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RESEARCH**

APPLICATION NUMBER:

125288Orig1s000

PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA 125288
Supporting document/s: 55
Applicant's letter date: 9/24/10
CDER stamp date: 9/27/10
Product: Belatacept
Indication: Prophylaxis of organ rejection in adult patients
receiving renal transplants
Applicant: Bristol-Myers Squibb
Review Division: Division of Special Pathogen and Transplant
Products
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Template Version: September 1, 2010

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1 Executive Summary

Belatacept is a selective T-cell costimulation blocker indicated for prophylaxis of organ rejection in adult patients receiving a kidney transplant. During clinical trials, patients treated with belatacept have shown an increased risk for post-transplant lymphoproliferative disease (PTLD) predominantly in the central nervous system (CNS) and progressive multifocal leukoencephalopathy (PML). In order to understand the potential mechanism behind the clinical CNS adverse events, additional investigative nonclinical studies in cynomolgus monkeys were conducted to evaluate whether belatacept penetrates the brain or affects brain permeability when administered with mycophenolate mofetil (MMF), an immunosuppressant used in combination with belatacept in transplant patients.

Low levels of belatacept were measured in the brain (due to residual blood in the microvasculature) and cerebrospinal fluid (due to blood contamination). Using immunofluorescence, belatacept measured in the brain was localized to the microvasculature. No test article related changes in brain morphology, immune cells, or CD80/86 were observed in the cynomolgus monkey brain. MHC Class II (expressed on macrophages, dendritic cells, granulocytes and B-cells) expression in the brain was increased with belatacept treatment. It is not known whether this change represents an up-regulation of MHC Class II on cells specific to the CNS, since markers on these specific cell types (e.g., microglia, astrocytes, or oligodendrocytes) were not examined. Since no adverse findings were associated with this change in monkeys, the clinical relevance and relationship to the CNS PTLD or PML cases observed in the clinic are unknown.

Belatacept administered with MMF did not cause morphologic changes in the brain or change the permeability of the brain based on measurement of belatacept and MMF metabolites in the brain.

Belatacept did not appear to penetrate the brain, alter brain permeability in the presence of MMF, or cause adverse effects in the brain in cynomolgus monkeys treated with belatacept for one month at up to 50 mg/kg or ~5.6-fold over the highest anticipated clinical exposures during the first month of treatment.

The results of the new nonclinical data support the use of belatacept in the prophylaxis of organ rejection in kidney transplant patients.

1.1 Recommendations

1.1.1 Approvability

The pharmacology and toxicology studies submitted to BLA 125288 support the approval of belatacept (BMS-224818) for the specified indication.

1.1.2 Additional Non Clinical Recommendations

No additional nonclinical studies are recommended.

1.1.3 Labeling

Nonclinical changes to the labeling are recommended for Sections 8.1 and 13 as follows:



(b) (4)



1.2 Brief Discussion of Nonclinical Findings

Low levels of belatacept were measured in the brain (due to residual blood in the microvasculature) and cerebrospinal fluid (due to blood contamination). Using

immunofluorescence, belatacept measured in the brain was localized to the microvasculature. No test article related changes in brain morphology, immune cells, or CD80/86 were observed in the cynomolgus monkey brain. MHC Class II (expressed on macrophages, dendritic cells, granulocytes and B-cells) was increased with belatacept treatment. It is not known whether this change is clinically meaningful or related to the clinical CNS PTLD or PML cases; no adverse findings were associated with this change. No other immune cell surface markers were changed.

Belatacept administered with MMF did not cause morphologic changes in the brain or change the permeability of the brain based on measurement of belatacept and MPA/MPAG levels in the brain.

1.2.1 Basis of Recommendation

The nonclinical pharmacology and toxicology study results did not identify safety issues that would preclude the approval of belatacept.

1.2.2 Clinical Implication

It is unclear whether the increase in MHC Class II expression in the brains of monkeys is clinically meaningful and related to the clinical CNS adverse events since no adverse findings were associated with this change in monkeys.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

749867-37-6

2.1.2 Generic Name

belatacept

2.1.3 Code Names

BMS-224818, LEA29Y

2.1.4 Trade Name

Nulojix[™]

2.1.5 Chemical Name

(1) CTLA-4 (antigen) [29-tyrosine, 104-glutamic acid] (human extracellular domain containing fragment) fusion protein with immunoglobulin G1 (human monoclonal Fc domain-containing fragment), bimol. (120→120^o)-disulfide.

(2) [Tyr₂₉, Glu₁₀₄, Gln₁₂₅, Ser₁₃₀, Ser₁₃₆, Ser₁₃₉, Ser₁₄₈](CTLA-4 (antigen)-[3-126]-peptide (human

extracellular domain-containing fragment) fusion protein with immunoglobulin G1-[233 C-terminal residues of the heavy chain]-peptide (human monoclonal Fc domain containing fragment)) bimolecular (120→120)-disulfide.

2.1.6 Molecular Weight

90,619 Da (determined by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry).

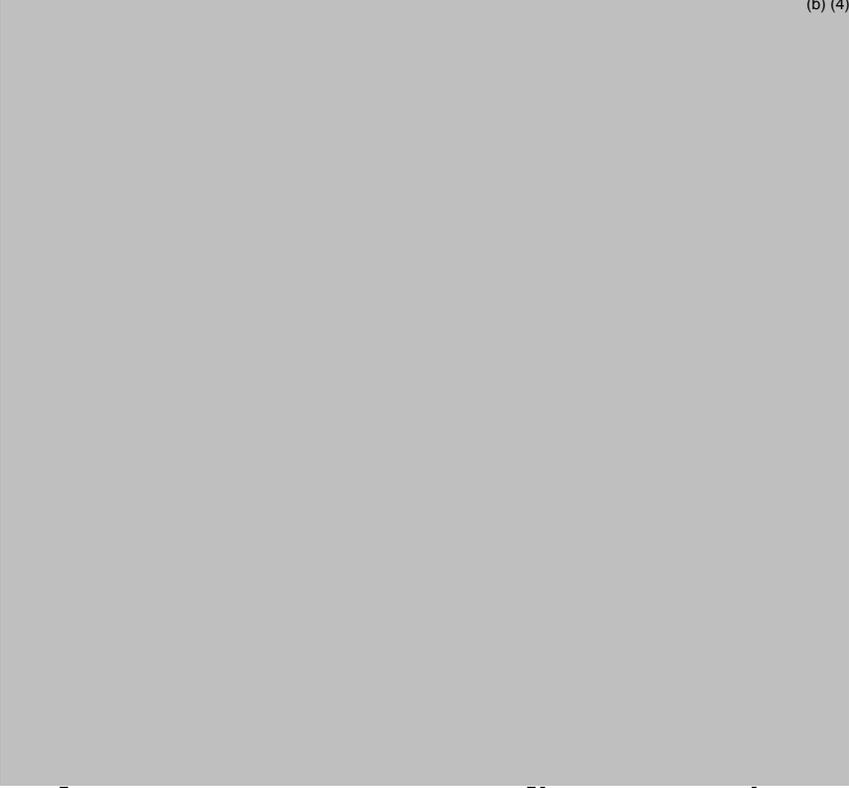
2.1.7 Pharmacological Class

T-cell co-stimulation blocker

2.1.8 Structure

Belatacept is a genetically engineered fusion protein consisting of the functional binding domain of modified human cytotoxic T-lymphocyte antigen-4 (CTLA-4) and the Fc domain of human IgG₁. Belatacept is made up of two polypeptide chains with 357 amino acids, and exists as a covalent homodimer (referred to as belatacept[™] "monomer") linked through an inter-chain disulfide bond.

(b) (4)



2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 9418 Belatacept

BLA 125288 Belatacept

BLA 125118 Orencia[®] (abatacept)

2.3 Drug Formulation

Belatacept drug substance is a clear to opalescent, colorless to pale yellow solution which contains [REDACTED] (b) (4)

2.4 Comments on Novel Excipients

See original review.

2.5 Comments on Impurities/Degradants of Concern

See original review.

2.6 Proposed Clinical Protocol

None.

2.7 Regulatory Background

A summary of the regulatory history highlights and issues relevant to the nonclinical development program are provided below.

- IND submitted October 19, 2000 to the Center for Biologics Evaluation and Research.
- End of Phase 2 meeting on September 21, 2004. The Pharmacology/Toxicology reviewer clarified that the abatacept reproductive toxicology program was not adequate to support belatacept because of the differences in binding and potency. The reviewer requested that belatacept reproductive and developmental toxicology studies be conducted to support Phase 3 clinical trials.
- Fast track designation was granted January 26, 2005.
- Special Protocol Assessment for clinical Protocol IM103008, FDA letter dated September 22, 2005.
- Pediatric Type C Meeting on December 18, 2007. The Division raised the concern that blocking CD80/86-CD28 costimulation of pre-T-cells in the thymus would allow for self-reactive thymocytes to escape, potentially leading to autoimmunity. The applicant responded that they were investigating autoimmunity in the context of the rat juvenile toxicology studies and would share the data with the Division once available.
- Orphan Drug designation granted on February 20, 2008.
- **Type B Guidance Meeting on December 15, 2008. BMS agreed to investigate the brain levels of belatacept in nonhuman primates.**
- **Pre-BLA Meeting on May 20, 2009. The study design to evaluate belatacept concentrations in the monkey brain was discussed (Study DS09027).**
- BLA 125288 submitted to FDA electronically on July 1, 2009.

Resubmission of BLA:

- August 16, 2010: Response to the proposed REMS deficiency
- September 9, 2010: Response to the CMC product quality deficiency

- September 24, 2010: Responses to the clinical, product quality microbiology, facility inspections, safety update, labeling, nonclinical study reports, clinic study reports, and Medication Guide deficiencies.
- December 15, 2010: Response to facilities inspection readiness
- PDUFA goal date June 16, 2011

3 Studies Submitted

3.1 Studies Reviewed

DS09027, BMS-224818: A One-Month Intravenous Investigative Study in Male Monkeys

DS09113, BMS-224818: One-Month Combination Investigative Study with Mycophenolate Mofetil and Cyclosporin A

Note: Figures/Tables used in this review were copied from the Applicant's Study Reports.

3.3 Previous Reviews Referenced

BLA Pharmacology/Toxicology Review - Mu, Y and Lansita, J 2010⁸.

4 Pharmacokinetics/ADME/Toxicokinetics

Study title: BMS-224818: One-Month Combination Investigative Study with Mycophenolate Mofetil and Cyclosporin A

Study no.: DS09113

Study report location: Same as conducting laboratory.

Conducting laboratory and location: (b) (4)

Date of study initiation: August 13, 2009

GLP compliance: No

QA statement: No

Drug, lot #, and % purity: BMS-224818, Lot no. 7M066, purity not provided
Cyclosporin A (CsA), Lot Nos. H5006 and H5008, purity not provided
mycophenolate mofetil (MMF), Lot no. U0328, purity not provided

The study was conducted to understand the mechanism behind the observed cases of CNS post-transplant lymphoproliferative disease (PTLD) in belatacept clinical trials and evaluate whether the co-administration of belatacept and MMF changed the brain permeability of belatacept or metabolites of MMF. Male monkeys (young adults, age

not specified, weighing 2.4 to 3.2 kg) were treated for one month according to the following 4 treatment groups: 1) belatacept (BMS-224818) alone, 2) mycophenolate mofetil (MMF) alone, 3) MMF and cyclosporine (CsA) in combination, and 4) belatacept and mycophenolate mofetil in combination. Doses used were as follows: belatacept was administered at 50 mg/kg, intravenously (IV), weekly for a total of 5 doses. MMF was dosed orally at 25 mg/kg/day and CsA was dosed orally at 100 mg/kg/day (Table 1).

Table 1 Study Design

Group No	No of Males	Belatacept - IV, weekly (mg/kg)	CsA - oral, daily (mg/kg)	MMF - oral, daily (mg/kg)
1	6	50	---	25
2	6	---	---	25
3	6	---	100	25
4	4	50	---	---

Total daily doses were divided into two equal daily doses; necropsies were 24 hours after the last dose on Day 30.

Belatacept, mycophenolic acid (MPA), the active metabolite of MMF, glucuronidated MPA (an inactive metabolite of MMF) and CsA were measured in the plasma (or serum for belatacept), brain and cerebrospinal fluid (CSF). Systemic exposures of belatacept, MPA, and MPAG were measured on Days 22 and 30. Plasma levels of CsA were measured on Days 5, 22, and 30. Brain tissues (parietal cerebrum, cerebellum, and brain stem) and CSF (collected 4 hours after the last dose from the cisterna magna) were collected at necropsy. CSF samples were analyzed for total protein, red blood cell counts, white blood cell counts, and absolute total nucleated cell counts to determine if contamination occurred. Total immunoglobulin G (IgG) levels were measured in the serum and brain tissues on Day 30. Histopathology of the brain was done to evaluate any potential test article related effects. Brain perfusion with saline was NOT done to remove residual amounts of drug in the vasculature because the applicant was concerned that any drug remaining in the brain would be washed out by moving down a concentration gradient. [Saline perfusion was performed in Study No. DS09027 (review below); this difference in methodology explains the ~10-fold difference in belatacept brain levels between the two studies.]

Additional endpoints included mortality, clinical observations, body weights, physical examinations, serum chemistry, hematology, gross-pathology, and organ weights for specific tissues (adrenal gland, brain, heart, kidney, liver, pituitary, prostate, seminal vesicles, spleen, testis, thyroid with parathyroid).

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The 50 mg/kg belatacept dose level was selected to provide a 5.6-fold exposure multiple over the highest exposure in patients during the first month of treatment; this

dose level was also used in the 6 month chronic monkey study. The 100 mg/kg CsA dose was selected based on the anticipated efficacious dose in monkeys. Finally the 25 mg/kg dose level for MMF was selected to achieve similar exposures to humans.

Results

All animals survived until the end of the study on Day 30. There were no test article related changes in body weights, physical examinations, clinical observations, serum chemistry, urinalysis, organ weights, or gross pathology. There were minor changes in hematology which included increases in reticulocytes in MMF (Day 15 and 30) and MMF+CsA (Day 15) treated animals that did not appear to be clinically significant.

Table 2 Toxicokinetics Summary (Day 22)

Day 22 Toxicokinetics Summary					
Parameter	Day	MMF 200mg/kg + CsA/MMF Doses (mg/kg)			
		50/25	0/25	0/100/25	50/0
MMF 200mg/kg					
C_{max} (ng/mL)	22	1,500	N/A	N/A	1,600
AUC(0-168h) (ng-h/mL)	22	65,000	N/A	N/A	66,900
CsA					
C_{max} (ng/mL)	22	N/A	N/A	1,140	N/A
AUC(0-24h) (ng-h/mL)	22	N/A	N/A	11,900	N/A
T_{max} (h)	22	N/A	N/A	2.5	N/A
MPA					
C_{max} (ng/mL)	22	4,210	111,500	3,630	N/A
AUC(0-4) (ng-h/mL)	22	30,700	42,100	15,500	N/A
T_{max} (h)	22	2.2	2.0	1.8	N/A
MPA/C					
C_{max} (ng/mL)	22	111,900	15,700	24,700	N/A
AUC(0-4) (ng-h/mL)	22	112,000	65,500	105,000	N/A
T_{max} (h)	22	1.7	1.5	2.8	N/A

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N/A = not applicable
T = 0, 2, 4, or 24 hours

Pharmacokinetic analyses showed that there was no difference in belatacept AUC or C_{max} for belatacept + MMF treated animals compared with belatacept-alone treated animals. Lower systemic MPA levels were observed in cynomolgus monkeys treated with belatacept + MMF (C_{max} 63% lower, AUC 27% lower) or CsA + MMF at 25 mg/kg (C_{max} 68% lower, AUC 63% lower) compared with MMF alone. The mechanism by which belatacept treatment results in lower MPA levels is unclear; it is possible that belatacept could alter the metabolism of MMF to MPA or the clearance of MPA by renal transporters resulting in lowered MPA exposures. However, the mean AUC of MPA

was 2-fold or 98% higher (C_{max} similar) in animals treated with belatacept and MMF compared to animals treated with CsA and MMF. In patients, MMF + belatacept resulted in systemic MPA levels that were higher (C_{max} 20% higher, AUC_{0-12} 40% higher) compared with CsA + MMF (USPI, Section 7.2, Use with Mycophenolate Mofetil). Systemic MPAG exposures were 70% higher for AUC and 24% lower for C_{max} in MMF + belatacept treated animals compared with MMF alone. Systemic MPAG levels were increased (C_{max} 57% higher, AUC 64% higher) in MMF + CsA treated animals compared with MMF alone (Table 2).

Belatacept, MPA and CsA were measured at low levels in the CSF and brain tissue (Table 3). The applicant uses the ratios of IgG in the brain to systemic exposures to argue that belatacept, MPA and CsA did not cross the BBB but were measurable because they were present in the microvasculature similar to IgG which does not cross the BBB. IgG levels from homogenized cerebellum were measured to account for the measured belatacept and MPA levels in the brains of monkeys. The study report notes that:

The low concentrations of BMS-224818, MPA, and CsA in CSF samples were consistent with low-level blood contamination, as demonstrated by presence of red- and white-blood cells in CSF hematology analysis samples. The range of brain to serum, plasma, or whole blood ratios of BMS-224818 (0.4% to 1.1%), MPA (0.1% to 0.8%), and CsA (1.2% to 2.0%) were similar to those ratios calculated for IgG (0.2% to 1.1%), indicating that the test articles did not cross the BBB. Furthermore, none of the combinatorial treatments affected BBB permeability of IgG as demonstrated by similar IgG brain-to-serum ratios amongst all treatment groups.

Table 3 Summary of Belatacept, MPA, MPAG and CsA in the CSF and Brain

Day 30 Bioanalysis Summary						
BMS-224818-CsA/MMF (mg/kg)	Analyte	Mean Concentrations			Mean Ratios^a	
		(ng/ml) or (ng/g for tissue)			CSF/Systemic	Brain/Systemic
		CSF	Brain	Systemic		
500/25 500/0	BMS-224818	272.10	3904.02	533212.90	0.000434	0.00755
		346.10	4152.27	564431.83	0.000613	0.00811
0/100/25	CsA	2.30	10.88	1307.33	0.00167	0.0147
500/25 0/0/25 0/100/25	MPA	8.06	10.56	3951.67	0.0024	0.00280
		7.19	8.18	3385.08	0.00230	0.00245
		7.41	9.45	3162.33	0.00233	0.00330
500/25 0/0/25 0/100/25	MPAG	N/A	N/A	4102.33	N/A	N/A
		N/A	N/A	4306.67	N/A	N/A
		N/A	N/A	27216.67	N/A	N/A

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Systemic = levels in serum (BMS-224818), blood (CsA), or plasma (MPA, MPAG); N/A = not available; MPAG levels in brain and CSF were below the lower detection limit of the bioanalytical assay.

^a Ratios were calculated for each monkey/analyte/group, and the mean is reported in the table.

CSF analyses showed contamination of samples with red blood cells (RBC), white blood cells (WBC), and protein. Three samples were clotted from Animal No.'s 1005, 3002, and 4004 and showed significant blood contamination. Red blood cell counts were high for animal No.'s 3003 (10,450 cells/ul) and 4003 (34,250 cells/ul). RBC counts for all other animals ranged from 0-1500 cells/ul. WBC counts (2-23 cells/ul) and protein levels (9-51 mg/dl) were low across animals with the exception of protein levels in samples that were clotted (>200-645 mg/dL). Cell counts were generally too low to analyze the differential cell counts. In three samples, differential counts showed that lymphocytes (72-99%) were present in the CSF at the highest percentage, followed by neutrophils (0-26%) and monocytes (1-2%). Although the applicant argues that the measurable levels of belatacept are due to contamination, the correlation between RBC contamination of the CSF and belatacept levels in the CSF was unclear; in the CSF sample with the highest belatacept levels, the RBC counts were also high (Animal No. 4003, belatacept - 2775.37 ng/ml, 34250 RBC cells/ml), however in a sample with no RBC counts, there was detectable belatacept at levels that were higher than samples with RBC counts (Animal No. 4001 belatacept - 384.17 ng/ml, 0 RBC cells/ml). A correlation was also unclear for contaminating WBC counts and protein levels.

Histopathology

A targeted histopathological evaluation of the brain was performed to understand whether brain permeability was affected with belatacept and MMF. Microscopic pathology of 5 regions (brain stem, cerebellum, parietal cerebrum, occipital cerebrum, and anterior cerebrum) of the brain were evaluated. Minimal focal gliosis was observed in 2/4 belatacept treated animals (anterior cerebrum), and 1/6 belatacept + MMF treated (cerebellum) animals. Focal perivascular infiltrates of the brainstem were seen in 1/6 belatacept + MMF animal. The brain findings appear to be incidental since they were not observed in the chronic 6 month monkey study or in the one month investigative study (DS09027) performed at the same lab.

At gross necropsy Animal No. 1104 showed enlargement of the mandibular lymph node. The mandibular lymph node was further evaluated microscopically and diagnosed with reactive lymphoid hyperplasia. The study report concludes that this is a common background finding.

Summary and Conclusion

Belatacept + MMF showed decreased systemic MPA exposures compared with MMF-alone treated animals and a corresponding increase in MPAG that was similar to CsA + MMF treated animals. Compared to animals treated with CsA + MMF, the mean AUC for MPA was 98% higher in animals treated with belatacept + MMF. In patients, belatacept + MMF showed higher systemic MPA levels (C_{max} 20%, AUC_{0-12} 40%) compared with CsA + MMF (USPI, Section 7.2 *Use with Mycophenolate Mofetil*).

No novel toxicities were observed in animals treated with belatacept + MMF. Low levels of belatacept, MPA, and CsA were measured in the CSF and brain; however, no morphological changes were associated with these low levels after one month of treatment. The applicant claims that these levels were due to sample contamination

which appears likely since Study No. DS09027 (review below) did not show evidence from immunofluorescent staining that belatacept penetrated non-vascularized brain tissues.

In conclusion, the co-administration of belatacept + MMF did not appear to change the levels of belatacept or metabolites of MMF in the brain or CSF. If belatacept does cross into brain tissues at low levels, it is not associated with morphological changes in the brain.

5 Special Toxicology Studies

Study title: BMS-224818: A One-Month Intravenous Investigative Study in Male Monkeys

Study no.: DS09027
Study report location: Same as conducting laboratory.
Conducting laboratory and location:  (b) (4)

Date of study initiation: April 8, 2009
GLP compliance: No
QA statement: No
Drug, lot #, and % purity: BMS-224818, Lot no. 7M066, purity not provided

Purpose

The study was conducted to evaluate the ability of BMS-224818 (belatacept) to cross the blood-brain barrier (BBB) in cynomolgus monkeys. The potential effects of belatacept on immune cells in the brain, as well as the expression of CD80 and CD86 (ligands of CTLA-4) in the brains of cynomolgus monkeys were also evaluated.

Methods

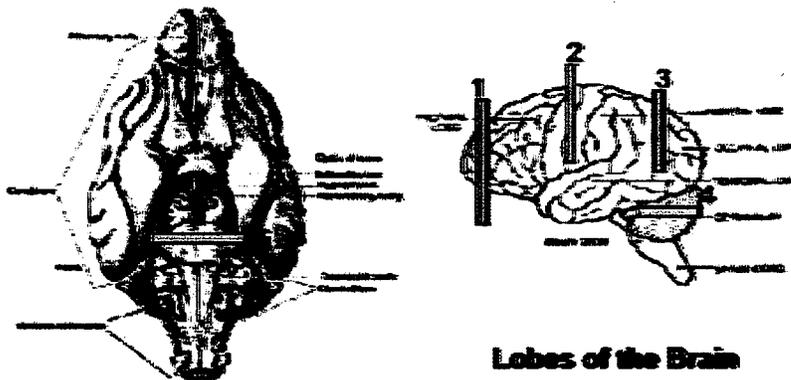
Male cynomolgus monkeys (4/group, 3.2-3.7 kg, young adult - adult, ages were not specified) were administered belatacept by slow intravenous (IV) bolus injection, once weekly for 5 weeks (Days 1, 8, 15, 22 and 29), at 0 (saline control), 10 or 50 mg/kg/week (dose volume 2.5 ml/kg) for a total of 5 doses. Animals were sacrificed 24 hours after the last dose on Day 30. At sacrifice, animals were perfused with saline in order to reduce the potential for blood contamination in the vasculature. BMS-224818 concentrations were measured in serum, homogenized brain tissues (parietal cerebrum (with hippocampus), cerebellum, brain stem (level of pons)), and cerebrospinal fluid (CSF, by puncture of the cisterna magna). The location of the brain sections collected is shown in Figure 2. Additional endpoints included toxicokinetics, clinical observations, body weights, physical examinations, clinical pathology, organ weights (adrenal gland, brain, heart, kidney, liver, pituitary gland, prostate gland, seminal vesicles, spleen, testis, thyroid gland with parathyroid), gross pathology and microscopic pathology of the brain.

Tissues were collected at necropsy on Day 30, formalin-fixed, and embedded in paraffin or OCT medium. Brain tissues were immunostained with the following reagents: fluorescein-conjugated mouse antihuman HLA DP DQ DR (MHC Class II, antigen-presenting cell marker) and biotinylated anti-human CD209 (dendritic-cell marker) antibodies; fluorescein-conjugated mouse anti-human CD68 (macrophage, dendritic-cell, and granulocyte marker) and biotinylated mouse anti-human CD80 (B7-1; macrophage, dendritic-cell, and B-cell marker) antibodies; fluorescein-conjugated sheep anti-human Von Willebrand Factor (endothelial-cell marker) and PE-Cy5-conjugated mouse anti-human CD86 (B7-2; macrophage, dendritic-cell, and B-cell marker) antibodies; fluorescein-conjugated antihuman CD3 (T-cell marker) and PE-Cy5 anti-human CD20 (B-cell marker) antibodies; and mouse-anti-BMS-224818 antibody. For detection of anti-BMS-224818 antibody, an AlexaFluor® 488-conjugated goat anti-mouse antibody was incubated following primary antibody incubation. Leukocyte analyses were performed on brain tissues from all monkeys. Belatacept analyses were performed on brain tissue from vehicle control and high-dose (50 mg/kg) monkeys. Positive controls included spleen for the leukocyte cell surface-markers and thymus from belatacept treated animals for detection of belatacept. Vehicle-control thymus was used as a negative control for evaluating the specificity of belatacept staining.

Figure 2 Location of Brain Sections Collected

Approximate location of brain sections to be collected:

- 1 Anterior cerebrum
- 2 Parietal cerebrum
- 3 Occipital cerebrum
- 4 Cerebellum
- 5 Brainstem (level of pons)



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Brain - Ventral Aspect

Take serial samples for microscopy sample collection:



Collect samples for drug level evaluation in areas adjacent to 2, 4, and 5 to enable potential correlation of histology, immunology, and drug level data.

From P. 274 of Final Study Report (P. 27 of Study Protocol)

For analysis of MHC class II and CD209, the entire tissue was evaluated by manually counting positive cells. The CD209 data showed no positive cells; therefore, the data were not recorded. Statistical analyses of the MHC class II data were performed. For

analysis of other cell surface markers, "25 congruent fields were captured at 40X magnification and tiled into 5 by 5 field analysis images. Cells positive for the markers were then counted from the images, with the exception of Von Willebrand Factor, of which a qualitative observation was deemed scientifically appropriate, and the cell count was divided by 25 to calculate the number of positive cells per field." For analysis of belatacept, 2 sections of tissue from all vehicle control and high-dose (50 mg/kg) monkeys were fully evaluated. Areas of immunofluorescence were imaged and a differential interference contrast (DIC, Nomarski) scan was used to identify areas of microvasculature. Images were captured using a Zeiss LSM 5 Pascal Laser Scanning Microscope, PC hardware, and Microsoft XP operating system.

IgG and albumin were measured in brain homogenates and serum from all animals in the vehicle control and 50 mg/kg dose groups. The applicant determined that "Brain tissue concentration ng/mL was considered equivalent to ng/g as the specific gravity of brain tissue is 1.03." Ratios of IgG and albumin levels in the brain:serum were analyzed to understand measurable protein levels in the brain for proteins that are not expected to cross the BBB, and compare these with the levels observed for belatacept.

Results

All animals survived until the end of the study. There were no test article related changes in clinical observations, body weights, physical examinations, clinical pathology, organ weights or gross or microscopic pathology of the brain.

Toxicokinetics on Day 22 confirmed belatacept exposure in treated animals (Table 4). Belatacept showed dose proportional systemic exposure by AUC and C_{max}. Belatacept showed less than dose proportional increases in the brain and CSF (Table 5). The belatacept brain:serum (0.06-0.07%) and CSF:serum (0.04-0.05%) ratios were very low.

Table 4 Toxicokinetic Summary (Day 22)

Parameter	BMS-224818	
	10 mg/kg/week	50 mg/kg/week
C(3 min) ^a (µg/mL)	380	1,890
AUC(0-168h) (µg·h/mL)	15,200	72,400

^a C(3 min) was the serum belatacept concentration at first postdose sampling time after IV dose administration.

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Low levels of belatacept (<0.07% of systemic exposure) were measured in the CSF and brain after administration of 10 and 50 mg/kg of belatacept. The applicant argues that the low levels of belatacept were due to blood contamination since residual blood was observed in the microvasculature by microscopic examination even though the animals were perfused with saline to remove residual blood from the vasculature. Levels of IgG and albumin were measured to make the argument that belatacept was observed at

similar levels to proteins that do not cross the BBB. Brain to serum ratios ranged from 0.03-0.014% for belatacept, 0.03-0.16% for IgG, and 0.02-0.03% for albumin. Immunofluorescent staining for belatacept showed infrequent staining in the microvasculature and no staining in non-vascular brain tissue. Albumin was similar in control and 50 mg/kg treated animals. Interestingly, total IgG was lower in 50 mg/kg belatacept treated animals in both the brain (-64%) and the serum (-37%) compared with vehicle control animals. As a result, the brain:serum ratio was 42% lower in belatacept treated animals (0.06%) compared with controls (0.10%). This reduction is likely due to the expected pharmacological effects of belatacept. Measurements of IgG were not conducted in cerebrospinal fluid (CSF) or in tissue samples using immunofluorescent staining procedures. Therefore it is unknown whether the measured total IgG is localized to the microvasculature rather than non-vascularized brain tissue. Literature reports have shown mAbs with an IgG Fc localize to brain tissues² and non-vascularized brain tissue³.

Table 5 Belatacept Levels in the CSF, Brain, and Serum (Day 30)

BMS-224818 (mg/kg/week)	Time	Mean Concentrations of BMS-224818 (µg/mL) or (µg/g for tissue)			CSF/Serum Ratio (%) ^b	Brain/Serum Ratio (%) ^c
		CSF	Brain ^a	Serum		
		10	Day 30	0.06		
50	Day 30	0.24	0.36	647.23	0.04	0.06

^a Brain tissue samples from the parietal cerebrum, cerebellum, and brain stem were homogenized and analyzed for BMS-224818 individually. The table shows mean concentrations with the data from the 3 areas of brain averaged per dose group, as there were no differences in BMS-224818 levels amongst the 3 areas.

