgG antibodies specific for viral capsid antigen (VCA), Epstein Barr nuclear antigen-1 (EBNA-1) and early antigen (EA). The titers of these antibodies vary depending on the immune status of the patient and/or past exposure (Figure 46).

Figure 46. Antibody titers to EBV antigens distinguish between primary and resolved infection

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*taken from Gulley ML, and W Tang, 2008, *J Mol Diagn.*, 10:279.

It is important to note that the results of a VCA IgM assay do not aid in determining past infection with EBV since the IgM assay is only specific in identifying acute infection at the time of blood draw. Also, a polymerase chain reaction (PCR) specific for EBV is typically used to measure viral load during active infection and is not useful in determining past infection. It should be noted that some patients in the trials were only tested for EBV-specific IgM or a PCR to determine serological status. It is assumed that the majority of the transplant patients enrolled in clinical trials will test negative for IgM (i.e. they do not have an active EBV infection at the time of testing) and those that do test positive are not enrolled until convalescence.

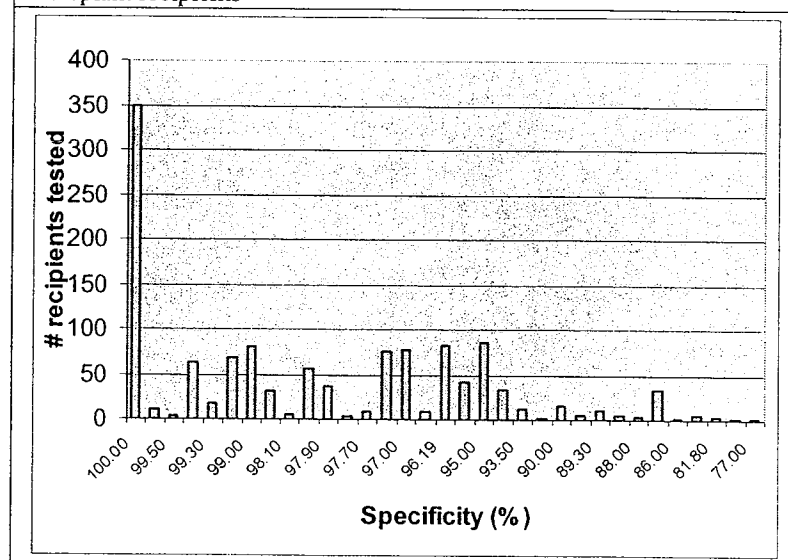
On the other hand, there are multiple IgG antibodies which can be measured in order to determine past infection with EBV. IgG antibodies to VCA and EBNA persist for life in most people. IgG antibodies to EA may also be present, but this titer tends to decrease to an undetectable level within months of clearing the primary infection. Reappearance or rising titers of antibody to EA is indicative of reactivation of EBV if noted in the presence of clinical signs of disease. False-positive VCA antibody titer results may occur on the basis of cross-reactivity with other herpes viruses, e.g. CMV. False positive results may also occur due to polyclonal activation of B-cells by CMV, *T. gondii*, rubella, adenovirus or an unknown agent. A panel which yields the results for more than one IgG antibody to one of the different antigens (e.g., VCA, EBNA, EA) may be more specific than the result of a single IgG antibody test for determining past infection. Typically EBV panels are interpreted as shown in Table 26. Patients who are negative for all antibodies are considered to have never been infected with EBV. For a patient to be considered as having a past infection, the patient should test positive for IgG antibodies against VCA and EBNA.

Table 26. Interpretive criteria for determining primary and past EBV infection				
VCA IgG	VCA IgM	EBNA IgG	EA IgG	Interpretation
-	-	-	-	No infection, past or present
-	+	-	-	Early phase of primary infection
+	+	-	-/+	Acute phase of primary infection
+	+	+	+/-	Late phase of primary infection/reactivation
+	-	+	-/+	Past infection

4.4.2 EBV Serostatus and Development of PTLD in the clinical trials of belatacept

Serology was performed to determine if the donor or recipient of the allograft had detectable levels of antibody to different EBV viral antigens. The central laboratory utilized 4 ELISA based assays to determine a recipient or donor EBV status. The central laboratory was (b) (4) and utilized Diasorin ELISAs for VCA-IgG, VCA-IgM (reverse ELISA), EA-IgG and EBNA IgG. However, only 55 of the 1425 recipients had EBV serology performed at the central laboratory. Overall, 81 different testing panels were used in the 3 clinical trials at 95 different laboratories. The sponsor provided product brochures for 31 of the assays. The sponsor also provides the specificity and sensitivity of many of the assays. As discussed below, the specificity of the assay will be an important performance characteristic since recipients who test as false positive and receive belatacept will be at increased risk for PTLD. Figure 47 shows the distribution of the assays' specificities and how many recipients were tested at each specificity. Please note that a single patient may have been tested with more than one assay as part of an assay panel to determine EBV serostatus. The results are only given for IgG tests since these are germane to determining past infection/immunity. The range of specificities was 77-100% and the median 96.1%.

Figure 47. Distribution of specificities of EBV IgG tests used on renal transplant recipients



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Table 27 lists a summary of PTLT cases across clinical trials IM103008, IM103027, and IM103100. Sixteen cases of PTLT were reported from these trials: IM103008 (n=6), IM103027 (n=6) and IM103100 (n=4). Fourteen of the PTLT cases were in belatacept treated arm, and 2 cases were in CsA treated arm. Of the fourteen PTLT cases in the belatacept arms, 8 occurred in patients receiving the MI regimen and 6 occurred in patients receiving the LI regimen. In sum, all three trials enrolled 477, 472, and 476 patients in the belatacept MI, belatacept LI, and CsA arms, respectively. Please note that this represents an incidence of PTLT in 14/949 (1.5%) and 2/476 (0.42 %) of the patients treated with belatacept (both regimens) and CsA respectively.

Table 27. Incidence of PTLT among all patients enrolled in clinical trials			
Clinical Trial	Treatment Arm	Total Enrolled	Patients with PTLT (%)
IM103008	Belatacept MI	219	3 (1.4%)
	Belatacept LI	226	2 (0.9%)
	Cyclosporine A	221	1 (0.5%)
IM103027	Belatacept MI	184	2 (1.1%)
	Belatacept LI	175	4 (2.3%)
	Cyclosporine A	184	0 (0%)
IM103100	Belatacept MI	74	3 (4%)
	Belatacept LI	71	0 (0%)
	Cyclosporine A	71	1 (1.4%)
Total		1425	16 (1.1%)

Of the fourteen PTLT cases in belatacept arms, 7 cases occurred in EBV negative recipients, 6 cases occurred in EBV positive recipients and 1 case was of unknown recipient status (Table 28). Of the 7 recipients treated with belatacept who were seronegative for EBV at the time of transplant, 2 received a kidney from an EBV seropositive donor, 4 were of unknown status and 1 was seronegative. A total of 96 recipients treated with belatacept were EBV negative prior to transplant. It appears that EBV negative recipients receiving belatacept were at the highest risk of developing PTLT (7/96 or 7.3% of EBV negative patients on belatacept compared to 1/57 or 2.3% for CsA treated patients). A comparison between belatacept and CsA can not be made for EBV positive patients who developed PTLT because no EBV positive recipients developed PTLT in the CsA arms. It is assumed that PTLT occurs in EBV positive recipients receiving CsA at some rate greater than 0%, however it appears that these trials did not enroll enough patients in the CsA arms to determine this rate. As it appears now, EBV positive recipients receiving belatacept are also at increased risk of developing PTLT (6/805 or 0.75% of EBV positive patients on belatacept compared to 0/399 or 0% for CsA treated patients). It should be noted that one recipient in the CsA arm was of unknown EBV status, as this patient represents fully one-half of the CsA patients who developed PTLT, knowing this patient's EBV status could critically affect the rates of PTLT listed in Table 28.

Table 28. Recipient EBV status prior to transplant and development of PTLT						
Treatment	EBV negative recipient		EBV positive recipient		Unknown Recipient Status	
	Total	Developed PTLT (% of total)	Total	Developed PTLT (% of total)	Total	Developed PTLT (% of total)
Belatacept MI	45	5 (11.1%)	404	2 (0.49%)	28	1 (3.6%)
Belatacept LI	51	2 (3.9%)	401	4* (1.0%)	20	0 (0%)
Cyclosporine A	57	1 (1.8%)	399	0 (0%)	20	1 (5%)
N.B.: MI = More intensive regimen; LI = Less intensive regimen						

Only a single PTLT case developed from a donor organ which was tested as EBV (-). The EBV serostatus of the donors to the recipients who developed PTLT for the remainder of the recipients were either EBV positive or of unknown status at the time of transplant (Table 29).

Table 29. Donor EBV status of post-transplant PTLT patients at time of transplantation				
Treatment	Total PTLT cases	Donor Status		
		Positive	Negative	Unknown
Belatacept MI	8	3	1	4
Belatacept LI	6	3	0	3
Cyclosporine A	2	1	0	1
Total	16	7	1	8

Of the fourteen PTLT cases in belatacept treated patients, 9 cases presented with CNS involvement (Table 30). Of these 9 cases, 6 occurred in belatacept MI regimen and 3 in the belatacept LI regimen. These represent 75% and 60% of the PTLT cases for the belatacept MI and LI regimens, respectively. All of the recipients who developed CNS PTLT were male. In total, the studies enrolled 450 females and 975 males. No patients in the CsA arm developed PTLT involving the CNS.

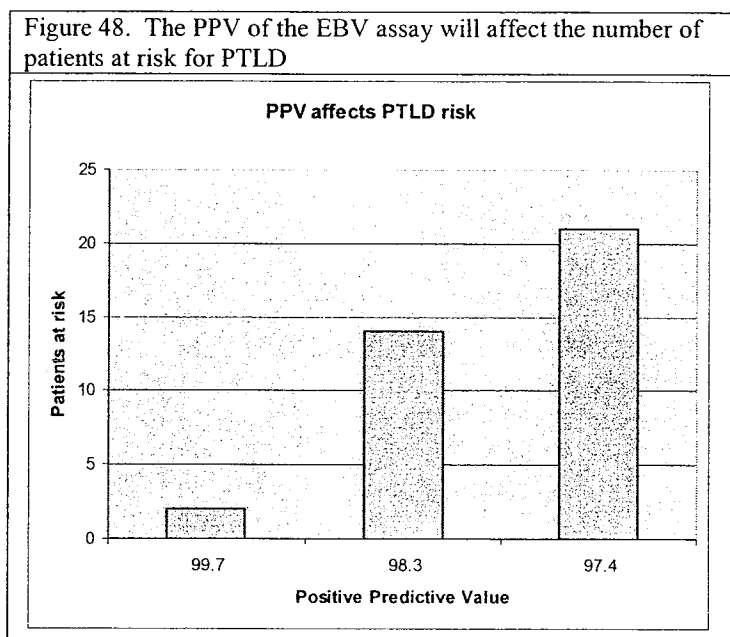
Table 30. PTLT cases with CNS involvement (% of PTLT cases involving CNS)		
Treatment	PTLT cases	Number of PTLT cases involving CNS (% of total)
Belatacept MI	8	6 (75%)
Belatacept LI	5	3 (60%)
Cyclosporine A	2	0 (0%)

It does not appear that EBV serostatus determined susceptibility as both EBV (+) and EBV (-) recipients developed CNS-PTLT (Table 31). Furthermore, both EBV (+) and EBV (-) donor organs were transplanted into recipients who would later develop CNS-PTLT.

Table 31. Recipient EBV status in PTLT cases with CNS involvement (% of PTLT cases involving CNS)						
Treatment	CNS PTLT cases	Recipient EBV status		Donor EBV status		
		Positive	Negative	Positive	Negative	Unknown
Belatacept MI	6	2	4	2	1	3
Belatacept LI	3	2	1	1	0	2
Cyclosporine A	0	0	0	0	0	0

The patients who appear to be most at risk for development of PTLT are EBV negative recipients. The applicant recommends that only patients who test as serologically positive for EBV antibodies which indicate past infection prior to transplant be treated with belatacept. If this recommendation is made, it appears that the performance of the assay used will be important. In particular, the specificity and PPV of the assay will be critical factors to consider.

As an example using the trials of belatacept which enrolled 805 EBV “positive” patients, more patients would have been put at risk of being a false positive if the specificity and hence PPV were lower (Figure 48). The PPV values of 99.7, 98.3 and 97.4 were calculated assuming 90% prevalence of EBV (+) serology and specificities of 98, 85 and 77 %, respectively. These false positive recipients, if administered belatacept, may have been at increased risk for EBV related PTLT since they are “true” negatives.



Less than 100% specificity allows the possibility of false positive results when screening EBV serology. A panel which yields positive IgG antibody results against different antigens (e.g. VCA, EBNA) may be more specific than the positive result of a single IgG antibody test for determining past infection against a single antigen. Patients who are negative for all antibodies are considered to have never been infected with EBV. For a

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patient to be considered as having a past infection, the patient should test positive for IgG antibodies against VCA and EBNA.

