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APPROVAL PACKAGE FOR:

APPLICATION NUMBER

BLA 125118/000

Pharmacology Review(s)

125118

07/21/05

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	**125118**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**11/16/04**
PRODUCT:	**Abatacept**
INTENDED CLINICAL POPULATION:	**Rheumatoid Arthritis**
SPONSOR:	**Bristol-Myers Squibb**
DOCUMENTS REVIEWED:	**Electronic Submission**
REVIEW DIVISION:	**Division of Therapeutic Biologic Internal Medicine Products (HFD-108)**
PHARM/TOX REVIEWER:	**Anita M. O'Connor, Ph.D.**
PHARM/TOX SUPERVISOR:	**Martin Green, Ph.D.**
DIVISION DIRECTOR:	**Marc Walton, M.D.**
PROJECT MANAGER:	**Eric Laughner**

Date of review submission to Division File System (DFS):

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

Approval is recommended.

B. Recommendation for nonclinical studies

No additional pharmacology studies are needed.

C. Recommendations on labeling.

Additional information to be added to the sponsor's proposed label:

i) Nine-fold increase in the T-cell dependent antibody (KLH) response in the F_1 generation of rat pups when F_0 dams are given ~20 times the human dose on day 6 of gestation through day 21 of lactation [see reproductive toxicity study number DN01060]
ii) Inflammation of the thyroid gland of one rat in the same (high dose) group as (i)

II. Summary of nonclinical findings

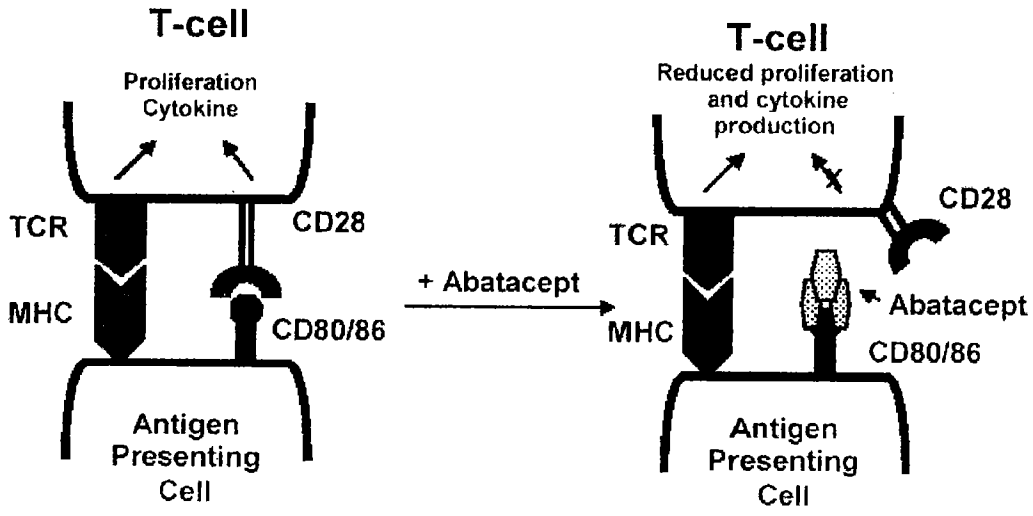
A. Brief overview of nonclinical findings

The pharmacokinetics of abatacept was studied in mice, rats, rabbits and monkeys. The drug exhibits linear pharmacokinetics in these species. Absorption (AUC) and C_{max} are generally proportional to dose and clearance is independent of dose. The half-life of abatacept is shorter in the preclinical model compared to humans. In monkeys, the most relevant animal model, the half-life is 5-8 days versus 13 days (average) in humans. Some accumulation occurred upon repeated dosing in animals. In monkeys given 10-50 mg/kg abatacept weekly for 12 months the accumulation ratio ranged from 1.3 to 3.1. Antibody formation to the drug increased clearance in some species. In a rat model, abatacept is passed via the placenta from the dam to the fetus and from the dam into the milk of a lactating rat. The drug was present in lactating pups (post natal day 21) of dams given abatacept daily. There were no gender differences in the pharmacokinetics of any species.

B. Pharmacologic activity

Abatacept binds to CD80/86 with higher affinity that CD28. It inhibits the binding of CD 80/86 on an antigen presenting cell (APC) to CD28 and reduces T cell proliferation and cytokine production.

Figure 2.1.1: Effect of Abatacept on T-Cell Costimulation



TCR = T-cell receptor; MHC = major histocompatibility complex

C. Nonclinical safety issues relevant to clinical use

The pharmacokinetic issues relevant to clinical use are:

- Presence of drug product in maternal and fetal sera via placental transfer (rat and rabbit models);
- Presence of drug product in milk of lactating dams (rat model);
- Presence of drug product in (post natal) sera of pups;
- Enhanced humoral immune response to KLH challenge in rat pups of dams repeatedly administered drug (~20 times human dose) during gestation and lactation.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

BLA number: 125118
Review number: 1
Sequence number/date/type of submission: 0/Nov.16, 2004/BLA
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Bristol-Myers Squibb
Manufacturer for drug substance: Bristol-Myers Squibb
Reviewer name: Anita M. O'Connor, Ph.D.
Division name: Division of Therapeutic Biologic Internal Medicine Products
HFD #:108
Review completion date: May 16, 2005

Drug:

Trade name: ORENCIA®
Generic name: Abatacept
Code name: BMS-188667, CTLA4Ig
Chemical name: *1-25-oncostatin M (human precursor) fusion protein with CTLA-4 (antigen) (human) fusion protein with immunoglobulin G1 (human heavy chain fragment)*
CAS registry number: 332348-12-6
Molecular formula/molecular weight: ~100 kD
Structure:

Figure 3.2.S.1.2.2.F01: Abatacept cDNA-Derived Amino Acid Sequence

Key:

Pro-sequence

CTLA4 Extracellular domain

Human IgG_1 fragment

O-Linked Glycosylation Sites (S129 and S139)