^b CSF/Serum Ratio = (mean CSF concentration/mean serum concentration) * 100

^c Brain/Serum Ratio = (mean brain concentration/mean serum concentration) * 100

Immunostaining

There were no belatacept related changes in the expression of CD 20, CD68, CD86, CD80, Von Willebrand factor, or CD3 by immunofluorescent analyses. CD86 was found not to be expressed on endothelial cells. A comparison of the median values in control animals compared with treated animals across the 5 regions of the brain that were evaluated (Table 6) showed an increase in MHC class II positive cells per field in belatacept treated animals at 10 mg/kg (1.3-3.8-fold, average 2.2-fold) and 50 mg/kg (2.2-5-fold, average 2.8-fold). The increases were not statistically significant. The range of MHC Class II cells was 0.0-0.3 cells/field in controls and 0.03-0.91 cells/field for animals dosed with belatacept at 10 and 50 mg/kg across all 5 regions of the brain that were evaluated (Figure 3 and Table 6).

The MHC Class II increase was not associated with increases in other immune cell surface markers. From the literature it appears that cell populations in the CNS such as microglia, oligodendrocytes, and astrocytes can also express MHC Class II under specific conditions^{4,5,6}. The study report notes that "... the apparent increase in MHC class II positive cells of less than 1/4th of a cell per field (difference between mean of

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vehicle-control and 50mg/kg BMS-224818-treated group data) was considered not to be biologically relevant (i.e., not indicative of an influx of immune cells) given that 202 to 1254 fields per each tissue sample approximately 1 cm by 1cm in size were evaluated and the range of total MHC class II cells present was only 0 to 370 cells.” This reviewer agrees that it is more likely that this change is an up-regulation of expression rather than an increase or influx of immune cells. Whether this change is clinically meaningful or not is unclear; however, it may be important for further understanding the pharmacological effects of belatacept treatment. Interestingly, the "Maximum" number of MHC class II positive cells per field in all 5 brain regions reported in Table 6 was observed in one animal (Animal No. 3003) out of 4 at 50 mg/kg.

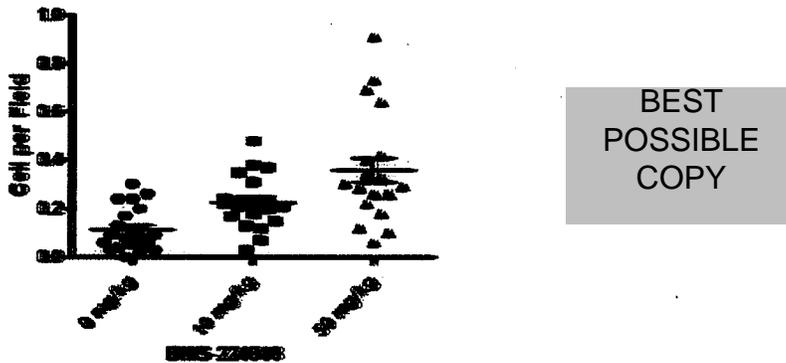


Figure 3 Effect of Belatacept on MHC Class II Cells in the Brain. Data are represented as cells per field. Each data point (25/group) results from 1 of each of the 5 tissues evaluated from each monkey. Mean and standard deviation for each group is shown.

Table 6 MHC Class II Positive Cells per Field in Brain Tissues

Test Table 1					
MHC class II Positive Cells per Field in Brain Tissues					
	Treatment	n	Median	Minimum	Maximum
ANTERIOR CEREBRUM	Vehicle	4	0.12	0.00	0.24
	BMS-224818 10 mg/kg	4	0.27	0.05	0.35
	BMS-224818 50 mg/kg	4	0.27	0.22	0.73
BRAIN STEM	Vehicle	4	0.11	0.09	0.20
	BMS-224818 10 mg/kg	4	0.21	0.13	0.37
	BMS-224818 50 mg/kg	4	0.36	0.30	0.91
CEREBELLUM	Vehicle	4	0.06	0.04	0.11
	BMS-224818 10 mg/kg	4	0.23	0.17	0.43
	BMS-224818 50 mg/kg	4	0.30	0.18	0.64
OCCIPITAL CEREBRUM	Vehicle	4	0.16	0.02	0.30
	BMS-224818 10 mg/kg	4	0.21	0.07	0.33
	BMS-224818 50 mg/kg	4	0.33	0.26	0.69
PARIETAL CEREBRUM	Vehicle	4	0.05	0.05	0.24
	BMS-224818 10 mg/kg	4	0.13	0.12	0.21
	BMS-224818 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 a Bonferroni correction ($p < 0.05$).

^an = number of male monkeys evaluated per Study Group.

Immunofluorescent staining of belatacept was analyzed in controls and 50 mg/kg belatacept treated animals. Belatacept was infrequently detected in the brain microvasculature (less than 3 fields out of 400 to 2500 fields per monkey) and was not seen in non-vascularized brain tissue (Figure 4).

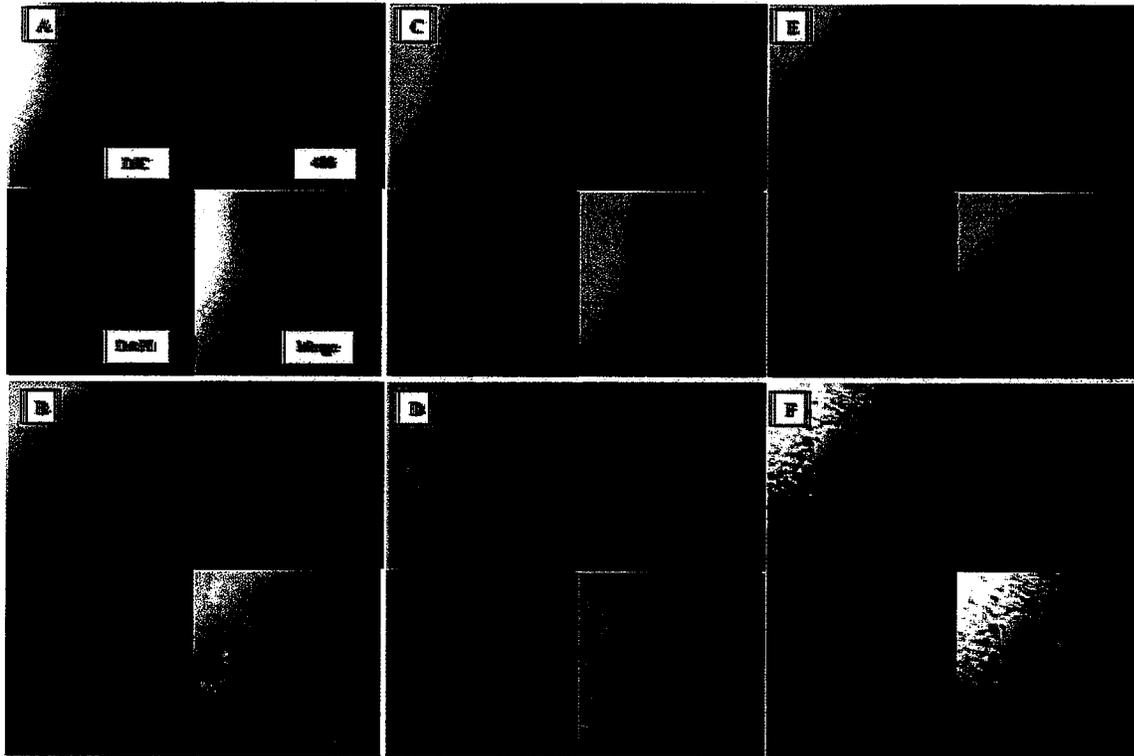


Figure 4 Analysis of BMS-224818 in Thymus (Positive Control) and Brain Tissue.

Tissues were immunostained with an anti-BMS-224818 antibody and imaged at 40X magnification with DIC (upper left), 458/488/514 laser (AlexaFluor® 488 conjugated-BMS-224818, upper right), and 405 laser (DAPI nuclear staining lower left) channels; merge of the three channels is in the lower left image of each panel. Immunostaining of thymus tissue from (A) a vehicle-control and a (B) monkey treated with 50 mg/kg BMS-224818 demonstrated specificity of the anti-BMS-224818 antibody. Cerebellum tissue from a (C) vehicle-control and a (D) high-dose monkey and parietal cerebrum tissue from a (E) vehicle-control and a (F) a high-dose monkey demonstrate BMS-224818 presence in microvasculature (from P. 217 of study report).

Two expert opinion memos from (b) (4) were submitted with the study report in order to support the argument that belatacept levels in the brain and CSF are due to contamination. The experts' main conclusions were that the low levels of belatacept detected in brain samples are likely due to either belatacept entrapped in brain microvessels, belatacept bound to endothelial cells in the brain vascular lumen, or residual blood in the microvessels. (b) (4) notes that belatacept in the CSF was observed at low levels similar to "very poorly penetrating compounds as well as short circulating vascular markers." The low CSF levels are likely due to blood contamination from the lumbar puncture procedure and "usually held to <0.05% with good procedure."

Summary and Conclusion

Belatacept was measured in the CSF and brain tissues at low levels that were likely due to contamination following weekly, IV doses up to 50 mg/kg (5 total doses). The immunofluorescent data confirm that belatacept was present in the brain microvasculature and not the brain tissue. Literature reports indicate that monoclonal antibodies can cross the BBB at low levels. Although belatacept is not a monoclonal antibody, it is a large protein with an IgG1 Fc portion. Whether or not belatacept crossed the BBB at levels that could not be detected in this study are unknown. However, no associated morphologic changes were seen.

Increases in MHC Class II positive cells in the brain of up to 2.8-fold, on average across 5 regions of the brain, were seen in belatacept-treated animals compared with controls. It is unclear whether MHC Class II was up-regulated on cells that were not specifically identified in this study such as microglial cells, astrocytes or oligodendrocytes. However, the up-regulation of MHC Class II was not associated with microscopic pathology changes (e.g., immune cell activation or infiltration) or changes in other immune cell markers normally associated with MHC Class II. In addition, no evidence of an influx of immune cells suggestive of altered immune cell trafficking was seen. Therefore, the clinical significance of this finding is unknown.

6 Integrated Summary and Safety Evaluation

Belatacept is a selective T-cell costimulation blocker indicated for the prophylaxis of organ rejection in adult patients receiving a kidney transplant. Belatacept is a fusion protein comprised of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) extracellular binding domain linked to the modified Fc domain of human immunoglobulin IgG1 (hinge-CH2-CH3 domains). CTLA-4 is an inhibitory receptor expressed on T-helper cells that binds with high affinity to CD80 and CD86, expressed on antigen presenting cells. The interaction between CTLA-4 and CD80/CD86 leads to a blockade of T-cell activation via CD28, a T-cell co-stimulatory molecule required for T-cell activation. Transplant therapies that inhibit T-cell activation and/or proliferation have been shown to enhance graft survival. Therefore, by blocking T-cell co-stimulation via CD28, belatacept therapy may result in the prevention of graft rejection.

Bristol-Myers Squibb submitted BLA for Nulojix (belatacept) to the FDA on July 1, 2009 and the agency issued a Complete Response letter on May 1, 2010. Since that time, the applicant completed and submitted two additional investigative, non-GLP, nonclinical studies in cynomolgus monkeys to further understand an increased risk for post-transplant lymphoproliferative disease (PTLD) and progressive multifocal leukoencephalopathy (PML) in the central nervous system (CNS) observed in kidney transplant patients treated with belatacept. The nonclinical studies were done to address whether belatacept penetrates the brain or affects brain permeability when administered with mycophenolate mofetil (MMF), an immunosuppressant to be used in combination with belatacept in kidney transplant patients.

Low levels of belatacept were measured in the brain (due to residual blood in the microvasculature) and cerebrospinal fluid (due to blood contamination) following IV,

weekly doses of up to 50 mg/kg for one month. These low levels appear to be due to residual blood or sample blood contamination since immunostaining for belatacept in the brain showed positive staining only within the brain microvasculature. Literature reports indicate that monoclonal antibodies can cross the BBB at very low levels. Although belatacept is not a monoclonal antibody, it is a large protein with an intact Fc. Whether or not belatacept crossed the BBB at levels that were not detected in this study is unknown; however, no morphologic changes were observed in the brain and no adverse findings were observed in animals.

The monkey studies showed no changes in CD80/86 (CTLA-4 ligands) expression in the brain. The number of cells positive for MHC Class II (expressed on macrophages, dendritic cells, granulocytes and B-cells) in the brain increased with belatacept treatment. It is not known whether this change represents an upregulation of MHC Class II on cells specific to the CNS, since markers specific for these cell types (e.g., microglia, astrocytes, or oligodendrocytes) were not used. This finding was not associated with morphologic changes or adverse consequences, therefore the clinical relevance and relationship to the observed clinical CNS PTLD and PML adverse events is unknown.

Belatacept administered with MMF did not show effects on brain morphology or brain permeability. The intravenous administration of belatacept + MMF did not change the levels of belatacept or MMF metabolites in the brain or CSF. Belatacept + MMF showed lower systemic MPA exposures and a corresponding increase in MPAG compared with MMF-alone treated animals. It is unclear whether belatacept treatment may alter the metabolism of MMF to MPA or affect the renal clearance of MPA. CsA + MMF treated animals showed similar changes. Compared to animals treated with CsA + MMF, MPA levels were 98% higher in animals treated with belatacept + MMF. In patients, belatacept + MMF showed higher systemic MPA levels (C_{max} 20%, AUC_{0-12} 40%) compared with CsA + MMF treatment⁷. No novel toxicities were observed in animals treated with belatacept + MMF.

The new nonclinical data show that belatacept did not appear to penetrate the brain, alter brain permeability in the presence of MMF, or cause adverse effects in the brain in cynomolgus monkeys treated with belatacept for one month at up to 50 mg/kg which is 5.6-fold over the highest anticipated clinical exposure during the first month of treatment.

The major safety signals previously observed in toxicology studies with belatacept or abatacept included a risk of infections, autoimmunity, and carcinogenicity, which are all described in the product package insert^{7,8}. The results of the new nonclinical data in addition to the previously submitted nonclinical package support the use of belatacept in the prophylaxis of organ rejection in kidney transplant patients.

Signatures:

Reviewer Signature  Date 03/28/2011
Janice Lansita, Ph.D., DABT
Pharmacology/Toxicology Reviewer

Supervisor Signature  Date 3/28/2011
William H. Taylor, Ph.D., DABT
Pharmacology/Toxicology Supervisor

References:

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- ⁵ De Keyser J, Laureys G, Demol F, Wilczak N, Mostert J, Clinckers R. Astrocytes as potential targets to suppress inflammatory demyelinating lesions in multiple sclerosis. *Neurochem Int.* 2010;57:446-50.
- ⁶ Itoh T, Horiuchi M, Itoh A. Interferon-triggered transcriptional cascades in the oligodendroglial lineage: a comparison of induction of MHC class II antigen between oligodendroglial progenitor cells and mature oligodendrocytes. *J Neuroimmunol.* 2009;212:53-64.
- ⁷ Nulojix US Package Insert, Draft 2011.
- ⁸ Ying M and Lansita J, Pharmacology/Toxicology BLA Review 2009

Comments on BLA 125288 Nulojix belatacept
From: Abigail Jacobs *A.J. 4/22/10*
Date : 4/19/10

There are no remaining pharm/tox issues

I have discussed some aspects of labeling with the reviewer/team leader

(b) (4)

2. The amount of detail in the labeling for autoimmunity and the placement of the material in the animal tox section vs the pregnancy section.
3. That the patients will be receiving other immunosuppressants in conjunction with the biologic

The reviewer has appropriately addressed these comments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: BLA125288
Supporting document/s: BLA 125118 Orenzia® (abatacept)
Applicant's letter date: June 30, 2009
CDER stamp date: July 1, 2009
Product: Nulojix™ (belatacept)
Indication: Prophylaxis of organ rejection in kidney transplant patients.
Applicant: Bristol-Myers Squibb Company
Review Division: Division of Special Pathogen and Transplant Products
Reviewer: Janice Lansita, Ph.D., DABT
YING MU, M.D., Ph.D.
Supervisor/Team Leader: William H. Taylor, Ph.D., DABT *William H Taylor* 5 01 2010
Division Director: Renata Albrecht, M.D.
Project Manager: June Germain, MS

Template Version: December 7, 2009

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Any data or information described or referenced below from a previously approved application that Bristol-Myers Squibb Company does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of BLA 125288.

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1 Executive Summary

Belatacept is a fusion protein comprised of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) extracellular binding domain linked to a modified Fc domain of human immunoglobulin IgG1 (hinge-CH2-CH3 domains). CTLA-4 is an inhibitory receptor expressed on T-helper cells and binds with high affinity to the B7 molecules, CD80 and CD86, expressed on antigen presenting cells. The interaction between CTLA-4 and CD80/CD86 leads to an inhibition or blockade of T-cell activation via CD28, a T-cell co-stimulatory molecule required for T-cell activation. The proposed mechanism of action of belatacept in kidney transplant is to block CD28:CD80/86 interactions, thereby preventing T-cell activation via CD28. Transplant therapies that inhibit T-cell activation and/or proliferation such as cyclosporine, tacrolimus, basiliximab and sirolimus have been shown to enhance graft survival. Therefore, by blocking T-cell co-stimulation or activation via CD28, belatacept therapy may result in the prevention of graft rejection.

Belatacept is a second generation variant of abatacept (Orencia[®]) and differs from abatacept by a two amino acid change in the CTLA-4 functional binding domain. Belatacept has pharmacological activity across rodents, rabbits and non-human primates. Belatacept has greater potency in humans and nonhuman primates and lower potency in rodents compared with abatacept. Abatacept and belatacept target the same pharmacological pathway and mechanism of action. Therefore, studies with abatacept in rodents are relevant to the belatacept clinical safety assessment. The nonclinical pharmacology and pharmacokinetic (PK) studies were reviewed by Janice Lansita, Ph.D., DABT. The toxicology studies were reviewed by Ying Mu, M.D., Ph.D.

Pharmacology and pharmacokinetic studies with belatacept were conducted in mice, rats, rabbits, and cynomolgus monkeys. The *in vitro* and *in vivo* pharmacology studies showed that belatacept binds to CD80 and CD86, as well as inhibits T-cell activation, proliferation, and the T-cell dependent antibody response *in vivo*. Belatacept did not induce complement mediated cytotoxicity or antibody-dependent cytotoxicity *in vitro*. The PK of belatacept was generally characterized across species by low clearance, low volume of distribution, and a long half-life that increased with dose.

The major safety signals observed in nonclinical toxicology studies with belatacept or abatacept included a risk of infections, autoimmunity, and carcinogenicity, which are all described in the product label. All findings except autoimmunity are related to the expected pharmacology of belatacept. No effects on reproductive function or teratogenicity were observed.

In summary, the pharmacology, PK, and toxicology studies support the proposed use of belatacept in the prophylaxis of organ rejection in kidney transplant patients.

1.1 Recommendations

1.1.1 Approvability

The pharmacology and toxicology studies submitted to BLA 125288 support the approval of belatacept (BMS-224818) for the specified indication.

1.1.2 Additional Non Clinical Recommendations

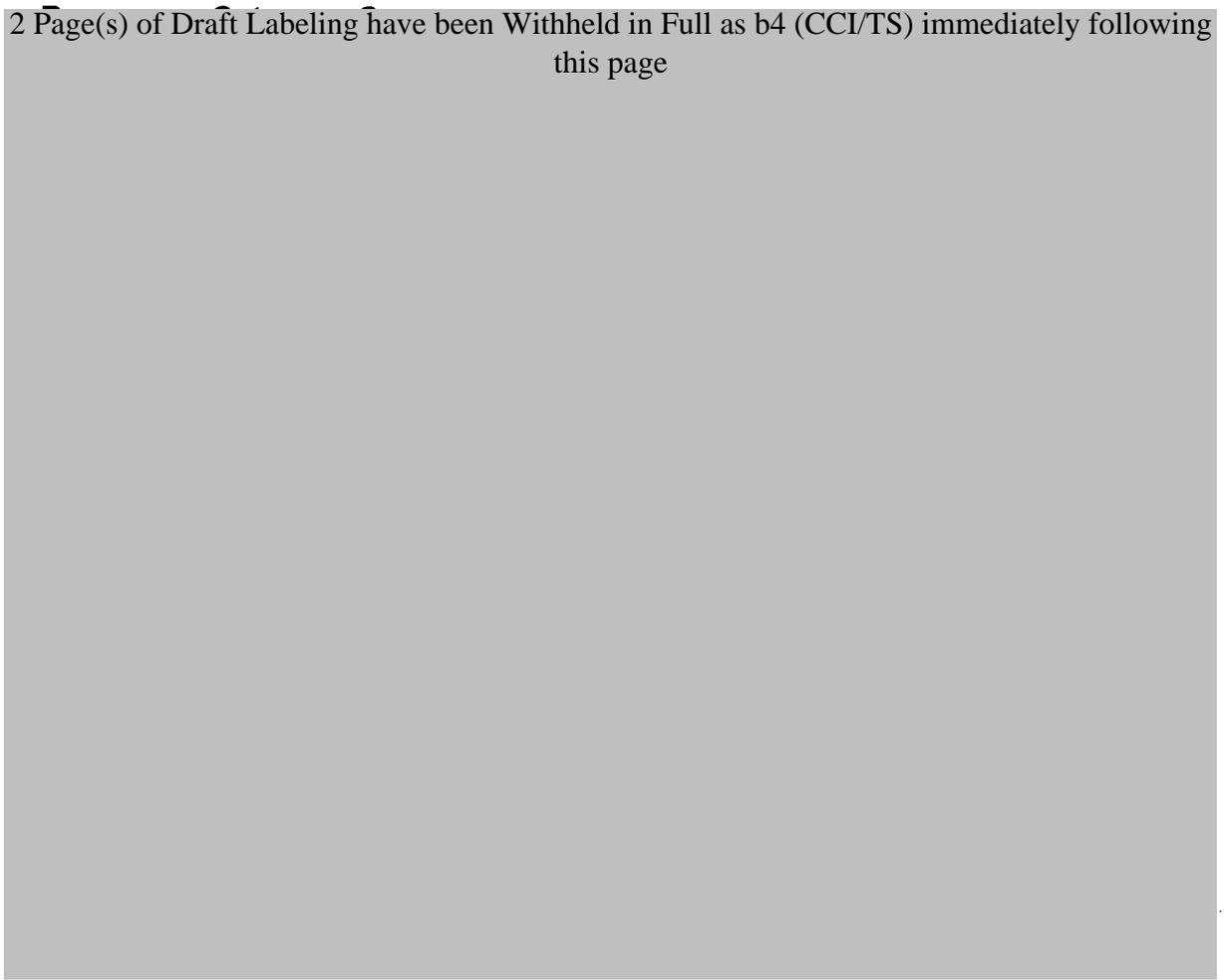
No additional nonclinical studies are recommended.

1.1.3 Labeling

Nonclinical labeling changes were recommended for Sections 8.1, 13.1, and 13.2 as follows ***(may be updated pending ongoing labeling discussions)***:

8.1 Pregnancy

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



1.2 Brief Discussion of Nonclinical Findings

1.2.1 Basis of Recommendation

The nonclinical pharmacology and toxicology study results did not identify safety issues that would preclude approval of belatacept. The nonclinical toxicology studies did identify the potential clinical risk for infections and autoimmunity. The current labeling includes cautionary language for autoimmunity and serious infections.

1.2.2 Clinical Implication

Serious infections associated with belatacept in the clinic have been observed, are described in the label and further discussed in Section 4.2 of this review.

The risk of autoimmunity to patients or a fetus exposed in utero to belatacept is currently unknown. Autoimmunity of the thyroid and pancreas (islet cells) was observed in adult and juvenile rats treated with abatacept. In addition, in a peri- post-natal study with abatacept, thyroiditis was observed in one out of twenty rat off-spring exposed *in utero* and during lactation, at the highest of three doses (200 mg/kg, a maternal abatacept exposure ~11-times higher than the estimated belatacept clinical exposure). The thyroids were not examined in maternal animals. The autoimmunity observed in juvenile rat studies with abatacept may be predictive of autoimmune effects to the human fetus. The juvenile rat immune system is less developed than in comparably aged humans and may be more similar to the human fetal immune system than the immune system in children. The relevance of these findings to patients or pregnant women treated with belatacept remains unclear. Autoimmunity has not been observed in the clinic to date or in a chronic monkey study with belatacept. Recommendations for labeling are included in Section 1.1.3 of this review. (See, also, the Toxicology Review)

Repeat-dose studies of belatacept in monkeys are considered most relevant to the clinic setting, and no significant adverse effects were noted in the pivotal repeat-dose studies in monkeys.

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

749867-37-6

2.1.2 Generic Name

belatacept

2.1.3 Code Name

BMS-224818

2.1.4 Trade Name

Nulojix™

2.1.5 Chemical Name

(1) CTLA-4 (antigen) [29-tyrosine, 104-glutamic acid] (human extracellular domain containing fragment) fusion protein with immunoglobulin G1 (human monoclonal Fc domain-containing fragment), bimol. (120→120')-disulfide.

(2) [Tyr₂₉, Glu₁₀₄, Gln₁₂₅, Ser₁₃₀, Ser₁₃₆, Ser₁₃₉, Ser₁₄₈](CTLA-4 (antigen)-[3-126]-peptide (human extracellular domain-containing fragment) fusion protein with immunoglobulin G1-[233 C-terminal residues of the heavy chain]-peptide (human monoclonal Fc domain containing fragment)) bimolecular (120→120')-disulfide.

2.1.6 Molecular Weight

90,619 Da (determined by matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry).

2.1.7 Pharmacological Class

T-cell co-stimulation blocker

2.1.8 Structure

Belatacept is a genetically engineered fusion protein consisting of the functional binding domain of modified human cytotoxic T-lymphocyte antigen-4 (CTLA-4) and the Fc domain of human IgG₁. Belatacept is made up of two polypeptide chains with 357 amino acids and exists as a covalent homodimer (referred to as belatacept "monomer") linked through an inter-chain disulfide bond.

(b) (4)



2.2 Relevant IND/s and NDA/s

IND 9418 belatacept

IND 9391 abatacept

BLA 125118 Orencia® (abatacept)

2.3 Clinical Formulation

2.3.1 Drug Formulation

Belatacept drug substance is a clear to opalescent, colorless to pale yellow solution which contains (b) (4)

Belatacept drug product for injection, 250 mg/vial, is a single-use, sterile, non-pyrogenic lyophilized powder to be reconstituted with 10.5 mls of sterile water, 0.9% sodium chloride, or 5% dextrose for IV administration for a final concentration of ~ 25 mg/ml. The composition of Belatacept for Injection is detailed in Table 1.

Table 1 Composition of Belatacept for Injection, 250 mg/Vial

Component	Quality Standard	Function	Amount per Vial ^a (mg)
Belatacept	BMS ^b Specification	Active Ingredient	(b) (4)
Sucrose	NF/Ph.Eur.	(b) (4)	(b) (4)
Sodium Phosphate Monobasic (b) (4)	USP/BP	(b) (4)	(b) (4)
Sodium Chloride ^c	USP/Ph.Eur.	(b) (4)	(b) (4)
(b) (4)			
a (b) (4)			
b Bristol-Myers Squibb			
c (b) (4)			
(b) (4)			

Table 2.3.P.1.T01 from Quality Overall Summary, 2.3.P.1 Description and Composition of the Drug Product, BLA submission²

2.3.2 Comments on Novel Excipients

No novel excipients were used in the final formulation of this product. The concentration of each excipient and its function are listed in Table 1. Based on the identity and low concentrations of the excipients, there does not appear to be a significant risk to patients. In addition, in the 6-month chronic toxicology study with belatacept in monkeys (Study No. 99655³) the same excipients in the clinical formulation were qualified using material from a comparable manufacturing process.

2.3.3 Comments on Impurities/Degradants of Concern

(b) (4) manufacturing processes for belatacept were used (b) (4) was used in all Phase 3 clinical trials and is the process that will be used to produce the marketed drug product. PK comparability studies in monkeys and humans indicated that the PK characteristics of belatacept from (b) (4) were comparable. A high molecular weight variant of belatacept was identified in the drug substance across (b) (4). Belatacept produced by (b) (4) was used in the 6-month belatacept cynomolgus monkey chronic toxicology study³. In this study, the high molecular weight variant was dosed IV, weekly for 6-months at ~ 1.5-fold higher dose levels than will be administered in the clinic. No adverse toxicities were identified in the belatacept 6-month chronic monkey study. Therefore, the high molecular weight variant was qualified in the nonclinical study.

See chemistry review for discussion of endotoxin.

2.4 Proposed Clinical Population and Dosing Regimen

Belatacept is proposed for use in the prophylaxis of organ rejection in adult patients receiving a kidney transplant.

Adult Kidney Transplant Recipients

Dosing of Nulojix

Initial Phase	Dose
Day of transplantation, prior to implantation (Day 1)	10 mg/kg
Day 5, Day 14, and Day 28 (1 month after transplantation)	10 mg/kg
Monthly, month 2 and 3 after transplantation	10 mg/kg
Maintenance Phase	Dose
Monthly, starting at Month 4 after transplantation	5 mg/kg

2.5 Regulatory Background

A summary of the regulatory history highlights and issues relevant to the nonclinical development program are provided below.

- IND submitted October 19, 2000 to the Center for Biologics Evaluation and Research.
- End of Phase 2 meeting on September 21, 2004. The Pharmacology/Toxicology reviewer clarified that the abatacept reproductive toxicology program was not adequate to support belatacept because of the differences in binding and potency. The reviewer requested that belatacept reproductive and developmental toxicology studies be conducted to support Phase 3 clinical trials.

- Fast track designation was granted January 26, 2005.
- Special Protocol Assessment for Protocol IM103008, FDA letter dated September 22, 2005.
- Pediatric Type C Meeting on December 18, 2007. The Division raised the concern that blocking CD80/86-CD28 costimulation of pre-T-cells in the thymus would allow for self-reactive thymocytes to escape potentially leading to autoimmunity. The sponsor responded that they were investigating autoimmunity in the context of the rat juvenile toxicology studies and would share the data with the Division once available.
- Orphan Drug designation granted on February 20, 2008.
- Type B Guidance Meeting on December 15, 2008 - BMS agreed to investigate the brain levels of belatacept in nonhuman primates.
- Pre-BLA Meeting on May 20, 2009. The study design to evaluate belatacept concentrations in the monkey brain was discussed (Study DS09027).
- BLA 125288 submitted to FDA electronically on July 1, 2009.

3 Studies Submitted

3.1 Pharmacology/PK Studies Reviewed

- Review of Historical *In vitro* Potency and Binding Data for BMS-224818 (LEA29Y) in Murine Cells and to Murine B7 Molecules. DCN 930023829.
- Binding of FcRn to PV Lots of Belatacept (STR-178). DCN 930032035.
- Assessment of Belatacept and Abatacept Mediated Co-stimulation Blockade *In Vitro*: Comparison of CD80/86 Receptor Saturation and Inhibition of Allo-responses. DCN 930028781.
- Belatacept Does Not Mediate Human Complement-dependent Cellular cytotoxicity. DCN 930015933.
- The Effect of Belatacept on Antibody-dependent Cellular Cytotoxicity (ADCC). DCN 930015715.
- Efficacy Comparison of Abatacept and Belatacept in Murine Primary Immune Response Model. DCN 930019020.
- Belatacept and Abatacept: Two-week intermittent-dose intravenous exploratory study in rats (Study No. DS04256). DCN 930009642.