Table 32 summarizes the EBV serological tests used on the 16 patients who developed PTLT. Performance of these assays has shown some variation in comparative studies. For example, the applicant lists the specificity of the Captiva VCA IgG test as 100%, however another study found the specificity of this assay to be 97% (Broo *et al*, 2000, *Clin Diag Lab Immunol*, 7:451). The specificity of the IgG antibody based EBV tests in the 14 recipients with known EBV status was $\geq 96\%$.

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Table 32. Tests used to determine EBV status in the 15 patients who developed PTLD. (unk = unknown or not provided by applicant)								
Clinical Trial - Patient ID	Treatment Arm	Donor EBV status	Recipient EBV status	EBV test used	Assay Type	Manufacturer	Specificity	Sensitivity
103008 - 20019	CsA	+	-	VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103008 - 20060	Belatacept - LI	+	+	Captiva EBV VCA IgG	ELISA	Trinity Biotech	100%	IgG: 95.5%
103008 - 20256	Belatacept - LI	+	+	VCA IgM, EBNA IgG	ELISA	Zeus Scientific	89.9-97.5%, 98-100%	70.8-98.9%, 90-97.3%
103008 - 20039	Belatacept - MI	+	-	VCA IgG	ELISA	Wampole Laboratories	98%	98%
103008 - 20734	Belatacept - MI	+	+	LIAISON EBV IgM, LIAISON VCA IgG, LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103008 - 20548	Belatacept - MI	-	-	VCA IgG, VCA IgM	ELISA	Demeditec Diagnostics	100% 97.4%	98% 97.2%
103027 - 10029	Belatacept - LI	Unknown	-	EBNA-1 IgG	ELISA	Diamedix		
103027 - 10093	Belatacept - LI	Unknown	+	ETI-VCA-IgG, ETI-EBV-IgM reverse, ETI-EA-IgG, ETI-EBNA-IgG	ELISA	DiaSorin, Inc.	97%, 86%, 95%, 99%	98% 96% 95% 92%
103027 - 10593	Belatacept - LI	+	-	EBV-VCA (P-18) IgG	ELISA	Trinity Biotech	100%	95%
103027	Belatacept - LI	Unknown	+	VCA-EBV IgG/IgM	IFA	In-house	100%	100%
103027 - 10185	Belatacept - MI	+	+	LIAISON EBV IgM, LIAISON VCA IgG, LIAISON EBNA IgG	ELISA	DiaSorin, Inc.	99.22% 96.19% 97.61%	97.83% 98.54% 98.84%
103027 - 10447	Belatacept - MI	Unknown	-	Unknown	Unknown	Unknown	Unknown	Unknown
103100 - 00025	CsA	Unknown	Unknown	EBNA-1 IgG	ELISA	Diamedix	Unknown	Unknown
103100 - 00006	Belatacept - MI	Unknown	-	Unknown	Unknown	Unknown	Unknown	Unknown
103100 - 00031	Belatacept - MI	Unknown	Unknown	EBNA-1 IgG	ELISA	Diamedix	Unknown	Unknown
103100 - 00004	Belatacept - MI	Unknown	-	VCA IgG, EBNA-1 IgG	ELISA	Medac Diagnostika	97.9%, 98%	96.3%, 100%

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4.5 Effect on lymphocyte subsets

Lymphocyte subset analysis was performed on blood obtained from patients enrolled in phase 2 and 3 clinical trials of belatacept (Bluestone *et al.*, 2008, *Am J Transplant*, 8:2086). Patients after short term exposure to a belatacept containing regimen (within six months of transplant) were compared to those exposed to a long term regimen (3 – 5 years post transplant). The regimen was the same as the regimen proposed for approval and were comprised of basiliximab induction, MPA and steroids. PBMC were obtained from the patients and stained with conjugated antibodies to detect cell surface expressed CD127, CD25, CD8, CD4 and intracellular Foxp3. Results are based on the results of analysis of 10 – 22 patients and showed a decrease in CD25⁺ CD4⁺ regulatory T-cells in the first month following transplantation in patients treated with either belatacept or CsA (CNI) in combination with MMF and basiliximab induction (Figure 49A). Presumably, recipients at this time are more effected by basiliximab induction (i.e. anti-CD25) compared to longer term patients who appear to begin recovery of this subset by 3 months. It appears that a subset of Tregs, those not expressing CD25, were spared depletion and represent a higher proportion of FoxP3⁺ cells in the first month (Figure 49B). These data were confirmed by examining the ratio of both CD127^{lo} and FoxP3⁺ cells (both markers of Tregs) in CD25⁺ CD4⁺ cells (Figure 49 C and D).

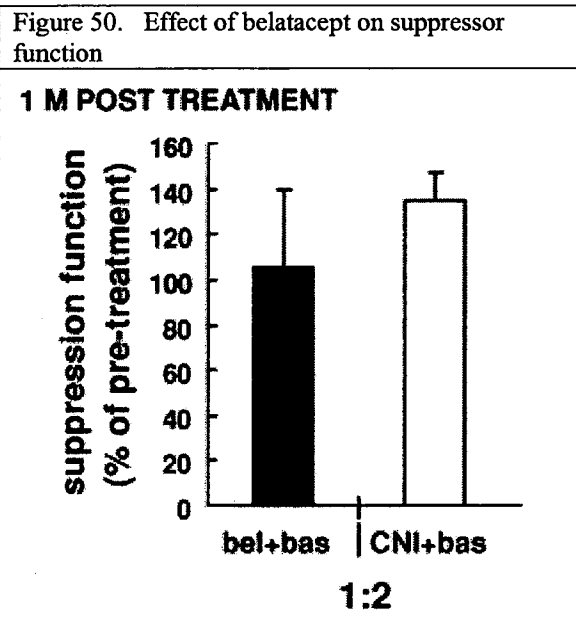
Figure 49. Short term effects of belatacept (bel) and basiliximab (bas) treatment on regulatory T-cells

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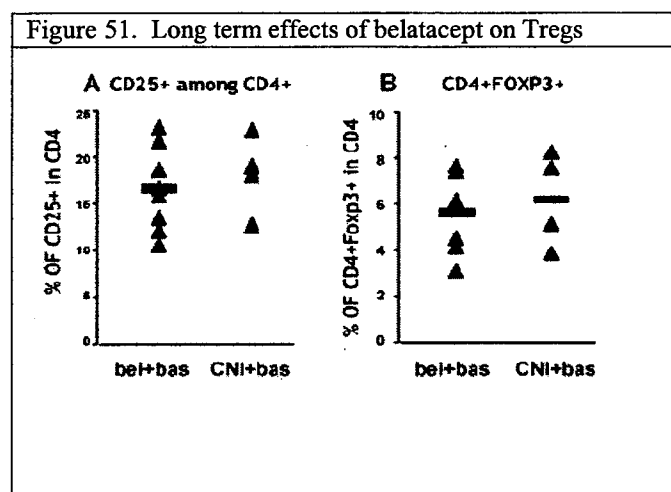
Another aliquot of cells was also analyzed in a Treg suppression assay. Regulatory T-cells (CD4⁺CD127^{lo}) were cultured with PBMC labeled with CFSE from an unrelated donor (1:2 ratio) and stimulated with anti-CD3 and anti-CD28. Cultures were incubated for 4 days and then CD8⁺ cells were analyzed by flow cytometry for CFSE dilution. In the absence of suppressor activity, the labeled T-cells will proliferate and dilute CFSE with each successive generation. Suppressor activity is seen as a

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inhibition of this proliferative response. The percentage of suppressive activity was compared in blood samples obtained 1 month following transplant to pre-transplant activity and show that Treg activity is not significantly affected by belatacept at this time point (Figure 50).



In long term patients 3 – 5 years post transplant (i.e long after basiliximab induction), the percentage of CD25⁺ and FoxP3⁺ regulatory cells among CD4⁺ cells appears comparable to CsA treated patients (Figure 51). The authors note this ratio is within normal limits and does not differ significantly from pre-transplant levels. This observation extends to the ratio of FoxP3⁺ cells among CD25⁺ cells. Suppressor activity also remained comparable to cells isolated from healthy controls.



Renal biopsy sections taken at times of acute rejection were stained for CD3 and FoxP3 using a streptavidin-peroxidase technique. The number of CD3⁺ and FoxP3⁺ T-cells in the biopsies were quantitated in a blinded fashion by a single investigator. Five cortical fields per patient were examined (40x magnification). The authors portend a representative result, Figure 52, that shows CD3⁺ cells

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staining blue and FoxP3 positive cells staining brown and noticeable results are an increased number of cells staining strongly positive for FoxP3. A significant difference was noted when the ratio of FoxP3⁺ to CD3⁺ cells was compared between belatacept and CsA treated subjects (Figure 53). The statistical method used for determining significance was not specified.

Figure 52. CD3 and FoxP3 staining of tissue biopsies of rejecting renal allografts

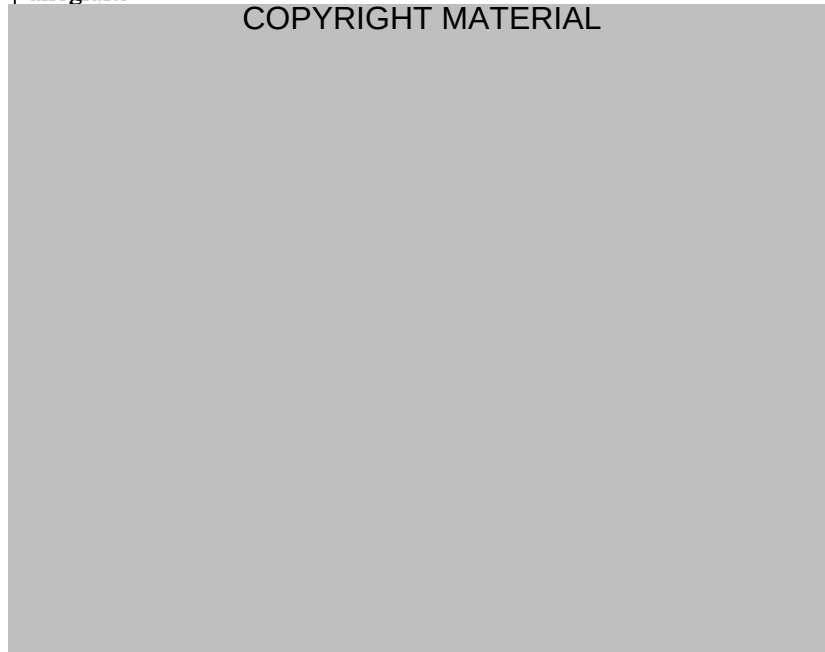
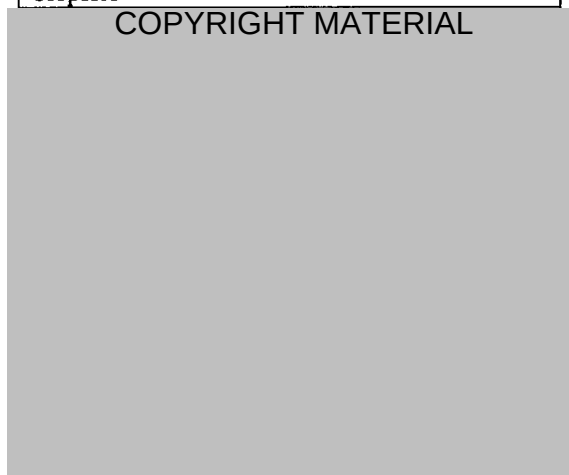


Figure 53. Ratio of FoxP3⁺ to CD3⁺ cells in biopsies

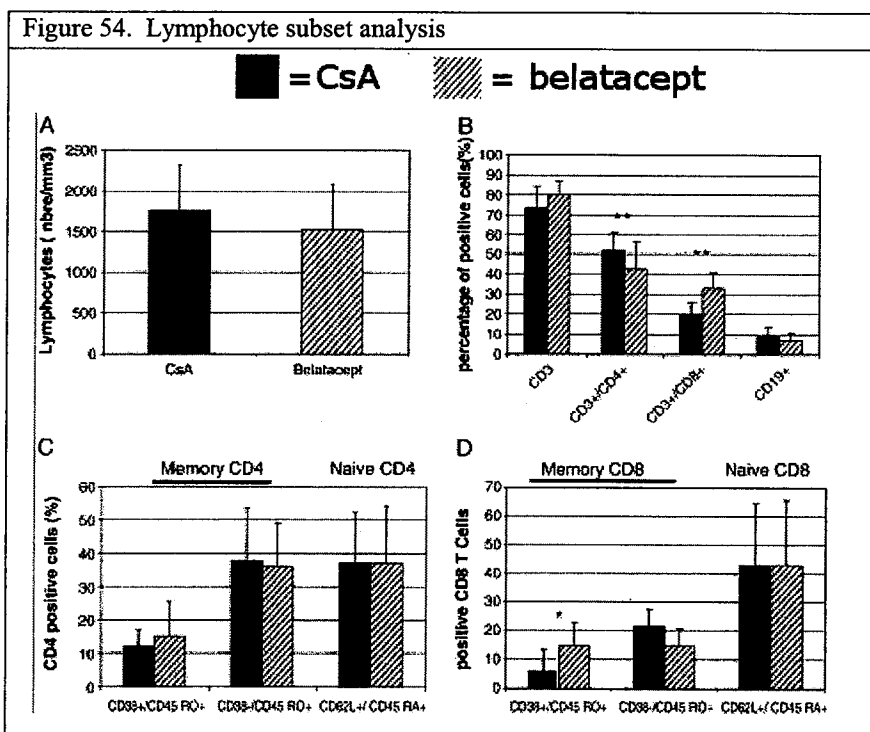


Another study examined Tregs 6 months after transplantation in the belatacept phase 2 trial 103100 (Chavez *et al.*, 2007, *Transplant Immunol*, 17:243). The lymphocyte subsets were analyzed within PBMC obtained from whole blood 6 months after transplant of eighteen patients in the belatacept arm and 10 patients in the CsA arm. Analysis was performed by flow cytometry. Foxp3 mRNA expression

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was measured by quantitative PCR. Results showed that the number of lymphocytes and the percentage of CD3+ T cells were similar in both arms (Figure 54); the percentage of CD3+ CD4+ T cells was lower in the belatacept arm than in the CsA arm ($42.5\% \pm 13.7$ vs $52.9\% \pm 9$), and the percentage of CD3+ CD8+ cells was higher in the belatacept arm than in the control ($32.9\% \pm 6.7$ vs $19.5\% \pm 8.2$). However, the absolute number of CD8+ cells was not significantly higher with belatacept than with CsA. The number of CD4 T cells was significantly less in the belatacept arm than in the CsA arm.