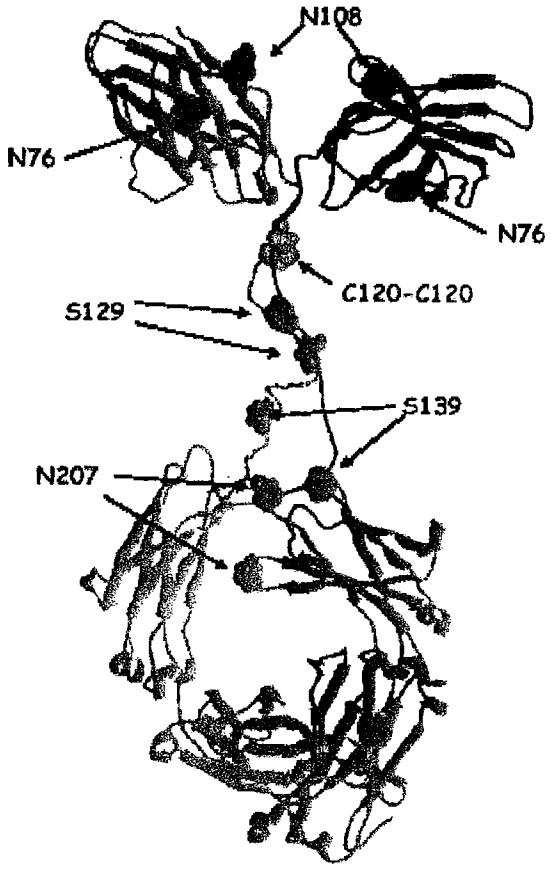
N-Linked Glycosylation Sites (N76, N108, and N207)

*Alanine, a product variant (8 to 10%) at the N-terminus

** Methionine, Experimentally determined primary N-terminus (predominant species)

***Lysine, C-terminus (cDNA)

****Glycine, C-terminus (predominant species)



A model of abatacept is shown with the N-linked glycosylation sites (N76, N108, and N207), O-linked glycosylation sites (S129 and S139), and the C120-C120 disulfide bond

Relevant INDs/NDAs/DMFs: IND 9391

Drug class: Fully humanized, recombinant, anti-inflammatory fusion protein that mediates CD28 initiated inflammatory responses to include T-cell dependent antibody production and cytokine release. The molecule is a homodimer of CTLA-4 connected to an IgG1 heavy chain (Fc).

Intended clinical population: Rheumatoid arthritis; the proposed dose is 500 mg intravenously once monthly for patients <60 kg (increasing to 750 mg for patients between 60 and 100 kg, and 1000 mg for those >100 kg in body weight). These doses produce trough levels of 20-30 *u*g/ml; average half-life is 13 days.

Clinical formulation:

Table 3.2.P.1.T01: Composition of Drug Product

Component	Quality Standard	Function	mg per Vial[a]
Abatacept	BMS Specification	Active Ingredient	
Maltose	BMS Specification		
Sodium Phosphate, Monobasic,	USP		
Sodium Chloride[b]	USP		

Route of administration: Intravenous

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

[For (b)(2) applications:

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of BLA 125118 are owned by Bristol Myers Squibb or are data for which Bristol Myers Squibb has obtained a written right of reference. Any information or data necessary for approval of BLA 125118 that Bristol Myers Squibb does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Bristol Myers Squibb 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 125118.

Studies reviewed within this submission:
Pharmacology: all primary pharmacodynamic studies (n=10), safety pharmacology (n=2), pharmacokinetic absorption (n=3), pharmacokinetic distribution (n=1), pharmacokinetic excretion (n=1) and other pharmacokinetic reports (n=19).

Studies not reviewed within this submission:
Toxicology

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary
See page 4, (II.A and II.B)

2.6.2.2 Primary pharmacodynamics

In *in vitro* experiments with human lymphocytes, abatacept inhibits the proliferation of human T-cells and the production of cytokines Il-2, IFN-γ, and TNF-α. The drug does not induce complement mediated cellular cytotoxicity. Abatacept reduces the clinical inflammation scores in a rat model of arthritis.

Mechanism of action:

The binding of abatacept to cell surface receptors CD 80 and CD86 on APC blocks the costimulatory signal thought to stimulate T-cell proliferation in response to an antigen. T cells are believed to need two signals to proliferate: the primary signal of the antigen/MHC complex and the costimulatory signal (i.e., CD80/86 binding to CD28 on a T-cell). *In vitro* and *in vivo* experiments in the submission suggest that abatacept is most efficacious at inhibiting CD4+ T cells in generating T-cell dependent humoral immune responses in humans and multiple animal species. In primates, for example, abatacept inhibited primary and secondary antibody responses to keyhole limpet hemocyania (KLH) and bacteriophage ϕX174.

Drug activity related to proposed indication:

Although the human antigen for RA is unknown, abatacept is efficacious against animal models of arthritis and human RA.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects: no effects

Cardiovascular effects: no effects

Pulmonary effects: no effects

Renal effects: no effects

Gastrointestinal effects: no effects

Abuse liability:

Other:

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Table 2.6.3.2: Primary Pharmacodynamics **Test Article: Abatacept**

Organ Systems Evaluated	Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study No./ Document Control No.
In vitro lymphocytes	Human	In vitro	0-100µg/ml	n/a	In vitro, abatacept causes a maximal 40-80% inhibition of proliferation of human T-cells.	No	930006438
In vitro lymphocytes	Human	In vitro	0-100µg/ml	n/a	In vitro, abatacept inhibits a tetanus memory recall response with a maximal inhibition of 40-60%.	No	930006461
In vitro lymphocytes	Human	In vitro	0-100µg/ml	n/a	In vitro, abatacept inhibits the production of the cytokines IL-2, IFN-γ, and TNF-α in a mixed lymphocyte assay.	No	930007790
In vitro monocytes	Human	In vitro	0-100µg/ml	n/a	In vitro, abatacept had no effect on LPS endotoxin induced TNF-α production from purified monocytes	No	930007832

Table 2.6.3.2: Primary Pharmacodynamics **Test Article: Abatacept**

Organ Systems Evaluated	Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study No./ Document Control No.
Whole animal	Rat	IP	1mg/kg days -1, 0, 2, 4, 6, 8, 10 or days10,12,14,16,18,20,22,24 or day 4 only	Female/8 per group	Abatacept reduced clinical inflammation scores in the rat model of arthritis. Anti-collagen antibody production, cytokine levels and bone erosion were inhibited when animals were treated prophylactically with abatacept.	No	930008117
Whole animal	Mouse	IV	200µg/dose	18 for MCMV study 10 for PC study Female	Abatacept treatment at time of infection or at 100 days prior to infection with MCMV or PC had no significant observable effects on host immune responses	No	51475/ 910044345
In vitro B-cells	Human	In vitro	n/a	n/a	Abatacept did not induce complement mediated cellular cytotoxicity	No	X021004.CDC/ 930006519