- Belatacept and Abatacept: Two-week Intermittent-dose Intravenous Exploratory Bioactivity and Pharmacokinetics Study in Rats (Study No. DS04284). DCN 930013223.
- BMS-224818 (Belatacept) and BMS-188667 (Abatacept): Two-week Intermittent-dose Intravenous Exploratory Bioactivity and Pharmacokinetics Study in Rats (Study No. DS05055). DCN 930015077.
- BMS-224818 and Abatacept: Two-Week Intermittent-Dose Intravenous Exploratory Study in Female Rabbits (Study No. DS04252). DCN 930009600.
- BMS-188667 and BMS-224818: Single-dose Intravenous Comparative Efficacy Study in Monkeys (Study No. 97607). DCN 910069206.
- Single-dose Intravenous Exploratory Bioactivity Study in Monkeys (Study No. DS06045). DCN 930017924.
- Review of Historical LEA29Y *in vitro* studies on human cells. DCN 930009365.
- Single-dose IV Comparability Study in Cynomolgus Monkeys (Study No. DS04106). DCN 930008157.
- Single-dose IV Exploratory Comparative PK Study in Monkeys (Study No. DS03204). DCN 930007628.
- Single-dose IV Comparability Study in Cynomolgus Monkeys (Study No. DS00129). DCN 930000262.
- Single-dose IV Comparability Study in Cynomolgus Monkeys (Study No. DS05207). DCN 930014911.
- Single-dose IV, Intraarterial, Paravenous Local Tolerance Study in Rabbits (Study No. DS05072). DCN 930012153.
- BMS-224818 and Abatacept: Two-week intermittent-dose intravenous exploratory study in rabbits (Study No. DS04287). DCN 930013910.

Pharmacology/PK Summary reviews were done for the following studies:

- Abatacept: Evaluation for Reactivation in Murine Model of Chronic Tuberculosis (Study No. DS04081). DCN 930011785.
- Effect of BMS-188667 on Host Resistance to *Pneumocystis carinii* and Murine Cytomegalovirus in Intact and Skin-grafted Mice (Study No. 51475). DCN 910044345.

3.1 Toxicology Studies Previously Reviewed and filed in DARRTS under IND 9418 (belatacept) or IND 9391 (abatacept). Review summaries are included in this BLA review.

- Study No. 98642 - Belatacept, Study Title: Single-dose intravenous toxicity and toxicokinetics study in monkeys
- Study No. 98699 - Belatacept, Study Title: One-Month Intermittent-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys
- Study No. DN06032 - Belatacept, Study Title: Belatacept (BMS-224818): Intravenous Study of Fertility and Early Embryonic Development in Rats
- Study No. DN06008 - Belatacept, Study Title: Belatacept (BMS-224818): Intravenous Study of Embryo-Fetal Development in Rats

- Study No. DN06056 - Belatacept, Study Title: Belatacept (BMS-224818): Intravenous Study of Embryo-Fetal Development in Rabbits
- Study No. DN06002 - Belatacept, Study Title: Belatacept (BMS-224818): Intravenous Study of Pre- and Postnatal Development in Rats
- Study No. DS07165 - Abatacept, Study Title: 3-Month Intermittent-Dose (Q3D) Subcutaneous and intravenous Immunotoxicity Study in Juvenile Rats
- Study No. DN07013 - Abatacept, Study Title: Thirteen-Week Subcutaneous/Intravenous Immunotoxicity Study in Juvenile Rats
- Study No. DS07166 - Abatacept, Study Title: Three-Month Intermittent-Dose (Q3D) Intravenous Immunotoxicity Study in Rats
- Study No. DS04027 - Abatacept, Study Title: Nine-Month Intermittent-Dose (QWX40) Subcutaneous Investigative Study of the Effects of Mouse Mammary Tumor Virus-Initiated Events in I/LnJ Mice
- Study No. DS04029 - Abatacept, Study Title: Repeat Intermittent (QW)-Dose Subcutaneous Investigative Study of Effects on an Established Anti-Mouse Mammary Tumor Virus Antibody Response in CD-1 Mice
- Study No. 930008605 - Belatacept, Study Title: The Effect of LEA29Y on Renal and Islet Allograft Survival in Rhesus Macaques

3.2 Studies Reviewed and Not Previously Submitted to INDs:

- Study No. 99722 - Belatacept, Study Title: Mono- and Combination-Therapy Repeat-Dose Intravenous Study in Renal Transplant Recipient Monkeys; Histopathological Evaluation

3.3 Studies Reviewed Previously by Other Reviewers:

BLA 125118 Abatacept, PK Pharmacology Review, Anita M. O' Connor, Toxicology Review, Hanan Ghantous, 11/16/04.

- Study No. 99655 - Belatacept, Study Title: Six-Month Intermittent-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys
- Study No. 97610 - Abatacept, Study Title: Subcutaneous Carcinogenicity Study in Mice
- Reviewer: Hanan Ghantous, Ph.D., DABT, Reviews are attached as Appendix 1 from Orenca BLA 125118 Pharmacology/Toxicology Review.

3.2 Pharmacology/PK/Toxicology Studies Not Reviewed

Studies not fully reviewed within this submission because the study was conducted with abatacept and reviewed previously, did not directly support the IV route of administration, or did not provide new or additional information include:

- Additional characterization testing of abatacept drug substance from BMS Syracuse and Lonza Biologics: Fc receptor binding and functional activity (Study No. STR-103) DCN 930016593.

- Development of a CD86 Receptor Competition Assay: A Method for Measuring CD86 Receptor Occupancy on Monocytes in Whole Blood. DCN 930023833.
- Review of Historical LEA29Y In vivo Study. DCN 930008556.
- Binding of Abatacept to Fc Receptors. DCN 930011015.
- Single-dose IV Exploratory Comparative PK Study in Monkeys (Study No. DS03028). DCN 930004893.
- Single-dose Subcutaneous Comparative Irritation Study in Rats (Study No. DS05071). DCN 930012152.
- Single-dose Subcutaneous Local Tolerance Study in Rats (Study No. DS07122). DCN 930023508.
- Single-dose Subcutaneous Exploratory Comparative Irritation Study in Rats (Study No. DS03019). DCN 930004100
- Study No. DS05071 - Belatacept, Study Title: Single-Dose Subcutaneous Comparative Irritation Study in Rats
- Study No. DS07122 - Belatacept, Study Title: Single-Dose Subcutaneous Local Tolerance Study in Rats
- Study No. 93601 - Abatacept, Study Title: Abatacept Cream Formations, Dermal Carcinogenicity Study in Mice

4 Pharmacology

Belatacept is a fusion protein comprised of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) extracellular binding domain linked to a modified Fc domain of human immunoglobulin IgG1 (hinge-CH2-CH3 domains). The interaction between CTLA-4, an inhibitory receptor, and CD80/CD86, expressed on antigen presenting cells, leads to an inhibition or blockade of T-cell activation via CD28, a T-cell co-stimulatory molecule required for T-cell activation. The proposed mechanism of action of belatacept in kidney transplant is to block CD28:CD80/86 interactions, thereby preventing T-cell activation via CD28 which may result in the prevention of graft rejection.

Belatacept is a second generation variant of abatacept (Orencia[®]) and differs from abatacept by a two amino acid change in the CTLA-4 functional binding domain. Abatacept and belatacept target the same pharmacological pathway and mechanism of action. Belatacept has pharmacological activity across rodents, rabbits and non-human primates. Belatacept is more potent than abatacept in *in vitro* human studies by 3-29-fold and *in vivo* cynomolgus monkeys by 2-11-fold as measured by the inhibition of T-cell mediated immune responses. In contrast, belatacept is 2-10-fold less potent in rodents compared with abatacept. Therefore, the abatacept studies in rodents, including those previously reviewed for the approval of Orencia[®], are supportive of the belatacept BLA based on potency and pharmacological activity.

In vitro pharmacology studies characterized the binding, receptor saturation, and activity of belatacept using human and mouse cells. *In vivo* pharmacology and pharmacokinetic studies were conducted in mice, rats, rabbits, and cynomolgus monkeys to compare belatacept with abatacept (Section 4.1).

The Fc portion of belatacept (and abatacept) was modified in order to reduce Fc receptor interactions. Studies to evaluate Fc-mediated binding to Fc receptors, as well as belatacept's ability to induce complement dependent cytotoxicity (CDC) and antibody dependent cytotoxicity (ADCC) were conducted (Section 4.1).

The secondary pharmacology of belatacept was evaluated in studies conducted by the applicant or through reports in the literature. A study in cynomolgus monkeys measured the effects of belatacept on the induction of indoleamine 2,3 dioxygenase (IDO), a tryptophan catabolism enzyme with potential regulatory effects on T-cells. The effects of CD28 co-stimulation blockade on host resistance were evaluated in mouse models using CTLA4-Ig or CD28 pathway blockade (Section 4.2).

4.1 Primary Pharmacology

The primary pharmacology of belatacept was characterized through *in vitro* binding affinity studies to human CD80-Ig, CD86-Ig, FcRn (the neonate Fc receptor), as well as to mouse CD80-Ig and CD86-Ig fusion proteins. Formal Fc receptor binding studies were not done with belatacept, with the exception of FcRn, as studies were previously done with abatacept and both belatacept and abatacept share the same Fc portion. Abatacept (Study No. STR-103: Fc Receptor Binding and Functional Activity⁴, Davis et al., 2007⁵) demonstrated Fc receptor binding to CD64 (high affinity FcγRII) but no binding to CD32 or CD16 (low affinity FcγRIIIa and FcγRIIIb). Abatacept binding to CD64 was lower compared to a CTLA4-Ig fusion protein with a non-modified Fc.

Additional *in vitro* pharmacology studies included CD80 and CD86 receptor saturation, Fc-mediated CDC and ADCC, as well as a comparison of the biological activity of belatacept to abatacept in both human and mouse cells. *In vivo* pharmacology studies compared the activity of belatacept to abatacept in mice, rats, and cynomolgus monkeys. In addition, a monkey renal transplant study with belatacept demonstrated efficacy by prolongation of graft survival (See Section 10 Special Toxicology Studies).

In Vitro Binding Affinity Studies

The binding kinetics of abatacept and belatacept (lot numbers not provided) to mouse CD80-Ig and CD86-Ig fusion proteins and human CD80 Ia fusion protein were determined using surface plasmon resonance detection (b)(4) (Table 2), Study No. 930023829⁶. Belatacept bound with higher affinity to HuCD80-Ig (2.8-fold) compared with abatacept. In contrast, belatacept bound with lower affinity to MuCD80-Ig (3.8-fold), and similar affinity to MuCD86-Ig. Binding to murine CD86-Ig was similar for abatacept and belatacept. The binding affinity differences are related to differences in the off-rates rather than a change in the on-rates.

Table 2 Belatacept and Abatacept Binding Properties to Mouse CD80 and CD86, and Human CD80

Fusion Protein	Ligand	On-rate k_{a1} (1/Ms)	Off-rate k_{d1} (1/s)	K_{d1} (nM)

Abatacept	HuCD80-Ig	3.2×10^5	0.0066	22
Abatacept	MuCD80-Ig	2.1×10^5	0.0085	39
Abatacept	MuCD86-Ig	1.4×10^5	0.014	107
Belatacept	HuCD80-Ig	3.3×10^5	0.0026	8
Belatacept	MuCD80-Ig	2.1×10^5	0.031	148
Belatacept	MuCD86-Ig	1.8×10^5	0.019	107

The binding of abatacept and belatacept to human CD86 was not determined in this study but was determined by Larsen et al., 2005⁷. Larsen et al., reports binding affinity values for CTLA4-Ig to CD86-Ig of 13.9 nM and for LEA29Y to CD86-Ig of 3.21 nM; a 4.3-fold higher binding affinity for belatacept compared with abatacept. However, a direct comparison between the values generated by Larsen et al. to the values generated in Study No. 930023829⁶ cannot be drawn since binding affinities for CTLA4-Ig and LEA29Y to CD80-Ig were 2-3-fold lower (i.e., higher affinity binding) in the Larsen paper. An important note is that the abatacept binding affinity to murine CD80-Ig is 4.9-fold lower than belatacept binding to human CD80-Ig. Although binding affinity studies were not done with non-human primate receptors, this difference in binding likely contributes to the belatacept potency differences between rodents and non-human primates *in vivo*.

The binding kinetics of belatacept (Lot LEC07026), abatacept (Lot 46375) and human IgG1 (ipilimumab, a fully human antibody to CTLA-4) to human FcRn were evaluated using surface plasmon resonance (b)(4) Study STR-178⁸. Fusion proteins and monoclonal antibodies with an Fc moiety can bind to Fc receptors such as FcRn (neonatal Fc receptor). FcRn is expressed on various cell types and thought to contribute to the long half-life of monoclonal antibodies and Fc-containing fusion proteins. Belatacept and abatacept bound with similar affinity to FcRn. Compared with an IgG1 mAb (average $K_D \sim 80$ nM), both belatacept and abatacept bound to FcRn with 3-4 fold lower affinity (average $K_D \sim 324.7$ -365.3 nM).

In Vitro Potency and Receptor Saturation

Belatacept showed greater potency in a human T-cell assay and lower potency in a mouse primary spleen cell assay compared with abatacept (Study No. 930023829⁶). The potency of belatacept and abatacept were compared by measuring the inhibition of T-cell co-stimulation in a human immortalized T-cell line (Jurkat) and in primary murine spleen cells. The half maximal effective concentration (EC₅₀) was 20 ng/ml for belatacept and 310 ng/ml for abatacept; belatacept relative potency was 16-fold higher than abatacept (**Error! Reference source not found.**). In contrast in an *in vitro* mouse spleen-derived T-cell co-stimulation assay, abatacept showed greater potency than belatacept at inhibiting a T-cell response; belatacept relative potency was 14% compared with abatacept, with EC₅₀ values of 3,500 ng/ml for belatacept and 480 ng/ml for abatacept.

Belatacept and abatacept binding to CD80 and CD86 were evaluated on CD14+ monocytes and Lin1-/Human Leukocyte Antigen (HLA)-DR+ dendritic cells to determine CD80 and CD86 receptor saturation, Study No. 930028781⁹.

5-fold higher concentrations of both abatacept and belatacept were required to saturate CD86 compared with CD80 in monocyte-derived dendritic cells. Saturation of CD86 and CD80 required ~8-fold higher abatacept concentrations compared with belatacept. The results are summarized in Table 3.

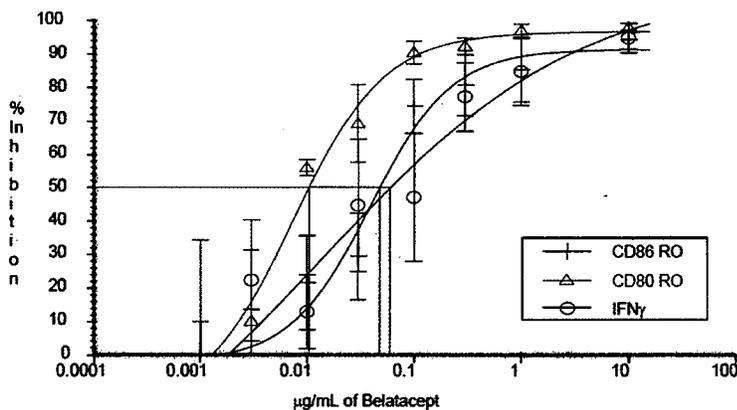
Table 3 CD80 and CD86 Comparative Receptor Saturation Values for Abatacept and Belatacept

Receptor	Abatacept IC50 (µg/ml)	Belatacept IC50 (µg/ml)	Fold Difference: Abatacept vs. Belatacept
CD80	0.076	0.010	7.6
CD86	0.387	0.048	8.1
Fold Diff. CD86 vs. CD80	5.1	4.8	

Belatacept was more potent than abatacept at saturating CD80 and CD86 receptors, inhibiting human T-cell proliferation and T-cell activation as measured by the inhibition of a cytokine response (Study No. 930028781⁹). Dendritic cells were treated with abatacept or belatacept, co-cultured with isolated T-cells for 72 hours, and supernatants collected for cytokine analysis. Based on IC50 values, the equivalent fold amount of abatacept needed to inhibit a cytokine response was higher than belatacept by 4.6-fold for IL-2, 22-fold for TNF- α , and 29-fold for IFN- γ . A 14-fold higher concentration of abatacept compared with belatacept was required to inhibit T-cell proliferation.

A comparison of the CD80 and CD86 receptor saturation IC50 values with the T-cell proliferation/activation IC50 values shows a correlation between CD86 receptor saturation and T-cell inhibition for abatacept and belatacept (Figure 2). A correlation was not evident for CD80. At a belatacept concentration of 0.1 µg/ml, CD80 was >90% saturated, CD86 was ~60% saturated, and IFN- γ inhibition was ~55%. The data indicate that saturation of CD80 alone is not sufficient to inhibit the T-cell response, and that as CD86 saturation increases, inhibition of the T-cell mediated cytokine response also increases. A similar correlation was observed for abatacept. The potential clinical significance of this difference is further discussed in the Clinical Pharmacology Review.

Figure 2 Comparison of IFN- γ Inhibition with CD80 and CD86 Receptor Saturation



IC50 values: CD80=0.010 µg/ml, CD86 0.048 µg/ml, IFN-γ=0.060 µg/ml, RO - Receptor Occupancy)
Figure from Study Report 9300287781⁹

Belatacept was a more potent inhibitor than abatacept for both CD80- and CD86-driven CD4+T cell co-stimulation using CD80- and CD86-expressing Chinese Hamster Ovary (CHO) cells after phorbol myristate acetate (PMA) stimulation (Study No. 930009365¹⁰). Based on the EC50s for inhibition, CD80-mediated T cell proliferation was 5-fold lower and for CD86-mediated T cell proliferation were 13-fold lower for belatacept compared with abatacept.

Belatacept was 6-fold more potent than abatacept at inhibiting a cytokine response in a primary allogeneic mixed lymphocyte reaction (MLR) assay and 5-7-fold more potent in the secondary MLR assay (Study No. 930009365¹⁰). EC50s for inhibition of proliferation in the primary MLR were 0.18 µg/ml for belatacept and >1.0 µg/ml for abatacept. In the secondary MLR, the EC50s were 0.20 µg/ml for belatacept and >1.0 µg/ml for abatacept. The EC50s for cytokine inhibition are summarized in Table 4.

Table 4 Belatacept (LEA29Y) and CTLA4-Ig Cytokine Inhibition in the Primary and Secondary MLR Assays

Cytokine Inhibition	EC 50 Belatacept (µg/ml)	EC 50 Abatacept (µg/ml)	Fold-potency Belatacept vs. Abatacept
Primary MLR			
IL-2	0.04	0.25	6
Secondary MLR			
IL-2	0.03	0.2	7
IL-4	0.2	1	5
IFN-gamma	0.14	1	7

ADCC and CDC

Fusion proteins containing the Fc moiety have the ability to induce complement dependent cytotoxicity (CDC) and antibody dependent cytotoxicity (ADCC) by cross-linking effector cells with target cells. The Fc portion of fusion proteins can interact with high affinity (Fc gamma RI [CD64]) and low affinity Fc gamma receptors (Fc gamma RII [CD32] and RIII [CD16]) expressed on effector cells. The CTLA4 portion can bind to CD80 and CD86 expressed on target cells which can lead to cross-linking and potential cell death/ cytotoxicity. Belatacept consists of a modified Fc hinge region (mutations in ^{(b) (4)}

Belatacept did not induce complement dependent cytotoxicity (CDC) at concentrations up to 30 µg/ml in an *in vitro* assay using an EBV-transformed human B-cell line expressing CD80/86 as target cells (Study No. 930015933)¹¹. The positive control antibodies, anti-CD80 and anti-CD86, showed complement mediated killing with a maximal response at 100 ng/ml.

Belatacept did not mediate ADCC in an *in vitro* assay using an immortalized human B-cell line expressing CD80 and CD86 as target cells and peripheral blood mononuclear cells (PBMC) from 13 normal human donors as effector cells were (Study No. 930015715¹²). The positive control, a CTLA4-Ig with a non-mutated Fc, induced ADCC as expected with cytotoxicity ranging from 5.0% to 55.4% across donors.

In Vivo Activity

A comparison of abatacept and belatacept on the inhibition of a T-cell dependent antibody response (TDAR) was evaluated in mice, rats, rabbits and cynomolgus monkeys.

Mouse

In female BALB/c mice, abatacept was more potent than belatacept and inhibited > 90% of the KLH response at doses 10-fold lower than belatacept after subcutaneous (SC) administration on Day 0, 7, and 14 with a control IgG (3, 10, 16, 30 or 100 mg/kg), abatacept (0.5, 1, 3, 5, 10, 16, 30 or 100 mg/kg) or belatacept (0.5, 1, 3, 5, 10, 16, 30 or 100 mg/kg; Study No. 930019020¹³). Abatacept inhibited the anti-KLH response >78% across doses whereas belatacept achieved similar inhibition at 10 and 30 mg/kg. Abatacept inhibited > 90% of the KLH response at doses 10-fold lower than belatacept (3 mg/kg abatacept vs. 30 mg/kg belatacept). In addition, belatacept serum concentrations were markedly lower than abatacept at equivalent dose levels. Higher anti-drug antibody (ADA) titers (2-2000 times greater) were observed after belatacept administration compared with abatacept at equivalent doses on Day 21. An inverse correlation between antibody titer and dose were seen for both abatacept and belatacept (i.e., higher titers at lower doses). This observation could either be due to immunosuppression at higher doses or drug interference at higher drug concentrations. The immunogenic response to belatacept correlated with the lower inhibition of the anti-KLH response and lower serum concentrations of belatacept compared with abatacept.

Rat

Inhibition of the TDAR response to KLH was evaluated in three studies in Sprague-Dawley rats. In the first study, $\geq 99\%$ suppression of anti-KLH Abs was seen in rats for both abatacept and belatacept across timepoints after 20 mg/kg, IV doses, weekly, for a total of two doses (Study No. DS04256¹⁴). Belatacept exposures in rats were 46-53% lower than abatacept exposures. Anti-drug antibody (ADA) samples were collected but were not analyzed. Although a conclusion cannot be made without the ADA data, the exposure differences between abatacept and belatacept were likely due to immunogenicity as observed in other belatacept rat studies.

A second rat anti-KLH study (Study DS04284¹⁵) evaluated belatacept inhibition of TDAR in rats at doses below 20 mg/kg. Female rats treated with belatacept or abatacept IV, once weekly, at doses of 0 (0.9% sodium chloride), 0.3, 1, 3, and 10 mg/kg for two weeks (a total of 2 doses) showed lower exposures (AUC_{0-168h} 51-67%), greater immunogenicity (earlier onset, higher incidence, and higher titers), and lower potency at the 0.3 mg/kg dose with belatacept compared with abatacept. Abatacept and belatacept both blocked the anti-KLH response at $\geq 98\%$ across all dose levels and

study days with the exception of the 0.3 mg/kg belatacept dose group. The potency difference was likely related to the higher incidence of immunogenicity with belatacept, resulting in lower exposures. Linear regression analyses performed by the applicant indicate that 24% higher belatacept serum concentrations (quantified as C_{trough} levels on Day 8) are needed to achieve similar ADA titers on Day 15 compared to abatacept. No treatment related effects were observed in female rats across dose groups for clinical signs, body weights, and physical exams (including neurological and respiratory).

A third rat study, Study No. DS05055¹⁶, was conducted at doses of belatacept below 0.3 mg/kg. 10 females/group, ages 9-10 weeks, weighing 192.5-241.0 g were administered belatacept or abatacept IV once weekly for two weeks (a total of 2 doses) at doses of 0.03, 0.1, 0.3, and 1 mg/kg. No treatment related effects were observed in female rats across dose groups for clinical signs, body weights, and physical exams (including neurological and respiratory).

Belatacept exposures compared with abatacept exposures were lower at 0.3 and 1 mg/kg (AUC_{0-168h} , 45-69% of abatacept), similar at 0.1 mg/kg, and slightly higher at the 0.03 mg/kg dose. Based on other belatacept studies in rats, exposures were likely impacted by immunogenicity which was not measured in this study. The PK parameters are tabulated in the PK section (Table 9).

The % suppression of a KLH-specific antibody response was calculated relative to controls as $[100 - (\text{treated group-mean end point titer/control group mean end point titer} \times 100)]$. Abatacept showed $\geq 90\%$ suppression at 0.3 and 1 mg/kg, a lower range of suppression at 0.1 mg/kg (59-81%), and no suppression at 0.03 mg/kg. Belatacept showed $\geq 89\%$ suppression at 1 mg/kg, lower to no suppression at 0.1 and 0.3 mg/kg (-57-62%), and very low to no suppression at 0.03 mg/kg (-269-15%). Neither abatacept nor belatacept showed significant activity at 0.03 mg/kg. The applicant conducted a linear regression analysis of the anti-KLH data which determined that 4.8-5.3 times more belatacept on a mg/kg basis would be needed to achieve a similar response with abatacept on Day 15 and 22. Using an exposure comparison, 6% higher serum concentrations (based on C_{trough} at 72 hours) of belatacept would be needed to achieve a similar abatacept anti-KLH antibody response on Day 15.

In conclusion, female rats treated with belatacept overall showed lower serum exposures with similar potency at 1 mg/kg and lower potency at 0.1 and 0.3 mg/kg, compared with abatacept at the same dose levels. Neither abatacept nor belatacept showed significant potency at 0.03 mg/kg.

Rabbit

Inhibition of TDAR was evaluated in female New Zealand White rabbits administered abatacept or belatacept by IV bolus injection at 10 mg/kg/dose (dose volume 0.4 ml/kg) once weekly for 2 weeks in 6 females/group, (total of 2 doses, Study No. DS04252¹⁷). Controls, 5/group, were administered 0.9% sodium chloride. Animals were monitored for 4 weeks and returned to the colony on Day 36.

No treatment related changes in body weights, clinical signs, or physical examinations including neurologic and respiratory function were identified. Systemic exposure was confirmed in treated animals (Table 5). Belatacept AUC_{0-inf} was 35% of abatacept, T_{1/2} was 67% of abatacept, and clearance was 3.5-fold higher compared with abatacept. V_{ss} was similar.

Table 5 Comparison of Abatacept and Belatacept PK Parameters in Female Rabbits After IV Administration (N= 6 females/group)

Test Article	AUC ₀₋₁₆₈ (ug/ml*h)	AUC _{0-840h} (ug/ml*h)	AUC _{0-inf} (ug/ml*h)	T _{1/2} (h)	MRT (h)	CL (mL/h)	CL (mL/h/kg)	V _{ss} (L)
Abatacept	7950	12100	12100	77.6	142	3.01	0.86	0.404
Belatacept	4000	3830	4250	52.2	69.1	11.34	3.01	0.543

A greater incidence of immunogenicity was first observed in belatacept treated animals compared with abatacept treated animals on Day 15. On Day 36, 5 out of 5 of the evaluated belatacept treated animals (5 samples available) were positive for anti-drug antibodies. After abatacept treatment, 1 out of 6 animals was positive at the end of the study. Immunogenicity likely impacted the AUC_{0-inf}, T_{1/2}, and clearance in belatacept treated rabbits.

Anti-KLH titers were measured to calculate the suppression of an anti-KLH immune response; % suppression = 100 – [100*(animal endpoint titer/control mean endpoint titer)]. On Days 8 and 15, abatacept and belatacept inhibited the anti-KLH response similarly at > 96%. On Day 22, abatacept was more potent than belatacept at inhibiting the anti-KLH response (99.2% vs. 66.5%). The high incidence of immunogenicity in belatacept treated animals likely impacted the exposure and potency of belatacept in this study.

Belatacept was highly immunogenic in rabbits from Day 15 until the end of the study which impacted potency (99.2% for abatacept vs. 66.5% for belatacept, as measured by an anti-KLH response) and exposure (35% of abatacept) compared with abatacept after a single-dose at 10 mg/kg IV.

In a second New Zealand White rabbit study, the inhibition of TDAR to KLH was evaluated in a two-week IV study in females at doses of 1, 3, and 10 mg/kg for abatacept and 3, 10, and 20 mg/kg for belatacept (Study No. DS04287¹⁸). Animals were monitored until Day 36 when they were euthanized. All rabbits survived until the terminal sacrifice. No drug-related clinical signs or body weight changes were seen.

Systemic exposure was confirmed across treatment groups. AUC values increased approximately dose-proportionally for both abatacept and belatacept. At equivalent doses of abatacept and belatacept (3 and 10 mg/kg), belatacept AUC(0-168h) exposures were 55-57% lower than abatacept exposures. T_{1/2} ranged from 45.9-83.2 hours for abatacept and 43.0-68.7 hours for belatacept. Clearance decreased with dose for abatacept and was similar for belatacept. Volume of distribution (V_{ss}) increased with dose for both abatacept and belatacept.

The specific antibody response to KLH was suppressed at all doses of belatacept and abatacept (Table 6). At 3 mg/kg and 10 mg/kg, belatacept showed lower percent suppression than abatacept (81-89% for belatacept vs. 99-100% for abatacept) on Days 15 (3 mg/kg dose only) and 22.

Table 6 Percent Suppression of a T-cell Dependent Antibody Response to KLH

Compound	Dose (mg/kg)	Study Day			AUC _(0-168 h) (µg•h/mL)	C _{trough} ^b (µg/mL) Day 8
		8	15	22		
BMS-188667	1	96	94	80	706	0.71
	3	100	99	99	2,530	4.57
	10	100	100	100	8,390	19.00
BMS-224818	3	96	89	81	1,450	1.26
	10	95	95	89	4,620	6.25
	20	99	98	92	9,290	13.30

^a Expressed as percent suppression of KLH-specific antibody response relative to vehicle-control group.

^b C_{trough} = serum concentration prior to dosing on Day 8 (refer to Section 7, Toxicokinetics Report Appendices 2 and 3).

From Applicant's Study Report No. DS04287¹⁸

Immunogenicity was observed by Day 8 for belatacept and Day 15 for abatacept. The incidence and onset of drug-specific antibodies showed an inverse correlation with dose which may be due to a drug-related suppression of an ADA response or drug interference with the assay. As drug concentrations decreased following the last dose on Day 8, the appearance of ADAs increased. By Day 36, all belatacept treated animals were positive for ADAs and abatacept treated animals were all positive at 1 and 3 mg/kg, with 2/6 animals positive at 10 mg/kg. Specific antibody titers were measured for each drug but the type (e.g., neutralizing) was not determined.

In conclusion, abatacept and belatacept were both immunogenic in rabbits and inhibited the TDAR to KLH. At equivalent doses, belatacept exhibited 55-57% lower exposures as well as slightly lower inhibition of the TDAR compared with abatacept.

Cynomolgus Monkey

In a single-dose IV comparative efficacy study in cynomolgus monkeys, the biological activity, immunogenicity and pharmacokinetics of abatacept and belatacept were compared after IV administration. Belatacept showed lower serum concentrations, a similar immunogenicity incidence, and increased potency compared with abatacept at inhibiting a T-cell mediated immune response to sheep red blood cells, but no difference in potency at suppressing an immune response to the murine L6 antigen (Study No. 97607¹⁹). A liquid ready-to-use solution of belatacept was used in this study which is representative, but a different formulation than the clinical formulation.

Cynomolgus monkeys, 2/sex/group, 2-4 years of age, weighing 2-2.8 kg, were administered a single-dose of abatacept IV at doses of 0.125, 0.5, and 2.0 mg/kg or belatacept at 0.03, 0.125, and 0.5 mg/kg (dose volume 0.25 ml/kg). Control animals received vehicle containing 4% maltose, 10mM sodium phosphate, and 20 mM sodium chloride (vehicle matched to abatacept formulation). Animals were observed for 6 weeks following treatment and returned to the colony at the end of the study on Day 42.