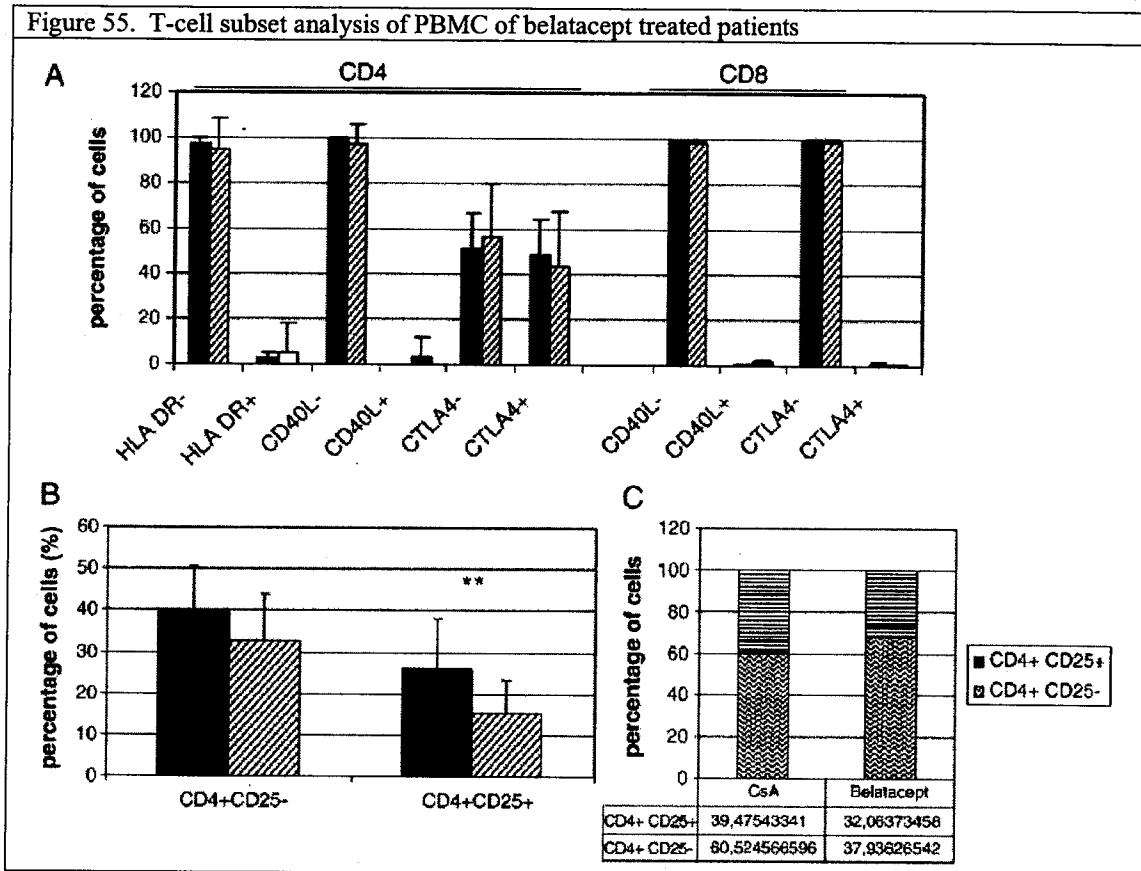
The percentage of CD19+ cells was similar in both groups. Among CD4 and CD8 T cells, the percentage of naïve cells (CD45RA+CD62L+) was similar in the two groups (for CD4 T cells: $38.5\% \pm 16.7\%$ vs $36.5\% \pm 16.3\%$; and for CD8 T cells: $77.5\% \pm 14.3\%$ vs $76.8\% \pm 10.9\%$) (Figure 54 C and D). The percentage of memory T cells CD45RO+ also did not vary significantly in both groups (for CD4 T cells: $34.7\% \pm 11\%$ vs $41.1\% \pm 12\%$; and for CD8 T cells: $29\% \pm 16.1\%$ vs $31.9\% \pm 15.3\%$). Among memory cells, expression of CD38, a marker of activation/differentiation, was similar in belatacept or CsA treated patients for CD4+ cells (26.2% vs 27%), while CD38 expression in CD8 T cells appeared higher in the belatacept arm than CsA arm (15.3% vs 6.6% , $p = 0.05$).



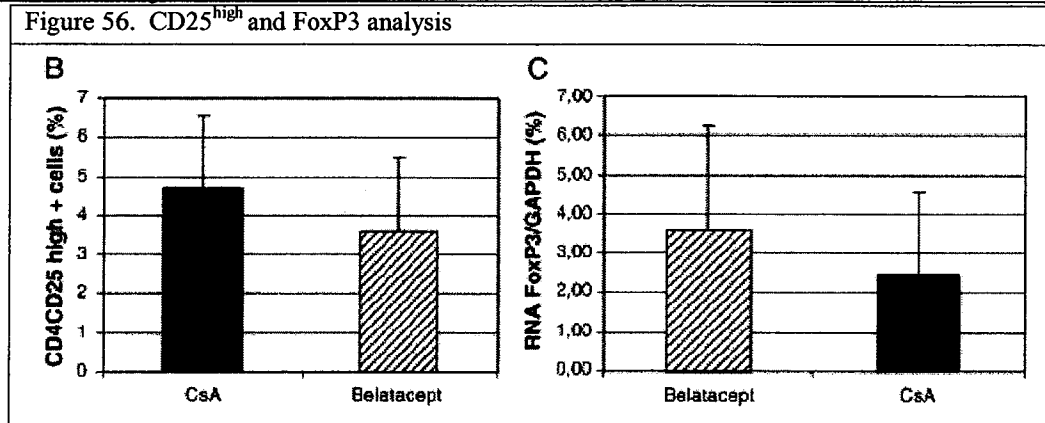
HLA DR, CD40L and CTLA4 were used as cell surface markers of T-cell activation. Patients in the belatacept arm had a lower number of HLA DR⁺ CD4⁺ T cells than the CsA arm ($725 \pm 320/\text{mm}^3$ vs $1241 \pm 404/\text{mm}^3$) although the proportion of HLA DR⁺ T cells among CD4⁺ cells and those expressing HLA DR⁺ was similar in both groups (Figure 55A). Patients treated with belatacept had fewer CD4⁺ T cells that did not express CD40L for belatacept, but the percentage between CD40L⁺ CD4⁺ cells ($B=97.8\% \pm 8.7\%$ vs $CsA=99.9\% \pm 0.08\%$, ns) and those expressing CD40L⁺ was similar in both groups (Figure 53A). The percentage of CD4⁺ T cells expressing CTLA4 was similar in the two groups. These

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data suggest that the number of CD4 T cells was lower but that the activation profile of CD4 T cells was similar in both arms. For CD8+ T cells both the number and the percentage of cells that did not express CD40L was similar in both groups. The number and the percentage of CD8+ CTLA4-cells were also similar in both groups indicating that the profile of activation of CD8 T cells was similar in both groups.



The percentage of regulatory CD4+CD25+ cells with bright CD25 staining was similar in both groups ($3.6 \pm 2.3\%$ vs $4.7 \pm 1.9\%$ for belatacept and CsA, respectively) as was the expression of FoxP3 (Figure 56). The results indicate that belatacept does not affect these cell populations in the peripheral blood, though does not rule out a lack of effect on extravascular Tregs. Indeed data from the above two citations may indicate that while peripheral blood Tregs are unaffected by belatacept, they do suggest re-distribution of Tregs.



4.5 Anti-HLA and anti-AT₁ antibody response

The presence of alloantibodies directed against donor HLA antigens has been associated with severe early acute rejection and chronic allograft rejection. The production of anti-donor HLA and anti angiotensin II type 1 (AT₁) receptor antibodies by recipients in the trials was examined to determine whether the belatacept-based regimen reduced the development of these antibodies compared with the CsA-based regimen. Antibodies against AT₁ receptor have been detected in renal transplant recipients with refractory vascular rejection in the absence of anti-HLA antibodies. These agonistic antibodies may stimulate AT₁ receptors and induce inflammatory mediators and thrombosis. Anti- AT₁ antibodies have been associated with malignant hypertension and vascular rejection. The presence of these antibodies was evaluated to assess their correlation with study treatment or the occurrence of acute rejection.

Study 103008

Blood samples for antibody testing were obtained at baseline and at pre-specified times after transplant. A sample was also obtained at the time of any suspected rejection episode(s). The assays were performed blindly at the (b) (4)

The proportion of subjects who had anti-donor HLA and anti-AT₁ receptor antibodies at baseline and by Month 6 was calculated for each treatment group.

Several different methods were used to measure HLA alloantibodies. Flow cytometric cross match (FCXM) and flow cytometric panel reactive antibody (PRA) screening were used to detect Class I and Class II HLA antibodies. Flow cytometric HLA antibody identification (Flow Specific Beads) and single antigen beads were used to determine the specificity of the antibodies. A Luminex-based assay was also used when samples were strongly positive in standard flow cytometry. Anti-AT₁ receptor antibody was assayed by ELISA (Celltrend GmbH). A serum sample with a result of > 2.0 Units was defined as a positive result.

At baseline, in the belatacept MI, belatacept LI, and CsA (CsA) arms, 5%, 6%, and 7%, respectively, of the patients had anti-HLA antibody (Table 33). After transplantation, the frequency of anti-donor HLA antibodies was slightly reduced compared to baseline in the MI and LI belatacept treatment arms (3% and 1%, respectively) but not in the CsA arm (5% - 7%). This reduction may suggest that belatacept inhibits the production of anti-donor HLA antibody.

Table 33. Summary of subjects with anti-donor HLA antibodies in ITT population in 103008				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	208	215	199
	Anti-donor HLA positive (%)	10 (4.8%)	12 (5.6%)	13 (6.5%)
Month 6	No. evaluated	206	213	196
	Anti-donor HLA positive (%)	6 (2.9%)	3 (1.4%)	10 (5.1%)
Month 12	No. evaluated	208	216	198
	Anti-donor HLA positive (%)	6 (2.9%)	3 (1.4%)	14 (7.1%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

The frequency of anti-donor HLA antibodies was similar at baseline in patients who did and did not experience an AR episode. Among subjects who had AR, the proportions of subjects who were positive for anti-donor HLA antibodies at any time by Month 6 were 5%, 0% and 7% in the belatacept MI, LI and CsA arms, respectively (Table 34 and Table 35). By Month 12, the proportions were the same as at 6 months. The proportions of subjects without AR who had post-transplantation donor-specific anti-HLA antibodies by Month 12 were 2%, 2% and 7% in the belatacept MI, LI and CsA arms, respectively. The applicant reports less episodes of rejection associated with antibodies in belatacept treated patients by Banff grading but specific comparative analysis results matching patients with and without antibodies to Banff were not included in the report.