Table 2.6.3.4 Safety Pharmacology

				Test Article: Abatacept or BMS-224818[a]			
Organ Systems Evaluated	**Species/ Strain**	**Method of Administration**	**Doses (mg/kg)**	**Gender and No. per Group**	**Noteworthy Findings**	**GLP Compliance**	**Study No./ Document Control No.**
Parameters of cardiovascular and respiratory function and clinical observations and physical exams evaluated within the 1-month intermittent-dose monkey toxicity study.[b,c]	Cynomolgus monkey	Intravenous injection	Abatacept 0, 10, 22.4, or 50 every other day for 15 doses	M3/F3	No findings related to treatment with abatacept	Yes	94704/ 910044347
Parameters on cardiovascular and respiratory function and clinical observations and physical exams evaluated within the 6-month intermittent-dose monkey toxicity study.[b,d]	Cynomolgus monkey	Intravenous injection	BMS-224818 0, 10, 22, or 50 once weekly for 26 doses[d]	M5/F5	No findings related to treatment with BMS-224818	Yes	99655/ 920007203
Parameters of cardiovascular and respiratory function, histamine, complement, TNF-α, and IL-6 levels in serum or plasma; and clinical observations and physical exams evaluated within the 1-year intermittent-dose monkey toxicity study.[b,e]	Cynomolgus monkey	Intravenous injection	Abatacept 0, 10, 22, or 50 once weekly for 52 doses[f]	M5/F5	No findings related to treatment with abatacept	Yes	DS02008/ 930002781

M= males; F = females; IL = interleukin; TNF = tumor necrosis factor

[a] BMS-224818 is a second generation molecule that differs from abatacept by two amino acid residues within the CD80/86 binding domains; this confers a significant increase in binding avidity to CD86 relative to that of abatacept in humans.

b Criteria for evaluation included measurement of heart sounds by thoracic auscultation, heart rate, femoral pulse rate, behavior, coordination/balance, muscle tone, spinal abnormalities, lung sounds by thoracic auscultation, and respiratory rate. In addition, 10-lead electrocardiograms (ECGs) were obtained from conscious monkeys and evaluated for drug-related changes in ECG wave-forms, amplitudes, or interval durations.

c In 1-month study with abatacept, safety and ECG assessments were conducted prior to the first dose, at the completion of dosing during time of high abatacept serum levels, and after approximately 6- and/or 11-week dose-free observation periods. Clinical observations were recorded daily.

d In the 6-month study with BMS-224818, clinical observations were recorded daily, physical exams were conducted prior to study start and prior to necropsies; electrocardiogram assessments were conducted prior to the first dose, 3 hr after dosing during months 3 and prior to scheduled necropsies; and histamine, complement (C3a), TNF-α, and IL-6 levels were evaluated on Days 1 and on day of dosing during weeks 4, 8, and 25, to assess for the potential release of mediators associated with hemodynamic changes and anaphylactoid responses. Clinical observations were recorded daily.

e In 1-year study with abatacept, safety and electrocardiogram assessments were conducted prior to the first dose, 3 hr after dosing on weeks 1, 4, 16, 24, 39, and 52, and following a 13-week dose-free observation period Histamine, complement (C3a), TNF-α, and IL-6 levels in serum or plasma were also evaluated prior to dosing and immediately following dosing on Day 1 and on the day of dosing during weeks 16, 32, and 52, to assess for the potential release of mediators associated with hemodynamic changes and anaphylactoid responses. Clinical observations were recorded daily.

f After week 8 in the high-dose group, number of monkeys examined was 4 males and 5 females due to animal sacrificed for humane reasons (broken femur)

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2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

2.6.4.2 Methods of Analysis

2.6.4.3 Absorption

Study title: Study 910049020 - Pharmacokinetics of BMS-188667 (CTLA4Ig) Following Intravenous and Subcutaneous Administration to Mice

Study no. 817-BMS-188667-01
Facility: Bristol–Myers Squibb, Princeton, NJ
Date: January 16, 1995
GLP: No
Dose & Formulation: 0.29 mg abatacept (BMS-188667, Lot # ACMIV-3 (Research Grade); PBS buffer (10 mM sodium phosphate, 50 mM sodium chloride, pH 8.0)
Animals: IV: F/8; SC: F/8;
Protocol: Two groups of female mice were given a single dose of product by either the SC or IV route (see Table 2.6.5.3A)

RESULTS: see table 2.6.5.3A
CONCLUSIONS: Subcutaneous absorption was 85% of IV absorption (F=85%) in female mice.

Study title: Pharmacokinetics and Pharmacodynamics of BMS-188667 (CTLA4Ig) in Mice Following a Single Intravenous and Subcutaneous Dose Administration

Study no. 910053989
Facility: Bristol–Myers Squibb, Princeton, NJ
Date: August 30, 1996
GLP: No
Dose & Formulation: 0.33 (16.5 mg/kg) IV, 0.5 mg (25 mg/kg) SC, 1.6 mg (80 mg/kg) SC, 3.3 mg (165 mg/kg) SC; abatacept (BMS-188667) Lot# C95157 (Process A), lyophile (5% maltose)
Animals: IV: F/6; SC: F/18 (6 mice per dose level)
Protocol: See Table 2.6.5.3B

RESULTS: See Table 2.6.5.3B
CONCLUSIONS: C_{max} values were dose proportional in the SC groups; AUC (INF) was not dose proportional. Bioavailability by the SC route ranged from 78-110% and half-life was 4-5 days.

Study title: Toxicokinetics of BMS-188667 in Rats Following Repeated Intravenous and Subcutaneous Administration of BMS-188667

Study no. 95676
Facility: Bristol–Myers Squibb, Princeton, NJ
Date: January 5, 1996

GLP: Yes
Dose & Formulation: abatacept (BMS-188667) Lot# C95201 (Process A)
SC: Lyophile (20% maltose, 100 mM sodium phosphate, 200 mM sodium chloride)
IV: Lyophile (5% maltose, 25 mM sodium phosphate, 50 mM sodium chloride)
Animals: Single Dose -SC: M/9, F/9 (3 per gender per dose group); IV: M/9, F/9 (3 per gender per dose group); Repeat Dose: M/6, F/6 (3 rats per gender per dose level)
Protocol: See Tables 2.6.5.4E and2.6.5.3C. For the repeat dose part of the study animals were given 10 mg/kg once every other day for 7 doses over 13 days.

RESULTS: See Tables 2.6.5.4E and2.6.5.3C
CONCLUSIONS:
Single Dose: Clearance of the drug by the IV route was dose independent (CLT was not determined for the SC group). Half-life ranged from 3 to 7 days for both routes of administration. Absorption was dose proportional for the IV groups (AUC). C_{max} was dose dependent for the IV group. The SC data for C_{max} and AUC had a trend towards dose dependency.
Repeat Dose: Accumulation occurred with both SC and IV dosing (3-5 fold). Half-life was 4-4.8 days for both routes of administration.