No treatment related effects were observed in cynomolgus monkeys across dose groups for clinical signs or body weights.

PK parameters were not calculated due to the limited timepoints collected. However, measured serum concentrations confirmed systemic exposure. C_{max} showed no gender differences and increased with dose. Slightly higher serum concentrations were observed with abatacept compared with belatacept at the same dose levels. Although clearance was not formally calculated, it appeared more rapid for belatacept at 0.5 mg/kg (no detectable drug by Day 21), compared with abatacept at 0.5 mg/kg (drug detected until the end of the study Day 42). The increased belatacept clearance was not observed at the 0.125 mg/kg dose level and did not appear to be related to immunogenicity incidence. However, due to low animal numbers it was difficult to make a definitive conclusion.

The total anti-drug antibody (ADA) response was measured on Days 8, 14, 21, 28, and 42. At equivalent doses of belatacept and abatacept, no clear differences in immunogenicity were observed. The applicant evaluated the anti-belatacept antibodies for cross-reactivity to abatacept and showed that the antibodies bound to abatacept and belatacept similarly. These data indicate that the anti- belatacept antibodies target the same portions of both abatacept and belatacept and do not specifically target the 2 amino acid substitutions of belatacept. Further characterization of the ADAs formed was conducted by evaluating whether they were anti-abatacept antibodies (anti-CTLA4tp, abatacept without the Fc or immunoglobulin domains), anti-belatacept antibodies (anti-LEA29Ytp, belatacept without the Fc or immunoglobulin domains), or non-specific antibodies to immunoglobulin or the Fc portion (antibodies to CD40Ig). ADA samples from animals treated with 0.125 mg/kg abatacept and animals treated with 0.03 mg/kg belatacept were further analyzed in these assays. The results from the antibody characterization assays did not show remarkable differences across the assays indicating the anti-drug antibody response likely targeted regions shared by abatacept, belatacept as well as the immunoglobulin or Fc portion of the fusion protein. Non-validated assays were used to make these determinations.

Belatacept suppressed the anti-SRBC response to a greater degree than abatacept as evidenced by the slightly lower incidence of antibody positive Anti-SRBC titers at the 0.5 mg/kg dose level across study days (Table 7). A difference between abatacept and belatacept suppression was not apparent for the anti-murine L6 antibody titer responses with the exception of the 0.5 mg/kg dose level; abatacept treated animals raised a detectable immune response by Day 8 whereas belatacept animals did not mount a response until Day 21. In conclusion, belatacept appeared to be more potent than

abatacept at inhibiting a T-cell mediated immune response to SRBCs but was similar to abatacept at inhibiting an immune response to murine L6.

Table 7 Incidence of Positive Anti-SRBCs Titers (Range) by Study Day and Treatment, N=2/sex/group

Compound	Dose (mg/kg)	Day 8	Day 14	Day 21	Day 28	Day 42
Control	0	4	4	3	3	4
Abatacept BMS-188667	0.125	4	4	3	1	3
	0.5	1	4	1	3	2
	2.0	1	2	1	2	2
Belatacept BMS-224818	0.03	4	4	3	3	3
	0.125	1	2	2	1	3
	0.5	0	1	1	2	0

The applicant calculated the potency of abatacept and belatacept as ID50 values (50% inhibition) and ID 90 values (90% inhibition) (Table 8). The inhibitory dose response for SRBCs was higher for belatacept, ID50 - 2-3-fold higher and ID90 - 6-11-fold higher, compared with abatacept. No major differences were observed for the inhibitory response to murine L6.

Table 8 Sheep Red Blood Cells and Murine L6 Antigen Inhibitory Dose Response at 50% (ID50) and 90 % (ID90)

Compound	ID50 (mg/kg)*			ID90 (mg/kg)*		
	SRBC		Murine L6	SRBC		Murine L6
	Day 8	Day 14	Day 21	Day 8	Day 14	Day 21
BMS-188667	0.065	0.188	0.167	0.664	1.91	0.583
BMS-224818	0.031	0.057	0.106	0.108	0.17	0.421

Belatacept in cynomolgus monkeys showed lower serum concentrations, a similar immunogenicity incidence, and increased potency compared with abatacept at inhibiting a T-cell mediated immune response to sheep red blood cells, but no difference in potency at suppressing an immune response to the murine L6 antigen. Anti-belatacept antibodies were not specific to the 2 amino acid substitution regions and cross-reacted with abatacept and belatacept similarly.

4.2 Secondary Pharmacology

Belatacept Induction of Indoleamine 2,3 dioxygenase (IDO) in Monkeys

This study was conducted to explore whether belatacept induces indoleamine 2,3 dioxygenase (IDO), an enzyme involved in tryptophan catabolism (Study No. DS06045²⁰) in cynomolgus monkeys. Kynurenine, a metabolite of tryptophan was measured as a marker of IDO induction. Increased IDO may be related to decreased T-cell proliferation due to tryptophan depletion and kynurenine accumulation.

Three female cynomolgus monkeys, aged 2-3 years, weighing 2.1-2.8 kg, were administered a single IV dose of belatacept at 10 mg/kg (dose volume 1 ml/kg). One

female (No. 1201) was found dead on Day 5; this animal's death was incidental and attributed to physical trauma. In the remaining two females on study, no treatment related effects were seen in clinical observations, body weights, physical examinations, neurologic examinations, cardiovascular examinations, or respiratory evaluations. Belatacept serum levels confirmed systemic exposure. Kynurenine concentrations increased to 2-fold over pre-dose values on Day 8 and were not detected on Day 27. Tryptophan levels were similar to pre-dose values with the exception of a transient elevation in values of 47% in one animal compared to the predose value. Tryptophan to kynurenine ratios decreased by 1.6-2.1-fold on Day 8 indicating a potential transient increase in IDO activity resulting in increases of kynurenine. IDO was not directly measured in this study. In conclusion, transient 2-fold increases in kynurenine, a tryptophan metabolite, were observed following a single-dose of belatacept at 10 mg/kg in female cynomolgus monkeys which may be related to an increase in IDO activity. A major limitation of this study is that these conclusions are based on a limited data set from two female monkeys.

Clinical evaluation of belatacept induction of IDO was conducted in 26 patients as part of the Phase 2 IM103100 long-term extension pharmacokinetic (PK) substudy (Report No IM103100 LTE²¹). IDO induction was determined pre-dose and post-dose by measuring serum kynurenine and tryptophan levels as markers of IDO activity. Although no significant changes were observed in 23/26 patients at more than 1 year post-transplant (serum kynurenine values <2.5 uM), there did appear to be an effect in 3 patients that interestingly had higher pre-dose kynurenine values than other patients evaluated. These results are summarized from Report No IM103100 LTE²¹:

Although there were no statistically significant differences for these comparisons, the subgroup of subjects in the eight week treatment regimen whose baseline kynurenin levels were > 3.0 µM show a marked increase in kynurenin levels to the 24-hour time point. The p-value of this change was 0.08, yet with only 3 subjects in this subgroup to evaluate this comparison, the difference remains of interest, and warrants further scrutiny in a larger sample set. For these 3 subjects only, the post-Week 4 samples (Days 42 and 56) were analyzed for kynurenin and tryptophan levels. The kynurenin values for these subjects were similar to the pre-Week 4 values (4.8 to 6.65 µM), and thus, remained elevated above the 3.0 µM level even for the extended period of time.

Literature publications using a CTLA4-Ig which does not contain a modified Fc region have demonstrated induction of IDO leading to effects on T-cell regulation (Boasso, et al, 2005²²). Interestingly, no induction of IDO was observed in *in vitro* studies with human monocyte derived dendritic cells treated with abatacept (Carman et al, 2009²³, Davis et al, 2008²⁴) and belatacept (Davis et al, 2008²⁴). Therefore, as detailed by Davis et al, 2008²⁴, the induction of IDO may actually be related to Fc effector activity rather than a CTLA4 mediated effect.

Host Resistance

Host defense or resistance studies with belatacept have not been conducted.

However, infections in rats leading to mortality have been observed in repeat-dose toxicology studies with both abatacept (juvenile rat studies) and belatacept (rat peri-post-natal study, described in Toxicology section). Additionally, lymphomas and mammary adenocarcinomas that appear to be related to the viral recrudescence of MMTV (mouse mammary tumor virus) and MLV (mouse leukemia virus) during treatment with abatacept were observed in a mouse carcinogenicity assay (see Toxicology review). The effects of abatacept or blockade of the CD28 pathway using other means (another CTLA4-Ig, transgenic models, or monoclonal antibodies) on host defense have been reported by the applicant and the literature. These studies are summarized below.

Host Resistance to Viruses

In viral infection models with vesicular stomatitis virus (VSV), lymphocytic choriomeningitis virus (LCMV), vaccinia virus (VV), influenza, HSV, and mouse polyoma virus (PYV) effects on delayed pathogen clearance, as well as impaired T cell proliferation and IgG antibody response were observed. An acute murine cytomegalovirus (CMV) model with abatacept did not show decreased host survival or delayed clearance (Study No. 910044345²⁵). Acute infection with VSV, LCMV, and VV were evaluated in transgenic mice expressing soluble CTLA4-Ig. VSV infection reduced the IgG response up to 30-fold with a “drastically impaired” cytotoxic T-cell response, LCMV infection reduced IgG up to 20-fold, and VV infection resulted in slight decreases in CD8+ T-cells (Zimmerman, 1997²⁶). An acute influenza model showed a 3 day delay in viral clearance, reduced CD8+ T cells, and decreased virus specific antibody titers after treatment with CTLA4-Ig (Lumsden, 2000²⁷). In a CD28 knock-out mouse, infection with LCMV showed a normal anti-LCMV CD8+ T cell response and infection with VSV showed normal IgM, but impaired IgG class-switching which is CD4+T-helper cell dependent (Shahinian, 1993²⁸). After primary acute HSV infection in wild-type mice, treatment with CTLA4-Ig reduced paralysis-free survival and near complete ablation of the CD4+ and CD8+ T-cell responses (anergy and reduced counts) were observed (Edelmann and Wilson, 2001²⁹). Kembell et al 2006³⁰ evaluated acute and persistent/chronic infection of mouse PYV with blockade of CD28 or CD40 using transgenic knock-outs and/or an anti-CD40 mAb and CTLA4-Ig (from BMS). Blockade of the CD28 pathway during acute infection resulted in a substantially reduced CD8+ T cell response and memory response as well as reduced serum IgG. After persistent infection with mouse PYV, CD8+ T cells declined but CD28 and CD40 were not required for maintaining or generating new CD8+T cells.

Williams et al., (2002)³¹ reported that after chronic LCMV infection, treatment of mice with both a CTLA4-Ig and an anti-CD40L mAb resulted in a reduced ability to control infection which was associated with decreases in activated CD8+ T-cells in the spleen.

Host Resistance to Bacteria

In CD28 knock-out mice infected with acute *listeria monocytogenes*, impaired T-cell activation and exacerbated listeriosis after primary and secondary infection were observed. Reduced CD8+ T-cells counts were seen after primary and secondary infection. However, after the secondary infection, CD8+ T-cells retained their effector

function and were able to differentiate into effector and memory T cells (Mittrucker, 2001³²).

Chronic tuberculosis was established in mice 4 months after infection by *M. tuberculosis* after aerosol inhalation (Study No. DS04081³³). Mice were treated for up to 16 weeks with abatacept, SC, weekly at 20 mg/kg. All animals survived abatacept treatment and controlled the *M. tuberculosis* infection in contrast to the positive control group (anti-TNF-alpha mAb) which had no survivors. No significant changes were observed in CD4+ cells, activated CD4+ cells, CD8+ cells, and activated CD8+ cells in the lung and lymph nodes of infected mice treated with abatacept compared to the vehicle control group. Abatacept did not impact host resistance in mice with chronic tuberculosis.

The effect of abatacept on the tetanus toxoid (TT) recall or memory response was conducted *in vitro* by isolating T-cells from normal human blood donors previously immunized against TT, treating the cells with abatacept or a control Ig, and then activating the cells with TT. T-cell proliferation was measured using 3H-thymidine incorporation. Maximal inhibition of a T-cell response was observed with abatacept at 10 µg/ml and saturation of the response between 10-100 µg/ml (Davis et al, 2008²⁴).

Host Resistance to Parasites

C57BL6 CD28^{-/-} mice infected with *Leishmania major* controlled their infection with a normal Th1 response. Balb/c mice were susceptible. The study demonstrated that CD28 was not required for generating Th1 or Th2 responses (Brown, 1996³⁴). In another study, CD28 knock-outs were treated with CTLA4-Ig and showed either a Th1 or Th2 response to *Leishmania major*, showing evidence for a B7 independent pathway for host resistance (Elloso and Scott, 1999³⁵).

Host Resistance to Fungi

Mice (BDF1) were acutely infected with *Pneumocystis carinii* (Study No. 910044345²⁵) after treatment with abatacept at 10 mg/kg, IV, twice weekly for three weeks. All abatacept treated animals survived until the end of the study with no evidence of infection in lung homogenates.

Clinical Risk of Infections

Nonclinical and *in vitro* host resistance studies demonstrate abatacept decreases host resistance to certain pathogens. In the clinic, belatacept has been associated with serious infections. Warnings are included in the labeling for post-transplant lymphoproliferative disease (PTLD) and serious infections including tuberculosis, herpes, BK virus-associated nephropathy (BKVAN) and progressive multifocal leukoencephalopathy (PML) caused by the JC virus, based on the clinical data to date. Risk factors associated with the development of PTLT include Epstein-Barr virus (EBV) and CMV status. From the current version of the label.

(b) (4)

(b) (4)

(b) (4) The label also recommends CMV and pneumocystis pneumonia prophylaxis following transplantation. The known

clinical risk of belatacept- associated serious infections is in the label to guide the monitoring and treatment of patients.

Host Resistance to Autoimmunity

Although there was no evidence of autoimmunity in toxicology studies with belatacept in monkeys, nonclinical studies with abatacept, showed evidence of inflammation and autoimmunity in rats. In studies with abatacept at clinically relevant doses, inflammation and autoimmunity (insulinitis and thyroiditis) were observed in juvenile^{36 37} and adult rats³⁸. The incidence and/or severity of the autoimmune findings in rats increased during the 2-3 month treatment-free recovery periods. In a peri- post-natal study of abatacept, thyroiditis was observed in one out of twenty rat off-spring exposed *in utero* and during lactation, at the highest of three doses (200 mg/kg, a maternal abatacept exposure ~11-times higher than the estimated belatacept clinical exposure)³⁹. The thyroids were not examined in maternal animals. Autoimmunity was not observed in chronic toxicology studies with abatacept in mice and monkeys; therefore, the autoimmunity described in rats may represent a rat-specific effect of abatacept. The mechanism of the autoimmunity is currently unknown. T-regulatory cells were decreased by up to 80% in the juvenile rat studies. Literature reports indicate that the CD28 pathway is essential for the development and homeostasis of T-regulatory cells that suppress autoimmune disease (Salomon, 2000⁴⁰). While these findings may indicate a need for vigilance when abatacept is used in patients including pregnant women, the relevance of these findings to patients or pregnant women treated with belatacept remains unclear since no evidence of autoimmunity has been observed in rat and monkey toxicology studies with belatacept (see Toxicology Review).

Adverse events related to autoimmune disease appeared evenly distributed across all treatment groups in the belatacept kidney trials: approximately 2% of patients in IM103100, IM103008, and IM103027 reported autoimmune events. Psoriasis was seen in three belatacept MI treated patients and three belatacept LI treated patients; hyperthyroidism was reported in three CsA treated patients and no belatacept treated patients. An episode of Guillain-Barré syndrome was reported in one patient in IM103027 in the belatacept MI group. Diabetes mellitus was reported in 5% of belatacept patients and 10% of CsA patients⁴¹.

4.3 Safety Pharmacology

Safety pharmacology endpoints were evaluated in the context of the toxicology studies (Toxicology Review) and the cynomolgus monkey PK comparability Studies (Study No.'s DS04106⁴² and DS00129⁴³). No belatacept treatment related effects were observed for respiratory, cardiovascular, or neurological function endpoints.

5 Pharmacokinetics

Belatacept pharmacokinetics was evaluated in rats, rabbits, and cynomolgus monkeys. Toxicokinetic data collected in the context of the toxicology program are included in

Toxicology Section 6. Since belatacept is a biologic, radiolabeled distribution, metabolism, and excretion studies were not conducted.

5.1 PK Analyses Across Species

Abatacept and Belatacept Quantification in Serum and Measurement of Immunogenicity

Abatacept and belatacept were quantified using a validated sandwich enzyme linked immunosorbent assay (ELISA). A monoclonal anti-CTLA4-Ig antibody was adsorbed to a microtiter plate to capture abatacept or belatacept in serum or milk. The captured CTLA4-Ig was detected in rats using a goat anti-human IgG_{FC} conjugated to horse radish peroxidase (HRP). Monkey and rabbit ELISA formats were similar except the detection antibody used was a biotinylated anti-belatacept or anti-abatacept antibody (to minimize interference), followed by streptavidin conjugated to HRP.

The immunogenicity assays utilized a direct format assay. For belatacept immunogenicity in mice and rabbits, a non-validated assay was utilized. Belatacept was coated onto microtiter followed by incubation with serum from mice or rabbits. Bound anti-belatacept antibodies were detected with alkaline phosphatase (AP)-conjugated goat anti-mouse IgG and IgM (heavy- and light- chain specific) or goat anti-rabbit antibodies. The criteria for a positive titer was an endpoint titer at least two serial dilutions or 9-fold greater than the pre-dose endpoint titer. A similar format was used to detect belatacept immunogenicity in rat except that the assay was validated and the detection reagent was a goat anti-rat IgG and IgM antibody. In the validated rat immunogenicity assay, a positive sample was defined as ≥ 30 . For the monkey, a similar ELISA platform to the mouse, rabbit and rat was used. The method was validated and used an AP-conjugated goat anti-human kappa and lambda antibody cocktail. A positive titer was defined as a 9-fold endpoint titer over predose values. The abatacept immunogenicity assays were similar to the belatacept assays but abatacept was used to capture the anti-abatacept antibodies across species.

Rats

Belatacept exposures in rats were determined in a two-week study after a total of two IV doses were administered once weekly in female Sprague-Dawley rats at doses of 0.03-1 mg/kg (Study No. DS05055¹⁶). Abatacept was also evaluated in this study at the same doses. Belatacept exposures compared with abatacept exposures after a single-dose was administered were lower at 0.3 and 1 mg/kg (AUC_{0-168h}, 45-69% of abatacept), similar at 0.1 mg/kg, and slightly higher at the lowest dose of 0.03 mg/kg dose. Exposures (AUC_{0-168h}) increased greater than dose-proportionally at 0.1 and 0.3 mg/kg and approximately dose-proportionally at 1 mg/kg for both abatacept and belatacept. The exposure ratios (comparing the AUC of belatacept to abatacept) showed an inverse correlation with dose; highest at the low dose and decreasing with dose. The mean residence time (MRT) and T_{1/2} increased with dose whereas clearance (CL) decreased with dose (Table 9). Based on other belatacept studies in rats, belatacept exposures were likely impacted by immunogenicity which was not measured in this study.

Table 9 Systemic Exposure of Belatacept (BMS-224818) or Abatacept (BMS-188667) in Female Rats (N=10/group) after IV Doses at 0.3, 1, 3, 10 mg/kg, Once Weekly for 2 Weeks

Pharmacokinetic Summary						
Compound	Dose (mg/kg)	AUC(0-168 h) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Exposure Ratio ^a (0-168 h)	MRT (h)	T _{1/2} (h)	CL (mL/min/kg)
BMS-188667	0.03	4.66		22.9	16.3	0.107
	0.1	43.4		33.3	28.5	0.0384
	0.3	260		60.9	50.9	0.0193
	1	1,130		103	83.4	0.0148
BMS-224818	0.03	7.64	1.64	37.9	31.5	0.0655
	0.1	37.9	0.873	43.7	41.5	0.0439
	0.3	179	0.688	59.5	44.7	0.0280
	1	511	0.452	71.1	63.1	0.0326

^a BMS-224818:BMS-188667 AUC ratio exposures determined over the specified time interval.

From Applicant's Study Report No. DS05055¹⁶

Rabbits

The PK of abatacept and belatacept were compared in rabbits after IV doses of 1, 3, and 10 mg/kg of abatacept and 3, 10 and 20 mg/kg of belatacept (Study No. DS04287¹⁸). Another study in rabbits, evaluated the PK of abatacept and belatacept after a 10 mg/kg IV dose (Study No. DS04252¹⁷). These studies were previously discussed in the Primary Pharmacology Section 4.1.

At 10 mg/kg, belatacept AUC_{0-inf} was 35% of abatacept, T_{1/2} was 67% of abatacept (52.2 h belatacept vs. 77.6 h abatacept), and clearance was 3.5-fold higher compared with abatacept (Study No. DS04252¹⁷). V_{ss} was similar. A greater incidence of immunogenicity was first observed in belatacept treated animals compared with abatacept treated animals on Day 15. On Day 36, 5 out of 5 of the evaluated belatacept treated animals (5 samples available) were positive for anti-drug antibodies compared with 1 out of 6 abatacept treated animals. Immunogenicity likely impacted the AUC_{0-inf}, T_{1/2}, and clearance in belatacept treated rabbits.

After IV doses of 1, 3, and 10 mg/kg of abatacept and 3, 10 and 20 mg/kg of belatacept (Study No. DS04287¹⁸), AUC values increased approximately dose proportionally for both abatacept and belatacept. At equivalent doses of abatacept and belatacept (3 and 10 mg/kg), belatacept AUC_{0-168h} values were 55-57% lower than abatacept exposures. T_{1/2} ranged from 45.9-83.2 hours for abatacept and 43.0-68.7 hours for belatacept. Clearance decreased with dose for abatacept and was similar for belatacept. Volume of distribution (V_{ss}) increased with dose for both abatacept and belatacept.

Monkeys

(See Toxicology Section 6.1 for Single-dose PK.)

PK Comparability

PK comparability studies in monkeys compared belatacept produced by different manufacturing processes, Process A, B and C (Study Numbers DS00129⁴³ and DS04106⁴²). The PK analyses showed that materials produced by the different processes were comparable in the cynomolgus monkey.

6 General Toxicology

6.1 Single-Dose Toxicity

Study title: Single-dose intravenous toxicity and toxicokinetics study in monkeys
Study no.: 98642, a full review is available in DARRTS referring to IND-9418, received 08/05/2009^{Error! Bookmark not defined.}
Study report location: Princeton, NJ 08543-4000
Conducting laboratory and location: BMS Pharmaceutical Research Institute
Princeton, NJ 08543-4000
Date of study initiation: July 20, 1998
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Belatacept, 43465-082, 98.1%

KEY FINDINGS

1. No belatacept-related toxicities were observed in monkeys given up to 100 mg/kg.
2. Mild decreases in mean serum IgG of approximately 30%, relative to predose, levels were observed on days 22 and 43 in high-dose monkeys.
3. Dose-dependent decreases of CD8+ T-cells were observed on day 15 in peripheral-blood lymphocytes (PBLs) and splenic lymphocytes.
4. Antibodies to belatacept developed 5 and 6 weeks after drug administration in low- and intermediate-dose monkeys, respectively.

RESULTS

Belatacept was administered to cynomolgus monkeys, 3/sex/group, aged 25 to 33 months, weighing 2.2-3.1 kg, at single intravenous doses of 10, 33 and 100 mg/kg to assess TK and immunological parameters for up to 45 days post-dose. Controls received vehicle containing sucrose, sodium phosphate, and sodium chloride. Necropsies were conducted on one monkey per sex in each group on Day 15. Additional parameters evaluated included toxicokinetics, clinical observations, clinical pathology, anatomic pathology, peripheral-blood lymphocyte (PBL) phenotyping [CD2 (pan-T-cell), CD4 (T-helper cell), CD8 (T-cytotoxic cell), and CD20 (B-cell)], splenic lymphocyte phenotyping, anti-belatacept and anti-Chinese Hamster Ovary Protein (CHOP) antibody formation, serum immunoglobulins (IgM, IgG, and IgA), histamine, complement C3a, TNF- α , and IL-6 levels.

Single-dose intravenous injections of belatacept were well tolerated in monkeys at doses up to dose 100 mg/kg. Belatacept-related changes included 1) anti-drug

antibody formation in low- and mid-dose monkeys at 5 and 6 weeks after drug administration; 2) a dose-dependent decrease of peripheral blood CD8+ T cells (in percentages and absolute numbers) and splenic CD8+ T cells on day 15; 3) mild decreases of IgG on days 22 and 43 at the high-dose. The mild decreases of IgG and CD8+ T cells were considered to be secondary to the pharmacology of the drug.

Toxicokinetics

C_{max} increased dose proportionally. AUC_{inf} increased dose proportionally in males and greater than dose proportionally in females. T_{1/2} was generally long, 75.2-85.6 h at 10 and 33 mg/kg, and increased with dose to 116-132 h at 100 mg/kg. The lower T_{1/2} at 10 and 33 mg/kg were attributed to the ADA response. Clearance was slightly higher in females at 10 mg/kg and males at 33 mg/kg. V_{ss} was low and generally similar across dose groups. The PK parameters are summarized in Table 10 and serum concentration-time curves are presented in Figure 3.

Table 10 PK Parameters in Male and Female Monkeys after a Single IV Dose of Belatacept

Dose (mg/kg)	Gender	C _{MAX} (µg/ml)	AUC(INF) (h.µg/ml)	T-HALF (h)	CLT (ml/h/kg)	VSS (ml/kg)
10	Male	256	11977	88.3	0.84	107
	Female	255	8503	85.6	1.18	144
33	Male	914	32950	75.2	1.01	117
	Female	1025	36133	80.6	0.91	117
100	Male	2575	107407	116	0.94	126
	Female	2850	124726	132	0.82	118

Table from Study No. 98642

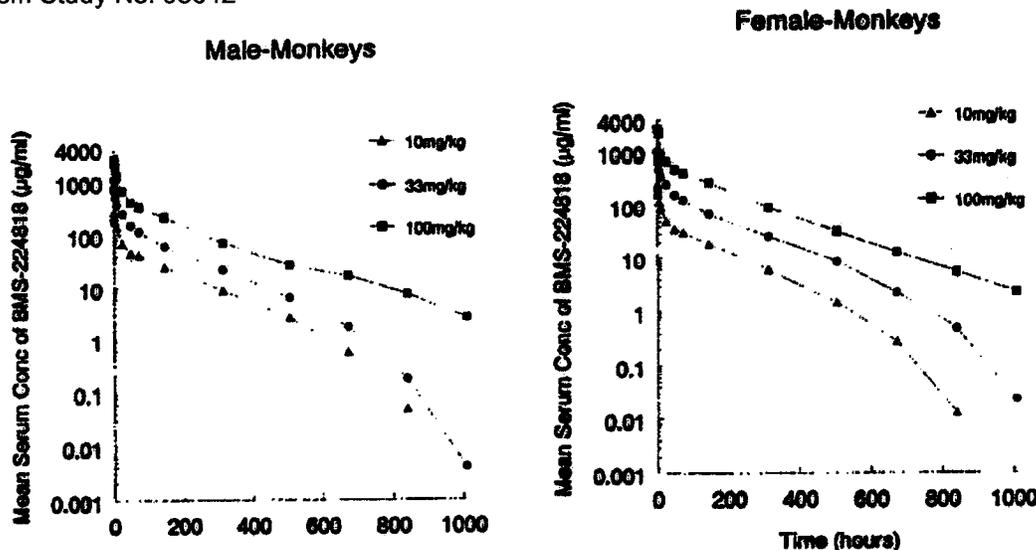


Figure 3 Mean serum concentration-time curve of BMS-224818 in male and female monkeys following administration of single-IV doses of belatacept at 10, 33, and 100 mg/kg. Figure from Study Report No. 98642

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6.2 Repeat-Dose Toxicity

Study title: One-Month Intermittent-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys

Study no.: 98699, a full review is available in DARRTS referring to IND-9418, received 08/05/2009.

Study report location: Princeton, NJ 08543-4000

Conducting laboratory and location: BMS Pharmaceutical Research Institute
Princeton, NJ 08543-4000

Date of study initiation: September 8, 1998

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Belatacept, 43465-082, 98.1%

Key Findings

1. Belatacept was well tolerated when given intravenously to healthy monkeys at repeat-doses up to 50 mg/kg.
2. Decreased serum IgG levels were partially reversible following a 6-week dose-free recovery period.
3. Lymphoid depletion of the germinal centers in the spleen and/or lymph nodes that occurred following belatacept administration were considered pharmacologic effects of the drug and did not recover during a 6-week recovery period.
4. The absence of an antibody response to the drug was related to the presence of immunosuppressive levels of drug or to assay interference.

Methods

Doses: 10, 22, 50 mg/kg

Frequency of dosing: Once every other day

Route of administration: intravenously

Dose volume: 0.4, 0.88, 2.0 mL/kg

Formulation/Vehicle: Lyophilized belatacept supplied in 100-mg vials containing 125 mg of belatacept, 250 mg sucrose, 2.8 mg sodium phosphate monobasic monohydrate, 14.9 mg sodium phosphate dibasic and 2.9 mg of sodium chloride / 0.9% Sodium Chloride Injection, USP (saline)

Species/Strain: Monkeys/Cynomolgus (*M. fascicularis*)

Number/Sex/Group: 3/Sex/Group

Age: 25 to 40 months

Weight: 2.7 and 4.1 kg (males) or 2.6 and 3.1 kg (females)

Experimental design:

Necropsies were conducted on the first two monkeys per sex in each group on day 31 at the end of the treatment period and on remaining monkeys on days 73/74 after a 6-week recovery period. Additional observations and measurements included toxicokinetics, clinical observations, clinical immunology: 1) peripheral-blood lymphocyte

(PBL) phenotypic analyses for percentages of CD2 (pan-T-cell), CD4 (T-helper cell), CD8 (T-cytotoxic cell), and CD20 (B-cell) positive cells by flow cytometry from peripheral-blood samples of all monkeys at prestudy, days 16 and 28, and in recovery monkeys on days 49/50 and 70/71; 2) splenic lymphocyte phenotypic analyses were performed on single cell suspensions obtained at necropsy on days 31 and 73/74; 3) anti-belatacept and anti-Chinese Hamster Ovary Protein (CHOP) antibody formation were assessed on serum obtained predose, day 29, and once weekly thereafter; 4) serum immunoglobulins (IgM, IgG, and IgA) predose and day 28, during recovery on days 49/50 and 70/71; 5) serum histamine, complement C3a, TNF- α , and IL-6 levels at predose, and 3 and 30 minutes postdose (for histamine and C3a) and at 1, 2, and 4 hours postdose (for TNF- α , and IL-6) on days 1, 15, and 21; clinical pathology: hematology, coagulation, serum chemistry and urinalysis; anatomic pathology: mortality, organ weights, gross pathology; and histopathology on representative sections of tissues collected at necropsy from all monkeys that were sacrificed as scheduled.

Results

There were no electrocardiographic alterations or changes in body temperatures, body weight, food consumption, pathology, histamine, complement C3a, TNF- α , or IL-6 levels that were clearly drug-related. In particular, the data suggest that belatacept does not activate complement or inflammatory responses.

The absence of an antibody response to the drug is considered due to the presence of immunosuppressive levels of the drug or could be related to assay interference.