Table 34. Summary of subjects with anti-donor HLA antibodies in patients <u>with no acute rejection</u> through month 12 in Study 103008				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	164	178	184
	Anti-donor HLA positive (%)	8 (4.9%)	10 (5.6%)	13 (7.1%)
Month 6	No. evaluated	162	176	182
	Anti-donor HLA positive (%)	4 (2.5%)	3 (1.7%)	9 (4.9%)
Month 12	No. evaluated	164	179	184
	Anti-donor HLA positive (%)	4 (2.4%)	3 (1.7%)	13 (7.1%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

Table 35. Summary of subjects with anti-donor HLA antibodies in patients <u>with acute rejection</u> by month 12 in Study 103008				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	44	37	15
	Anti-donor HLA positive (%)	2 (4.5%)	2 (5.4%)	0
Month 6	No. evaluated	44	37	14
	Anti-donor HLA positive (%)	2 (4.5%)	0	1 (7.1%)
Month 12	No. evaluated	44	37	14
	Anti-donor HLA positive (%)	2 (4.5%)	0	1 (7.1%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

Table 36 summarizes anti-AT₁ receptor antibodies at baseline and by Week 2 and Month 6. At baseline, the percentage of subjects in the belatacept MI, LI groups and the CsA group who had anti-AT₁

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antibodies were 20%, 18%, and 21%, respectively. However, the proportions who were post-transplantation antibody positive by Week 2 had decreased to 8%, 9%, and 12%, respectively. This began to increase by Month 6 with 12%, 13%, and 15% having antibodies.

Table 36. Summary of subjects with anti- AT1 receptor antibodies in patients with acute rejection by month 12 103008

		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	209	224	215
	Anti-donor HLA positive (%)	42 (20.1%)	40 (17.9%)	45 (20.9%)
Week 2	No. evaluated	210	217	203
	Anti-donor HLA positive (%)	17 (8.1%)	19 (8.8%)	25 (12.3%)
Month 6	No. evaluated	216	226	213
	Anti-donor HLA positive (%)	26 (12.0%)	29 (12.8%)	31 (14.6%)

N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.

The data from this study suggest that the proportion of subjects with anti-AT₁ antibodies at baseline was similar between subjects who experienced an AR episode and subjects who did not experience an AR episode in all treatment groups (Table 37 and Table 38). This suggests that the presence of anti-AT₁ antibodies prior to transplant was not predictive of AR.

Table 37. Summary of subjects with anti- AT1 receptor antibodies in patients with no acute rejection through month 12 103008

		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	163	185	199
	Anti-donor HLA positive (%)	31 (19%)	35 (18.9%)	40 (20.1%)
Week 2	No. evaluated	165	179	187
	Anti-donor HLA positive (%)	12 (7.3%)	16 (8.9%)	22 (11.8%)
Month 6	No. evaluated	169	187	197
	Anti-donor HLA positive (%)	21 (12.4%)	24 (12.8%)	28 (14.2%)

N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.

Table 38. Summary of subjects with anti- AT1 receptor antibodies in patients with acute rejection by month 12 103008

		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	46	39	16
	Anti-donor HLA positive (%)	11 (23.9%)	5 (12.8%)	5 (31.3%)
Week 2	No. evaluated	45	38	16
	Anti-donor HLA positive (%)	5 (11.1%)	3 (7.9%)	3 (18.8%)
Month 6	No. evaluated	47	39	16
	Anti-donor HLA positive (%)	5 (10.6%)	5 (12.8%)	3 (18.8%)

N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.

Study 103027

A similar analysis of anti-HLA antibody was performed with recipients enrolled in Study 103027. Table 39 summarizes the subjects who had anti-donor HLA antibodies at baseline and by Month 6, and Month 12. At baseline, in the MI, LI, and CsA groups, the proportions of subjects who had detectable anti-donor HLA antibodies were 6%, 6%, and 8%, respectively. By Month 6, the proportions were 3%,

Prevention of acute rejection

3%, and 7%, respectively and by Month 12, the proportions of subjects who were antibody-positive were 5%, 4%, and 9%, respectively.

Table 39. Summary of subjects with anti-donor HLA antibodies in ITT population in 103027				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	173	165	174
	Anti-donor HLA positive (%)	10 (5.8%)	9 (5.5%)	13 (7.5%)
Month 6	No. evaluated	179	163	166
	Anti-donor HLA positive (%)	6 (3.4%)	5 (3.1%)	12 (7.2%)
Month 12	No. evaluated	179	168	173
	Anti-donor HLA positive (%)	9 (5%)	7 (4.2%)	15 (8.7%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

The frequency of anti-donor HLA antibodies was similar among belatacept-treated patients who developed AR and those who did not. For CsA treated patients, anti-HLA antibody was present in a higher proportion of recipients with acute rejection than belatacept treated patients. Among subjects who had an acute rejection (AR) episode within the first 12 months of the study, the proportions of subjects who had anti-donor HLA antibodies by Month 6 were 6%, 3% and 17% in the belatacept MI, LI and CsA arms, respectively (Table 40 and Table 41). By Month 12, the proportions were 6, 3 and 20% respectively. The proportions of subjects without AR who had antibodies by month 12 were 5%, 4% and 7% in the belatacept MI, LI and CsA arms, respectively. Most AR episodes in all treatment arms were Banff Grade IIa and IIb indicating the presence of vascular inflammation. Vascular inflammation has been associated with antibody mediated rejection.

Table 40. Summary of subjects with anti-donor HLA antibodies in patients <u>with no acute rejection</u> through month 12 in Study 103027				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	141	136	149
	Anti-donor HLA positive (%)	9 (6.4%)	7 (5.1%)	11 (7.4%)
Month 6	No. evaluated	147	133	143
	Anti-donor HLA positive (%)	4 (2.7%)	4 (3%)	8 (5.6%)
Month 12	No. evaluated	147	137	148
	Anti-donor HLA positive (%)	7 (4.8%)	6 (4.4%)	10 (6.8%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

Table 41. Summary of subjects with anti-donor HLA antibodies in patients <u>with acute rejection</u> by month 12 in Study 103027				
		Belatacept MI	Belatacept LI	CsA
Baseline	No. evaluated	32	29	25
	Anti-donor HLA positive (%)	1 (3.1%)	2 (6.9%)	2 (8.0%)
Month 6	No. evaluated	32	30	23
	Anti-donor HLA positive (%)	2 (6.3%)	1 (3.3%)	4 (17.4%)
Month 12	No. evaluated	32	31	25
	Anti-donor HLA positive (%)	2 (6.3%)	1 (3.2%)	5 (20.0%)
N.B. This is a cumulative summary (including specimens collected at all times except baseline), and that once a subject is positive, that subject remains positive for the later time points.				

Prevention of acute rejection

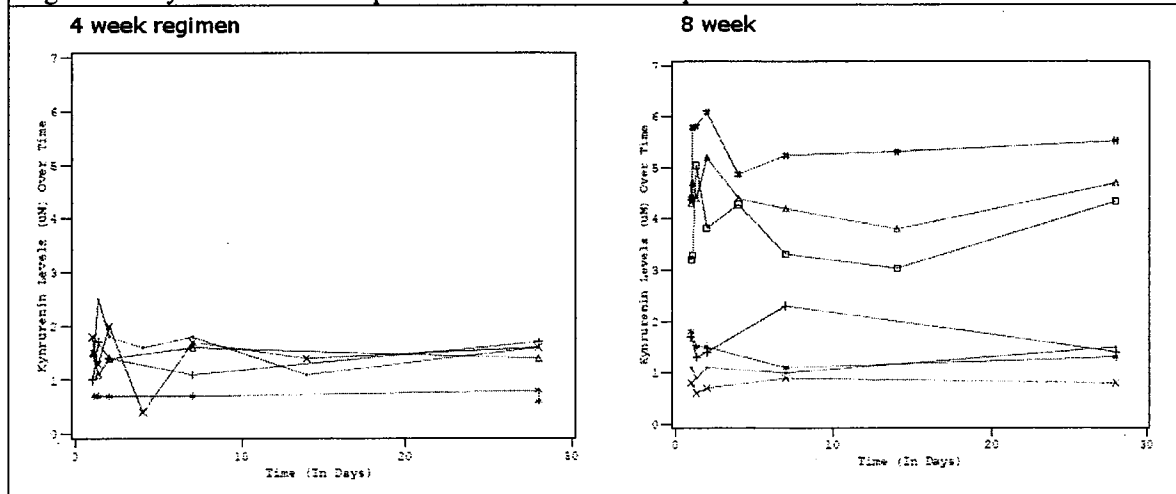
4.6 Effect on lymphoproliferation

Another experiment performed on samples from patients enrolled in the long term extension to study IM103100 investigated the effect of belatacept on the synthesis of ATP in CD4 T-cells after *in vitro* stimulation. The Immunknow[®] (Cylex Inc, Columbia, MD) was utilized to determine ATP production. No significant changes over time in ATP levels following belatacept administration were observed for any of the subjects. The mean ATP levels in the 8- and 4-week treatment groups were 282.3 and 333.0 ng/mL which is close to value proposed by the applicant of ~280 ng/mL for normal ATP levels in transplant patients. No data was available to correlate alteration in ATP levels with belatacept concentrations or clinical response.

4.7 IDO activity assay results

A sub-study of the long term extension to study IM103100 investigated the effect of belatacept treatment on IDO activity in the APC. As discussed above in section 2, belatacept may induce signaling in the APC (its target cell) after interaction with CD80 or CD86 expressed on the APC. One enzyme induced by CTLA-4:CD80/86 ligation is IDO which acts to deplete local tryptophan levels and inhibit rapid proliferation by nearby T-cells and pathogens. Blood samples were taken before and on days 1, 2, 7, 14 and 28 after belatacept dosing to determine tryptophan (the substrate of IDO) and kynurenine (a metabolite of tryptophan produced by IDO) levels. It is thought that stimuli resulting in IDO activation will cause tryptophan levels to drop and kynurenine levels to rise compared to pretreatment levels. The results suggested no drop in tryptophan or rise in kynurenine levels after treatment with belatacept. Tryptophan levels remained within a narrow range of ~40 – 43 μ M for all patients. However, there is a variability in the kynurenine levels among the small number of patients tested (Figure 57). The applicant comments on a dichotomy in which 3 patients with high kynurenine levels are consistently higher across the study period than other patients with low levels but no consistent rise in kynurenine was observed following belatacept treatment.

Figure 57. Kynurenine levels in patients treated with belatacept



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5 Discussion

Belatacept was derived from abatacept, a human CTLA4-IgG1 fusion protein which binds to CD80/CD86 on antigen presenting cells (APCs). Studies of binding kinetics showed that belatacept binds to human CD80 and CD86 with relatively equal avidity. Belatacept binds human CD80 and CD86 with greater avidity than abatacept, however, abatacept binds to murine CD80 and CD86 with a higher avidity than belatacept. These species specific differences are also reflected by both *in vitro* and *in vivo* studies comparing belatacept to abatacept. In *in vitro* functional assays, the IC_{50} of belatacept was lower than that of abatacept *in vitro* when human cells were utilized, while conversely, the IC_{50} of abatacept was lower than that of belatacept when murine cells were used. *In vitro*, belatacept prevented proliferation of T-cells in a primary allogeneic mixed lymphocyte reaction (MLR). In similar reactions, belatacept prevented the production of IL-2, IL-4, TNF- α , and IFN- γ . Belatacept almost completely inhibited CD4+ proliferation whereas a residual subpopulation of CD8+ T cells remained resistant to blockade with belatacept.

Results of immunophenotyping of CD4⁺FoxP3⁺ T-cells in blood from juvenile rats treated with high doses of abatacept showed an increase in CD4⁺ T-cells (2-3-fold over controls) and a decrease in regulatory T-cells (CD4⁺CD25⁺ FoxP3⁺; 50-70% less than controls). Such an effect was reversible after discontinuation of treatment. In lymphoid organs, B-cell areas were decreased including the size and number of germinal centers in spleen and lymph nodes. T-cell areas were increased in size. Autoimmune findings including thyroiditis and pancreatitis were also reported. These studies showed that abatacept can cause the development of autoimmunity in experimental adult and juvenile rodents. While similar experiments were performed with belatacept in adult nonhuman primates and alteration in cell phenotype was not observed, it does not appear that juvenile NHP studies were performed to determine correlation between changes in cell phenotype and development of autoimmunity or susceptibility to infection (for more extensive details please see Pharmacology/Toxicology review). Similar observations were not made in a small number of patients, treated with the belatacept regimen with basiliximab induction for the long term in the clinical trials; regulatory (FoxP3⁺) and CD8⁺ T-cells were not decreased in peripheral blood T-cells. A decrease in CD4⁺ T-cells was reported. Also, an increase in FoxP3⁺ T-cells in renal biopsy sections taken during episodes of acute rejection was reported.