2.6.4.4 Distribution

Study title: Study of Embryo-Fetal Development in Rats
Study no. 95024
Facility: Bristol-Myers Squibb, Princeton, NJ
Date: December 15, 1995
GLP: Yes
Dose & Formulation: abatacept (BMS-188667), Lot #C95201 (Process A); lyophile
Animals: F/75 (25 pregnant rats per dose group)
Protocol: The drug was given intravenously once daily to pregnant rats on days 6 through 15 of gestation at doses of 10, 45, or 200 mg/kg. On day 20 of gestation, the dams and fetuses were sacrificed and evaluated. At cesarean sectioning, material and fetal blood samples were collected from 10 dams and 10 litters in each group for serum product concentration.
RESULTS: See Table 2.6.5.8
CONCLUSIONS: Abatacept concentrations in maternal and fetal sera were approximately dose related. Fetal sera contained 40-60% of the amount of abatacept in maternal sera. The concentration in fetal sera relative to maternal sera slightly decreased with dose (see table on page 19, top).

Exposure on Day 20				
Dose (mg/kg)	Route	Maternal sera (ug/mL)	Fetal sera (ug/mL)	Ratio Fetal: Maternal
10	IV	8.4	5.0	.60
45	IV	26.7	14.7	.55
200	IV	81.0	33.1	.40

2.6.4.5 Metabolism

2.6.4.6 Excretion

Study title: Intravenous Study of Pre-and Post-Natal Development in Rats

Study no. DN01060

Facility: [redacted]

Date: October 30, 2002

GLP: Yes

Dose & Formulation: abatacept (BMS-188667) Lot #: C00196 (Process D)

Animals: F/30 (10 pregnant rats per dose group)

Protocol: See Table 2.6.5.13. This study is part of the larger pre and post natal development study in rats. Pregnant rats were dosed intravenously once every 3 days from day 6 of gestation through day 21 of lactation with 10, 45, or 200 mg/kg of drug. Milk and serum were sampled on day 12 of lactation.

RESULTS: See Table 2.6.5.13

CONCLUSIONS: The maternal sera data in this study are much higher than the values in the previous study because animals are still being administered drug in this study at time of sampling. In the preceding study (DN01060) drug had not been administered for 5 days (i.e., serum levels dropped due to clearance for 5 days). There were no gender differences in pup sera concentrations of abatacept. The maternal and fetal sera, and milk concentration data are dependent on dose. The ratio of maternal abatacept sera concentration to milk abatacept concentration is constant regardless of dose (see table below).

Exposure on Day 12				
Dose (mg/kg)	Route	Maternal sera (ug/mL)	Milk (ug/mL)	Ratio Maternal sera: Milk
10	IV	69.6	6.2	11.2
45	IV	299	28.1	10.6
200	IV	1726	135	12.8

2.6.4.7 Pharmacokinetic drug interactions

2.6.4.8 Other Pharmacokinetic Studies

Study 910048944 - Single dose-dose proportionality and multiple dose pharmacokinetics of BMS-188667 (CTLA4Ig) following intravenous administration to skin intact and skin grafted mice	No significant differences were found in the pharmacokinetics of abatacept in mice with intact skin compared to mice with skin grafts. Cmax and AUC increased in a dose proportional manner. Clearance was dose independent. Half-life was ~20-70 hours.
Study 910049073 - Pharmacokinetics of BMS-188667 (CTLA4Ig) in mice following a 2 mg single intravenous bolus dose	Cmax and AUC increased in a dose dependent manner when combined with data from the previous study (910048944).
Study 96615 - Two-week intermittent-dose subcutaneous irritation and comparative toxicokinetic study in rats	Two formulations were compared; the pharmacokinetics of the two formulations differed by Cmax (↑15-21%) and AUC (↑43-49%).
Study 910061964 - A comparative pharmaco-kinetic study of BMS-188667 in rats using materials made from current and scale-up processes	Ten rats per group were used to compare the current versus the scale-up processes. Three of the pK parameters were significantly different between the two processes (AUC, CLT, VSS) and three were not different (Cmax, MRT, T1/2)
Study DS01166 - Single-dose intravenous exploratory efficacy and pharmacokinetic study in rabbits	Abatacept has pharmacological activity in rabbits as shown by decreased KLH specific IgG and IgM responses in treated animals.
Study 94703 - Verification of exposure and toxicokinetics of BMS-188667 (CTLA4Ig) in a single dose intravenous toxicity study in monkeys	In a single IV dose study where monkeys were given either 10 or 33 mg/kg, AUC and Cmax were dose dependent. There were no gender differences. Clearance was not affected by dose.
Study 95654 - Single-dose intravenous comparative pharmacokinetic study of BMS-188667 (CTLA4Ig) in monkeys after administration of a ready-to-use solution and lyophilized formulations	Pharmacokinetics were comparable between the two formulations.
Study DS02051 - A single-dose intravenous exploratory comparative pharmacokinetic study in monkeys	Formulation comparison study: the pharmacokinetics of BMS-188667 from the [redacted] processes was not comparable to BMS-188667 obtained from the [redacted] process; however they were comparable if galactose was added to the medium of the [redacted] process.
Study DS02003 - Single-dose intravenous comparability study in monkeys	Formulation comparability study: BMS-188667 derived from the [redacted] process is comparable to that obtained from the [redacted] process
Study 96633 - Six-month intermittent-dose (QWx26) subcutaneous toxicity study in mice	There were no gender differences in the TK. Overall, mice given product for 26 weeks showed minimal accumulation (range: 1.5 to 2.0). The absorption was not dose proportional between the 1st and week 26th dose.
Study 910048945 - Preliminary pharmacokinetics of BMS-188667 (CTL4Ig) in a mouse skin transplant study: "Effects of Dose and Schedule of BMS-188667 on Efficacy"	This was a pharmacokinetic study done for a separate department of BMS (Autoimmunity/Transplantation). Sparse sampling diminished the validity of the results.
Study 97610 - Toxicokinetic analysis of BMS-	This is the TK satellite study for the two year