Toxicokinetics confirmed systemic exposure after repeat-doses of belatacept (Table 11).

Table 11 TK Parameters in Male and Female Cynomolgus Monkeys After IV Repeat-doses of Belatacept at 10, 22, and 50 mg/kg

Dose [mg/kg]	Study Day	C _{MAX} [μ g/mL]		AUC(TAU) [h \cdot μ g/mL]	
		Male	Female	Male	Female
10	1	260	273	4381	4379
	29	385	432	11892	12329
22	1	589	558	9860	9170
	29	961	855	24765	20165
50	1	1299	1006	22137	19901
	29	1995	2008	53208	49336

Compared to individual monkey predose levels, no drug-related changes in serum levels of IgM or IgA were observed. However, beginning on day 28, decreases in mean serum IgG levels in the 10, 22, and 50 mg/kg dose groups (10%, 34%, and 32%, respectively), relative to mean pretreatment levels were seen. The effect on serum IgG is consistent with the immunosuppressive activity of the drug. Serum immunoglobulin levels were partially restored to predose levels during the recovery period. Minimal lymphoid depletion of germinal centers of spleen and/or lymph nodes was observed in one male and female at 22 mg/kg and two males and one female at 50 mg/kg. This finding was observed at the end of a 6-week, dose-free observation period in one of two monkeys at 10 mg/kg and in both animals at 22 and 50 mg/kg. These changes

(decreased serum IgG levels and lymphoid depletion of germinal centers) were observed at all time points during the study. Lymphoid depletion of germinal centers did not recover during a 6-week dose-free recovery period.

The repeat-dose intravenous injections of belatacept were well tolerated up to the high dose of 50 mg/kg during the dosing and recovery periods. The observations are consistent with previous studies' outcomes (Study 930008605 and Study 98624) which showed that belatacept inhibits alloantibody production, decreases IgG levels, and induces lymphoid depletion in monkeys.

7 Genetic Toxicology

Not applicable to biologics. However, genetic toxicity studies previously conducted with abatacept and reviewed in BLA 125118 were negative.

8 Carcinogenicity

Carcinogenicity studies with belatacept were not conducted. In a carcinogenicity study in mice with abatacept, an increased incidence of lymphomas and mammary gland tumors were observed at exposures 1-3-fold over the anticipated belatacept clinical dose. After further investigation, the mice on the study were found to be infected with mouse mammary tumor virus (MMTV) and murine leukemia virus (MLV). Therefore, the tumors may be a secondary effect of the drug due to viral reactivation. In a 1-year chronic toxicity study in cynomolgus monkeys with abatacept, no evidence of lymphomas or preneoplastic changes were observed at exposures 7-11X-fold over the anticipated belatacept clinical dose, despite the presence of lymphocryptovirus known to cause these lesions in immunosuppressed monkeys within the time frame of this study. These studies were previously reviewed in BLA 125118 Abatacept.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: Belatacept (BMS-224818): Intravenous Study of Fertility and Early Embryonic Development in Rats

Study no.: DN06032, a full review is available in DARRTS referring to IND-9418, received 10/06/2009.

Study report location: Princeton, NJ 08543-4000

Conducting laboratory and location:

(b) (4)

Date of study initiation: November 7, 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Belatacept, 6E12688, 99.8%

Key Findings

There were no effects of belatacept on male or female rat reproductive function or early embryonic development of the offspring at doses of 20, 65, or 200 mg/kg/day.

Experimental Design

Belatacept was administered intravenously once daily to groups of male and female rats at doses of 20, 65, or 200 mg/kg/day. Control groups of male and female rats were given 0.9% sodium chloride. A dose volume of 10 mL/kg was used for all groups. Each group consisted of 25 rats/sex assigned to evaluations of fertility and reproductive function and an additional 10 rats/sex were assigned to toxicokinetic evaluations on Day 10 of dosing (Table 12).

Table 12 Rat Fertility and Early Embryonic Development Study Design

Dose Group	Rats Assigned to Study	Dose ^a (mg/kg/day)	Dose Volume ^b (mL/kg)
	Fertility and Early Embryonic Development Evaluations		
1	25 /sex	0 (Vehicle)	10
2	25 /sex	20	10
3	25 /sex	65	10
4	25 /sex	200	10
	Toxicokinetic Evaluations		
5	10/sex	0 (Vehicle)	10
6	10/sex	20	10
7	10/sex	65	10
8	10/sex	200	10

^a All doses and concentrations were expressed in terms of belatacept content. Belatacept was considered 100% pure for the purpose of dose calculations.

^b Administered as a slow bolus injection of approximately 1 minute duration

The applicant conducted standard measurements and reproductive parameter observations including toxicokinetics (Groups 5 to 8), mortality and clinical signs, fertility and early embryonic development evaluations (Groups 1 to 4), necropsies, body weights, food consumption, and estrous cycling, mating, and fertility and caesarean-sectioning and litter observations.

Results

Belatacept was administered intravenously once daily to groups of male and female rats at doses of 0, 20, 65, or 200 mg/kg/day. Endpoints evaluated in this study included toxicokinetics, mating, fertility, estrous cycling, maternal uterine contents and early embryonic development, as well as viability, clinical signs, body weights, and food consumption parameters.

Toxicokinetics

Systemic exposure of rats to Belatacept was dose related. Increases in mean AUC_(0-24 h) and maximum concentration (C_{max}) values for both male and female rats after repeated doses appear to be proportional to the dose increment between 65 and 200

mg/kg/day. The increase in AUC value was less proportional to the dose increment between 20 and 65 mg/kg/day, while the C_{max} value was approximately proportional to the dose increment between 20 and 65 mg/kg/day. In general, systemic exposures were slightly higher in male rats than in females (Table 13).

Table 13 Mean Toxicokinetic Values Following Intravenous Administration of 20, 65, and 200 mg/kg/day in Male and Female Rats

Parameter	Belatacept Dose (mg/kg/day)					
	20		65		200	
	Male	Female	Male	Female	Male	Female
C _{max} (µg/mL)	679	530	1,770	1,560	5,410	4,800
AUC _{0-24h} (µg·h/mL/mL)	9,210	7,270	20,300	15,100	52,600	42,300

There were no effects of belatacept on male or female reproductive function (mating and fertility; estrous cycling in females) or early embryonic development of the offspring at doses as high as 200 mg/kg/day, the highest dose tested in this study. At the no-observable effect level of 200 mg/kg/day, AUC values in male and female rats were 52,600 µg·h/mL and 42,300 µg·h/mL, respectively. The study characterized fertility and early embryonic developmental toxicity of intravenously administered belatacept in both sexes of adult rats. On the basis of these data, belatacept is not a reproductive toxicant in the fertility and early embryonic development study in rats.

9.2 Embryonic Fetal Development

Study title: Belatacept (BMS-224818): Intravenous Study of Embryo-Fetal Development in Rats

Study no: DN06008, a full review is available in DARRTS referring to IND-9418, received 10/06/2009.

Study report location: Princeton, NJ 08543-4000

Conducting laboratory and location: (b) (4)

Date of study initiation: March 13, 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Belatacept, 5K01055, 99.4%

Key Findings

Body-weight gains in dams dosed at 200 mg/kg/day were transiently reduced on day of gestation (DG) 6 to 9. There were no belatacept-related effects on fetuses at all doses.

Experimental outline

Belatacept was administered intravenously (dose volume - 10 mL/kg) to groups of mated female Crl:CD(SD) rats, ~9 weeks of age, weighing 204 to 256 g, once daily on DG 6 through 15 at doses of 20, 65, or 200 mg/kg. Rats were randomly assigned to 8 groups, with 22 animals/group in Groups 1-4 (teratological evaluation) and 10 animals/group in Groups 5-8 (toxicokinetic evaluation), see Table 14. Dams were

evaluated for toxicokinetics (Groups 5 to 8 on DG 15), pregnancy status, viability, clinical signs, body weights, food consumption, gross necropsy ((Groups 1 to 4; of the thoracic, abdominal, and pelvic viscera) at caesarean-section on DG20. The uterus of each dam in Groups 1-4 was examined for pregnancy, number and distribution of implantation sites, live and dead fetuses, and early and late resorptions. Placentas were examined for size, color, and shape. Fetus parameters included belatacept exposure, weight, sex, external, soft tissue, and/or skeletal alterations. Approximately one-half of the fetuses in each litter were examined for soft tissue alterations. The thoracic and abdominal viscera were examined (ex vivo) as fresh tissue, and the heads subsequently examined by free-hand sectioning of fixed tissue. The remaining fetuses (approximately one-half of the fetuses in each litter) were stained using KOH-alizarin red S for skeletal alterations.

Table 14 Rat Intravenous Belatacept Study of Embryo-Fetal Development Study Design

Group	Rats Assigned	Dose ^a (mg/kg/day)	Dose Volume ^b (mL/kg)
		Teratological Evaluations	
1	22	saline	10
2	22	20	10
3	22	65	10
4	22	200	10
		Toxicokinetic Evaluations	
5	10	saline	10
6	10	20	10
7	10	65	10
8	10	200	10

^a All doses and concentrations were expressed in terms of belatacept content. Belatacept was considered 100% pure for the purpose of dose calculations.

^b Administered as a slow bolus injection of approximately 1 minute duration

Results

No belatacept-related toxicity was observed in dams at 20 or 65 mg/kg/day or in the fetuses at any dose administered. A reduction of maternal body-weight gain (25% less than controls) was observed at 200 mg/kg/day during DG 6 to 9. There were no other drug-related changes in this study.

Toxicokinetics

Maternal exposures to belatacept following 10 daily doses of 20, 65 and 200 mg/kg/day were verified on DG 15 and the fetal serum concentrations were determined on DG16. The data are summarized in Table 15.

Table 15 Rat Maternal and Fetal Exposures to Belatacept

Dose (mg/kg/day)	Maternal Exposures to Belatacept on DG 15		Fetal Exposure to Belatacept on DG 16	
	C _{max} (µg/mL)	AUC _{0-24h} (µg·h/mL)	Maternal ^a (µg/mL)	Fetal ^b (µg/mL)
20	646	5,380	144	11.2
65	1,790	12,400	283	24.4
200	4,170	28,200	442	38.4

^a Maternal concentration of belatacept 24 hours following administration of the tenth daily dose on DG 15
^b Pooled fetal samples per litter.

Maternal belatacept AUC exposures increased in a less than dose proportional manner at 65 and 200 mg/kg/day. C_{max} values were dose proportional between 20 and 65 mg/kg/day and less than dose proportional between 65 and 200 mg/kg/day. Fetal exposures to belatacept were determined in all treated groups, demonstrating that belatacept crosses the placenta. Fetal serum concentrations were approximately 8 to 9% of maternal circulation and are consistent with the data reported in other belatacept reproductive toxicology studies (DS07166 and DN06056).

9.3 Embryonic Fetal Development

Study title: Belatacept (BMS-224818): Intravenous Study of Embryo-Fetal Development in Rabbits

Study no: DN06056, a full review is available in DARRTS referring to IND-9418, received 10/08/2009.

Study report location: Princeton, NJ 08543-4000

Conducting laboratory: (b) (4)

Date of study initiation: October 13, 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Belatacept, 5C04183, 99.5% and 5B08488, 99.3%

Key Findings

Rabbits did not show drug-related effects in dams or their fetuses after once daily doses of 10, 30, or 100 mg/kg/day to dams on GD 7 through 19.

Experimental outline

Belatacept was administered intravenously to mated rabbits, ~7 months of age, weighing 2.8-4.3 kg, once daily on GD 7 through 19 at doses of 10, 30, or 100 mg/kg (Table 16). Rabbits (Rabbit/Hra:(NZW)SPF) were randomly assigned to 8 groups, with 22 animals/group for Groups 1 to 4 (for teratological evaluation), and 5 animals/group for Groups 5 to 8 (for toxicokinetic evaluation), see table 1. Study endpoints and observations for embryo-fetal development in pregnant rabbits included toxicokinetics (Groups 5 to 8 on GD 19), mortality, clinical signs, body weights, and food consumption.

Table 16 Rabbit Intravenous Belatacept Study of Embryo-Fetal Development Study Design

Group	Rabbits Assigned	Dose ^a (mg/kg/day)	Dose Volume ^b (mL/kg)
		Teratological Evaluations	
1	22	saline ^c	4
2	22	10	4
3	22	30	4
4	22	100	4
		Toxicokinetic Evaluations	
5	5	saline	4
6	5	10	4
7	5	30	4
8	5	100	4

^a All doses and concentrations were expressed in terms of belatacept content. Belatacept was considered 100% pure for the purpose of dose calculations.

^b Administered as a slow bolus dose delivered by injection pump at an approximate rate of 2 mL/min.

^c 0.9% Sodium Chloride Injection, USP.

All dams assigned to the teratology portion (maternal and developmental toxicity) of the study were euthanatized on GD 29, caesarean-sectioned, and subjected to a gross necropsy of the thoracic, abdominal, and pelvic viscera. The number and distribution of corpora lutea in each ovary were recorded. The uterus of each rabbit was excised and examined for pregnancy, number and distribution of implantation sites, live and dead fetuses, and early and late resorptions. The uteri of the apparently nonpregnant rabbits were confirmed by the absence of implantation sites and retained with ovaries in neutral buffered 10% formalin (NBF). Placentas were examined for size, color, and shape. Gross lesions were retained in NBF for possible future evaluation (corresponding tissues were retained from 2 rabbits in the control group). Dams assigned to the toxicokinetic portion of the study and their fetuses were evaluated for belatacept exposures on GD19 or GD20. Caesarean-section and gross necropsy were performed on the remaining study dams on GD 29.

Fetal weight, sex, external, soft tissue, and skeletal alterations were evaluated. Each fetus was removed from the uterus, examined for gross external alterations, and examined internally to identify sex. Cavitated organs were evaluated in all fetuses by dissection. A single cross-section was made between the parietal and the frontal bones, and the brain was examined *in situ*. All fetuses were examined for skeletal alterations as well.

Results

No belatacept-related changes were observed in rabbit reproductive and developmental parameters in either the dams or the fetuses.

Toxicokinetics

Maternal exposures to belatacept following 13 consecutive daily doses of 10, 30 and 100 mg/kg/day were quantified on GD 19 and the fetal serum concentrations were measured on GD20. The data are summarized in the Table 17.

Table 17 Rabbit Belatacept Maternal and Fetal Exposures

Dose (mg/kg/day)	Maternal Exposures to Belatacept on GD19		Fetal Exposure to Belatacept on GD20	
	C _{max} (µg/mL)	AUC _{0-24 h} (µg·h/mL)	Maternal ^a (µg/mL)	Fetal ^b (µg/mL)
10	383	4,330	119	1.24
30	955	10,500	290	3.74
100	2,950	31,800	794	5.88

^a Maternal concentration of belatacept 24 hours following administration of the thirteenth daily dose on GD19

^b Pooled fetal samples per litter.

Increases of maternal AUC(0-24 h) and C_{max} values were approximately dose-proportional. Fetal exposures to belatacept were quantified in all treated groups, confirming that belatacept (humanized IgG1) crosses the placenta. Fetal serum concentrations were observed to be approximately 0.7 to 1.3% of maternal concentrations, which is consistent with data reported in other reproductive studies (DN06008 and DN06002 in rats).

9.4 Prenatal and Postnatal Development

Study title: Belatacept (BMS-224818): Intravenous Study of Pre- and Postnatal Development in Rats

Study no: DN06002, a full review is available in DARRTS referring to IND-9418, received 10/23/2009.

Conducting laboratory and location:

(b) (4)

Date of study initiation: January 17, 2006

GLP compliance: Yes

QA statement: Yes

Drug, lot #, and % purity: Belatacept, 5K01047 - 99.4% and 5K01055 - 99.3%

Key Findings

1. Administration of belatacept to pregnant rats (F₀-generation) from gestation day (GD) 6 to lactation day (LD) 20 did not affect neurobehavioral or reproductive development in their offspring (F₁-generation) at any dose tested. There were no significant changes or adverse effects on development of the immune system.
2. Belatacept had no effects on duration of gestation, number of pups delivered, or pup viability at birth at any dose tested.
3. Microbial infections (3.8-9.6%) were observed in all treated groups of F₀-generation dams following belatacept administration.
4. Belatacept dosing was associated with drug-related maternal toxicity with secondary pup mortality on postnatal days (PNDs) 1 to 24.

Experimental outline

Belatacept was administered intravenously (dose volume -10 mL/kg), to timed-mated Crl:CD(SD) female rats (55/group) once daily at doses of 20, 65, or 200 mg/kg from

gestation day (GD) 6 through lactation Day (LD) 20 to determine the potential reproductive toxicity.

Healthy mated female Crl:CD(SD) rats (F₀-generation) were assigned to 4 dose groups in the main study (45 rats/group) with an additional 10-15 F₀ rats/group assigned to an extension study for conduct of the T-cell-dependent antibody response and potential histopathology evaluations (Table 18). On PND 21, pups (F₁-generation rats) were assigned to 1 of 5 subsets for various evaluations described further below.

F₀-Generation Rats: Female rats were given the test article and/or vehicle once daily from GD 6 to lactation day (LD) 12 (for measurements of drug levels in maternal serum/milk determination and maternal toxicokinetic evaluation), to GD 24 (for rats that did not deliver a litter), or to LD 20 for examination of maternal toxicity and pre-/postnatal development evaluations. In the study extension, 15 F₀ rats were assigned to Group 1 and 10 F₀ rats were assigned to Groups 2 to 4.

For F₀-generation rats, endpoints included clinical observations, milk/serum levels of belatacept on LD 12, immunogenicity, toxicokinetics on LD12, gross necropsies, and histopathology after completion of the 21-day lactation period. In addition, to investigate the possible cause(s) for clinical toxicity and necropsy changes noted in some F₀-generation dams, the maternal sera were analyzed for the presence of Tyzzer's disease using an immunofluorescent assay and Polymerase Chain Reaction (PCR) of intestinal tissues. Tyzzer's disease is a bacterial disease caused by the organism *Clostridium piliforme* and is commonly observed in laboratory animals.

Table 18 Rat IV Belatacept Pre- and Postnatal Development Study Design

Dose Group	Number of Female Rats Assigned to Study	Belatacept Dose ^a (mg/kg/day)	Concentration (mg/mL)	Dose Volume ^b (mL/kg)
1	25 ^c + 10 ^d + 10 ^e + 15 ^f	0	0	10
2	25 ^c + 10 ^d + 10 ^e + 10 ^f	20	2	10
3	25 ^c + 10 ^d + 10 ^e + 10 ^f	65	6.5	10
4	25 ^c + 10 ^d + 10 ^e + 10 ^f	200	20	10

^a All doses and concentrations were expressed in terms of belatacept content. Belatacept was considered 100% pure for the purpose of dose calculations.

^b Administered as a slow bolus injection of approximately 1 minute in duration.

^c Assigned to maternal toxicity and pre-/postnatal development evaluations.

^d Assigned to drug levels in maternal serum and milk determination on LD 12.

^e Assigned to maternal toxicokinetic evaluations on LD 12.

^f Study Extension- for conduct of the T-cell-dependent antibody response and potential histopathology evaluations.

F₁-Generation Pups: F₁ animals were evaluated in both the main study and the study extension. The main study consisted of Subsets 1 through 4, and the study extension included Subset 5 (Table 19). 10-40 F₁ animals/sex from each dosed F₀ group were assigned to Subsets 1 through 4 (Table 18). 25 F₁ animals/sex from each dosed F₀ group were assigned to Subset 5, resulting in 200 F₁-generation rats (100 per sex). At least 1 male pup and 1 female pup per litter, when possible, were selected.

Table 19 F₁ Rats Subset Assignments

	Evaluation	PND	Rats Assigned
Subset 1	•Serum Analysis (belatacept levels and belatacept-specific antibodies)	21	10/sex/group (when possible)
Subset 2	•T-cell-dependent antibody response following immunization with keyhole limpet hemocyanin (KLH) Positive control group treated with cyclophosphamide •Histopathology ^a •Peripheral blood lymphocyte phenotyping	56 & 64 (Cohort 1) 100 & 108 (Cohort 2)	10/sex/group/cohort Additional 10/sex/cohort from Group I served as positive controls.
Subset 3	•Serum analysis (immunoglobulin levels, belatacept levels, and belatacept-specific antibodies) •Histopathology ^a •Lymphoid organ weights (thymus and spleen) •Histopathology	62 to 64	10/sex/group
Subset 4	•Clinical pathology •Serum analysis (immunoglobulin levels and anti-nuclear antibodies)	112	10/sex/group
Subset 5	•Sexual Maturation •Reproductive Performance	21 to 130	1/sex/litter (when possible)

^a Selected tissues were retained in 10% neutral buffered formalin (NBF) for possible future histopathologic evaluation.

- **Clinical Observations:** Each litter was evaluated for viability, and each pup was observed for clinical signs, general appearance, body weights, food consumption, and sexual maturation.
- **Subset-1 Evaluations:** On PND 21, blood samples of surviving Subset-1 pups were collected for serum levels of belatacept and belatacept-specific antibodies evaluations. Following blood collection, the rats were necropsied and examined for gross lesions.
- **Subset-2 Evaluations (PNDs 56 to 108):** Each group in the main study and study extension had 2 cohorts for T-Cell-Dependent Antibody Response (TDAR) assay. Rats in each cohort were immunized subcutaneously with 1 mg of KLH (0.2 mL of a 5 mg/mL KLH solution using a 22 gauge needle) on PND 50 (Cohort 1 in the Main Study), 50/51 (cohort 1 in the study extension), or 94 (cohort 2 in the main study and study extension). In addition, positive-control rats in group 1 were given an intraperitoneal dose of 25 mg/kg of cyclophosphamide immediately after KLH administration and continuing daily until the day before necropsy. Blood samples (approximately 1.5 to 2.0 mL each) were collected on days 6 and 14 after KLH immunization. Following final blood collection, the thymus, spleen, mesenteric lymph nodes, kidneys, thyroid, pancreas, stomach, right femur, and testes/ovaries from all groups were collected.
- **Subset-3 Evaluations:** On PNDs 62 to 64, Blood samples were collected from surviving Subset-3 rats for serum levels of belatacept and belatacept-specific antibodies, immunoglobulins evaluations, and peripheral-blood lymphocyte phenotyping including the absolute number of lymphocytes expressing CD3+ (T cells), CD4+CD8- (T-helper cells), CD8+CD4- (T-cytotoxic cells), CD4+CD25+Foxp3+ (Treg cells), CD45RA+ (B cells), and CD3-CD161+ (NK cells).

- Subset-4 Evaluations: On PND 112, Blood samples were collected from surviving Subset-4 rats for clinical chemistry, serum belatacept, immunoglobulin, and anti-nuclear antibody levels, hematology, coagulation, lymphoid organ weights and histopathology evaluation.
- Subset-5 Evaluations (PNDs 28 to 130): For observation of sexual maturation, rats were evaluated for the age of vaginal patency (beginning on PND 28 for females) and preputial separation (beginning on PND 39 for males). One female and one male rat per litter were evaluated for behavioral parameters including motor activity, acoustic startle habituation, and water-filled M-maze for overt coordination, swimming ability, learning, and memory. On PNDs 93 to 97, 1 male rat per female F₁-generation rats within each dose group were assigned to cohabitation for reproductive capacity evaluation. On GD 20, surviving Subset-5 females were euthanatized by CO₂ asphyxiation, caesarean-sectioned, and a gross necropsy of the thoracic, abdominal, and pelvic viscera was performed.

Results

In the dams, belatacept had no effect on duration of gestation, number of pups delivered, or pup viability at birth at any dose tested. In contrast, 3.8% - 9.6% of the dams in all treated groups presented with dose-dependent clinical toxicities. The toxicities were noted exclusively during peripartum and lactation and were attributed to opportunistic infection, secondary to pharmacologically mediated immunosuppression. The observations imply a minimal increase of susceptibility of infection in maternal rats. However, susceptibility of infection might be associated with the combination of pregnancy and the length of belatacept administration during pregnancy and through lactation, because there is no evidence of infections in nonpregnant adult rats with similar exposure duration of abatacept (Study No. DS07166) or in pregnant rats and rabbits with a shorter dose duration of belatacept (Study No. DN06032, DN06008, and DN06056). In the F₁-generation offspring, increased pup mortality occurred at all doses. This finding was noted during the preweaning period (8 litters with 44-100% pup losses on PNDs 1-18; 2 litters with 33% weanlings died/euthanatized on PNDs 22-24); and was restricted to offspring of the dams that exhibited pronounced toxicity. Therefore, the mortality is considered a secondary effect of impaired maternal health.

Administration of belatacept to pregnant rats did not affect neurobehavioral or reproductive development in the offspring at any dose tested (mean maternal AUC values $\leq 32600 \mu\text{g}\cdot\text{h}/\text{mL}$). There were no toxicological effects on the development of the immune system due to the relatively low exposure of belatacept (refer to table 3 and 4) in the offspring. No drug-related clinical observations occurred at any dose tested in the F₁-generation rats. Specifically, belatacept did not affect the absolute numbers of peripheral-blood lymphocytes expressing CD3+ (T cells), CD4+CD8- (T-helper cells), CD8+CD4- (T-cytotoxic cells), CD4+CD25+Foxp3+ (a subset of T-regulatory cells), CD45RA+ (B cells), or CD3-CD161+ (NK cells) at any dose on PNDs 62 to 64. The findings in the F₁-generation were limited to decreases of serum IgG concentrations at all doses on PNDs 62-64 (53-98% of controls; except the F₁ litters born of the F₀ females dose receiving 20 mg/kg/day) and PND 112 (61-82% of controls). The

reduction of IgG may be associated with the exposure to belatacept from dams during gestation and lactation; and are considered secondary pharmacological effects without dose dependency or clinical signs. Furthermore, the incidence and severity were attenuated from PNDs 62-64 to 112. There were no drug-related effects on sexual maturation, behavioral evaluations, mating or fertility parameters, in F₁ dams or their litters (F₂) at any dose tested. There were also no drug-related gross external alterations in the F₂-generation fetuses.

Toxicokinetics

On LD 12, belatacept C_{max} and AUC values in F₀-generation dams generally increased in a dose-proportional manner from 20 to 200 mg/kg/day (Table 20).

Table 20 Maternal Rat Belatacept Toxicokinetics and Serum and Milk Concentrations

Belatacept Dose (mg/kg/day)	Toxicokinetics Parameter		Serum and Milk Levels or Ratio		
	C _{max} (µg/mL)	AUC (µg·h/mL)	Maternal Serum (µg/mL)	Maternal Milk (µg/mL)	Milk/Serum Ratio
20	539	4630	224 ± 21.4	29.0 ± 4.92	0.129
65	1700	11900	920 ± 172	81.8 ± 8.50	0.0889
200	4700	32600	2,640 ± 295	270 ± 75.3	0.102

As shown in Table 21, belatacept was present in maternal milk, and the concentrations were dose related at approximately 9 to 13% of those in the serum. Belatacept-specific antibodies (evidence of immunogenicity) were not detected in serum or milk of drug-treated dams. Additionally, serum samples were collected from F₁-generation offspring on PNDs 21, 62-64, and 112 to determine the extent of pup drug exposure and immunogenicity. As outlined in table 4, serum concentrations of belatacept in PND-21 pups were dose-related and comparable between males and females, although the values were low (1.4 to 3.2% of maternal C_{max} on LD 12).

Table 21 F₁-Generation Belatacept Serum Levels in Male and Female Offspring on PND 21

Dose (mg/kg/day) in F ₀	Serum Levels of Belatacept (µg/mL) in F ₁ -Generation Offspring on PND 21	
	Male	Female
20	17.3 ± 6.73	15.9 ± 5.56
65	38.3 ± 12.6	38.9 ± 13.9
200	66.6 ± 22.9	79.2 ± 28.6

10 Special Toxicology Studies

Local Tolerance in Rabbits

No signs of irritation were observed after IV, intraarterial, or paravenous belatacept administration using three different formulations per route in female New Zealand White rabbits (Study Number DS05072⁴⁴).

Juvenile Toxicology Studies in Rats

Study No. DN07013, a full review is available in DARRTS referring to IND 9418, received 10/16/2009.

Study Title: Thirteen-Week Subcutaneous/Intravenous Immunotoxicity Study in Juvenile Rats

Applicant: Bristol-Myers Squibb Company

Study Facility: [REDACTED] (b) (4)

Date of Study Initiation: March 13, 2007

GLP Compliance: Yes

QA Report: Yes

Drug: Abatacept

KEY FINDINGS

1. Partially reversible decreases in serum IgG concentrations (0.03 to 0.44x controls)
2. Suppression of T-cell-dependent IgG ($\leq 0.04x$ controls) and IgM ($\leq 0.16x$ controls) antibody responses to KLH during the dosing period with full reversibility of IgM at all doses and partial reversibility of IgG.
3. Fully reversible decreases in absolute numbers of peripheral-blood T-regulatory (CD4+CD25+Foxp3+) cells (0.14 to 0.29x controls).
4. Partially (males) to fully (females) reversible increases in absolute numbers of peripheral-blood T-helper (CD4+CD8-; 1.61 to 3.60x controls) and total T cells (CD3+; 1.53 to 3.10x controls).
5. 4.6% dose-independent death attributed to opportunistic infections under secondary pharmacologically mediated immunosuppression.
6. No belatacept-related effects on body weight, food consumption, sexual maturation, behavioral parameters, sperm parameters, estrous cyclicity, cesarean-section parameters among litters sired by treated males, in litters of treated females, and mating or fertility in males or females were observed at any dose tested.

METHODS

Experimental Design

Abatacept was given once every 3 days at doses of 0 (vehicle control, group 1), 20 (group 2), 65 (group 3), and 200 mg/kg (group 4) from PND 4 through PND 94 (31 doses total). The test and control articles were given subcutaneously on PNDs 4 through 28 and intravenously from PNDs 31 through 94. Table 22 shows the study design. All doses were expected to be pharmacologically active because a single intravenous dose of 2.5 mg/kg in rats effectively inhibits both primary and secondary alloantibody responses.

Table 22 Juvenile Rat Thirteen-Week Subcutaneous/Intravenous Abatacept Immunotoxicity Study Design

Dose Group	Number of F ₁ - Generation Rats	Dose(mg/kg)	Dose Volume ^a (mL/kg)
1	82 /sex	0 (Vehicle)	5
2	77 /sex	20	5
3	77 /sex	65	5
4	77 /sex	200	5

^a Administered subcutaneously during the preweaning period (PNDs 4 to 28) and intravenously (as a bolus injection) during the post weaning period (PNDs 31 to 94).

RESULTS

Administration of abatacept to rats from post-natal days (PNDs) 4 to 94 did not affect neurobehavior or reproduction at any dose tested. Abatacept at all doses was associated with altered immunological parameters (including lymphoid-tissue changes) and inflammation in non-lymphoid organs. Severity and incidence of these findings were generally independent of dose (20, 65, and 200 mg/kg) in the study. Following a 3-month treatment-free period, most immunological changes showed evidence of partial to complete recovery that are consistent with similar studies. The major findings in this juvenile toxicity study were lymphocytic thyroiditis and insulinitis, and infections (leading to moribundity/death).