CD28 may also play a role in the deletion of self reactive thymocytes and in the development of regulatory T-cells (T_{reg}) in the thymus (reviewed Palmer, 2003, *Nat Rev Immunol*, 3:383 and Nomura *et al.*, 2007, *Nat Immunol*, 8:333). It appears that the interaction of CD28 expressed on immature T-cell and CD80/CD86 expressed on thymic APC mediates this effect during the process of negative selection. The effect of blocking this interaction could potentially lead to the escape of self-reactive T-cells or the inhibition of T_{reg} development and promote autoimmunity. This may be of higher importance in juvenile or young adult patients in whom the thymus is more active. In the patients, signs and symptoms of autoimmunity were not alarming and may not be relevant to patients on active immunosuppression since autoimmune effector mechanisms may be suppressed by the immunosuppressive drug regimen as well. However, it is unknown whether autoimmunity can or will develop in patients who cease or decrease immunosuppression. A correlation of belatacept to cause autoimmunity in human patients at the doses tested has not been substantiated by clinical observations, however a potential risk may theoretically exist in patients who discontinue immunosuppression (i.e. return to dialysis).

Ex vivo data suggest that the saturation level of CD86 on the APC correlates with the activity of belatacept in inhibiting T-cell response to stimulation in a mixed lymphocyte reaction. Clinical specimens from patients in the MI regimen in Study IM103100 (>1 year post transplant), and Studies IM103027 and IM103008 (2 – 6 months post transplant) were assayed to determine the extent of CD86 saturation. Approximately 90% saturation of CD86 receptors on the surface of APCs in the peripheral blood was observed following the initial administration of belatacept. During the first month post-transplantation, 85% saturation of CD86 is maintained. During months 2 to 4 post-transplantation at the recommended dose, the applicant states that the level of CD86 saturation is expected to be maintained at approximately 70%. A similar analysis was not performed on blood samples from patients in the LI arm.

It is interesting to note the saturation kinetics of belatacept on human dendritic cells (Figure 19). It appears that on monocyte derived, LPS activated DC, belatacept saturates CD80 at lower concentrations than those required for CD86 saturation. While the sponsor did not determine the absolute expression ratio of CD80 and CD86 on these cells, it appears that at some concentrations of belatacept and under certain conditions, different proportions of CD80 could be bound by belatacept compared to CD86 even though both have been shown *in vitro* to have relatively the same kinetic avidity for each receptor. While the exact nature of the differences between CD80 and CD86 are not completely elucidated, early evidence suggested that CD86 mediates a more activating signal in naïve T-cells (reviewed: Sperling and Bluestone, 1996, *Immunol Rev*, 153:155). The clinical relevance of saturating or more proportionally inhibiting one receptor over another is unknown, but it may be interesting to correlate the relative ratio of CD80/86 expression to periods such as those preceding or during infection or acute rejection. Also, while preclinical studies suggested an effect of belatacept on the APC (IDO assays), similar results were not found in the human trials.

In a primate transplant model, belatacept alone or as part of an immunosuppressive regimen extended the survival of kidney allografts. Belatacept in combination with sirolimus also extended the survival of transplanted pancreatic islets. The observation made in clinical trials of belatacept patients having a lower incidence of antibody mediated rejection is supported by extensive preclinical experiments showing belatacept prevents a humoral response (i.e. antibody production) to different antigens.

In clinical trials, recipients treated with belatacept had a lower incidence of testing positive for anti-donor HLA antibodies. No correlation between patients with and without episode(s) of acute rejection and anti-HLA antibodies was found. The clinical observations of prevention of acute rejection and lower anti-HLA antibody production are reflected by the mechanism of action of belatacept. It appears that by binding CD80 and CD86 on the APC, belatacept prevents a T-cell response to the allograft. Through inhibition of CD4-T helper cell function, antibody production was decreased.

A higher incidence of post-transplant lymphoproliferative disorder (PTLD) was found in patients treated with belatacept compared to the control arm. Recipients who were serologically negative for EBV antibody were the most susceptible to PTLD. The applicant recommends that only patients who test as serologically positive for EBV be treated with belatacept. Many different tests were employed across the clinical trial sites and laboratories to determine EBV status. The performance of these assays was variable with some assays being less specific than others (range 77 – 100%). Less than 100% specificity allows the possibility of false positive results when screening EBV serology. These false

Prevention of acute rejection

positive recipients, if administered belatacept, may be at increased risk for EBV related PTLT. It is recommended that the labeling for belatacept specifically state that practitioners and clinical laboratory technicians employ the most specific assay possible when screening recipients for EBV status to ensure a high positive predictive value. Tests which employ a panel of antibodies that determine the stage of patient EBV infection based on the presence and absence of antibodies of different isotypes and antigen specificities may allow for a more specific result.

Many unknowns remain regarding the literal effect of belatacept treatment on the immune system and its function. B-cells express CD80 and CD86 and act as important APC in the blood. B-cells are also the target of EBV infection where even following clearance of the primary infection, the virus may remain dormant and later become reactivated. During this period of latency, there may be complex interactions between EBV antigen specific T-cells and latent EBV infected B-cells that require an intact CD28:CD80/86 signaling pathway. It is possible that in some patients belatacept binds CD80/86 on the latent EBV infected B-cell and inhibits this homeostatic maintenance of latency. Furthermore, primary infection of the B-cell with EBV in previously serologically negative patients treated with belatacept may not allow requisite CD28:CD80/86 ligation to occur between the infected B-cell and a naïve cytotoxic or CD4 T-cell specific for EBV antigen. Such a lack of active immunity in either case may potentially contribute to the increased susceptibility to PTLT observed in the clinical trials. One may wonder whether the increased incidence of EBV reactivation, PTLT and CNS involvement are associated with B-cell expression of CD80 or CD86.

The immune system has developed evolutionarily such that activation is regulated and typically only occurs when multiple signals converge. This is true for T-cell biology where multiple signals and their amplitude control activation, memory, tolerance and deletion. CD28 and CTLA-4 expressed on T-cells and CD80/CD86 expressed on APCs play a major role in regulating tolerance or anergy versus an activated response. It is interesting to note that blockade of CD28 signaling by belatacept will predominantly prevent activation of naïve T-cells, since memory T-cells are not entirely dependent on a CD28 costimulatory signal for activation. Therefore, memory T-cell responses may still be functionally intact in belatacept treated patients which in the case of a reinventing pathogen would be beneficial but may have consequences in regards to activation of alloreactive memory T-cells such as may be present postpartum in women or in former recipients of transfusions or allografts.

6 Labeling

6.1 Sponsor's version of the label

(b) (4)

2 Page(s) of Draft Labeling have been
Withheld in Full as b4 (CCI/TS) immediately
following this page

6.2 Comments

1. In a section which will be overt to prescribers (i.e. MedGuide, Black Box, or Warnings and Precautions), a text which recommends that prescribers utilize the most specific panel of assays possible to determine EBV IgG serology. A high specificity should reduce the number of false positive results. Recipients who test as false positive are seronegative for EBV and thus at increased risk of PTLT.
2. The text was rearranged to avoid repetition and to describe mechanism of action first followed by activity *in vitro* and *in vivo*.
3. The phrase (b) (4) should be changed. Other treatment regimens that do not include belatacept are equally effective in preventing acute rejection. (b) (4)
(b) (4), therefore the phrase should be changed to "extends allograft survival".
4. Remove reference to (b) (4).
5. Change all instances of the word (b) (4) to T-lymphocyte(s) in keeping with consistency of Prograf labeling.

6.1 FDA's version of the label

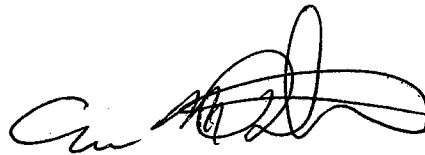
Additons are double underlined and deletions striked out

(b) (4)



7. Recommendations:

The BLA is approvable from a microbiological/immunological standpoint pending an acceptable version of the labeling.



Aaron M. Ruhland, Ph.D.
Microbiologist

CONCURRENCES:

Microbiology Team Leader Shukel Bab Signature 3/26/10 Date

CC:

Original BLA

Project Manager/GermainJ

Appendix 1-A: EBV assays used in Study IM103008

Appendix 1-A. EBV Assay panels used in IM103008						
Kit/Panel	Manufacturer	Assay Type	Specificity	Sensitivity	FDA cleared?	Number of patients tested
not given	not given	X-map (Luminex)	100%	96.90%	not given	2
ARTUS	Qiagen	rtPCR	20,500,000 copies/ml	300 copies/ml	no	7
Athena Multi-Lyte EBV IgG Plus Test System and EBV VCA IgM ELISA II	Zeus Scientific or Wampole	Multiplex Immunoassay or ELISA	78.9%, 77% 82.1%	91.8%, 97.7% 92.9%	yes	1
BioPlex 2200	BioRad	Multiplex Flow Immunoassay	IgG: 97.8% IgM: 92.8% EBNA: 81.8%	IgG: 91.8% IgM: 94.2% EBNA: 98.9%	yes	3
BioPlex 2200 EBV IgG Kit	BioRad	Immunoassay	not given	not given	yes	7
Captiva EBV VCA IgG	Trinity Biotech	ELISA	IgG:100%	IgG: 95.5%	yes	8
Captiva: EBV VCA IgG EBNA-1 IgG EBV VCA IgM	Trinity Biotech	ELISA	not given	n/a	yes	7
COPALIS EBV-M ANTIBODY ASSAY (IgM)	Diasorin, Inc.	ELISA	not given	n/a	yes	11
EBNA IgG or VCA-p18 IgG, EBV-VCA IgM or VCA-p18 IgG, EBV-VCA IgM	Panbio Diagnostics, Panbio Diagnostics or Trinity Biotech	ELISA	EBNA IgG: 96.4% VCA-p18 IgG: 96% EBV-VCA IgM: 100%, 87.5% VCA-p18 IgG: 96.4% EBV-VCA IgM: 96.4%	EBNA IgG: Past: 97.1%; Acute: 92.3% VCA-p18 IgG: Past 100%; Acute:73.9% EBV-VCA IgM: 100%, 85.7% VCA-p18 IgG: 97.4% EBV-VCA IgM: 97.4%	yes	8

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

BLA/NDA Number:
STN125288

Applicant: Bristol-Myers Squibb **Stamp Date:**
Company

Established/Proper Name:
belatacept

BLA/NDA Type:
Orphan drug designation.

On initial overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?	If not, justification, action & status
Cover Letter	Y	
Form 356h completed	Y	
☐ including list of all establishment sites and their registration numbers	Y	
Comprehensive Table of Contents	Y	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y	
Labeling:	Y N	
☐ PI –non-annotated	Y N	
☐ PI –annotated	Y N	
☐ PI (electronic)	Y N	
☐ Medication Guide	Y N	
☐ Patient Insert	Y N	
☐ package and container	Y N	
☐ diluent	Y N	
☐ other components	Y N	
☐ established name (e.g. USAN)	Y N	
☐ proprietary name (for review)	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y	
☐ legible	Y	
☐ English (or translated into English)	Y	
☐ compatible file formats	Y	
☐ navigable hyper-links	Y	
☐ interpretable data tabulations (line listings) & graphical displays	Y	
☐ summary reports reference the location of individual data and records	Y	
☐ all electronic submission components usable (e.g. conforms to published guidance)	Y	

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

Examples of Filing Issues	Yes?	If not, justification, action & status
Companion application received if a shared or divided manufacturing arrangement	Y N	Not applicable.

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y	
Introduction to the summary documents (1 page) [2.2]	Y	
Quality overall summary [2.3]	Y	
☐ Drug Substance	Y	
☐ Drug Product	Y	
☐ Facilities and Equipment	Y	
☐ Adventitious Agents Safety Evaluation	Y	
☐ Novel Excipients	Y N	Not applicable.
☐ Executed Batch Records	Y	
☐ Method Validation Package	Y	
☐ Comparability Protocols	Y	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	Y N	
Drug Substance [3.2.S]		
☐ general info	Y	
☐ nomenclature		
☐ structure (e.g. sequence, glycosylation sites)		
☐ properties		
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y	
☐ description of manufacturing process and process control	Y	
☐ batch numbering and pooling scheme		
☐ cell culture and harvest		
☐ purification		
☐ filling, storage and shipping		
☐ control of materials	Y	
☐ raw materials and reagents		
☐ biological source and starting materials		
☐ cell substrate: source, history, and generation		

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
○ cell banking system, characterization, and testing □ control of critical steps and intermediates ○ justification of specifications ○ stability □ process validation (prospective plan, results, analysis, and conclusions) □ manufacturing process development (describe changes during non- clinical and clinical development; justification for changes) □ characterization of drug substance □ control of drug substance ○ specifications ○ justification of specs. ○ analytical procedures ○ analytical method validation ○ batch analyses □ reference standards □ container closure system □ stability □ summary □ post-approval protocol and commitment □ pre-approval ○ protocol ○ results ○ method validation	Y Y Y Y Y Y Y	
Drug Product [3.2.P] [Dosage Form] □ description and composition □ pharmaceutical development ○ preservative effectiveness ○ container-closure integrity □ manufacturers (names, locations, and responsibilities of all sites involved) □ batch formula □ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at	Y Y Y Y Y Y	Not applicable.