188667 in a subcutaneous carcinogenicity study in mice	bioassay. There were no gender differences in the toxicokinetics. The pharmacology was roughly dose dependent over a lifetime administration, however, the small animal numbers left after the 53rd and 79th dose administration diminish the study conclusions.
Study DS04016 - Seven-Week intermittent dose (QWX7) subcutaneous exploratory pharmacokinetic/pharmacodynamic study in mice	Pharmacokinetics in the mice was dose linear overall, but less so at the lower doses due to antibody development
Study 910049007 - Dose proportionality and multiple dose pharmacokinetics of BMS-188667 (CTLA4Ig) after intravenous administration to cynomolgus monkeys	In monkeys given 1, 2.9 or 8.7 mg/kg abatacept by IV on days 1, 4, 8, 11, 15 and 18 pharmacokinetics were linear.
Study 94704 - Toxicokinetics of BMS-188667 (CTLA4Ig) in a one-month intermittent-dose intravenous toxicity study in monkeys	Monkeys were dosed IV with 10, 22, or 50 mg/kg once every other day for 29 days. AUC (24), half-life and C_{max} were proportional to dose over the range tested. Antibodies developed at 6-9 weeks.
Study DS02008 - One year intermittent-dose intravenous toxicity and toxicokinetic study in monkeys	Monkeys were dosed with 10, 22, or 50 mg/kg once weekly for 52 weeks. Compared to humans dosed monthly at 10 mg/kg the exposure was ~2, 4, or 9-fold respectively.
Study DN03068 - Intravenous toxicokinetics study in pregnant and lactating rats	AUC in rats dosed every day at 45 or 200 mg/kg during gestation had an exposure multiple of 8.99 or 29.51, respectively, compared to the exposure in humans with rheumatoid arthritis dosed monthly at the proposed dose of 10 mg/kg. In lactating rats, given the same doses of Abatacept, AUC had an exposure multiple of 2.99 or 10.91, compared to AUC in RA patients dosed monthly with 10 mg/kg.
Study DN02003 -Intravenous study of embryo-fetal development in rabbits	This study show that BMS-188667 was transferred from the doe to the fetus over a dose range of 10-200 mg/kg given to the doses intravenously on days 7, 10, 13, 16 and 19 of gestation.
Study DN03069 - Thirteen-day intravenous toxicokinetics study in pregnant rabbits	Pregnant rabbits dosed with 200 mg/kg every 3 days from day 7 to 19 of gestation had a mean exposure (AUC) of ~30 times the exposure in humans doses monthly with 10 mg/kg

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Table 2.6.5.3B: Pharmacokinetics: After A Single Dose (Study 910053989)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C95157 (Process A)
Compliance:	Non-GLP
Study Number:	Not applicable
Document Control Number:	910053989
Location in Dossier:	Section 4.2.2.2

Species	**Mouse**
Sex (M/F) / Number of Animals	IV: F/6; SC: F/18 (6 mice per dose level)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile (5% maltose)
Method of Administration	Single IV, single SC
Dose (mg)	IV: 0.33 (corresponding to 16.5 mg/kg) SC: 0.5, 1.6, and 3.3 (corresponding to 25, 80, and 165 mg/kg, respectively)
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values					
Dose (mg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**	**F (%)**
0.33	IV	323	9490	2.8	1.5	0.17	NA
0.5	SC	96	15800	3.6	NC[a]	NC	110
1.6	SC	315	45200	5.2	NC	NC	98
3.3	SC	726	73800	4.0	NC	NC	78

[a] NC = Not calculated

Additional Information:

- Absorption after subcutaneous administration was essentially complete
- Cmax values appeared to increase dose proportionally, however, increment of AUC(INF) values was less than proportional
- Apparent T-HALF values were comparable between IV and SC routes of administration

Table 2.6.5.3C: Pharmacokinetics: After A Single Dose (Study 95676)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C95201 (Process A)
Compliance:	GLP
Study Number:	95676
Document Control Number:	910053669 [Appended to Toxicology Report No. 910051540]
Location in Dossier:	Section 4.2.3.2

Species	**Rat**
Sex (M/F) / Number of Animals	SC: M/9, F/9 (3 per gender per dose group); IV: M/9, F/9 (3 per gender per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	SC: Lyophile (20% maltose, 100 mM sodium phosphate, 200 mM sodium chloride) IV: Lyophile (5% maltose, 25 mM sodium phosphate, 50 mM sodium chloride)
Method of Administration	Single SC, IV
Dose (mg/kg)	10, 80, and 200
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values					
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**	**F (%)**
10	SC	26.0	5536	3.1	NC[a]	NC	63
80	SC	133.3	35153	5.5	NC	NC	56
200	SC	262.6	56900	7.0	NC	NC	41
10	IV	243.4	8857	2.7	1.2	0.15	NC
80	IV	2162	63167	4.5	1.3	0.19	NC
200	IV	4610	138608	7.1	1.5	0.24	NC

[a] NC = Not calculated

Additional Information:

- No gender differences. Thus, results are combined from male and female animals for calculation of mean PK parameters.
- CLT values were dose independent
- T-HALF values were comparable between IV and SC routes of administration

Table 2.6.5.3D: Pharmacokinetics: After A Single Dose (Study 817-BMS-188667-02)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	ACMIV-3 (Research Grade)
Compliance:	Non-GLP
Study Number:	817-BMS188667-02
Document Control Number:	910048944
Location in Dossier:	Section 4.2.2.7

Species	**Mouse**
Sex (M/F) / Number of Animals	Skin-intact (SI): F/9 (3 mice per dose level); Skin-grafted (SG): F/3
Feeding Conditions	Non-fasted
Vehicle/Formulation	PBS buffer (10 mM sodium phosphate, 50 mM sodium chloride, pH 8.0)
Method of Administration	Single IV
Dose (mg)	Skin-intact: 0.07, 0.29, and 0.57 (corresponding to 3.6, 14, and 29 mg/kg) Skin-grafted: 0.29 (corresponding to 14 mg/kg)
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
0.07 (SI)	IV	56.8	1594	0.8	2.0	0.11
0.29 (SI)	IV	290.3	8705	2.5	1.5	0.14
0.57 (SI)	IV	563.4	15322	2.9	2.0	0.16
0.29 (SG)	IV	305.3	9124	2.7	1.5	0.13

Additional Information:

- Cmax and AUC(INF) values increased in a dose proportional manner
- Single dose PK parameters obtained for skin-grafted mice were comparable to those obtained from skin-intact mice

Table 2.6.5.3E: Pharmacokinetics: After A Single Dose (Study 744/188667/001)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	ACMIV-3 (Research Grade)
Compliance:	Non-GLP
Study Number:	744/188667/001
Document Control Number:	910049073
Location in Dossier:	Section 4.2.2.7

Species	**Mouse**
Sex (M/F) / Number of Animals	F/8
Feeding Conditions	Non-fasted
Vehicle/Formulation	PBS buffer (10 mM sodium phosphate, 50 mM sodium chloride, pH 8.0)
Method of Administration	Single IV
Dose (mg)	2.0 (corresponding to 100 mg/kg)
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
2.0	IV	2409	65762	6.9	1.5	0.22

Additional Information:

- Dose proportionality was assessed across studies 817-188667-02 (Table 2.6.5.3D) and 744/188667/001 (Table 2.6.5.3E).
- The results showed that both Cmax and AUC(INF) values appeared to increase in a dose proportional manner over the dose range of 0.29 to 2 mg

Table 2.6.5.3F: Pharmacokinetics: After A Single Dose (Study 96615)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C96021(Process B) C95157 (Process A)
Compliance:	GLP
Study Number:	96615
Document Control Number:	910056843 [Appended to Toxicology Report No 910056020]
Location in Dossier:	Section 4.2.3.6

Species	**Rats**
Sex (M/F) / Number of Animals	M/6; F/6 (3 rats per gender per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	100 mg/mL solution (20% maltose, 50 mM sodium phosphate, 100 mM sodium chloride) 40 mg/mL solution (20% maltose, 100 mM sodium phosphate, 200 mM sodium chloride)
Method of Administration	SC
Dose (mg/kg)	100
Dosing schedule	Single SC
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

			Mean Pharmacokinetic Parameter Values		
Dose (mg/kg)	**Route**	**Formulation**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**
100	SC	100 mg/mL solution	226	40900	4.8
100	SC	40 mg/mL solution	273	47200	6.7

Additional Information:

- No gender differences. Thus, data from male and female rats were combined for calculation of mean PK parameters.