Mortality

Death or moribundity necessitating euthanasia occurred in 27 abatacept-treated rats from PNDs 34 to 161. The overall incidence of mortality/moribundity was 4.6%; unrelated to dose (10, 5, and 12 rats at 20, 65, and 200 mg/kg, respectively); predominantly affected males (20 males versus 7 females); and occurred primarily during the postdose period (21/27 rats died or euthanatized on PNDs 100 to 161). Most affected rats exhibited signs of clinical toxicity, initially characterized by persistent weight loss of approximately 1 to 3 weeks duration, with cumulative weight losses of 19% to 37%. Subsequently, the onset of adverse clinical signs was acute; these included dehydration, ptosis, ungroomed coat, hunched posture, decreased motor activity, cold to touch, soft or liquid feces, dyspnea, and chromodacryorrhea. Death or euthanasia followed shortly thereafter. Some affected rats also exhibited intermittent moderate to severe decreases in food consumption. All other rats survived to scheduled termination. Observed infections leading to moribundity or deaths occurred in all treated groups. The incidence of moribundity/death due to infections was low (approximately 4.6%), the severity and incidence were independent of dose, and attributed to pharmacologically mediated immunosuppression.

In a follow-up similar study (DS07165), the severity and the incidence (1.47%) were significantly improved by employing a more microbiologically-controlled facility. The mortality and moribundity predominantly affected males (20 males versus 7 females); and occurred primarily during the postdose period (21/27 rats died or euthanatized on PNDs 100 to 161). The cause of death and moribundity in these rats was attributed to infection, secondary to pharmacologically mediated immunosuppression. Findings consistent with this interpretation consisted primarily of: **1)** clinical observations of

persistent weight loss, diarrhea (soft or liquid feces), dehydration, and hypothermia (cold to touch); **2)** positive blood cultures for opportunistic pathogens (*Klebsiella oxytoca*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, or *Escherichia coli*) in 4/11 moribund rats euthanatized on PNDs 118 to 161; **3)** the presence of many adult pinworms or eggs (*Syphacia obvelata*) in rectal/perianal samples from 2/2 moribund rats euthanatized on PND 118; **4)** gross lesions indicative of microbial infections in the gastrointestinal tract of early decedents (dark red/red areas or mucosal surfaces, thickened or pale intestinal walls, firm/dark red masses, white raised areas, enlarged cecum, and gas/mucus distension); and **5)** microscopic findings of acute inflammation with hemorrhage/edema, and/or bacterial invasion (small cocco-bacilli) of the intestinal wall, lymph node, and prostate. These data, in conjunction with decreased antibody responses and serum immunoglobulins, strongly suggest that pharmacologic immunosuppression lowered host resistance and increased susceptibility to infections, leading to moribundity/death in affected rats. Importantly, the incidence of death (1.47%) was improved in study DS07165. Moreover, in a similar study DN07166, conducted in adult rats, there were comparable changes in all evaluated parameters without any evidence of drug-related infections. Thus, the infections observed in juvenile rats are considered a juvenile-rat-specific finding that predominantly affects males and is dose independent. The juvenile-rat-specific infections may be due to immaturity of the immune system compared with adult rats (PNDs 56) when immunosuppressive treatment was initiated at PNDs 4. Furthermore, the male-juvenile-rat-predominant infections may imply sex hormone-control of adaptive immunity after a 3-month dosing period.

Clinical Immunology

There were no drug-related antinuclear antibodies or effects on serum IgM concentrations at any dose tested. In contrast, abatacept altered peripheral-blood T-cell population profiles, serum IgG concentrations, and T-cell-dependent antibody responses to keyhole limpet hemocyanin (KLH) at all doses (summarized in Table 23). These effects were first noted during the late-juvenile phase (PNDs 42 to 56, the earliest interval evaluated), and were generally more pronounced at the end of the dosing period (PND 93). The overall recovery of these changes was incomplete following a 3-month postdose period, and was likely related to the presence of drug over the same period.

Descriptions of these findings are outlined below:

Peripheral-Blood T-Helper and total T Cell Counts: Time-dependent increases in T-helper (CD4+CD8-) and total T (CD3+) cell counts were noted in the peripheral blood on PNDs 47 (1.61-2.24x controls and 1.53-1.97x controls, respectively) and PND 93 (2.60-3.60x controls and 2.17-3.10x controls, respectively). These findings were consistent with clinical-pathology findings of elevated lymphocyte counts during the same period and correlated, microscopically, with expansion or increased prominence of T-helper/T cell areas in the spleen and mesenteric/mandibular lymph nodes noted on PND 93. During recovery, the effects resolved in females, but were partially reversed in males by PND 183. T-helper and total T cells in males were 1.86-2.07x and 1.65-1.89x controls, respectively.

Table 23 Summary of Immune Parameter Changes in Juvenile Rats

Parameter	PND	Males			Females		
		Group 2	Group 3	Group 4	Group 2	Group 3	Group 4
T-Helper (CD4+CD8-) Cell Counts	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 (CD3+) Cell Counts	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+) Cell Counts	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*
IgM	48	0.15x	0.14x	0.15x	0.16x*	0.10x*	0.15x*
KLH-Specific Antibody	176	2.01x	0.45x	0.42x	0.59x	0.89x	0.67x
	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

Group 2 = 20 mg/kg; Group 3 = 65 mg/kg; Group 4 = 200 mg/kg

"x" = Treated group mean ÷ control group mean (for peripheral-blood lymphocyte phenotypes)

"x" = Treated group geomean ÷ control group geomean (for IgG and KLH-specific antibody response)

Italicized data were not analyzed statistically due to lack of variability in individual results.

Statistical significance (* = $p \leq 0.05$) was based on the actual data.

- Peripheral-Blood T-regulatory Cell Counts:** Abatacept decreased T-regulatory (CD4+CD25+Foxp3+) cell counts in the peripheral blood on PNDs 47 (0.14 to 0.24x controls) and 93 (0.20 to 0.29x controls). However, these reductions were completely recovered by PND 183.
- Serum Immunoglobulin (Ig) Isotyping:** There were no abatacept-related changes on serum IgM concentrations at any dose tested. Age-related increases in serum IgG concentrations were observed among control animals from PNDs 42 through 183 (0.75 to 5.77 mg/mL). Among treated animals, abatacept consistently reduced these concentrations on PNDs 42 (0.14 to 0.44x controls) and 93 (0.03 to 0.09x controls). The reductions were attenuated during recovery, but remained evident on PND 183 (0.21 to 0.42x controls). IgG levels of a few treated animals were comparable in magnitude to those on PND 93.
- T-Cell-Dependent Antibody Responses to KLH:** Consistent with pharmacologically mediated inhibition of CD28 costimulation, serum anti-KLH IgM and IgG levels in all drug-treated groups were suppressed to less than 16% and 4% of respective control titers following KLH immunization on PND 42. By PND 184, complete reversal was observed on IgM responses at all doses and IgG responses at 20 mg/kg. Conversely, some 65-mg/kg and many 200-mg/kg rats failed to mount IgG responses to KLH immunization, which is likely due to the presence of biologically active drug levels when the animals were immunized with KLH on PND 170.
- Abatacept Immunogenicity:** In drug-treated groups, consistent with pharmacologically mediated immunosuppression, abatacept-specific antibodies (immunogenicity) were not detected until 1 month after cessation of dosing (PND 125).

Although the incidence and magnitude of immunogenicity generally increased as serum drug concentrations declined, these antibodies did not affect T1/2 values for abatacept (Table 25).

Histopathology

At the end of the dosing period (PND 93), drug-related microscopic pathology was noted in males and females at all doses. Findings included alterations to the architectures of spleen and mesenteric and mandibular lymph nodes; lymphocytic inflammation of the thyroid and pancreas; and mononuclear-cell infiltration and inflammation of the Harderian glands and prostate. The incidence and severity of these findings were generally unrelated to dose. Reversibility assessed on PND 183 was complete to partial for splenic and lymph node findings; while inflammatory changes persisted (in Harderian glands and prostate and expanding to seminal vesicles) or progressed (in thyroid and pancreas). Descriptions of these drug-related changes are outlined below (Table 24):

Table 24 Summary of Abatacept-related Histopathology Findings

Histopathological Finding	PND	Males Group				Females Group			
		1	2	3	4	1	2	3	4
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	-*
	93	-	9	10	9	-	10	10	10
Spleen PALS Prominent	183	-	8	9	9	-	-	-	-
Marginal Zone Expansion	93	-	8	10	9	-	8	7	10
	183	-	-	-	1	-	-	-	-
Thyroid Gland Thyroiditis: lymphocytic	93	-	2	3	2	-	2	1	4
	183	-	7	7	5	-	8	8	6
Pancreas Islet inflammation, lymphocytic	93	-	2	2	-	-	-	-	1
	183	-	3	6	4	-	-	2	1
Harderian Glands Infiltration: mononuclear cell	93	-	2	6	6	1	4	5	4
	183	-	6	4	6	-	4	3	2
Prostate Gland Infiltration: mononuclear cell	93	4	9	10	9				
	183	3	7	9	8				
Inflammation	93	1	-	-	2				
	183	1	3	3	2				
Seminal Vesicle Infiltration: mononuclear cell	93	-	-	-	-				
	183	-	5	4	5				

Group 1= Vehicle; Group 2= 20 mg/kg; Group 3= 65 mg/kg; Group 4= 200 mg/kg
n = 8 to 10 rats evaluated/sex/group, except where indicated, * = 7 rats evaluated,
'-' = 0; PALS = Periarteriolar lymphoid sheaths,

The numbers in each group indicate the incidences of findings at the endpoints.

- **Spleen and Mesenteric/Mandibular Lymph Nodes:** In the H&E stained sections, drug-related changes on PND 93 were characterized by expansion or increased prominence of the marginal zone and periarteriolar lymphoid sheaths (PALS) in the spleen; and expansion of paracortical zones in mesenteric and mandibular lymph nodes. Immunocytochemical staining further demonstrated that expanded PALS and paracortical zones were comprised primarily of T cells (particularly T-helper cells in the PALS). This observation is consistent with those of increased T-helper and total T cells in the peripheral blood. Additional drug-related changes included decreased lymphoid germinal centers, with disrupted B-cell organization and absence of active germinal centers, also revealed by immunocytochemical staining. These effects on B-cell areas reflected pharmacological immunosuppression. Upon evaluation on PND 183, these findings were fully resolved in females. Residual effects on lymphoid germinal centers and PALS were still evident in males. Although the incidence and/or severity of these lesions were generally reduced, PALS severity scores in a few animals were comparable to those recorded on PND 93.
- **Thyroid Gland:** Minimal to marked lymphocytic thyroiditis, consistent with an autoimmune etiology, was observed in both sexes on PND 93; the overall incidence was 24%. By PND 183, the incidence was increased to 75% with severities ranging from slight to marked. Some affected glands on PND 183 were grossly enlarged; these correlated microscopically with mild to marked lymphocytic infiltration/inflammation of the glandular tissue. In severe cases, this was accompanied by effacement of thyroid follicular architecture. The parathyroid glands were unaffected.
- **Pancreas:** Minimal to moderate lymphocytic inflammation of islets (insulitis), also consistent with an autoimmune etiology, was observed on PND 93. The overall incidence was 9% on PND 93 and increased to 29% on PND 183 with greater incidence in males than females. The acinar tissue of the exocrine pancreas was unaffected.
- **Harderian Glands, Prostate, and Seminal Vesicles:** Mononuclear-cell infiltration and chronic inflammation were noted in Harderian glands and prostate on PND 93. Although the nature and severity of these changes are consistent with reported incidental lesions in this species and strain, the incidences in drug-treated animals were clearly increased, suggesting exacerbation of normal background inflammation that is likely associated with the immunomodulatory action of abatacept. These effects persisted to PND183, with seminal vesicles also affected. These ophthalmic findings occurred exclusively during the postdose period and were consistent with conjunctivitis (improving with administration of Neosporin ophthalmic solution).

The reversibility of drug-related immune parameter changes was variable. The changes were generally reversible following the 3-month postdose period independent of dose in the study. Effects on hematology/clinical-immunology parameters and lymphoid organs were generally reversible, and the degree of reversal was often inversely related to dose or serum level of abatacept during the postdose period. As such, females which displayed lower drug levels and a shorter T_{1/2} (Table 2) for abatacept exhibited greater recoveries. Therefore, by PND 184, changes were still evident on the following parameters: 1) serum IgG levels (at all doses) and IgG responses to KLH (at 65 and 200 mg/kg) in both sexes; 2) lymphoid germinal centers and serum globulin levels in males at 65 and 200 mg/kg; and 3) splenic PALS and peripheral-blood T-helper and

total T cells (and corresponding lymphocyte and leukocyte counts) in males at all doses. The incidences of mononuclear-cell infiltrations in Harderian gland, prostate, and seminal vesicles persisted, and lymphocytic infiltrations of thyroid and pancreatic islet cells persisted or progressed. Lymphocytic insulinitis and thyroiditis were exacerbated, as evidenced by increased incidence (20% and 75%, respectively) and severities on PND 183; thyroids of many affected rats were grossly enlarged at scheduled termination.

Toxicokinetics

Abatacept toxicokinetic parameters are summarized in Table 25.

Table 25 Abatacept Toxicokinetic Parameters in Juvenile Rats

Parameter	PND	Dose (mg/kg Q3D)					
		20		65		200	
		Male	Female	Male	Female	Male	Female
Cmax (µg/mL)	28	203	195	499	559	1,470	1,510
	88	635	550	2,420	2,050	5,810	5,310
AUC _{0-72h} (µg·h/mL)	28	10,300	10,100	26,800	29,600	67,000	69,800
	88	25,200	20,100	68,100	52,900	148,000	117,000
CLT _{ss} (mL/h/kg)	28	1.94	1.98	2.42	2.20	2.98	2.87
	88	0.793	0.994	0.954	1.23	1.36	1.71
V _{ss} (L/kg)	28	0.0613	0.0637	0.0792	0.0712	0.0917	0.0868
	88	0.0225	0.0279	0.0261	0.0325	0.0315	0.0407
MRT _{ss} (h)	28	31.6	32.2	32.7	32.4	30.7	30.3
	88	28.4	28.1	27.3	26.5	23.2	23.8
T _{1/2} ^a (h)	88 to 183 ^b	199	149	223	183	245	164
	88 to 183 ^c	200	150	230	176	244	120
Serum Abatacept (µg/mL)	93	246	231	583	497	1120	833
	125	24.5	21.0	51.9	42.5	61.7	45.5
	155	3.07	1.08	7.51	4.39	9.48	3.76
	183	0.16	0.01	0.69	0.15	1.19	0.10

^a Elimination half-life; calculated using combined data from PNDs 88-91, 125, 155, and 183.

^b Includes all samples regardless of the immunogenicity status.

^c Excluded samples with the presence of abatacept antibodies.

The abatacept-related changes were generally partially reversible following a 3-month postdose period, independent of selected dose. The findings were also consistent with most of the changes in adult mice, rats and monkeys. This study confirmed the observed changes associated with abatacept are not juvenile-rat-specific with the exception of infections.

Study No. DS07165, a full review is available in DARRTS referring to IND-9418, received 10/16/2009

Study Title: Abatacept 3-Month Intermittent-Dose (Q3D) Subcutaneous and intravenous Immunotoxicity Study in Juvenile Rats (b) (4)

Study Facility: [REDACTED]

Date of Study Initiation: November 01, 2007

GLP Compliance: Yes

QA Report: Yes

Drug: Abatacept

KEY FINDINGS

1. Decreases in serum IgG levels in males at PND 48 (0.50 to 0.77x control) and in males and females at PND 98 (0.04 to 0.08x controls) were partially reversible.
2. Decreases in the counts of a subset of peripheral-blood T-regulatory cells (CD4+CD25+FoxP3+) (0.33 to 0.50x controls) were fully reversible.
3. Decreases in the T-cell-dependent IgG (0% of controls) and IgM (\leq 6% of controls) antibody responses to KLH were partially reversible (only males assessed), with greater reversibility for groups dosed from PND 28.
4. Increases in the counts of peripheral-blood T cells (CD3+) and T-helper cells (CD4+CD8-; 1.29 to 3.46x controls) were partially reversible.
5. The severity and incidence of opportunistic infections (1.47% drug-related death) were improved (compare with Study No. DN07013, 4.6% dose-independent death) by employing a more microbiologically-controlled facility.
6. The study confirmed that in general, the abatacept-induced alterations in immunological parameters are not juvenile-specific findings in rats.

METHODS

Experimental Design

CrI:CD(SD) rats were selected since this strain of rat had been used in a previous juvenile toxicity study (Study# DN07013) with abatacept and the test article is pharmacologically active in this strain.

Abatacept was given once every 3 days at doses of 0 (vehicle control) or 65 mg/kg from PND 4 through 97; or at 20 mg/kg or 65 mg/kg from PND 28 through 97. The test and control articles were given subcutaneously on PNDs 4 through 28 and intravenously from PNDs 31 through 97.

Table 26 summarizes the study design.

Table 26 Abatacept Juvenile Rat 3-Month Subcutaneous/Intravenous Immunotoxicity Study Design

Group	Treatment & Dose (mg/kg)	Dose Volume (mL/kg)	Dosing Duration (PND)	Number of F-1 Generation Animals Assigned to Study			Total
				Cohort A	Cohort B	TK ^e	
1	Vehicle ^a 0 ^b	5	4-97	10/sex	10/sex	40/sex	120
2	Abatacept 65 mg/kg ^b	5	4-97	10/sex	10/sex	40/sex	120
3	Abatacept 20 mg/kg ^c	5	28-97	10/sex	10/sex	NA	40
4	Abatacept 65 mg/kg ^c	5	28-97	10/sex	10/sex	NA	40
5	Cyclosporine 15 mg/kg ^d	5	39-47 or 162-170	5/sex	5/sex	NA	20

^a 0.9% Sodium Chloride for Injection, USP.

- ^b Administered Q3D: Administered SC during the preweaning period (PNDs 4 to 28) and IV as a bolus injection during PNDs 31 to 97.
- ^c Administered Q3D: Administered SC on PND 28 and IV as a bolus injection during PNDs 31 to 97.
- ^d Cyclosporine is the positive control group for the TDAR. Each rat received one daily dose for 9 consecutive days. Dosing began on PND 39 for Cohort A and on PND 162 for Cohort B.
- ^e PND 28 toxicokinetic (TK) animals.

Additional observations and measurements included:

- Toxicokinetics
- Mortality and Clinical Observations
- Immunogenicity (Anti-Drug Antibody, ADA)
- Serum Immunoglobulin (Ig) Isotyping
- T-Cell-Dependent Antibody Response (TDAR) to KLH
- Peripheral-Blood Lymphocyte (PBL) Phenotyping
- Anatomic Pathology

RESULTS

Mortality and Clinical Observations

A low percentage (1.7%) of drug-related deaths was observed in 2 animals (1/sex) at 65 mg/kg (from each dose initiation day). Tail skin lesions were the only clinical signs (rash, reddened skin, tail biting, and/or irritation) considered drug-related. The lesions were observed for 5 of 40 rats dosed at 65 mg/kg (or of 120 total treated rats). The earliest and latest onsets of these observations were PNDs 119 and 161, respectively; and two animals were sacrificed on PNDs 134 or 155 with severe localized clinical signs (ulceration or red/inflamed with scab and exudate) and histopathological findings, i.e., surface bacteria (1 animal) and moderate chronic inflammation. The tail lesions are considered to be infection(s) based on positive tail swab and/or tail tissue cultures for several pathogens including *Pantoea agglomerans*, *Pasteurella haemolytica*, and *Proteus mirabilis* among other organisms. In addition, although the microbiologically-controlled facility improved the incidence and severity of infection (1.7%) compared with the regular animal facility (study# DN07013, 4.6% drug-related deaths were associated with infections) the infections further confirmed the pharmacological effect of the drug in compromising the host immune response. Compared with adult rats (study# DS07166), the finding also implies that juvenile rats are more susceptible to infection with abatacept treatment. A possible mechanism of infection may be that juvenile rats were treated with abatacept prior to development of memory immune responses to potential infectious pathogens and were therefore more susceptible to opportunistic infections.

Immunogenicity (ADA Levels)

On PND 98, at the end of the dosing phase, no abatacept-treated animals (Groups 2 to 4) were positive for ADAs. Following dose cessation the incidence of abatacept treated animals positive for ADAs increased over time (0/60 on PND 123, 16/48 on PND 155, and 31/53 on PND 189 – i.e., 0, 33, and 58%, respectively) with similar overall incidence in males and females. The magnitude of the responses also increased over time in abatacept-treated animals.

Serum Ig Isotypes

Drug-related Ig changes were limited in serum IgG concentrations that were down regulated on PND 98. Following drug withdrawal, partial or complete recoveries were observed on PND189.

T-Cell-Dependent Antibody Responses (TDAR) to KLH

The positive-control rats generally exhibited significantly decreased (where statistical evaluations were conducted) TDARs to KLH; KLH-specific IgM responses were decreased from 2 to 4% of control at 6 days post-KLH immunization (PKI) and IgG responses were decreased from 0 to 1% of control at 14 days PKI. Based on the data from the male animals, the ability of vehicle-control rats to mount TDARs improved following immunization with KLH on PND 165 compared to PND 42.

- **KLH-Specific IgG Antibody Response (Figure 1):** There were drug-related decreases in the T-cell-dependent IgG antibody response to KLH at ≥ 20 mg/kg of abatacept on PND 56. On PND 56, KLH-specific IgG concentrations were substantially decreased (0% of controls) in both sexes regardless of dose or dose-initiation day. Based on the KLH-specific IgG antibody response on PND 179 (males only), following a 3-month postdose period, the ability to mount a KLH-specific IgG antibody response partially recovered, with greater recovery in animals dosed at 20 mg/kg (from PND 28) than at 65 mg/kg (from PND 4 or 28).
- **KLH-Specific IgM Antibody Response:** There were drug-related decreases in the T-cell-dependent IgM antibody response to KLH at ≥ 20 mg/kg of abatacept on PND 48. On PND 48, KLH-specific IgM concentrations were substantially decreased ($\leq 6\%$ of controls) in both sexes regardless of dose or dose-initiation day. There were no drug-related effects on the kinetics of the TDAR. In general, the KLH-specific IgM response was higher at 6 days PKI than at 14 days PKI. Based on the KLH-specific IgM antibody response on PND 171 (males only), following a 3-month postdose period, the ability to mount a KLH-specific IgM antibody response partially recovered with greater recovery in animals dosed at either dose (20 or 65 mg/kg) from PND 28.

Peripheral-Blood Lymphocyte (PBL) Phenotypes

There were drug-related effects on counts of T (CD3+), T-helper (CD4+CD8-), and a subset of T-regulatory cells (CD4+CD25+FoxP3+) at ≥ 20 mg/kg of abatacept on both PNDs 42 and 98. At both timepoints, counts of T- and T-helper cells were significantly increased (T-helper cell counts 1.29 to 3.46x controls; generally $p \leq 0.05$); whereas, counts of T-regulatory cells were significantly decreased (0.33 to 0.50x controls) in both sexes regardless of dose or dose-initiation day. In addition, the decreases of T-regulatory cell counts in abatacept-treated rats, independent of dose and dose-initiation day, were similar to or greater than control counts following the 3-month postdose period indicated full reversibility. The drug-related effect on T-regulatory cells is expected based on the pharmacological inhibition by abatacept of the CD28-CD80/86 interactions that are required for T-regulatory cell survival. It is not known whether the decreases in T-regulatory cells and the increases in T and T-helper cells are biologically related. Increases in T and T-helper cells were not previously reported in mice (spleen) or monkeys (peripheral blood) dosed as adults with abatacept for up to 6-months or 1-

year, respectively (T-regulatory cells were not evaluated); however, qualitatively similar effects on peripheral-blood T, T-helper, and T-regulatory cell counts have been observed in rats dosed as adults.

Histopathology

Drug-related histopathologic changes were observed in the mesenteric and mandibular lymph nodes, spleen, pancreas, and thyroid glands at the end-of-dose (PND 98). These generally occurred at all doses with no dose proportionality in terms of incidence or severity. The lymphoid tissue changes were characterized by expansion or increased prominence of the T-cell areas (periarteriolar lymphoid sheaths in the spleen and paracortical regions in the mandibular lymph nodes) and a general paucity of germinal centers (B-cell areas) in the lymph nodes. Active germinal centers were not present in drug-treated rats. In the pancreas, the abatacept-related lesion was characterized by minimal to mild lymphocytic inflammation of the islets consistent histopathologically with a possible autoimmune etiology. Only male rats were affected and the incidence for all drug-treated groups combined at PND 98 was ~ 21%. Drug-related lymphocytic thyroiditis was present in both male and female rats; the overall incidence (sexes combined) was ~ 16% at PND 98. Although T-regulatory cell counts were completely recovered, the findings of thyroiditis and insulinitis were persisted or progressed throughout the 3-month recovery period. Therefore, the relationship between T-regulatory cell and thyroiditis or insulinitis is unclear.

Toxicokinetics

Abatacept toxicokinetic parameters are summarized in **Table 27**.

Table 27 Abatacept Juvenile Rat Toxicokinetic Parameters

Parameter	PND	Dose (mg/kg)					
		65 ^a		20 ^b		65 ^b	
		Male	Female	Male	Female	Male	Female
C _{max} (µg/mL)	28	405	387	NA	NA	NA	NA
	88	2,100	1,910	603	638	2020	1760
AUC(0-72 h)	28	23,000	22,500	NA	NA	NA	NA
(µg•h/mL)	88	49,700	38,900	20,200	17,900	49,400	41,100
CLT _{ss} /F	28	2.83	2.89	NA	NA	NA	NA
(ml/h/kg)	88	1.31	1.67	0.992	1.12	1.32	1.58
V _{ss} /F (L/kg)	28	0.0930	0.0977	NA	NA	NA	NA
	88	0.0352	0.0442	0.0298	0.0333	0.0351	0.0426
MRT _{ss} (h)	28	32.9	33.8	NA	NA	NA	NA
	88	26.9	26.5	30.1	29.8	26.7	27.0
T _{1/2} (h)	88 ^c	215	165	214	170	230	198
	88 ^d	215	160	205	161	246	204

^a Includes all samples regardless of the immunogenicity status.

^b Dosed Q3D by the subcutaneous route on PND 28, and by the intravenous route on PNDs 31-97.

^c T_{1/2} during the post-dose phase was calculated by including the samples with anti-abatacept antibodies detected.

^d T_{1/2} during the post-dose phase was calculated by excluding the samples with anti-abatacept antibodies detected.

Study No.

DS07166, a full review is available in DARRTS referring to IND-9418, received 10/06/2009

Study Title: Abatacept Three-Month Intermittent-Dose (Q3D) Intravenous Immunotoxicity Study in Rats
Applicant: Bristol-Myers Squibb Company
Location of Applicant: Princeton, NJ 08543-4000
Study Facility: Syracuse, New York, Bristol-Myers Squibb
Date of Study Initiation: October 9, 2007
GLP Compliance: Yes
Drug: Abatacept

KEY FINDINGS

1. Decreased serum total globulin secondary to pharmacologically decreased serum IgG concentration (0.07 to 0.30x controls).
2. Increased peripheral-blood total T cell counts (CD3+) that correlated with increased counts of total lymphocytes, primarily due to increases in T-helper cell counts (CD4+CD8-; 1.32 to 2.01x controls).
3. Decreased absolute numbers of a subset of peripheral-blood T-regulatory cells (CD4+CD25+Foxp3+; 0.23 to 0.60x controls).
4. In mesenteric and mandibular lymph nodes and spleen, increased size of predominantly T-cell areas and decreased number and size of germinal centers (B-cell areas).
5. Lymphocytic inflammation of the pancreatic islets at weeks 14 (20%, males only) and 21 (45% males and 5% females).
6. Lymphocytic thyroiditis at weeks 14 (5%, females only) and 21 (10% in both sexes).
7. The abatacept-related changes were generally partially reversible following a 3-month postdose period independent of selected dose. The findings are also consistent with most of the changes in adult mice, rats, and monkeys.
8. No drug-related infections were observed in the adult rat.

METHODS

Experimental Design

Abatacept was given intravenously to Crl:CD(SD) rats, ~8-9 weeks of age, weighing 254.8-342.8 g (males) and 171.3-219.6 g (females) once every 3 days at doses of 0 (0.9% sodium chloride, vehicle control, group 1), 65 mg/kg (group 2), and 200 mg/kg (group 3). Table 28 summarizes the study design.

Table 28 Abatacept Rat Three-Month Intermittent-Dose (Q3D) Intravenous Immunotoxicity Study Design

Group Number	Daily Dose abatacept (mg/kg)	Volume (mL/kg)	Concentration abatacept (mg/mL)	Number of Animals ^b
1	0 ^a	5	0	20 M, 20 F
2	65	5	13	20 M, 20 F
3	200	5	40	20 M, 20 F

^a Vehicle control was 0.9% sodium chloride for injection, USP.

^b The first 10 rats per sex in each group were used for hematology evaluation and peripheral-blood lymphocyte phenotyping during the dosing phase. The last 10 rats per sex in each group, if available, were used for toxicokinetic, immunogenicity, and postdose evaluations.

Additional observations and measurements included:

- Toxicokinetics
- Clinical Observations
- Clinical Immunology: Immunotoxicological evaluation included serum immunoglobulin (Ig) isotypes, peripheral-blood lymphocyte phenotypes, and abatacept immunogenicity. Analyses were conducted during the dosing phase (Weeks 4, 8, and/or 13/14) and following the 2-month post-dose evaluation phase (Week 21). These serum samples were analyzed for IgG and IgM antibody concentrations. The whole blood was analyzed for the percent of lymphocytes expressing CD3 (T cells), CD4+CD8- (T-helper cells), CD8+CD4- (T-cytotoxic cells), or CD4+CD25+Foxp3+ (a subset of T-regulatory cells) using a validated method. Absolute numbers of lymphocytes were calculated using the lymphocyte counts obtained from the hematology analysis. Abatacept-specific antibodies were analyzed.

RESULTS

The study in adult rats (dosing initiated between 8 and 9 weeks of age) was conducted to determine whether changes in immune parameters observed in a juvenile rat study (Study# DN07013, dosing initiated at 4 days of age) were a function of age at the time of dose initiation. The changes observed in the juvenile rat study included altered peripheral-blood lymphocyte profiles (increased counts of total T and T-helper cells and decreased counts of a subset of T-regulatory cells), lymphocytic inflammation of the thyroid and pancreatic islets, and infections (leading to moribundity/death). None of these findings were previously observed or evaluated (in the case of peripheral-blood lymphocyte profiles in mice and T-regulatory cells in mice and monkeys) in sub-chronic studies with adult mice or monkeys. The results of the current study demonstrated that abatacept dosing in adult rats resulted in inflammation of the thyroid and pancreatic islets and qualitatively similar effects on peripheral-blood lymphocyte profiles. No drug-related infections were observed in the adult rat study.

Abatacept-related effects on peripheral-blood lymphocyte profiles appear to be of similar magnitude in adult rats and in juvenile rats. In addition, the overall incidence of lymphocytic inflammation of the thyroid in adult rats (6%) was lower than the incidence in juvenile rats (48%). Although it is uncertain whether these quantitative differences represent true differences in sensitivity or biological variation across studies, the collective data suggest a qualitatively similar response profile to sub-chronic abatacept administration in rats regardless of the age of treatment initiation except the infection profile. The autoimmunity observed in rats suggests that this species, for reasons that are not understood, is very sensitive to the effects of abatacept exposure as compared to mice, monkeys, or humans. No evidence of autoimmunity has been observed following long-term treatment for up to 6 or 20 months in mice, and 1 year in monkeys,

and no evidence of drug-related autoimmunity has been noted in human clinical trials of adults or juveniles or in rats, monkeys and humans with belatacept treatment. Furthermore, decreases in T-regulatory cells have not been observed in patients treated with belatacept (second-generation molecule) in combination with other immunomodulatory agents in a kidney transplant trial.