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ outside [e.g., contract] facilities ☐ controls of critical steps and intermediates ☐ process validation including aseptic processing & sterility assurance: ☐ Filter validation ☐ Component, container, closure depyrogenation and sterilization validation ☐ Validation of aseptic processing (media simulations) ☐ Environmental Monitoring Program ☐ Lyophilizer validation ☐ Other needed validation data (hold times) ☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin) ☐ control of drug product (justification of specifications; analytical method validation; batch analyses, characterization of impurities) ☐ reference standards or materials ☐ container closure system [3.2.P.7] ☐ specifications (vial, elastomer, drawings) ☐ availability of DMF & LOAs ☐ administration device(s) ☐ stability ☐ summary ☐ post-approval protocol and commitment ☐ pre-approval ☐ protocol ☐ results ☐ method validation	Y Y Y Y Y Y Y	
Diluent (vials or filled syringes) [3.2P'] ☐ description and composition of diluent ☐ pharmaceutical development ☐ preservative	Y N Y N Y N	Not applicable.

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?		If not, justification, action & status
effectiveness			
○ container-closure integrity	Y	N	
❑ manufacturers (names, locations, and responsibilities of all sites involved)	Y	N	
❑ batch formula	Y	N	
❑ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y	N	
❑ controls of critical steps and intermediates	Y	N	
❑ process validation including aseptic processing & sterility assurance:	Y	N	
○ Filter validation			
○ Component, container, closure depyrogenation and sterilization validation	Y	N	
○ Validation of aseptic processing (media simulations)			
○ Environmental Monitoring Program	Y	N	
○ Lyophilizer sterilization validation	Y	N	
○ Other needed validation data (hold times)			
❑ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)	Y	N	
❑ control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)	Y	N	
❑ reference standards	Y	N	
❑ container closure system	Y	N	
○ specifications (vial, elastomer, drawings)			
○ availability of DMF & LOAs			
❑ stability	Y	N	

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ summary ☐ post-approval protocol and commitment ☐ pre-approval ☐ protocol ☐ results		
Other components to be marketed (full description and supporting data, as listed above): ☐ other devices ☐ other marketed chemicals (e.g. part of kit)	Y N	DMF information will be requested. Not applicable.
Appendices for Biotech Products [3.2.A] ☐ facilities and equipment ☐ manufacturing flow; adjacent areas ☐ other products in facility ☐ equipment dedication, preparation, sterilization and storage ☐ procedures and design features to prevent contamination and cross-contamination ☐ adventitious agents safety evaluation (viral and non-viral) e.g.: ☐ avoidance and control procedures ☐ cell line qualification ☐ other materials of biological origin ☐ viral testing of unprocessed bulk ☐ viral clearance studies ☐ testing at appropriate stages of production ☐ novel excipients	Y Y Y Y Y N	Not applicable.
USA Regional Information [3.2.R] ☐ executed batch records ☐ method validation package ☐ comparability protocols	Y Y Y	
Literature references and copies [3.3]	Y	

Examples of Filing Issues	Yes?	If not, justification, action & status
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PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)

Examples of Filing Issues	Yes?	If not, justification, action & status
Includes production data on drug substance and drug product manufactured in the facility intended to be licensed (including pilot facilities) using the final production process(es)	Y	
Includes data demonstrating consistency of manufacture	Y	
Includes complete description of product lots and manufacturing process utilized for clinical studies	Y N	Under OBP's purview.
Describes changes in the manufacturing process, from material used in clinical trial to commercial production lots	Y N	Under OBP's purview.
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y N	Under OBP's purview.
Certification that all facilities are ready for inspection	Y	
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test methods used and time intervals for product assessment.	Y N	Under OBP's purview.
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: ☐ LAL instead of rabbit pyrogen ☐ mycoplasma ☐ sterility	Y N Y N Y N Y	Information on rabbit pyrogen test will be requested. Under OBP's purview.
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	Y	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR ORIGINAL BLA/NDA (OBP & DMPQ)**

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? Y

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. Please provide information and summary data for the rabbit pyrogen test for belatacept.
2. Please provide information and summary data for the (b) (4) sterilization of the co-packaged (b) (4) syringe. Alternatively, a Letter of Authorization referencing the relevant DMF file and the exact locations of the validation information and data in the DMF should be provided.

Michelle Clark-Stuart
Bo Chi

Bo Chi for MCR-S
Bo Chi

Product Quality Reviewer(s)

8/18/09
Date

Patricia Hughes
Team Leader

[Signature]

8/18/09
Date

Barry Rothman
Acting Branch Chief

Barry Rothman

8/19/09
Date

MICROBIOLOGY FILING CHECKLIST

BLA Number: 125288

Applicant: Bristol Myers Squibb Stamp Date: 7/1/2009

**Product Name: Nulojix
(Belatacept)**

BLA Type: Original

On initial overview of the BLA application for filing:

	Content Parameter	Yes	No	Comments
1	Is the microbiology information (preclinical/nonclinical and clinical) described in different sections of the BLA organized in a manner to allow substantive review to begin?	✓		
2	Is the microbiology information (preclinical/nonclinical and clinical) indexed, paginated and/or linked in a manner to allow substantive review to begin?	✓		
3	Is the microbiology information (preclinical/nonclinical and clinical) legible so that substantive review can begin?	✓		
4	On its face, has the applicant <u>submitted</u> <i>in vitro</i> data in necessary quantity, using necessary clinical and non-clinical strains/isolates, and using necessary numbers of approved current divisional standard of approvability of the submitted draft labeling?	✓		
5	Has the applicant <u>submitted</u> any required animal model studies necessary for approvability of the product based on the submitted draft labeling?	✓		
6	Has the applicant <u>submitted</u> all special/critical studies/data requested by the Division during pre-submission discussions?			Not applicable.
7	Has the applicant <u>submitted</u> the clinical microbiology datasets in a format which intends to correlate baseline pathogen with clinical and microbiologic outcome?			Not applicable.
8	Has the applicant <u>submitted</u> draft/proposed interpretive criteria/breakpoint along with quality control (QC) parameters and interpretive criteria, if applicable, in a manner consistent with contemporary standards, which attempt to correlate criteria with clinical results of NDA/BLA studies, and in a manner to allow substantive review to begin?			Not applicable.
9	Has the applicant <u>submitted</u> a clinical microbiology dataset in an appropriate/standardized format which intends to determine resistance development by correlating changes in the phenotype (such as <i>in vitro</i> susceptibility) and/or genotype (such as mutations) of the baseline pathogen with clinical and microbiologic outcome?			Not applicable.
10	Has the applicant used standardized or nonstandardized methods for measuring microbiologic outcome? If nonstandardized methods were used, has the applicant included complete details of the method, the name of the laboratory where actual testing was done and performance characteristics of the assay in the laboratory where the actual testing was done?		✓	EBV assay was done at different sites. At the time of the pre-BLA meeting, the applicant stated the information was to be provided following initial

MICROBIOLOGY FILING CHECKLIST

2

	Content Parameter	Yes	No	Comments
				submission of BLA. An additional reminder request is to be sent to the applicant (for details see Additional Comment below).
11	Has the applicant submitted draft labeling consistent with current regulation, divisional and Center policy, and the design of the development package?	✓		
12	Has the applicant submitted annotated microbiology draft labeling consistent with current divisional policy, and the design of the development package?	✓		
13	Have all the study reports, published articles, and other references been included and cross-referenced in the annotated draft labeling or summary section of the submission?	✓		
14	Are any study reports or published articles in a foreign language? If yes, has the translated version been included in the submission for review?	✓		

IS THE MICROBIOLOGY SECTION OF THE APPLICATION FILEABLE? YES

If the BLA is not fileable from the microbiology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

The following comment should be communicated to the sponsor immediately.

1. At the time of pre-BLA meeting on May 20, 2009 the issue of assays used for determination of EBV status were discussed (see meeting minutes dated 7/1/09). You were also requested to provide the following information in the BLA submission.
 - Specify the assay used for determining EBV status in the BLA submission. If the assay is FDA approved, then the product brochure should be included. If the assay is not FDA approved, please provide details of the method and the performance characteristics for the assay.
 - Details of the method used, including storage, shipping and handling of samples.
 - Specify whether testing was done at the site or a central laboratory.
 - Specify the time of testing.
2. It will be helpful for our review if the datasets are formatted as shown in the template (Table 1). If EBV titer results were quantified, then please include the results in the table as well.

MICROBIOLOGY FILING CHECKLIST

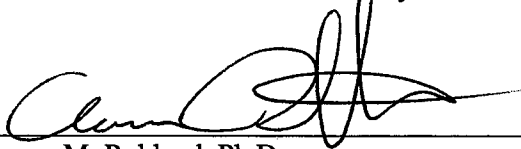
3

Table 1. EBV testing and status data								
Study ID	Subject ID	Site ID	Laboratory name and location	EBV Screening Assay Used	Donor EBV Status (Day)*	Recipient EBV Status (Day)*	Developed PTLT (Day)*	Developed CNS-PTLT (Day)*
IM103100	12345	City, State, Country	Lab X, City, State, Country	Company X ELISA	Positive (Day -1)	Negative (Day -5)	Yes (Day 100)	No

*Day of observation or measurement relevant to transplantation

3. It is unclear if the EBV test was repeated post-transplant or at different time points for the allograft recipients. Please clarify and provide the results of these tests in the above table as suggested in comment #2 if they were performed.

Please submit this information by 9/18/09 for our review.


 Aaron M. Ruhland, Ph.D.,
 Microbiology Reviewer, DSPTP

8/12/09
 Date

Shukal Bala
 Shukal Bala, Ph.D.
 Microbiology Team Leader, DSPTP

8/12/09
 Date

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Appendix N

BLA 125288/0 (original submission- Container closure review

Product : Belatacept (BMS-224818)

Applicant: BMS-Syracuse

Submission Date: 6/30/09

Action due: 3/22/10

Formulation Lyophilized powder for (b) (4) and injection

Strength: 250 mg/vial

Indications: prophylaxis of organ rejection and preservation of a functioning allograft in adult patients receiving renal transplants.

Clinical Division: Division of Neurological products

RPM: Tamy Kim

Product Chair: Jack Raghreb

CMC team: Jack Raghreb

Joao Pedras-Vasconcelos (container closure system/viral clearance)

Norihisa Sakamoto (MCB)

Edward Max (contract)

Susan Kirshner

Daniela Verthelyi

Milestones:

Filling Meeting: 8/12/09

D74 deficiency letter: 9/13/09

Mid-cycle meeting: 12/14/09

Inspection: Early 11/09

AC recommendations: 3/3/09

Wrap up: 3/15/10

Decision letter: 4/1/10

From: Joao Pedras-Vasconcelos, Ph. D.

Through: Susan Kirshner, Ph.D.

Daniela Verthelyi, MD/Ph. D.

Comment to the file:

This report analyzes the container closure portion of BLA 125288 Belatacept (BMS-22418) for both Drug substance and Drug Product. Data on suitability, compatibility and integrity of the container closure system were obtained through simulated in-use stability testing. Compatibility of container closure system with the drug product is tested as part of the on-going stability studies. Container closure integrity was demonstrated by methylene blue dye immersion. The container closure integrity is also indirectly monitored by microbiological monitoring for sterility. Complete leachable and extractable test results were provided. Overall, the data suggest the container closure system is adequate to ensure the sterility and integrity of the lyophilized product on long term storage, and the integrity of the reconstituted solution for recommended short term storage at 2-8 °C for 24 hours. The container closure system testing data and description appear adequate for ensuring product safety and efficacy.

Container-closure Summary

Belatacept for injection is a single use, sterile, (b) (4) lyophilized powder for intravenous (IV) administration after its constitution with either sterile water for injection (SWFI), 0.9% sodium chloride injection (NS) or 5% dextrose solution (D5W) to obtain a solution with a protein concentration of 25 mg/mL and subsequent dilution to concentrations between (b) (4) mg/mL and 10 mg/mL with either NS or D5W. The proposed commercial presentation for Belatacept for Injection, 250 mg/Vial, is a 20-cc Type I (b) (4) glass vial, a 20-mm gray (b) (4) stopper and an aluminum flip-off seal. Belatacept for Injection, 250 mg/ Vial, will be co-packaged with one silicone-free 10/12 ml (b) (4) syringe for each vial. The 10/12 ml syringe is single-use, sterile, silicone-free, graduated, with a (b) (4) finish. The syringe is pre-packaged in a plastic and (b) (4) paper-blister. The vial will be placed into a paperboard-folding carton to protect the product from light exposure, since it is susceptible to photodegradation.

This report analyzes the container closure portion of BLA 125288 Belatacept for both Drug substance and Drug Product. The results provided herein support the integrity of the container and closure system. On the basis of the available stability data, the container closure system appears to be suitable for protection of the drug product from moisture and the materials of construction can be considered to be compatible with the dosage form.