Table 2.6.5.3G: Pharmacokinetics: After A Single Dose (744/188667/001)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C96118 (Process B) C96335 (Process C)
Compliance:	GLP
Study Number:	744/188667/001
Document Control Number:	910061964
Location in Dossier:	Section 4.2.2.7

Species	**Rat**
Sex (M/F) / Number of Animals	M/20 (10 per group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile, Abatacept from Current Process B (lot # C96118) Lyophile, Abatacept from Scale-Up Process C (lot # C96335)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (S; Scale-up)	IA[a]	282.2	9938	2.6	1.03	0.10
10 (C; Current)	IA	276.3	6847	2.3	1.47	0.14
Statistics[b]		NS[c]	S > C	NS	S < C	S < C

[a] IA = Intra-arterial; [b] $p \leq 0.05$; [c] NS = Not significant

Additional Information:

- Cmax and T-HALF values were comparable
- Significantly higher AUC(INF) (~31%) and lower CLT a(~30%) and Vss (~29%) values were obtained for the scale-up process material compared to corresponding values from current process material

Table 2.6.5.3H: Pharmacokinetics: After A Single Dose (Study DS01166)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C00196 (Process D)
Compliance:	Non-GLP
Study Number:	DS01166
Document Control Number:	930001267
Location in Dossier:	Section 4.2.3.7.7

Species	**Rabbit**
Sex (M/F) / Number of Animals	F/4
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	10
Dosing schedule	Single IV
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10	IV	288.9	5939.9	2.4	1.73	0.13

Table 2.6.5.3.J: Pharmacokinetics: After A Single Dose (Study 95654)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	95046-30 and C95157 (Process A)
Compliance:	GLP
Study Number:	95654
Document Control Number:	910051756 [Appended to Toxicology Report No. 910051104]
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	M/4; F/4 (2 monkeys per gender per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Ready-to use (RTU) solution (25 mM sodium phosphate, 50 mM sodium chloride, pH 7.5) (Lot #95046-30) Lyophilized (LYO) formulation (5% maltose) (Lot #C95157)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (RTU)	IV	333.9	16902	6.0	0.60	0.13
10 (LYO)	IV	336.3	13173	5.4	0.77	0.16
Statistics [a]		NS [b]	NS	NS	NS	p=0.044

[a] T-test; [b] NS = Not significant

Additional Information:

- No gender differences. Thus, data from male and female monkeys were combined for calculation of mean PK parameters.
- Comparable pharmacokinetics were obtained between the ready-to-use (RTU) and lyophilized (LYO) formulations

Table 2.6.5.3K: Pharmacokinetics: After A Single Dose (Study DS02051)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	010920-112 (Process D) 020211-409 ([redacted] without galactose)
Compliance:	Non-GLP
Study Number:	DS02051
Document Control Number:	930002917 [Appended to Toxicology Report No. 930003063]
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	F/6 (3 monkeys per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile - Process D (Lot #010920-112) Lyophile - [redacted] without galactose (Lot #020211-409)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (#010920-112)	IV	353.8	17059.7	6.4	0.59	0.09
10 (#020211-409)	IV	360.0	8831.5	4.7	1.18	0.12

Additional Information:

- Abatacept from Process D (Lot #010920-112) and [redacted] without galactose (Lot #020211-409) was not pharmacokinetically comparable in monkeys

Table 2.6.5.3K: Pharmacokinetics: After A Single Dose (Study DS02051)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	010920-112 (Process D), 188667-2002-002; NB2589PO39-B; NB2589PO39-C (3 lots)
Compliance:	Non-GLP
Study Number:	DS02051
Document Control Number:	930002917 [Appended to Toxicology Report No. 930003063]
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	M/12 (3 monkeys per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile - Process D (Lot #010920-112) Lyophile - (3) without galactose (Lot #188667-2002-002) Lyophile - (3) with galactose (NB2589PO39-B) Lyophile - (3) with low sialic acid (NB2589PO39-C)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (#010920-112)	IV	339.6	15752.9	6.6	0.67	0.12
10 (#188667-2002-002)	IV	311.1	7765.3	5.4	1.31	0.17
10 (NB2589PO39-B)	IV	248.3	20445.0	8.2	0.50	0.10
10 (NB2589PO39-C)	IV	274.6	7266.1	4.4	1.40	0.18

Additional Information:

- Results indicated that abatacept from (3) without galactose (#188667-2002-002) and (3) with low sialic acid (NB2589PO39-C) was not comparable pharmacokinetically in monkeys to the reference Process D abatacept (#010920-112).
- Abatacept from (3) with galactose (NB2589PO39-B) was comparable pharmacokinetically to reference Process D (#010920-112).

Table 2.6.5.3K: Pharmacokinetics: After A Single Dose (Study DS02051)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	010920-112 (Process D) 020611-409; NB2589PO31; NB2620P100 ([redacted] lots)
Compliance:	Non-GLP
Study Number:	DS02051
Document Control Number:	930002917 [Appended to Toxicology Report No. 930003063]
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	F/12 (3 monkeys per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile - Process D (Lot #010920-112) Lyophile - [redacted] with galactose (Lot #020511-409; NB2589PO31; NB2620P100)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (#010920-112)	IV	321.4	15459.8	6.4	0.67	0.11
10 (#020611-409, Day 12)	IV	244.9	18750.9	6.2	0.53	0.10
10 (NB2589PO31, Day 14)	IV	274.7	20707.8	5.6	0.50	0.08
10 (NB2620P100; Day 16)	IV	254.8	15779.9	5.9	0.65	0.10

Additional Information:

- All 3 abatacept [redacted] with galactose lots harvested on days 12, 14, and 16, respectively, were pharmacokinetically comparable to the reference Process D material