Toxicokinetics

Abatacept toxicokinetic parameters are summarized in Table 29.

Table 29 Male and Female Rat Abatacept TK Parameters Following Dosing Every 3 Days for up to 3 Months

Parameter	Day	Dose (mg/kg once every 3 days)			
		65		200	
		Male	Female	Male	Female
C _{max} (µg/mL)	31	1,860	1,500	3,530	3,180
	91	1,750	1,350	10,400	3,270
AUC _(0-72 h) (µg•h/mL)	31	44,600	27,500	82,600	60,900
	91	39,000	25,000	92,700	63,900
AUC _(0-1344 h) (µg•h/mL)	91 ^a	140,000	84,300	314,000	193,000
	91 ^b	139,000	84,100	314,000	193,000
CLT _{ss} (mL/h/kg)	31	1.46	2.37	2.42	3.28
	91	1.67	2.60	2.16	3.13
V _{ss} (L/kg)	31	0.0393	0.0642	0.0609	0.0794
	91	0.0436	0.0651	0.0504	0.0725
MRT _{ss} (h)	31	26.9	27.1	25.1	24.2
	91	26.2	25.0	23.4	23.2
T _{1/2} (h)	91 ^a	242	187	218	184
	91 ^b	241	187	219	185
Concentrations of BMS-188667 (µg/mL)	119	33.3 ^c	15.0 ^c	52.0 ^c	27.3 ^c
		32.2 ^d	14.7 ^d	NA ^{d,e}	NA ^{d,e}
	147	8.74 ^c	1.45 ^c	11.8 ^c	3.70 ^c
		8.69 ^d	1.47 ^d	12.0 ^d	3.78 ^d

^a AUC(0-1344 h) or T_{1/2} during the postdose phase was calculated by including the samples detected with anti-abatacept antibodies.

^b AUC(0-1344 h) or T_{1/2} during the postdose phase was calculated by excluding the samples detected with anti-abatacept antibodies.

^c The samples detected with immunogenicity were included in the mean concentrations.

^d The samples detected with immunogenicity were excluded in the mean concentrations.

^e No immunogenicity was detected. NA: not applicable.

Abatacept was pharmacologically active at all doses, and all doses were associated with altered immunological parameters including lymphoid-tissue changes and inflammation in non-lymphoid organs in a dose-independent manner. Compared to juvenile rats, the observations of immunotoxicological parameters are generally comparable either in magnitude or in pattern of changes including the reversibility. However, there was a significant difference between juvenile and adult rats in infection profile.

Study No.

930008605, a full review is available in DARRTS referring to IND-9418, received 07/15/2009

Study Title: The Effect of LEA29Y on Renal and Islet Allograft Survival in Rhesus Macaques
Applicant: Bristol-Myers Squibb Company
Study Facility: [REDACTED] (b) (4)
Date of Study Initiation: November 2002
GLP Compliance: No
Drug: Belatacept, LEA29Y, BMS-224818

KEY FINDINGS:

1. LEA29Y in combination with other drugs provides significant protection from rejection and prolongs the survival of renal and islet allografts in non-human primate models.
2. None of the animals treated with belatacept developed anti-donor antibodies during therapy.

OVERALL OUTLINE OF STUDIES

To investigate the immunosuppressive potential of belatacept in blocking the CD28/B7 mediated co-stimulatory immune response (signal 2), the applicant conducted studies in non-human primate transplant models.

Study endpoints included the following:

- Allograft function
- Recipient survival
- The serum levels of belatacept, and anti-belatacept antibody
- The development of anti-donor antibodies
- Peripheral blood leukocyte subsets
- Renal allograft histology
- Necropsy

The study was conducted in two Phases, Phase I and Phase II:

Phase I

Studies were conducted to determine the efficacy of belatacept in monotherapy and in combination with other conventional immunosuppressive agents in the non-human primate renal allograft model. Rhesus macaques in this study received an allogeneic renal transplant, underwent simultaneous bilateral nephrectomy, and were treated as follows:

- Group 1: Historical albumin control
- Group 2: Belatacept monotherapy
- Group 3: MMF/Steroids
- Group 4: Belatacept / MMF/Steroids
- Group 5: Basiliximab monotherapy
- Group 6: Belatacept /basiliximab.

Phase II

Studies were conducted to explore the optimization of the dosing strategy for LEA29Y. The applicant studied belatacept in combination with basiliximab, MMF and steroids to prolong renal allograft survival in the non-human primate renal allograft model. Rhesus macaques in this phase also received an allogeneic renal transplant and underwent simultaneous bilateral nephrectomy. In addition all recipients in both treatment groups received basiliximab, MMF and steroids using the dosing regimens as described above for groups 3 and 5 in phase I. Two dosing strategies for belatacept were tested:

- Group 1: Low belatacept maintenance dose: Belatacept dosing to maintain serum trough concentrations ~20 µg/ml for 30 days, ~7 µg/ml to day 30, ~0.25 µg/ml to day 365. Dosing was per the following schedule:
Day 0: 10 mg/kg
Day 4: 15 mg/kg
Day 14: 20 mg/kg
Days 30, 49, 68: 20 mg/kg (every 19 days)
Days 101, 134, 167, 200, 233, 266, 299, 332: 20 mg/kg (every 33 days)
- Group 2: High belatacept maintenance dose: Belatacept dosing to maintain serum trough concentrations at ~20 µg/ml for 30 days, ~7 µg/ml to day 90, ~2 µg/ml to day 365. Dosing was per the following schedule:
Day 0: 10 mg/kg
Day 4: 15 mg/kg
Day 14: 20 mg/kg
Days 30, 49, 68: 20 mg/kg (every 19 days)
Days 91, 114, 137, 160, 183, 206, 229, 252, 275, 298, 321, 344: 20 mg/kg (every 23 days)

Phase III

Studies were conducted to determine whether belatacept can provide effective immunosuppression as a replacement for the calcineurin inhibitor tacrolimus in the non-human primate islet-cell-allograft model. The applicant selected a regimen designed to maintain serum trough belatacept concentrations of ~20 µg/ml. Diabetes was induced by surgical pancreatectomy of recipient animals and confirmed by pre-transplant intravenous glucose tolerance test. Donor-recipient pairings were defined based on molecular typing using a panel of previously defined MHC alleles. Pairings maximized disparity at both class I and II loci. Allogeneic islet cell transplant was via an infusion in the portal vein. Rejection was defined as two consecutive fasting blood glucoses >125 mg/dl on subsequent days. Immunosuppressive therapy in the two experimental groups was as follows:

- Group 1: Belatacept/sirolimus/basiliximab. Belatacept was administered intravenously intra-operatively (10 mg/kg) and on post-operative days 4 (15 mg/kg). Additional doses of 20mg/kg were given on post-operative days 14, and every two weeks until day 154. Basiliximab (0.3 mg/kg iv) was administered intra-operatively and on post-operative day (POD) 4. Sirolimus was administered orally in a Prima-burger treat, 1.25 mg/kg bid POD 0-50 (target plasma drug levels 8-12 ng/ml), 1 mg/kg qd POD 50-100, and then tapered to terminate dosing by POD 121.

Group 2: Sirolimus/basiliximab. Basiliximab (0.3 mg/kg iv) was administered intra-operatively and on POD 4. Sirolimus was administered orally in a Primaburger treat, 1.25 mg/kg bid POD 0- 50 (target plasma drug levels 8-12 ng/ml), 1 mg/kg qd POD 50-100, and then tapered to terminate dosing by POD 121.

RESULTS

The applicant conducted a series of functional studies to explore the immunosuppressive potential of belatacept. The results suggest that belatacept plays a role in the inhibition of solid tissue allograft (Renal Transplantation) rejection and prolongs the allograft survival in nonhuman primate model by blocking CD28 mediated T-cell co-stimulation.

Renal Transplantation-Phase I

The immunosuppressive effect of belatacept is additive when combined with MMF/steroids or basiliximab. This is particularly apparent when comparing allograft function in the recipients treated with belatacept monotherapy with those treated with belatacept/MMF/steroids or belatacept/basiliximab. The outcomes of the Phase I study are summarized in Table 30.

Table 30 Phase I Survival Summary

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

In the belatacept monotherapy group all of the recipients experienced severe allograft dysfunction between postoperative days 20 and 45. In contrast, 3/5 recipients in the belatacept /MMF/steroid group maintained excellent renal function for >84 days. Similarly, 5/6 recipients in the belatacept/basiliximab group maintained excellent function for >50 days. Importantly, none of the animals treated with belatacept developed anti-donor antibodies during therapy. This finding suggests that LEA29Y inhibits alloantibody production and the combination therapies provide improved clinical effectiveness compared to belatacept monotherapy. Moreover, there is no evidence that belatacept antagonizes the conventional immunosuppressive agents (or that they antagonize belatacept) in the nonhuman primate model. The applicant also concluded there were no consistent effects on total white blood cell count (WBC), absolute lymphocyte counts, CD3, CD4, CD8 or CD20 in any treatment group on day 28 compared to day 0. However, it would be interesting to know the long-term effects, such as at day 70, because the immune cells have relative long half-life in the peripheral blood.

Renal Transplantation-Phase II

The Phase II results (Table 31) indicate that belatacept has compatible immunosuppressive activity when combined with conventional immunosuppressive agents. There was not a clear difference between the high and low maintenance dosing regimens for belatacept.

Islet Transplantation-Phase III

The steroid-free immunosuppressive regimens result in successful insulin independence following islet transplantation. The applicant identified a novel calcineurin inhibitor/steroid-free, receptor-specific immunosuppressive regimen that provides significant protection from rejection and prolongs the survival of islet allografts in non-human primates.

Table 31 Phase II Survival Summary

Experimental Group	Survival(days)
Basiliximab/MMF/Steroids	8, 9, 36, 36
Belatacept Hi maintenance	32, 51, 112, 198
Belatacept Low maintenance	28, 51, 118, 189, >625

The following review is the histopathology evaluation for the previous study above (Report No. 930008605 Effect of LEA29Y on Renal and Islet Allograft Survival in Rhesus Macaques. (b) (4) November 2002) reviewed by Janice A. Lansita, Ph.D., DABT.

Study No. 99722
Study Title: Mono- and Combination-Therapy Repeat-Dose Intravenous Study in Renal Transplant Recipient Monkeys; Histopathological Evaluation
Study Facility: Drug Safety Evaluation, Bristol-Myers Squibb
Date of Study Initiation: November 01, 2007
GLP Compliance: No
QA Report: No
Drug: Belatacept

Key Findings: The histopathology of transplanted kidneys in rhesus monkeys after treatment with belatacept alone or in combination, with MMF + solumedrol or Simulect[®], showed a greater incidence and severity of microscopic kidney changes compared with MMF + solumedrol alone or Simulect[®] alone.

Methods

Following a bilateral nephrectomy, 28 male rhesus monkeys received allogeneic (antigenically distinct) renal transplants of the right kidney on Day 0. Monkeys were treated with belatacept alone or in combination with mycophenolate mofetil (MMF) + solumedrol (methylprednisolone), or with Simulect[®] (basiliximab, an antagonist chimeric monoclonal antibody to IL-2Ralpha). Animals treated with MMF + solumedrol, and with

Simulect® alone were included as controls (Table 32). Animals were necropsied between Day 0 and Day 375 after renal transplantation.

Formalin-fixed sections of the transplanted kidney, adrenal gland, brain, colon, duodenum, eye, gall bladder, heart, ileum, inguinal lymph node, jejunum, liver, lung, mesenteric lymph node, ovary, pancreas, parathyroid, pituitary, prostate, rib, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle, skin, spleen, stomach, testis, thymus, thyroid, trachea, and urinary bladder from all monkeys were processed, stained with hematoxylin and eosin, and examined by histopathology.

Table 32 Kidney Allotransplantation Study Design and Survival

Group	No. Males	Belatacept (mg/kg) ^a	MMF (mg/kg) ^b	Solumedrol (mg/kg) ^c	Simulect® (mg/kg) ^d	Survival Range (days)
1 Belatacept Alone	6	10-20	0	0	0	11-134
2 MMF + Solumedrol	5	0	15	1-20	0	8-50
3 Belatacept, MMF, Solumedrol	8	10-20	15	1-20	0	9-375
4 Belatacept + Simulect	6	10-20	0	0	0.3	28-145
5 Simulect	3	0	0	0	0.3	8-9

^a Administered IV at 10 mg/kg at the time of transplant, 15 mg/kg on day 5, and 20 mg/kg on days 15, 29, 43, 57, and 71.

^b Administered twice daily IM at time of transplant and for the first 14 days, and once daily thereafter through 6 months post-transplant.

^c Administered IV at 12 mg at the time of transplant, and daily thereafter IM in order of decreasing dose as follows: 20 mg on day 1, 16 mg on day 2, 12 mg on day 3, 8 mg on day 4, 4 mg on day 5, 3 mg on days 6-14, and 1 mg on days 15-182.

^d Administered IV within 2 hours of transplant and again on day 4.

Summary of Results/Discussion/Conclusions

Histopathology findings that appeared at a higher incidence or severity in belatacept treated animals included minimal to moderate mineralization of the adrenal gland, minimal to mild germinal center depletion of the lymph nodes (mesenteric, inguinal), spleen, and thymus (minimal-moderate severity in thymus). In the kidney, inflammation, dilatation, infarct, thrombus, and vasculitis were observed. Additional findings of unknown relationship to treatment included immature prostate, testes and seminal vesicles, as well as thyroid cysts which appeared to a greater extent in belatacept treated groups (Group 1, 3 and 4) compared with other groups but may be related to animal maturity and not treatment; the ages of the animals were not provided.

The germinal center depletion of the lymph nodes (inguinal and mesenteric) and lymphoid organs (spleen and thymus) was related to the expected pharmacology of belatacept. Interestingly, lymph node and lymphoid organ cellular depletion were not observed in the belatacept + Simulect® combination group; this may be related to the fact that 5 out of 6 of these animals survived longer than 100 days thus allowing the germinal center depletion to reverse. However, the effect may also be related to Simulect® treatment because animals surviving greater than 100 days treated with belatacept alone or with belatacept + MMF did show lymph node/lymphoid organ

depletion, which is an expected pharmacological effect of belatacept based on toxicology study results.

The most noteworthy findings were observed in the kidney. A greater incidence and severity of kidney histopathology findings was observed in animals treated with belatacept alone or in combination with MMF + Solumedrol or Simulect[®]. The transplanted kidney findings are summarized in Table 33. One animal in Group 3 (RMP-5) treated with belatacept, MMF, and solumedrol survived until Day 375. This animal showed notable histopathology changes in the spleen (minimal hypertrophic/hyperplastic) and kidney. In the transplanted kidney, minimal histopathology findings consisted of chronic lymphocytic inflammation (tubulo-interstitial nephritis), glomerular changes (thickening of Bowman's capsule and mesangium), fibroplasia/fibrosis, edema of interstitium, partial hydronephrosis/dilatation, and extratubular Tamm-Horsfall Protein deposition. The most severe change in the kidney was moderate vasculopathy. The changes were consistent with graft rejection and with the exception of the moderate vasculitis were overall similar in severity to the animals that died earlier in the study. A major caveat of this study is the low animal numbers across groups, especially with regard to the Simulect[®] alone group (N=2).

Table 33 Incidence of Transplanted Kidney Histopathology Findings

		Belatacept (N=6)	MMF + Solumedrol (N=4)	Belatacept + MMF + Solumedrol (N=7)	Belatacept + Simulect [®] (N=6)	Simulect [®] (N=2)
Inflammation, Chronic, Interstitial Tissue	Mild	2	1	4	1	0
	Moderate	2	1	2	5	0
	Marked	1	2	0	0	0
	TOTAL	5 (83%)	4 (100%)	6 (86%)	6 (100%)	0
Dilatation Renal Tubules	Minimal	2	0	1	3	0
	Mild	3	2	4	0	0
	Moderate	1	0	1	0	0
	TOTAL	6 (100%)	2 (50%)	6 (86%)	3 (50%)	0
Infarct	Minimal	0	0	0	1	0
	Mild	0	0	0	1	0
	Moderate	1	0	0	0	0
	Marked	0	0	0	0	2
	TOTAL	1 (17%)	0	0	2 (33%)	2 (100%)
Thrombus	Minimal	1	0	0	0	0
	Mild	3	0	1	1	0
	Moderate	0	0	1	0	0
	TOTAL	4 (67%)	0	2 (29%)	1 (17%)	0
Vasculitis	Minimal	1	0	0	0	1
	Mild	1	1	2	5	0
	Moderate	1	1	1	0	0
	TOTAL	3 (50%)	2 (50%)	3 (43%)	5 (83%)	1 (50%)
Increased Thickness Bowman's Capsule	Minimal	3	1	2	3	0
	Mild	0	0	1	1	0
	TOTAL	3 (50%)	1 (25%)	3 (43%)	4 (67%)	0
Increased	Minimal	0	0	3	1	0

Thickness Mesangium	Mild	0	0	0	1	0
	TOTAL	0	0	3 (43%)	2 (33%)	0

In conclusion, after treatment with belatacept alone or in combination with MMF+solumedrol or with Simulect® the transplanted kidney histopathology in rhesus monkeys showed a greater incidence and severity of microscopic kidney changes compared with MMF + solumedrol alone or with Simulect® alone. All other histopathology changes were incidental or were related to the pharmacology of belatacept.

Study No. DS04027, a full review is available in DARRTS referring to IND-9418, received 12/07/2009

Study Title: Nine-Month Intermittent-Dose (QWX40) Subcutaneous Investigative Study of the Effects of Mouse Mammary Tumor Virus-Initiated Events in I/LnJ Mice

Study Facility: Syracuse, New York, Bristol-Myers Squibb

Date of Study Initiation: March 23, 2004

GLP Compliance: No

QA Report: No

Trade Name: Orencia

Drug: Abatacept, BMS-188667, CTLA4-Ig

KEY FINDINGS

1. Abatacept at 200 mg/kg significantly suppressed anti-mouse polyvalent and IgG2a mammary tumor virus (MMTV) antibody responses, but was not sufficient to inhibit MMTV-antibody activity and cause infection.
2. Abatacept failed to promote MMTV mediated initiation of mammary tumors in I/LnJ mice. Thus, the relationship between abatacept and exogenous MMTV-mediated tumor development remains unclear.
3. Abatacept increased the incidence of malignant lymphoma and moderate lymphoid hyperplasia in the thymus on Day 285.

METHODS

Experimental Design

The doses of MMTV were prepared as equivalent to the virion isolated from 100 µL of milk from an infected BALB/cLA mother and were injected subcutaneously into 2 mammary gland regions of I/LnJ mice on day -28 to facilitate the normal course of mammary tissue infection. Abatacept was given subcutaneously to the I/LnJ mice at doses of 0 (saline, group 1) and 200 mg/kg (group 2), beginning on day 1 and once weekly through day 281 (40 doses).

Female I/LnJ mice were assigned to two groups (18 mice in Group 1 and 19 mice in Group 2). On Day -28, the mice were 5 to 7 weeks old and weighed 13 g to 22 g. For the neutralizing-antibody evaluations, 8 to 11 week old female BALB/cJ mice were used based on their high susceptibility to MMTV infection and mammary tumor formation.

On Day 63 (end of week 9), interim necropsies were performed for 8 mice in each group. Final (end-of-dose) necropsies were performed on Day 285 for 9 mice in Group 1 and 7 mice in Group 2.

Additional observations and measurements included:

- Toxicokinetics
- Mortality and Clinical Observations
- Body Weights
- Physical Examinations
- Immunology:

MMTV-Specific Antibody Responses: Blood samples were collected from all animals before MMTV infection (Day -29); prior to initiating drug treatment on Day -1; and then approximately every 2 to 4 weeks for the first 6 months following the initiation of drug treatment (through Day 181). Thereafter, samples were collected on Days 252 and 285. Blood samples (approximately 150 μ L) were collected from the orbital sinus under anesthesia. Samples were assayed for total (Ig) anti-MMTV antibody and MMTV-specific IgG2a antibody responses.

Bioassay for Neutralizing-Antibody Responses: serum samples collected over Weeks 4 to 14 from individual I/LnJ mice were evaluated. Diluted serum samples pre-incubated with 50% of MMTV(LA), were administered into the footpads of naive BALB/cJ mice as single 70 to 100- μ L injections. After five days, the mice were euthanatized and popliteal lymph nodes (PLN) were removed, separated into single-cell suspensions, and stained for CD4 and V β T-cell receptors for flow cytometric analyses. An increase in CD4+V β + T cells is expected with MMTV infection by this route, whereas neutralization of active virus by antibodies during the pre-incubation period prevents a rise in this T-cell subset.

Anatomic Pathology:

Gross Pathology: Examinations were performed on all animals that were euthanatized at the 2-month interim necropsy (Day 63) as well as the end-of-dose necropsy (Day 285). At approximately 2 hours prior to schedule euthanatization, I/LnJ mice were administered a single intraperitoneal injection of 100-mg/kg bromodeoxyuridine (BrdU) to facilitate assessment of cell proliferation. All animals except 3 found dead were euthanatized and necropsies were conducted to include gross examination of the following organs and tissues: mammary glands (right and left 3rd and 4th glands), lymph nodes (mesenteric, mandibular, and regional mammary gland nodes in the 4th mammary glands), spleen, thymus, duodenum, uterus, and vagina. Approximately one-half of the spleen and the left 4th mammary gland were snap frozen in liquid nitrogen for potential investigative analyses. The following tissues were fixed in 10% neutral buffered formalin: 1) tissue whole mounts prepared from the right and left 3rd mammary glands; 2) right 4th mammary gland (with its node); 3) approximately one-half of spleen; 4) mesenteric and mandibular lymph nodes; 5) thymus; 6) duodenum (used for BrdU control); and 7) uterus and vagina (to evaluate estrus).

Microscopic Pathology: The following tissues were processed and stained with hematoxylin and eosin: mammary glands (right 4th), spleen, lymph nodes (mesenteric and mandibular), thymus, and duodenum from all animals and the uterus and vagina from end-of-dose animals. In addition, the whole mounts of the right and left 3rd mammary gland were stained with Carmine alum. Mammary glands were not collected for potential electron microscopic investigation because the presence of MMTV in the mammary tissue was determined by in situ hybridization (ISH) and by immunohistochemistry (IHC).

RESULTS

This study was conducted to investigate the impact of immunomodulatory effects of abatacept on the susceptibility for carcinoma development in groups of 18 or 19 I/LnJ mice following mammary injection (on study Day -28) of MMTV. Abatacept was subcutaneously administered once weekly for 40 weeks at doses of 0 (vehicle) or 200 mg/kg. The study particularly focused on the MMTV levels, and anti-MMTV antibody responses. Scheduled interim (8/group) and terminal (all surviving mice) necropsies were conducted on Days 63 and 285, respectively.

Toxicokinetics

Serum drug concentrations on Days 50 and 280 confirmed the systemic exposure to abatacept (Table 34). Although drug levels were generally higher on Day 50 than on Day 280, results from both days were within or above the range of serum concentrations at a similar sample-collection timepoint in the carcinogenicity study in mice.

Table 34 Mouse Abatacept Serum Drug Concentrations on Days 50 and 280

Dose	Study Day	Mean Concentration (µg/mL)	SD
200 mg/kg/week	50	190	50.3
	280	119	59.8

Compared to CD-1 mice (study DS-04029), the exposures of abatacept are comparable across MMTV mediated carcinogenicity studies. As expected, there were no drug related deaths, clinical observations or body-weight changes. In contrast, the increases of anti-MMTV polyvalent and IgG2a (anti-MMTV-specific) antibody productions were suppressed following the drug administration. However, a response of anti-MMTV antibody (IgG2a) was induced by MMTV injection during the 4 weeks prior to abatacept initiation. The serum antibodies from both vehicle and abatacept-treated mice posed a sufficient neutralizing activity to inhibit the infectivity of naive BALB/cJ mice by MMTV. Functional characterization of these antibodies was confirmed by the neutralizing antibody bioassay. Furthermore, there was no evidence of increased viral levels, parenchymal proliferation, preneoplastic lesions, or tumor development in the mammary tissue of abatacept-treated I/LnJ mice, because the residual neutralizing antibody levels were still sufficient to interfere with amplification of the mammary gland infection and MMTV-induced tumor development. The data indicate that the IgG2a isotype plays a

critical role in the mechanism of resistance. Interestingly, at an incidence comparable to that observed in the abatacept carcinogenicity study, 2 of 7 abatacept-treated I/LnJ mice had malignant lymphomas in the thymus, and an additional abatacept-treated mouse showed moderate lymphoid proliferation in the thymus.

Finally, abatacept was able to suppress the production of MMTV-specific antibody responses in I/LnJ mice following viral infection, but failed to promote MMTV infection and mammary tumor formation in the particular strain. The failure may be attributed to the low-level anti-MMTV neutralizing-antibody response that was sufficient to block MMTV bioactivity. In addition, there was no positive control for the tumor formation in this study or in a similar study in CD-1 mice (Study DS04029). In contrast, endogenous MMTV infection during 88 weeks abatacept administration in CD-1 mice increased the incidence of mammary tumor formation. Therefore, the present study failed to characterize the impact of immunomodulatory abatacept on the MMTV-mediated carcinogenicity, and the relationship between abatacept and exogenous MMTV-mediated tumor development remains unclear.

Study No. DS04029, a full review is available in DARRTS referring to IND-9418, received 12/07/2009

Study Title: Abatacept Repeat Intermittent (QW)-Dose Subcutaneous Investigative Study of Effects on an Established Anti-Mouse Mammary Tumor Virus Antibody Response in CD-1 Mice

Study Facility: Syracuse, New York, Bristol-Myers Squibb

Date of Study Initiation: January 27, 2004

GLP Compliance: No

QA Report: No

Trade Name: Orencia

Drug: Abatacept, BMS-188667, CTLA4-Ig

KEY FINDINGS

1. MMTV (LA) induced anti-MMTV IgG2a responses in 50% (10 of 20) of CD-1 mice, and anti-MMTV polyvalent Ig responses in 90% (18 of 20) of CD-1 mice.
2. Abatacept partially suppressed the production of antibodies previously generated following MMTV challenge.
3. The residual MMTV-specific IgG2a apparently suppressed MMTV infectivity.
4. Due to the absence of a positive control as a reference in the study and the short dosing duration, the relationship between abatacept and exogenous MMTV (LA) mediated mammary tumor initiation was incompletely addressed.
5. Neither drug-related, nor MMTV-challenge related toxicity was observed.

METHODS

Experimental Outline

Female Crl:CD-1 (ICR) mice, 10 to 11 weeks old and weighing 29.4 g to 33.3 g, were initially divided into two groups (i.e., 10 mice in Groups 1 and 20 mice in Group 2) from day 1 through day 106 for the phase I study. On day 107, the 2 groups were further

divided into 4 subgroups (1A, 1B, 2A, and 2B) through day 282 as the phase II study. Phase I established the anti-MMTV antibody response, whereas phase II evaluated the effect of abatacept on MMTV-induced anti-MMTV antibody responses (Table 35).

Table 35 Study Design of Abatacept in an Established Anti-Mouse Mammary Tumor Virus Antibody Response in CD-1 Mice

Group	Phase I (Day 1-106)			Phase II (Day 107-282)		
	MMTV	Volume (mL/animal) ^a	n (Designations)	Group	Abatacept (mg/kg) weekly	n (Designations)
1	0	0.4	10 (1201-1210)	1A	0	4 (1201-1204)
				1B	200	5 (1205, 1207-1210)
2	1x	0.4	20 (2201-2220)	2A ^b	0	6 (2207, 2215-2219)
				2B ^b	200	6 (2201, 2203, 2208-2210, 2212)

^a The total 0.4-mL volume per animal was split into two (2) 0.2-mL subcutaneous injections administered into each of the 2 selected mammary gland regions.

^b Selection and designation of Group 2 animals for Phase II treatment was based upon week 12 anti-MMTV antibody analyses. Only animals with documented anti-MMTV antibody responses, primarily an IgG2a isotype response, were included in Phase II evaluations. Although animal 2207 (Group 2A) did not show a positive IgG2a response, its high total Ig response generally matched the low IgG2a and high total Ig response of animal 2208 (Group 2B), and was, therefore, being included in the Phase II analyses to allow equal numbers of animals in each Group 2 subdivision.

The doses of MMTV were prepared as equivalent to the virion isolated from 100 μ L of milk from MMTV positive BALB/cJ mice and were injected subcutaneously into 2 mammary gland regions of CD-1 mice (Group 2) on day 1 to facilitate the normal course of mammary tissue infection. Group 1 mice were similarly treated using saline alone. On day 107 after saline (Group 1) or MMTV (Group 2) injection, subdivisions of each group was treated once weekly with subcutaneous injections of saline (subdivisions 1A and 2A) or abatacept (subdivisions 1B and 2B) at doses of 200 mg/kg through day 282. On day 114, interim necropsies were performed for animals that were not selected to continue on with Phase II of the study. Terminal and end-of-dose necropsies were performed on all remaining mice on day 288. Final (end-of-dose) necropsies were performed on day 285 for 9 mice of Group 1 and 12 mice of Group 2.

Additional observations and measurements included:

- Mortality and Clinical Observations
- Body Weight
- Physical Examinations
- Clinical Pathology
- Immunology:

MMTV-Specific Antibody Responses: Blood samples were collected from all animals prior to dosing with or without MMTV and then at weekly intervals beginning approximately 2 weeks after MMTV injection (day 15). Weekly blood collections continued for both phases of the study through to day 267, and then a final blood

sample was collected prior to necropsy on day 288. Samples were assayed for total (Ig) anti-MMTV antibody and MMTV-specific IgG2a antibody responses.

Bioassay for Neutralizing-Antibody Responses: Serum samples collected over weeks 6 to 12 from individual CD-1 mice were evaluated. Diluted serum samples, which contained 50% of a MMTV(LA) preparation with pre-incubation, as a single 50- μ L injection was administered into the footpads of naive BALB/cJ mice. Five days after injection of the MMTV/serum mixtures, the mice were euthanatized and popliteal lymph nodes (PLN) were removed, separated into single-cell suspensions, and stained for CD4 and V β T-cell receptors for flow cytometric analyses. An increase in CD4+V β + T cells is expected with MMTV infection by this route, whereas neutralization of active virus by antibodies during the pre-incubation period prevents a rise in this T-cell subset.

Anatomic Pathology:

Gross Pathology: Examinations (interim and terminal/end-of-dose necropsies) were performed on all except 3 CD-1 mice that died or were euthanatized because of poor physical condition. Animals were fasted overnight prior to scheduled necropsies. Approximately 2 hr prior to the scheduled terminal/end-of-dose necropsy on day 288, animals to be euthanatized were administered a single intraperitoneal injection of 100-mg/kg bromodeoxyuridine (BrdU) to facilitate assessment of cell proliferation. With the exception of the mice found dead (Animals 1202, 1205, and 1206), all animals were euthanatized, and a limited necropsy was conducted to include gross examination of the following organs and tissues: mammary glands (right and left, 3rd and 4th glands), lymph nodes (mesenteric, mandibular, and regional mammary gland nodes in the 4th mammary glands), spleen, thymus, and duodenum (at end-of-dose necropsy only; positive control for BrdU).