(b) (4)

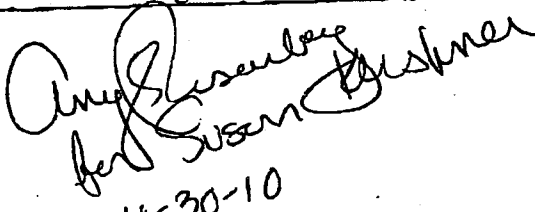


Appendix O

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ARY BLA 125288 - Nulioix (Belatacept; CIE)

VII. SIGNATURE BLOCK

Name and Title	Signature and Date
Susan Kirshner, Ph.D. Associate Laboratory Chief Laboratory of Immunology Division of Therapeutic Proteins	 4-30-10
Barry Cherney, Ph.D. Deputy Director Division of Therapeutic Proteins	 4-29-10
Jack A. Ragheb, M.D., Ph.D. Primary Reviewer Senior Regulatory Research Officer Principal Investigator Division of Therapeutic Proteins	 4-29-10

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

BLA/NDA Number: 125288 Applicant: BMS

Stamp Date: 07-01-09
 8-12-09

Drug Name: Belatacept BLA/NDA Type: BLA INITIAL

On initial overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?		If not, justification, action & status
Cover Letter	Y	N	
Form 356h completed	Y	N	
☐ including list of all establishment sites and their registration numbers	Y	N	
Comprehensive Table of Contents	Y	N	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y	N	
Labeling:	Y	N	
☐ PI –non-annotated	Y	N	
☐ PI –annotated	Y	N	
☐ PI (electronic)	Y	N	
☐ Medication Guide	Y	N	
☐ Patient Insert	Y	N	
☐ package and container	Y	N	
☐ diluent	Y	N	
☐ other components	Y	N	
☐ established name (e.g. USAN)	Y	N	
☐ proprietary name (for review)	Y	N	

labeled medication guide
 can't locate. Will IR

n.a.

Examples of Filing Issues	Yes?		If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y	N	
☐ legible	Y	N	
☐ English (or translated into English)	Y	N	
☐ compatible file formats	Y	N	
☐ navigable hyper-links	Y	N	
☐ interpretable data tabulations (line listings) & graphical displays	Y	N	
☐ summary reports reference the location of individual data and records	Y	N	
☐ all electronic submission components usable (e.g. conforms to published guidance)	Y	N	
Companion application received if a shared or divided manufacturing	Y	N	

n.a.

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

Examples of Filing Issues	Yes?	If not, justification, action & status
arrangement		

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	☒ Y N	
Introduction to the summary documents (1 page) [2.2]	☒ Y N	
Quality overall summary [2.3]	☒ Y N	
☐ Drug Substance	☒ Y N	
☐ Drug Product	☒ Y N	
☐ Facilities and Equipment	☒ Y N	
☐ Adventitious Agents Safety Evaluation	☒ Y N	
☐ Novel Excipients	Y ☒ N	n.a.
☐ Executed Batch Records	☒ Y N	
☐ Method Validation Package	☒ Y N	
☐ Comparability Protocols	☒ Y N	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	☒ Y N	
Drug Substance [3.2.S]		
☐ general info	☒ Y N	
☐ nomenclature		
☐ structure (e.g. sequence, glycosylation sites)		
☐ properties		
☐ manufacturers (names, locations, and responsibilities of all sites involved)	☒ Y N	
☐ description of manufacturing process	☒ Y N	
☐ batch numbering and pooling scheme		
☐ cell culture and harvest		
☐ purification		
☐ filling, storage and shipping		
☐ control of materials	☒ Y N	
☐ raw materials and reagents		
☐ biological source and starting materials		
☐ cell substrate: source, history, and generation		

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CED Module 3 Contents	Present?	If not, justification, action & status
○ cell banking system, characterization, and testing	(Y) N	
□ control of critical steps and intermediates ○ justification of specifications ○ analytical method validation ○ reference standards ○ stability	(Y) N	Can't locate. Will IR.
□ process validation (prospective plan, results, analysis, and conclusions)	(Y) N	
□ manufacturing process development (describe changes during non-clinical and clinical development; justification for changes)	(Y) N	
□ characterization of drug substance	(Y) N	
□ control of drug substance ○ specifications ○ justification of specs. ○ analytical procedures ○ analytical method validation ○ batch analyses ○ consistency (3 consecutive lots) ○ justification of specs.	(Y) N	
□ reference standards	(Y) N	
□ container closure system	(Y) N	
□ stability □ summary □ post-approval protocol and commitment □ pre-approval ○ protocol ○ results ○ method validation	(Y) N	Can't locate. Will IR.
Drug Product [3.2.P]		
□ description and composition	(Y) N	
□ pharmaceutical development ○ preservative effectiveness ○ container-closure integrity	(Y) N	n.a.
□ manufacturers (names, locations, and responsibilities of all sites involved)	(Y) N	
□ batch formula	(Y) N	

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y N Y N	
☐ controls of critical steps and intermediates		
☐ process validation including aseptic processing & sterility assurance: - o 3 <u>consecutive</u> lots - o Filter validation - o Component, container, closure depyrogenation and sterilization validation - o Validation of aseptic processing (media simulations) - o Environmental Monitoring Program - o Lyophilizer sterilization validation - o Other needed validation data (hold times)	Y N Y N Y N Y N	2 pair consecutive lots
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin)	Y N	(b) (4)
☐ control of drug product (justification of specifications; analytical method validation)		
☐ container closure system [3.2.P.7] - o specifications (vial, elastomer, drawings) - o availability of DMF & LOAs - o administration device(s)		
☐ stability ☐ summary ☐ post-approval protocol and commitment ☐ pre-approval - o protocol - o results - o method validation		DP no method validation same as DS?
Diluent (vials or filled syringes) [3.2P']		N.A.

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?		If not, justification, action & status
☐ description and composition of diluent	Y	N	<i>not applicable</i>
☐ pharmaceutical development	Y	N	
☐ preservative effectiveness	Y	N	
☐ container-closure integrity	Y	N	
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y	N	
☐ batch formula			
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y	N	
	Y	N	
☐ controls of critical steps and intermediates			
☐ process validation including aseptic processing & sterility assurance:	Y	N	
☐ 3 consecutive lots			
☐ Filter validation			
☐ Component, container, closure depyrogenation and sterilization validation	Y	N	
☐ Validation of aseptic processing (media simulations)	Y	N	
☐ Environmental Monitoring Program	Y	N	
☐ Lyophilizer sterilization validation			
☐ Other needed validation data (hold times)	Y	N	
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)			
☐ control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)			
☐ reference standards			

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

[illegible]

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ method validation package	☒ Y N	
☐ comparability protocols	☒ Y N	
Literature references and copies [3.3]	☒ Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization sufficient to permit substantive review?	☒ Y N	
☐ legible	☒ Y N	
☐ English (or translated into English)	☒ Y N	
☐ compatible file formats	☒ Y N	
☐ navigable hyper-links	☒ Y N	
☐ interpretable data tabulations (line listings) & graphical displays	☒ Y N	
☐ summary reports reference the location of individual data and records	☒ Y N	
☐ all electronic submission components usable	☒ Y N	
Includes appropriate process validation data for the manufacturing process at the commercial production facility	Y ☒ N	
Includes production data on drug substance and drug product manufactured in the facility intended to be licensed (including pilot facilities) using the final production process(es)	☒ Y N	
Includes data demonstrating consistency of manufacture	☒ Y N	
Includes complete description of product lots and manufacturing process utilized for clinical studies	☒ Y N	
Describes changes in the manufacturing process, from material used in clinical trial to commercial production lots	☒ Y N	
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	☒ Y N	
Certification that all facilities are ready for inspection	Y ☒ N	Amendment to be filed
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test	☒ Y N	

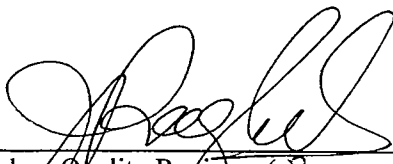
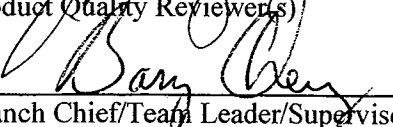
**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
methods used and time intervals for product assessment.		
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: ☐ LAL instead of rabbit pyrogen ☐ mycoplasma ☐ sterility	Y N ☒ Y ☒ N ☒ Y ☒ N ☒ Y ☒ N	
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	☒ Y N	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	☒ Y N	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	☒ Y N	
Information and data supporting validity of sterilization processes for sterile products and aseptic manufacturing operations	☒ Y N	

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? _____

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

	JACK RAGHEB	8-12-09
Product Quality Reviewer(s)		Date
		8-12-09
Branch Chief/Team Leader/Supervisor		Date

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

BLA/NDA Number: 125288

Applicant: Bristol-Myers Squibb Stamp Date:

Drug Name: Belatacept

BLA/NDA Type: BLA-125288

Initial

07-01-09
8-12-09

On initial overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?		If not, justification, action & status
Cover Letter	Y	N	
Form 356h completed	Y	N	
☐ including list of all establishment sites and their registration numbers	Y	N	
Comprehensive Table of Contents	Y	N	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y	N	
Labeling:	Y	N	
☐ PI –non-annotated	Y	N	
☐ PI –annotated	Y	N	
☐ PI (electronic)	Y	N	
☐ Medication Guide	Y	N	
☐ Patient Insert	Y	N	
☐ package and container	Y	N	
☐ diluent	Y	N	
☐ other components	Y	N	
☐ established name (e.g. USAN)	Y	N	
☐ proprietary name (for review)	Y	N	

Examples of Filing Issues	Yes?		If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y	N	
☐ legible	Y	N	
☐ English (or translated into English)	Y	N	
☐ compatible file formats	Y	N	
☐ navigable hyper-links	Y	N	
☐ interpretable data tabulations (line listings) & graphical displays	Y	N	
☐ summary reports reference the location of individual data and records	Y	N	
☐ all electronic submission components usable (e.g. conforms to published guidance)	Y	N	
Companion application received if a shared or divided manufacturing	Y	N	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
arrangement		

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y N	
Introduction to the summary documents (1 page) [2.2]	Y N	
Quality overall summary [2.3]	Y N	
☐ Drug Substance	Y N	
☐ Drug Product	Y N	
☐ Facilities and Equipment	Y N	
☐ Adventitious Agents Safety Evaluation	Y N	
☐ Novel Excipients	Y N	
☐ Executed Batch Records	Y N	
☐ Method Validation Package	Y N	
☐ Comparability Protocols	Y N	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	Y N	
Drug Substance [3.2.S]		
☐ general info	Y N	
☐ nomenclature		
☐ structure (e.g. sequence, glycosylation sites)		
☐ properties		
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
☐ description of manufacturing process	Y N	
☐ batch numbering and pooling scheme		
☐ cell culture and harvest		
☐ purification		
☐ filling, storage and shipping		
☐ control of materials	Y N	
☐ raw materials and reagents		
☐ biological source and starting materials		
☐ cell substrate: source, history, and generation		

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
○ cell banking system, characterization, and testing	Y ✓	
□ control of critical steps and intermediates ○ justification of specifications ○ analytical method validation ○ reference standards ○ stability	Y N	
□ process validation (prospective plan, results, analysis, and conclusions)	Y N	
□ manufacturing process development (describe changes during non-clinical and clinical development; justification for changes)	Y N	
□ characterization of drug substance		
□ control of drug substance ○ specifications ○ justification of specs. ○ analytical procedures ○ analytical method validation ○ batch analyses ○ consistency (3 <u>consecutive</u> lots) ○ justification of specs.	Y N Y N	
□ reference standards		
□ container closure system	Y N	
□ stability	Y N	
□ summary	Y N	
□ post-approval protocol and commitment		
□ pre-approval ○ protocol ○ results ○ method validation		
Drug Product [3.2.P]		
□ description and composition	Y N	
□ pharmaceutical development ○ preservative effectiveness ○ container-closure integrity	Y N Y N Y N	
□ manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
□ batch formula		

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y N Y N	
☐ controls of critical steps and intermediates		
☐ process validation including aseptic processing & sterility assurance: ☐ 3 <u>consecutive</u> lots ☐ Filter validation ☐ Component, container, closure depyrogenation and sterilization validation ☐ Validation of aseptic processing (media simulations) ☐ Environmental Monitoring Program ☐ Lyophilizer sterilization validation ☐ Other needed validation data (hold times)	Y N Y N Y N Y N	
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin)		
☐ control of drug product (justification of specifications; analytical method validation)		
☐ container closure system [3.2.P.7] ☐ specifications (vial, elastomer, drawings) ☐ availability of DMF & LOAs ☐ administration device(s)		
☐ stability ☐ summary ☐ post-approval protocol and commitment ☐ pre-approval ☐ protocol ☐ results ☐ method validation		
Diluent (vials or filled syringes) [3.2.P']		