Table 2.6.5.3L: Pharmacokinetics: After A Single Dose (Study DS02003)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	2G55374 (Process D) MQJ611 (Process E)
Compliance:	GLP
Study Number:	DS02003
Document Control Number:	930002770
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	F/12 (6 monkeys per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile - Process D (Lot #2G55374) Lyophile - Process E (Lot #MQJ611)
Method of Administration	Single IV
Dose (mg/kg)	10
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values				
Dose (mg)	**Route**	**Cmax (µg/mL)**	**AUC(0-T) [a] (µg.h/mL)**	**T-HALF (days)**	**CLT (mL/h/kg)**	**Vss (L/kg)**
10 (#2G55374)	IV	322.0	16588.0	5.1	0.6	0.09
10 (#MQJ611)	IV	330.2	19916.8	5.1	0.5	0.07

[a] T = 28 days

Additional Information:

- Abatacept from Process D and Process E demonstrated comparable pharmacokinetics in monkeys

Table 2.6.5.4A: Pharmacokinetics: After Repeated Doses (Study 910048945)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	ACMIV-3 (Research Grade)
Compliance:	Non-GLP
Study Number:	Not applicable
Document Control Number:	910048945
Location in Dossier:	Section 4.2.2.7

Species	**Mouse**
Sex (M/F) / Number of Animals	F/36 (6 mice per dose and schedule)
Feeding Conditions	Non-fasted
Vehicle/Formulation	PBS buffer (10 mM sodium phosphate, 50 mM sodium chloride, pH 8.0)
Method of Administration	IV
Dosing Schedule	q2d x 7, q3d x 7, q4d x 7
Dose (mg)	0.07, 0.29 (corresponding to 3.6 and 14 mg/kg)
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values		
Dose (mg)	**Schedule**	**Cmax (µg/mL)**	**AUC(INF) (µg.h/mL)**	**T-HALF (days)**
0.07	q2d x 7	93.2	7026	2.7
	q3d x 7	92.4	6737	2.5
	q4d x 7	70.5	3717	1.9
0.29	q2d x 7	421.8	49903	5.7
	q3d x 7	426.0	50058	4.7
	q4d x 7	382.4	47596	4.5

Table 2.6.5.4B: Pharmacokinetics: After Repeated Doses (Study 96633)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C96118 (Process B)
Compliance:	GLP
Study Number:	96633
Document Control Number:	910064489 [Appended to Toxicology Report 910066124]
Location in Dossier:	Section 4.2.3.2

Species	**Mouse**
Sex (M/F) / Number of Animals	M/27, F/27 (9 mice per gender per dose level)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile (4% maltose, 10 mM sodium phosphate, 20 mM sodium chloride)
Method of Administration	SC
Dosing Schedule	q7d x 26
Dose (mg/kg)	20, 65, 200
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values					
Dose (mg/kg)	**Gender**	**Cmax (µg/mL)**		**AUC(TAU)[a] (µg.h/mL)**		**T-HALF (days)**	
		Week 1	Week 26	Week 1	Week 26	Week 1	Week 26
20	Male	110.1	115.2	6735	11878	3.4	4.9
	Female	91.0	99.6	6851	8187	3.0	6.5
65	Male	171.1	264.7	14964	31102	4.6	7.5
	Female	188.6	242.2	15217	28060	3.8	2.7
200	Male	316.2	463.1	34658	51624	5.3	7.6
	Female	412.8	676.0	40479	57332	5.7	6.9

[a] TAU = 7 days

Additional Information:

- No gender differences
- Minimal accumulation (~ 1.5 - 2.0) after repeated once-a week dosing

Table 2.6.5.4C: Pharmacokinetics: After Repeated Doses (Study 97610)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C96335 (Process C)
Compliance:	GLP
Study Number:	97610
Document Control Number:	920003036 [Appended to Toxicology Report No. 920007076]
Location in Dossier:	Section 4.2.3.4.1

Species	**Mouse**
Sex (M/F) / Number of Animals	M/15; F/15 (5 mice per gender per dose level)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile (4% maltose, 10 mM sodium phosphate, 20 mM sodium chloride)
Method of Administration	SC
Dosing Schedule	Once a week for 21 months
Dose (mg/kg)	20, 65, 200
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values			
Dose (mg/kg)	**Week**	**Cmax (µg/mL)**		**AUC(TAU) [c] (µg.h/mL)**	
		Male	Female	Male	Female
20	53	75	81	8669	8954
	79	88	[a]	12169	[a]
65	53	177	230	21787	23412
	79	287	273	31818	NC [b]
200	53	341	390	34343	35507
	79	424	[a]	39872	[a]

[a] No surviving animals; [b] NC = Not calculated due to insufficient number of animals; [c] TAU = 7 days

Additional Information:

- No gender differences observed.

Table 2.6.5.4D: Pharmacokinetics: After Repeated Doses (Study DS04016)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	3A64965 (Process E)
Compliance:	Non-GLP
Study Number:	DS04016
Document Control Number:	930007629
Location in Dossier:	4.2.3.7.7

Species	**Mouse**
Sex (M/F) / Number of Animals	F/45 (9 mice per dose level)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	SC
Dosing Schedule	Once-a-week for 5 weeks (QW x 5)
Dose (mg/kg)	2.5, 5, 10, 20, and 65
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values on Day 29		
Dose (mg/kg)	**Schedule**	**Cmax (µg/mL)**	**AUC(TAU) [a] (µg.h/mL)**	**Tmax (Hours)**
2.5	QW x 5	0.29	NR [b]	6
5	QW x 5	22.4	890	12
10	QW x 5	70.6	4300	36
20	QW x 5	164	16700	12
65	QW x 5	802	39400	12

[a] TAU = 7 days, [b] NR = Not reported as N = 2

Additional Information:

- Lower than expected serum concentrations were obtained in mice dosed at 2.5 and 5 mg/kg. Presence of abatacept-specific antibodies in these mice could have contributed to increased clearance of abatacept and therefore to the low Cmax and AUC values.
- Over the dose range from 10 to 65 mg/kg, as the dose increased in the ratio 1: 2: 6.5, the mean Cmax values increased in the ratio 1: 2.3: 11.4 and the mean AUC(TAU) values increased in the ratio 1: 3.9: 9.2

Table 2.6.5.4E: Pharmacokinetics: After Repeated Doses (Study 95676)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C95201 (Process A)
Compliance:	GLP
Study Number:	95676
Document Control Number:	910053669 [Appended to Toxicology Report No. 910051540]
Location in Dossier:	Section 4.2.3.2

Species	**Rat**
Sex (M/F) / Number of Animals	M/6, F/6 (3 rats per gender per dose level)
Feeding Conditions	Non-fasted
Vehicle/Formulation	SC: Lyophile (20% maltose, 100 mM sodium phosphate, 200 mM sodium chloride) IV: Lyophile (5% maltose, 25 mM sodium phosphate, 50 mM sodium chloride)
Method of Administration	IV and SC
Dose (mg/kg)	10
Dosing schedule	q2d x 7
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values on Day 13		
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(TAU) [a] (µg.h/mL)**	**T-HALF (days)**
10	SC	99.3	4339.8	4.0
10	IV	414.8	9411.5	4.8

[a] TAU = 2 days

Additional Information:

- No gender differences. Thus, data from male and female rats were combined for calculation of mean PK parameters.
- The bioavailability and the single dose pharmacokinetics data from this study are reported in Table 2.6.5.3C.