Microscopic Pathology: The following tissues were processed, sectioned, and stained with hematoxylin and eosin: mammary glands (right 3rd and right 4th), spleen, lymph nodes (mesenteric and mandibular), thymus, and duodenum (terminal/end-of-dose necropsy only). In addition, the whole mounts of the left 3rd mammary gland were stained with Carmine alum.

IHC staining was performed on the right 3rd and 4th mammary gland and spleen (other tissues on the slide were also evaluated). Automated ISH was conducted with digoxigenin (DIG)-labeled MMTV ribonucleic probes using Ventana's Discovery Autostainer on sections of the right 4th mammary gland and spleen. The MMTV super antigen (MMTV-Sag) probe at the MMTV genome was amplified from genomic DNA isolated from mouse spleen by PCR. IHC staining for BrdU was performed on the spleen, duodenum, and right 3rd and 4th mammary glands.

RESULTS AND DISCUSSION

An increased incidence of mammary tumors was observed in an abatacept carcinogenicity study (Study No. 97610) in which CD-1 mice received 88 weeks of abatacept treatment subcutaneously. The mammary gland adenocarcinomas were statistically significantly increased in females at 65 and 200 mg/kg/week and were attributed to endogenous murine oncovirus MMTV and long-term abatacept-induced immunosuppression. The applicant conducted additional studies to investigate the

relationship between abatacept and exogenous MMTV (LA) mediated mammary tumor development. Two similar studies were conducted in female mice during 40 weeks study period (i.e., DS04027 using I/LnJ mice and DS04029 using CD-1 mice). In the present study, two groups of female CD-1 mice (total of 30 mice) were assigned to two consecutive study phases. In phase I (i.e., from day 1 to 106), 10 mice were assigned to group-1 with saline treatment as a negative control and the other 20 were assigned to group-2 and treated with bioactive MMTV injections for establishing an MMTV infective model and observing spontaneous anti-MMTV antibody responses. In phase II (i.e., from day 107 to 282), each group was further divided into two subgroups (i.e., group-1A, -1B, and group-2A, -2B, respectively). 1A and 2A received saline as negative controls, whereas, 1B and 2B received abatacept subcutaneously once weekly for 26 weeks at doses of 200 mg/kg.

The systemic exposures of abatacept were comparable among studies and resulted in sufficient immunosuppression to study the MMTV-mediated mammary tumor development. In general, there was no evidence that either MMTV infection or abatacept related deaths was associated with clinical alterations, body-weight changes or clinical-pathologic changes in this study.

MMTV-specific antibody polyvalent (total Ig) responses developed in 19 of 20 CD-1 mice over weeks 6 to 12 following the MMTV (LA) injection. Compared to study DS-04027 (the anti-MMTV antibody IgG2a were induced in 100% individual mice by the MMTV injection), approximately 50% of mice (10 of 20) showed increase of MMTV-specific IgG2a antibody compared to pre-infection. Inter-individual responses were variable (i.e., the intensity of the response ranging from minimal to mild was lower than that seen in I/LnJ mice in study DS-04027), but indicative of a persistent antibody response. Functional analyses demonstrated that the antibody responses in CD-1 mice were neutralizing and sufficient to prevent the infectivity of MMTV. For instance, an increase of CD4+Vβ+ T cells was observed (the same bioassay was conducted in study DS04027); whereas neutralization of active virus by antibodies during the pre-incubation period would prevent a rise in this T-cell subset (data not shown in this review). At week 16, abatacept significantly reduced, but did not eliminate the effectiveness of established anti-MMTV antibodies. As a result, there was no evidence of an increase in viral level or tumor proliferation indices in abatacept-treated mice, nor any drug-related preneoplastic lesions or tumors development, presumably because of the remaining functionality of residual anti-MMTV antibodies.

Retroviruses (MLV and MMTV) have been reported to cause lymphoma and mammary tumors in mice, respectively, and the CD-1 mice from (b) (4) not retrovirus free. Endogenous MMTV in both control- and abatacept-treated mice adds to the complexity of the studies with both studies (DS04027 and DS04029) presenting negative findings. The duration of dosing may have been insufficient to develop positive results. However, even if the duration of dosing was prolonged to 88 weeks which was the duration of the carcinogenicity study in which tumors were observed, it would be difficult to distinguish whether tumors derive from endogenous or exogenous MMTV. Nevertheless, there was no positive control as a reference in the

study, which might help rule out issues with the model (i.e., should choose MMTV free strain), viral load, dosing duration, animal maturation, viral integration, or insertion near a proto-oncogene. Regardless, the study was not optimized to promote the exogenous MMTV(LA) -mediated mammary tumor effectively; therefore, the relationship between abatacept and exogenous MMTV mediated carcinogenicity was insufficiently addressed.

11 Integrated Summary and Safety Evaluation

In Vitro and In Vivo Pharmacology

Belatacept is a fusion protein comprised of the cytotoxic T-lymphocyte antigen-4 (CTLA-4) extracellular binding domain linked to a modified Fc domain of human immunoglobulin IgG1 (hinge-CH2-CH3 domains). CTLA-4 is an inhibitory receptor expressed on T-helper cells and binds with high affinity to the B7 molecules, CD80 and CD86, expressed on antigen presenting cells (APCs). The interaction between CTLA-4 and CD80/CD86 leads to an inhibition or blockade of T-cell activation via CD28, a T-cell co-stimulatory molecule expressed on T-cells and required for T-cell activation. The proposed mechanism of action in kidney transplants is belatacept will bind to CD80/CD86 on APCs, thereby blocking CD28:CD80/86 interactions between T-cells (CD28) and APCs (CD80 or CD86), and preventing T-cell activation via CD28.

The *in vitro* and *in vivo* potency of belatacept was characterized in primary pharmacology studies across mouse, rat, rabbit, monkey, and human. Many of these studies include a comparison to abatacept as a reference since belatacept was originally developed to have greater biological activity than abatacept.

A comparison of belatacept to abatacept activity in the context of the *in vitro* and *in vivo* studies demonstrated that belatacept was 2.5-29-fold more potent than abatacept in the *in vitro* human cell assays (Table 36). In contrast, belatacept bound with lower affinity to murine CD80-Ig (MuCD80-Ig, 3.8-fold), and with similar affinity to MuCD86-Ig compared with abatacept; the binding difference to MuCD80-Ig likely contributes to the lower *in vivo* potency of belatacept in rodents.

Table 36 Human In Vitro Comparative Potency Studies of Belatacept Vs. Abatacept

Study	Potency Belatacept > Abatacept
Binding to CD86-Ig (Larsen et al 2005)	4.3X
Binding to CD80-Ig	2.8X
CD80 and CD86 saturation	8X
Primary Mixed Lymphocyte Reaction IL-2	6X
Secondary Mixed Lymphocyte Reaction IL-2, IL-4, IFN-gamma	5-7X
Inhibition of T-cell Proliferation (co-culture w/ dendritic cells)	14X
Inhibition of T-cell Co-stimulation - Jurkat cells	16X
Inhibition of T-cell Proliferation via CD80 Expressing CHO cells	5X

Inhibition of T-cell Proliferation via CD86 Expressing CHO cells	13X
T-cell Cytokine Inhibition	4.6-29X

Belatacept and abatacept share the same modified Fc portion. Abatacept studies showed Fc receptor binding to the high affinity receptor (CD 64) and no binding to the low affinity receptors (CD 32 and CD 16); belatacept is assumed to yield similar results since it has the same Fc portion as abatacept. Belatacept bound to FcRn with 3-4 fold lower affinity than an IgG1 mAb. Belatacept did not induce Fc-mediated complement dependent cytotoxicity (CDC) or antibody dependent cell-mediated cytotoxicity (ADCC) *in vitro*.

In vivo pharmacology studies measured the belatacept and abatacept inhibition of a T-cell dependent antibody response (TDAR) to a foreign antigen. In the *in vivo* monkey study, belatacept was 3-11-fold more potent than abatacept. In contrast, in the *in vivo* mouse, rat and rabbit studies, abatacept was 2-10-fold more potent than belatacept. Interestingly, abatacept in mice and rabbits was approximately 10-fold less potent than abatacept in rats (Table 37). Finally, a monkey renal transplant study with belatacept demonstrated efficacy in the nonclinical setting by prolongation of graft survival.

Table 37 Summary Table of Comparative In Vivo Belatacept and Abatacept Inhibition of a T-cell Dependent Antibody Response Across Mouse, Rat, Rabbit and Monkey

Species	Antigen	Study Day	IC90 ^a Abatacept (mg/kg)	IC90 Belatacept (mg/kg)	Dose (mg/kg) - Fold Difference	Exposure (AUC) - Fold Difference
Mouse	KLH*	14	3	30	10X	NA
Rat	KLH	15	0.3	1	3X	1.9-2.3X
Rabbit	KLH	22	3	10-20	3-7X	2-4X
Cynomolgus Monkey	Sheep Red Blood Cells	14	1.91 (IC90) 0.188 (IC50 ^b)	0.17 (IC90) 0.057 (IC50)	11X (IC90) 3X (IC50)	NA

* Keyhole limpet hemocyanin

^aIC90 - 90% inhibitory concentration

^bIC50 - 50% inhibitory concentration

Note: IC90 and IC50 values were provided for cynomolgus monkey.

Since abatacept is more potent in rodents compared with belatacept, studies with abatacept in rats and mice are relevant to the belatacept clinical safety evaluation. An extrapolation of the relative potency of abatacept in rodents to belatacept in monkeys or humans cannot be precisely determined. Nevertheless, based on the available *in vitro* and *in vivo* data, this reviewer estimates that abatacept in rodents may be approximately 2-30-fold less potent than belatacept in monkeys or humans. Therefore, adverse safety findings in rodent studies of abatacept may be conservatively considered as potential hazard indicators for belatacept in humans.

Secondary Pharmacology

Infection Risk

Serious infections have been observed in the clinic with belatacept. The label currently warns against the potential for serious bacterial, viral, fungal, and protozoal infections, including opportunistic infections with specific warnings for PTLD, PML, and BK virus-associated nephropathy (BKVAN). In addition, serious adverse reactions included a higher incidence of herpes and TB with belatacept treatment.

Host defense or resistance studies with belatacept have not been conducted. However, infections have been associated with abatacept and belatacept in the nonclinical setting (see Toxicology section, below). The effects of abatacept (or blockade of the CD28 pathway by other means, such as another CTLA4-Ig, transgenic models, or monoclonal antibodies) on host defense have been reported by the applicant, as well as the literature. In these studies, CTLA4-Ig or CD28 blockade generally resulted in the host clearing the infection with no impact on survival with the exception of acute studies of herpes simplex virus (HSV) and listeria. Blockade of the CD28 pathway appeared to increase susceptibility to clinical infection if the pathogen is more dependent on a CD4+ T-helper cell mediated clearance response (such as HSV).

Indoleamine 2,3 dioxygenase Induction

A transient 2-fold increase in kynurenine was observed following a single-dose of belatacept at 10 mg/kg in female cynomolgus monkeys. Kynurenine is a tryptophan metabolite and marker of indoleamine 2,3 dioxygenase (IDO) induction. Increased IDO may be related to T-cell regulation and decreased T-cell proliferation. Clinical evaluation of belatacept induction of IDO in 26 patients (Report No IM103100 LTE⁴⁵) showed no significant changes in 23/26 patients at more than 1 year post-transplant (serum kynurenine values <2.5 uM), however, there did appear to be an effect in 3 patients that also had higher pre-dose kynurenine values compared with other patients.

Pharmacokinetics

Belatacept pharmacokinetics was evaluated in rats, rabbits, and cynomolgus monkeys. Since belatacept is a biologic, radiolabeled distribution, metabolism, and excretion studies were not conducted. The PK of belatacept was generally characterized across species by a long half-life that increased with dose, low clearance, and low volume of distribution. In mice, rats and rabbits, exposure (AUC) to belatacept was lower than abatacept at equivalent doses, which was likely due to immunogenicity since ADA titers were higher for belatacept than abatacept in rodents.

PK comparability studies in cynomolgus monkeys showed that materials produced by the different manufacturing processes (A, B and C) throughout clinical development were comparable.

Immunogenicity

An anti-drug antibody (ADA) response to belatacept was measured in mice, rats, rabbits and cynomolgus monkeys. The ADA response was observed as early as Day 8 for rats, rabbits and monkeys. Immunogenicity likely impacted the AUC_{0-inf}, T_{1/2}, and clearance

in belatacept-treated mice, rats, and rabbits. As drug concentrations decreased, the appearance of ADAs increased. Across species, the incidence and onset of drug-specific antibodies showed an inverse correlation with dose which may be due to a drug-related suppression of an ADA response or drug-related assay interference.

Drug-specific antibodies were detected in repeat-dose toxicology studies only during the recovery periods when the drug concentrations dropped below the pharmacological effective levels or did not interfere with the immunogenicity assay. Belatacept-specific antibodies were not associated with any acute or target-organ toxicity in any species.

Toxicology

The applicant conducted appropriate nonclinical studies of abatacept and belatacept for general toxicity, reproductive and developmental toxicity, carcinogenicity, and immunotoxicity in rodents, rabbits and monkeys. Toxicology studies of abatacept in rodents are considered relevant to the belatacept safety assessment because abatacept is more potent than belatacept in rodents.

General toxicology studies were conducted in monkeys with belatacept administered intravenously with durations ranging from a single-dose to six months of repeat-dosing. No belatacept-related toxicities were observed after a single-dose of belatacept up to 100 mg/kg (Study No. 98642). Secondary pharmacological effects included minimal decreases in serum IgG at the high-dose; and decreases of CD8+ T-cells in peripheral-blood lymphocytes (PBLs) and splenic lymphocytes. ADAs were detected during weeks 5 and 6 in low- and mid-dose animals.

In the repeat-dose toxicity and toxicokinetics studies in monkeys, belatacept was administered at doses up to 50 mg/kg every other day for 30 days (Study# 98699) or weekly for 6 months (Study No. 99655). 50 mg/kg of belatacept is ~400 times higher than the lowest effective single dose (0.108-0.170 mg/kg, IV) that results in >90% inhibition of the T-cell dependent antibody response (TDAR) in monkeys. Belatacept was not associated with treatment-related adverse findings. Pharmacologically mediated findings in both studies included: minimal decreases in serum IgG and mild to moderate decreases in the number and diameter of lymphoid germinal centers in the spleen and/or lymph nodes. These findings were overall reversible. No anti-drug antibodies (ADA) were detected during the dosing period of the studies. Minimal ADAs were detected in all dose groups during the 3-month recovery period (week 38) of the 6-month study. The immunologic parameter changes were not considered clinically significant.

Based on the findings of the pivotal six-month monkey study, the NOAEL for belatacept was 50 mg/kg/week, which was estimated to be 5.8, 13, and 21 times (AUC base) higher than patients' exposure of belatacept during the first month, fourth month, and maintenance phases of the clinical dosing regimen (exposures provided by the applicant, Table 38).

Table 38 Safety Margins Based on the 6-month Monkey Belatacept Chronic Toxicology

Species (Study#)	Dosing Regimen	NOAEL (mg/kg)	AUC (TAU) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	AUC (28/30 days) ($\mu\text{g}\cdot\text{h}/\text{mL}$)	Multiple of Human Exposure (x)g
Human	multiple doses 10 or 5 mg/kg iv, belatacept	10 ^a 10 ^b 5 ^c	--	48,257 ^a 21,241 ^b 13,587 ^c	--
Monkey (99655)	6-month, once weekly, iv, belatacept	50	69,816	279,266 ^d	5.8, 13.1, 20.6

^a Exposure (AUC 28 days) in LI regimen during first month of treatment when administered at 10 mg/kg on Days 1, 5, and 14; source of human AUC data - Population PK data.

^b Exposure (AUC 28 days) in LI regimen during fourth month when administered at 10 mg/kg once every 28 days; source of human AUC data - Study IM103047.

^c Exposure (AUC 28 days) in LI regimen at steady state during maintenance phase when administered at 5 mg/kg once every 4 weeks; source of human AUC data - Study IM103100 long-term extension.

^d AUC (TAU), where TAU = 7 days, was multiplied by 4 to normalize for 4 weeks of exposure.

A complete battery of reproductive toxicity studies was conducted with belatacept and abatacept administered intravenously in both rats and rabbits. Belatacept was given at doses up to 200 mg/kg/day in rats or up to 100 mg/kg/day in rabbits. Abatacept was given at doses up to 200 mg/kg every 3 days in rats. Belatacept and abatacept treatment did not result in toxicities related to fertility, gestation, parturition, lactation, or mating. Additionally, the offspring (F₁ generation) of treated maternal rats and rabbits did not show effects on fertility or early embryo-fetal developmental parameters. However, infections and autoimmunity were observed in reproductive and developmental toxicity and general toxicology studies as further described below. A schematic summarizing these findings is included in Figure 4 below.

Infections Observed in Toxicology Studies

In the pre- and postnatal development study (Study No. DN06002) in female rats, belatacept was given at doses of 20, 65 and 200 mg/kg/day, IV, from gestation day (GD) 6 to lactation day (LD) 20. In all treated groups, 3.8% - 9.6% of the dams presented with opportunistic infections, leading to moribundity or death, impairment of maternal care and partial or complete litter losses.

Infections were not observed in pregnant rats and rabbits treated with belatacept over a shorter dosing duration (Study No. DN06032, DN06008, and DN06056), or in pregnant rats treated with abatacept (Study No. DS07166, DN01060).

A small percentage of pathogenic bacterial infections leading to moribundity or death was also observed in two 3-month juvenile toxicity studies with abatacept (study# DN07013 and DS07165). Results suggest that pregnant rats and juvenile rats treated with these compounds are more susceptible to infections than nonpregnant adult rats. Susceptibility to infections might be exacerbated with pregnancy, dose frequency, dose duration or dose volume (10 mL/kg, IV). In juvenile rats, a potential mechanism may include suppressed development of memory immune response.

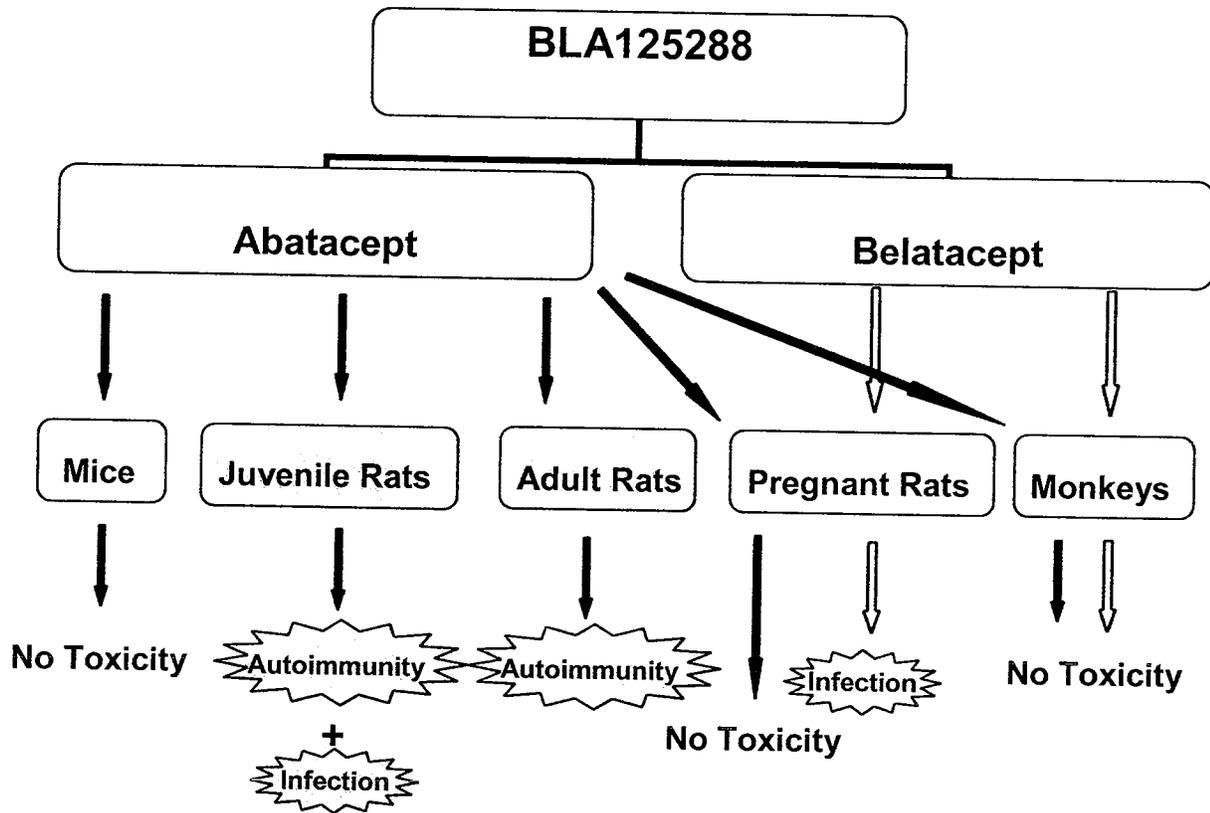


Figure 4: A summary of the significant toxicities observed in each animal study by treatment. The solid black arrows signify the abatacept studies and outcomes. The open-hatched arrows signify the belatacept studies and outcomes.

Autoimmunity Observed in Abatacept Toxicology Studies

Autoimmunity was observed pups exposed to abatacept in utero and during lactation in a peri- post-natal study (Study No. DN01060), in two abatacept 3-month juvenile rat toxicity studies (Study No. DN07013 and DS07165), and in an abatacept 3-month adult rat toxicity study (Study No. DS07166).

Autoimmunity of the thyroid (thyroiditis) was identified in one out of twenty rat off-spring at 112 days of age exposed to abatacept in utero and during lactation from a dam dosed at 200 mg/kg (Study No. DN01060). The dam's thyroid status was not examined, therefore, it is unknown whether the thyroiditis originated in the maternal animal (and may have been transferred to the off-spring via anti-thyroid antibodies). Additionally, female pups from dams dosed at 200 mg/kg in this study showed a 9-fold increase in TDAR.

In the rat juvenile toxicity studies abatacept was administrated SC from postnatal day (PND) 4 to 28 and IV from PND 31 to 94 at doses up to 200 mg/kg every 3 days. The autoimmunity was identified by histopathology as lymphocytic infiltration of the thyroid (thyroiditis) and pancreatic islet cells (insulinitis). Other treatment-related findings

included infections (described above), significant decreases in TDAR, decreases in serum IgG, increases in T-helper cells, decreases in T-regulatory cells, and morphological changes in lymphoid organs. The severity and incidence of findings were generally not dose-dependent. Although most immunological changes were partially or completely reversible, including a complete recovery of T-regulatory cell counts, the thyroiditis and insulinitis persisted and progressed in incidence and/or severity throughout the 3-month recovery period. T-regulatory cells are important in maintaining immune self-tolerance (Salomon, 2000⁴⁶). However, the relationship between decreased T-regulatory cells and the observed autoimmunity remains unclear. Additionally, although anti-CTLA4 antibodies have been associated with autoimmunity, no correlation between immunogenicity and autoimmunity could be determined in the nonclinical studies with abatacept (Sanderson et al., 2005⁴⁷, Blansfield et al., 2005⁴⁸).

Carcinogenicity

The applicant did not conduct carcinogenicity studies with belatacept. However, a carcinogenicity study in mice with abatacept (Study No. 97610) showed increased incidences of mammary tumors (female only) and lymphomas (both sexes). The tumors were associated with endogenous mouse mammary tumor virus (MMTV) and murine leukemia virus (MLV), respectively. In the clinic, belatacept is associated with malignancy through an increased risk of post-transplant lymphoproliferative disease (PTLD), specifically in the central nervous system (CNS) and is described in the product label.

Overall the toxicities observed with belatacept or abatacept were related to immune modulation and immune suppression and were generally secondary pharmacological effects. A summary of the immune parameter changes as well as the autoimmunity, infection, and mortality outcomes by study is provided in Table 39.

Table 39 A Comprehensive Summary of Immune Parameter Changes and Immunotoxicities Across Belatacept and Abatacept Toxicology Studies

Study No. /Treatment	Species	Start Age	Dose (mg/kg) /Route	Dose Frequency /N-value	IgG (%)	CD4	CD8	B-cell	Regulatory T-cell	Autoimmunity /Infection /Mortality
98642 Belatacept	Monkey	2-3 y	~100 iv	Single n=4-6	▼31	▼14%	▼28%	n/a	n/a	-/-/-
98699 Belatacept	Monkey	2-3.5 y	~50 iv	1 mon./2d n=6	▼32	△35%	▼26%	No change	n/a	-/-/-
99655 Belatacept	Monkey	1.5-3.5 y	~50 iv	6 mon./wk n=10	▼33	▼23%	▼27%	▼45%	n/a	-/-/-
DS02008 Abatacept	Monkey	4-8 y	~50 iv	12 mon./wk n=10	▼51	No change	No change	No change	n/a	-/-/-
96633 Abatacept	Mouse	6 wk	~200 sc	6 mon./wk n=40	▼72	No change	No change	▼61%	n/a	-/-/-
DS07166 Abatacept	Rat	8-9 wk	~200 iv	3 mon./3d n=17-20	▼91	△50%	No change	n/a	▼70%	++/-/-
DN06002 Belatacept	Pregnant Rat	8-9 wk	~200 iv	5 wk./d n=12-18	▼53 F ₁	No change F ₁	No change F ₁	No change F ₁	▼38% F ₁	F ₁ *-/-/-, F ₀ +++/++/2.2%
DN01060 Abatacept	Pregnant Rat	10 wk	~200 iv	5 wk./3d n=35	▼34 F ₁	No change	No change	No change	n/a	F ₁ *+/-/-, F ₀ -/-/-

DN07013 Abatacept	Juvenile Rat	PND4	~200 sc/iv	3 mon./3d n=144	▼95	F ₁ Δ2.6- 3.6X	F ₁ No change	F ₁ No change	▼80-90%	++/+++/6.9%
DS07165 Abatacept	Juvenile Rat	PND4 PND28	~65 sc/iv	3 mon./3d n=20	▼95 ▼95	Δ2.7X Δ3.2X	No change No change	Δ 1.9X Δ 2.7X	▼56% ▼51%	++/+++/1.7%

Dr. Ying Mu generated this table based on the average value of data. The value of (+) is based on the reviewer's judgment, (-): negative signal, ▼: decrease, Δ: increase, X: fold, n/a: not available, *: cross placenta and secrete into milk.

Summary/Conclusion

In summary, infections were observed in rats treated with belatacept or abatacept and observed in the clinic with belatacept. These adverse findings were anticipated as a possible consequence of the intended immunosuppression of these products. Warnings in the current version of the belatacept label caution patients and providers of the potential risk of serious infections.

The risk of autoimmunity was identified in rats with abatacept treatment at clinically relevant exposure levels. Autoimmunity was not observed in nonclinical studies of belatacept or in chronic adult mouse and monkey studies with abatacept. It is currently unclear whether patients or a fetus exposed in utero are at risk for developing autoimmunity. The risk of autoimmunity is described in the label to guide the monitoring of patients.

In conclusion, the nonclinical studies of belatacept and abatacept did not reveal any safety signals that would preclude approval. The pharmacology, PK and toxicology studies support the approval of belatacept for the prophylaxis of organ rejection in adult kidney transplant patients.

12 Appendix

The pivotal 6-month chronic toxicity study with belatacept in monkeys and the 88-week carcinogenicity study with abatacept in mice were previously reviewed in BLA 125118 Orencia® (abatacept).

The key findings from these studies are included for reference below.

Study title: Six-Month Intermittent-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys

Study no.: 99655

Volume #, and page #: Electronic submission

Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Thompson Road, Syracuse, New York, USA

Date of study initiation: June 8, 1999

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: BMS-224818, Lot # C99061, purity, NA

Key study findings: BMS-224818 (LEA29Y) is a second generation molecule that differs from BMS-188667 by 2 amino acid residues within CD80/86 binding domains resulting in a significant increase in binding activity to CD86 relative to that of BMS-188667 in humans, thus greater biologic activity. No significant drug-related toxicity was observed in monkeys when 10, 22 or 50 mg/kg of BMS-224818 was administered by IV, once per week for 6 months. BMS-224818 was well tolerated. Reversible minimal decreases in serum IgG levels and reversible minimal to moderate depletion of germinal centers in the spleen and/or lymph nodes at the end of the dosing period were seen and considered pharmacologic effects of the drug. Based on these results, the NOAEL was 50 mg/kg/week.

Study Title: Subcutaneous Carcinogenicity Study in Mice

Study No.: 97610

Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Thompson Road, Syracuse, New York, USA

Date of study initiation: February 12, 1997

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: Lyophilized BMS-188667 in 200 mg vials, lot # C96335, purity, NA

CAC concurrence: The protocol was not submitted to CAC

Key study findings: Subcutaneous administration of 20, 65 and 200 mg/kg of BMS-188667 once a week for 84 to 88 weeks to male and female mice respectively increased mortality and incidences of enlarged lymphoid tissues, lymphomas and renal tubular karyomegaly at all dose levels. In addition, incidences of mammary gland tumors were increased in females at the intermediate and high dose levels. In mice, retroviruses (murine leukemia [MLV] and mouse mammary tumor viruses [MMTV]) have been reported to cause lymphoma and mammary tumors, respectively. Endogenous ecotropic-specific MLV DNA was detected in the genome of CD-1 mice used in this study, and ^{(b) (4)} personnel informed the applicant that CD-1 mice are not retrovirus free (verbal communication to the applicant). Results from transmission electron microscopic evaluation of mammary tumors from this study identified large numbers of viruses in the cytoplasm, budding from the plasma membrane, and in the extracellular space. Significant chronic immunosuppression was observed at every dose level in this study demonstrated by the absence of any drug-specific antibody response to BMS-188667. Since BMS-188667 was negative when tested for genotoxicity, the applicant considered the increased lymphomas and mammary tumors in mice secondary to long-term immunosuppression by BMS-188667, likely due to the activation of endogenous retroviruses with resulting viral-associated malignancies in these organs. This conclusion is consistent with increased incidences of neoplasms in humans and mice on long-term immunosuppressants as azathioprine and cyclosporine, and enhanced expression of endogenous retroviruses following prolonged exposure to immunosuppressants.

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PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 125288 Applicant: Bristol Myers Squibb Stamp Date: 6/30/09

Drug Name: Belatacept NDA/BLA Type:

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Mouse carc study previously performed with abatacept (supported registration of Orencia).
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Yes, the 6-month monkey chronic study utilized a similar formulation (however, the control groups were administered saline not a matched vehicle); the formulation tested in monkeys provides a ~ 1.7-fold margin over the GMP batch formulation excipients.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?		X	Studies requested by DSPTP to evaluate belatacept BBB penetrance are ongoing but does not impact filing.

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
NDA/BLA or Supplement**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		Segment 3 study results need to be cross-checked with label (increased mortality in dams due to infections led to loss of pups).
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		A high MW variant has been qualified; ~2-fold margin in 6-month monkey.
11	Has the applicant addressed any abuse potential issues in the submission?		X	This is generally not applicable to a biologic product.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		X	Does not apply.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? X YES

If the NDA/BLA is not fileable from the pharmacology/toxicology 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.

	<u>08-12-2009</u>
Reviewing Pharmacologist	Date
	<u>08-12-2009</u>
Reviewing Pharmacologist	Date
<u>William H Taylor</u>	<u>8/12/09</u>
Team Leader/Supervisor	Date