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?		If not, justification, action & status
☐ description and composition of diluent	Y	N	
☐ pharmaceutical development	Y	N	
☐ preservative effectiveness	Y	N	
☐ container-closure integrity	Y	N	
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y	N	
☐ batch formula			
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y	N	
☐ controls of critical steps and intermediates			
☐ process validation including aseptic processing & sterility assurance:	Y	N	
☐ 3 consecutive lots			
☐ Filter validation			
☐ Component, container, closure depyrogenation and sterilization validation	Y	N	
☐ Validation of aseptic processing (media simulations)	Y	N	
☐ Environmental Monitoring Program	Y	N	
☐ Lyophilizer sterilization validation			
☐ Other needed validation data (hold times)	Y	N	
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)			
☐ control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)			
☐ reference standards			

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ container closure system ○ specifications (vial, elastomer, drawings) ○ availability of DMF & LOAs ☐ stability □ summary □ post-approval protocol and commitment □ pre-approval ○ protocol ○ results		
Other components to be marketed (full description and supporting data, as listed above): ☐ other devices ☐ other marketed chemicals (e.g. part of kit)	Y N Y N	
Appendices for Biotech Products [3.2.A] ☐ facilities and equipment ○ manufacturing flow; adjacent areas ○ other products in facility ○ equipment dedication, preparation, sterilization and storage ○ procedures and design features to prevent contamination and cross-contamination ☐ adventitious agents safety evaluation (viral and non-viral) e.g.: ○ avoidance and control procedures ○ cell line qualification ○ other materials of biological origin ○ viral testing of unprocessed bulk ○ viral clearance studies ○ testing at appropriate stages of production ☐ novel excipients	Y N Y N Y N	
USA Regional Information [3.2.R] ☐ executed batch records	Y N Y N	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ method validation package	Y N	
☐ comparability protocols	Y N	
Literature references and copies [3.3]	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization sufficient to permit substantive review?	Y N	
☐ legible	Y N	
☐ English (or translated into English)	Y N	
☐ compatible file formats	Y N	
☐ navigable hyper-links	Y N	
☐ interpretable data tabulations (line listings) & graphical displays	Y N	
☐ summary reports reference the location of individual data and records	Y N	
☐ all electronic submission components usable	Y N	
Includes appropriate process validation data for the manufacturing process at the commercial production facility	Y N	
Includes production data on drug substance and drug product manufactured in the facility intended to be licensed (including pilot facilities) using the final production process(es)	Y N	
Includes data demonstrating consistency of manufacture	Y N	
Includes complete description of product lots and manufacturing process utilized for clinical studies	Y N	
Describes changes in the manufacturing process, from material used in clinical trial to commercial production lots	Y N	
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y N	
Certification that all facilities are ready for inspection	Y N	
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test	Y N	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

Examples of Filing Issues	Yes?	If not, justification, action & status
methods used and time intervals for product assessment.		
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: ☐ LAL instead of rabbit pyrogen ☐ mycoplasma ☐ sterility	Y N Y N Y N	
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y N	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y N	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	Y N	
Information and data supporting validity of sterilization processes for sterile products and aseptic manufacturing operations	Y N	

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? _____

If the application is not fileable from product quality perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Jonhisa Sakamoto
Product Quality Reviewer(s)

Aug 11, 2007
Date

Bang Chen
Branch Chief/Team Leader/Supervisor

Aug 11, 2007
Date

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

(b) (4)

BLA/NDA Number: 125288

Applicant: BMS-Syracuse

Stamp Date: July 1, 2009

Drug Name: belatacept

BLA/NDA Type: 10-month *INITIAL*

On initial overview of the BLA/NDA application for filing:

CTD Module 1 Contents	Present?	If not, justification, action & status
Cover Letter	Y N	
Form 356h completed	Y N	
☐ including list of all establishment sites and their registration numbers	Y N	
Comprehensive Table of Contents	Y N	
Environmental assessment or request for categorical exclusion (21 CFR Part 25)	Y N	
Labeling:	Y N	
☐ PI –non-annotated	Y N	
☐ PI –annotated	Y N	
☐ PI (electronic)	Y N	
☐ Medication Guide	Y N	
☐ Patient Insert	Y N	
☐ package and container	Y N	
☐ diluent	Y N	
☐ other components	Y N	
☐ established name (e.g. USAN)	Y N	
☐ proprietary name (for review)	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization of paper and electronic components sufficient to permit substantive review?: Examples include:	Y N	
☐ legible	Y N	
☐ English (or translated into English)	Y N	
☐ compatible file formats	Y N	
☐ navigable hyper-links	Y N	
☐ interpretable data tabulations (line listings) & graphical displays	Y N	
☐ summary reports reference the location of individual data and records	Y N	
☐ all electronic submission components usable (e.g. conforms to published guidance)	Y N	
Companion application received if a shared or divided manufacturing	Y N	

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

Examples of Filing Issues	Yes?	If not, justification, action & status
arrangement		

CTD Module 2 Contents	Present?	If not, justification, action & status
Overall CTD Table of Contents [2.1]	Y N	
Introduction to the summary documents (1 page) [2.2]	Y N	
Quality overall summary [2.3]	Y N	
☐ Drug Substance	Y N	
☐ Drug Product	Y N	
☐ Facilities and Equipment	Y N	
☐ Adventitious Agents Safety Evaluation	Y N	
☐ Novel Excipients	Y N	
☐ Executed Batch Records	Y N	
☐ Method Validation Package	Y N	
☐ Comparability Protocols	Y N	

CTD Module 3 Contents	Present?	If not, justification, action & status
Module Table of Contents [3.1]	Y N	
Drug Substance [3.2.S]		
☐ general info	Y N	
o nomenclature		
o structure (e.g. sequence, glycosylation sites)		
o properties		
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
☐ description of manufacturing process	Y N	
o batch numbering and pooling scheme		
o cell culture and harvest		
o purification		
o filling, storage and shipping		
☐ control of materials	Y N	
o raw materials and reagents		
o biological source and starting materials		
o cell substrate: source, history, and generation		

PRODUCT QUALITY (BIOTECHNOLOGY)
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CTD Module 3 Contents	Present?	If not, justification, action & status
○ cell banking system, characterization, and testing		
☐ control of critical steps and intermediates	Y N	
○ justification of specifications		
○ analytical method validation		
○ reference standards		
○ stability		
☐ process validation (prospective plan, results, analysis, and conclusions)	Y N	
☐ manufacturing process development (describe changes during non- clinical and clinical development; justification for changes)	Y N	
☐ characterization of drug substance		
☐ control of drug substance	Y N	
○ specifications	Y N	
○ justification of specs.		
○ analytical procedures		
○ analytical method validation		
○ batch analyses		
○ consistency (3 <u>consecutive</u> lots)		
○ justification of specs.		
☐ reference standards		
☐ container closure system	☒ N	
☐ stability	Y N	
☐ summary	Y N	
☐ post-approval protocol and commitment		
☐ pre-approval		
○ protocol		
○ results		
○ method validation		
Drug Product [3.2.P]		
☐ description and composition	Y N	
☐ pharmaceutical development	Y N	
○ preservative effectiveness	Y N	
○ container-closure integrity	☒ N	
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y N	
☐ batch formula		

PRODUCT QUALITY (BIOTECHNOLOGY)
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CTD Module 3 Contents	Present?		If not, justification, action & status
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y	N	
☐ controls of critical steps and intermediates	Y	N	
☐ process validation including aseptic processing & sterility assurance: ☐ 3 <u>consecutive</u> lots ☐ Filter validation ☐ Component, container, closure depyrogenation and sterilization validation ☐ Validation of aseptic processing (media simulations) ☐ Environmental Monitoring Program ☐ Lyophilizer sterilization validation ☐ Other needed validation data (hold times)	Y	N	
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin)	Y	N	
☐ control of drug product (justification of specifications; analytical method validation)	Y	N	
☐ container closure system [3.2.P.7] ☐ specifications (vial, elastomer, drawings) ☐ availability of DMF & LOAs ☐ administration device(s)	Y	N	
☐ stability ☐ summary ☐ post-approval protocol and commitment ☐ pre-approval ☐ protocol ☐ results ☐ method validation	Y	N	
Diluent (vials or filled syringes) [3.2.P']			

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?		If not, justification, action & status
☐ description and composition of diluent	Y	N	
☐ pharmaceutical development	Y	N	
☐ preservative effectiveness	Y	N	
☐ container-closure integrity	Y	N	
☐ manufacturers (names, locations, and responsibilities of all sites involved)	Y	N	
☐ batch formula			
☐ description of manufacturing process for production through finishing, including formulation, filling, labeling and packaging (including all steps performed at outside [e.g., contract] facilities)	Y	N	
☐ controls of critical steps and intermediates	Y	N	
☐ process validation including aseptic processing & sterility assurance:	Y	N	
☐ 3 <u>consecutive</u> lots			
☐ Filter validation			
☐ Component, container, closure depyrogenation and sterilization validation		N	
☐ Validation of aseptic processing (media simulations)	Y	N	
☐ Environmental Monitoring Program	Y	N	
☐ Lyophilizer sterilization validation			
☐ Other needed validation data (hold times)	Y	N	
☐ control of excipients (justification of specifications; analytical method validation; excipients of human/animal origin, other novel excipients)			
☐ control of diluent (justification of specifications; analytical method validation, batch analysis, characterization of impurities)			
☐ reference standards			

PRODUCT QUALITY (BIOTECHNOLOGY) FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
☐ container closure system ○ specifications (vial, elastomer, drawings) ○ availability of DMF & LOAs ☐ stability ☐ summary ☐ post-approval protocol and commitment ☐ pre-approval ○ protocol ○ results		
Other components to be marketed (full description and supporting data, as listed above): ☐ other devices ☐ other marketed chemicals (e.g. part of kit)	Y N Y N	
Appendices for Biotech Products [3.2.A] ☐ facilities and equipment ○ manufacturing flow; adjacent areas ○ other products in facility ○ equipment dedication, preparation, sterilization and storage ○ procedures and design features to prevent contamination and cross-contamination ☐ adventitious agents safety evaluation (viral and non-viral) e.g.: ○ avoidance and control procedures ○ cell line qualification ○ other materials of biological origin ○ viral testing of unprocessed bulk ○ viral clearance studies ○ testing at appropriate stages of production ☐ novel excipients	Y N Y N Y N Y N	

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

CTD Module 3 Contents	Present?	If not, justification, action & status
	Y N	
USA Regional Information [3.2.R]		
☐ executed batch records	Y N	
☐ method validation package	Y N	
☐ comparability protocols	Y N	
Literature references and copies [3.3]	Y N	

Examples of Filing Issues	Yes?	If not, justification, action & status
Content, presentation, and organization sufficient to permit substantive review?	Y N	
☐ legible	Y N	
☐ English (or translated into English)	Y N	
☐ compatible file formats	Y N	
☐ navigable hyper-links	Y N	
☐ interpretable data tabulations (line listings) & graphical displays	Y N	
☐ summary reports reference the location of individual data and records	Y N	
☐ all electronic submission components usable	Y N	
Includes appropriate process validation data for the manufacturing process at the commercial production facility	Y N	
Includes production data on drug substance and drug product manufactured in the facility intended to be licensed (including pilot facilities) using the final production process(es)	Y N	
Includes data demonstrating consistency of manufacture	Y N	
Includes complete description of product lots and manufacturing process utilized for clinical studies	Y N	
Describes changes in the manufacturing process, from material used in clinical trial to commercial production lots	Y N	
Data demonstrating comparability of product to be marketed to that used in clinical trials (when significant changes in manufacturing processes or facilities have occurred)	Y N	
Certification that all facilities are ready for inspection	Y N	

**PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)**

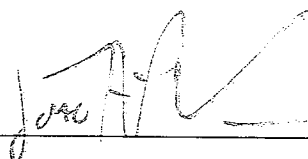
Examples of Filing Issues	Yes?	If not, justification, action & status
Data establishing stability of the product through the proposed dating period and a stability protocol describing the test methods used and time intervals for product assessment.	Y N	
If not using a test or process specified by regulation, data is provided to show the alternate is equivalent (21 CFR 610.9) to that specified by regulation. List: ☐ LAL instead of rabbit pyrogen ☐ mycoplasma ☐ sterility	Y N Y N Y N	
Identification by lot number, and submission upon request, of sample(s) representative of the product to be marketed; summaries of test results for those samples	Y N	
Floor diagrams that address the flow of the manufacturing process for the drug substance and drug product	Y N	
Description of precautions taken to prevent product contamination and cross-contamination, including identification of other products utilizing the same manufacturing areas and equipment	Y N	
Information and data supporting validity of sterilization processes for sterile products and aseptic manufacturing operations	Y N	

IS THE PRODUCT QUALITY SECTION OF THE APPLICATION FILEABLE? _____

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Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

Joao A Pedras-Vasconcelos
Product Quality Reviewer(s)



8/11/09
Date

PRODUCT QUALITY (BIOTECHNOLOGY)
FILING REVIEW FOR BLA/NDA (OBP & DMPQ)

Barry Chen

8/12-09

Branch Chief/Team Leader/Supervisor

Date