Table 2.6.5.4F: Pharmacokinetics: After Repeated Doses (Study 96615)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C96021 (Process B) C95157 (Process A)
Compliance:	GLP
Study Number:	96615
Document Control Number:	910056843 [Appended to Toxicology Report No. 910056020]
Location in Dossier:	Section 4.2.3.6

Species	**Rat**
Sex (M/F) / Number of Animals	M/6, F/6 (3 rats per gender per formulation)
Feeding Conditions	Non-fasted
Vehicle/Formulation	100 mg/mL solution (20% maltose, 50 mM sodium phosphate, 100 mM sodium chloride) 40 mg/mL solution (20% maltose, 100 mM sodium phosphate, 200 mM sodium chloride)
Method of Administration	SC
Dose (mg/kg)	100
Dosing schedule	q2d x 7
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values on Day 13			
Dose (mg/kg)	**Route**	**Formulation**	**Cmax (µg/mL)**	**AUC(0-T)[a] (µg.h/mL)**	**T-HALF (days)**
100	SC	100 mg/mL	361	87200	8.5
100	SC	40 mg/mL	515	130000	9.6

[a] T = 1032 h

Additional Information:

- No gender differences. Thus, data from male and female rats were combined for calculation of mean PK parameters
- The single dose pharmacokinetics data from this study are reported in Table 2.6.5.3F

Table 2.6.5.4G: Pharmacokinetics: After Repeated Doses (Study 94648)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	ACMV1-1 (Research Grade)
Compliance:	Non-GLP
Study Number:	94648
Document Control Number:	910049007
Location in Dossier:	Section 4.2.3.7.7

Species	**Monkey**
Sex (M/F) / Number of Animals	M/6, F/6 (2 monkeys per gender per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	10 mM sodium phosphate, 50 mM sodium chloride
Method of Administration	IV
Dose (mg/kg)	1.0, 2.9, and 8.7
Dosing schedule	Day 1, 4, 8, 11, 15, and 18
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values on Day 18		
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**AUC(0-T)[a] (µg.h/mL)**	**T-HALF (days)**
1.0	IV	31.7	2783.4	3.8
2.9	IV	104.1	8811.5	6.7
8.7	IV	269.5	25765	5.6

[a] T = 720 h

Additional Information:

- No gender differences. Thus, data from male and female monkeys were combined for calculation of mean PK parameters
- Steady state was achieved by Day 11 of dosing
- Abatacept exhibits linear pharmacokinetics at steady state over the dose range of 1 to 8.7 mg/kg

Table 2.6.5.4H: Pharmacokinetics: After Repeated Doses (Study 94704)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	940922-J (Research Grade)
Compliance:	GLP
Study Number:	94704
Document Control Number:	910049066 [Appended to Toxicology Report No. 910044347]
Location in Dossier:	Section 4.2.3.2

Species	**Monkey**
Sex (M/F) / Number of Animals	M/9, F/9 (3 monkeys per gender per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	25 mM sodium phosphate, 50 mM sodium chloride, pH 7.5
Method of Administration	IV
Dose (mg/kg)	10, 22.4, and 50
Dosing schedule	Dosed once every other day for 15 doses over 29 days
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values on Day 29			
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**		**AUC(0-T)[a] (µg.h/mL)**	**T-HALF[b] (days)**
		Day 1	**Day 29**		
10	IV	258.9	483.2	7104	10.1
22.4	IV	586.2	1164.3	16828	8.2
50	IV	1290.3	2078.5	29688	11.7

[a] T = 24 h; [b] T-HALF calculated within the timeframe of 1032 h, prior to the formation of anti-BMS-188667 antibody.

Additional Information:

- No gender differences. Thus, data from male and female monkeys were combined for calculation of mean PK parameters
- Accumulation of 1.6 - 2.0 fold based on comparison of Cmax values obtained on day 29 and day 1

Table 2.6.5.41: Pharmacokinetics: After Repeated Doses (Study DS02008)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	MQJ611 (Process E)
Compliance:	GLP
Study Number:	DS02008
Document Control Number:	930002781
Location in Dossier:	Section 4.2.3.2

Species	**Monkey**
Sex (M/F) / Number of Animals	M/15, F/15 (5 monkeys per gender per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	10, 22, 50
Dosing schedule	Once weekly for 12 months
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Pharmacokinetic Parameter Values											
Dose (mg/kg)	**Route**	**Cmax (µg/mL)**				**AUC(TAU)[a] (µg.h/mL)**				**Accumulation Ratio**			
Male	**Day**	1	78	267	358	1	78	267	358	1	78	267	358
10	IV	311.4	354.0	409.6	494.5	10645.4	16366.2	20703.1	24781.8	NA[b]	1.5	1.9	2.3
22	IV	738.9	871.3	862.5	1041.9	21190.7	35769.9	42818.3	49864.9	NA	1.7	2.0	2.4
50	IV	1330.4	1860.8	1953.9	2488.2	44449.3	79200.8	99178.6	134648.7	NA	1.8	2.3	3.1
Female													
10	IV	279.7	300.7	299.7	511.3	8803.6	14321.9	14693.2	19401.6	NA	1.6	1.7	2.2
22	IV	607.9	679.4	789.8	1073.8	21354.5	27133.3	36362.9	44641.8	NA	1.3	1.7	2.1
50	IV	1456.6	2213.5	1990.4	2173.9	47854.8	74225.1	76285.8	85605.5	NA	1.5	1.6	1.8

[a] TAU = 7 days; [b] NA = Not applicable

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Table 2.6.5.7A: Pharmacokinetics: Studies in Pregnant or Nursing Animals (Study DN03068)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	3A64965 (Process E)
Compliance:	GLP
Study Number:	DN03068
Document Control Number:	930006845
Location in Dossier:	Section 4.2.3.5.4

Species	**Rat**
Sex (M/F) / Number of Animals	F/64 (16 rats per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	Gestation: 45, 200 Lactation: 45, 200
Dosing schedule	Gestation: Once daily on gestation days 6 through 15 Lactation: Once every 3 days on lactation days 3, 6, 9, and 12
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

Dose (mg/kg)		**Route**	**Cmax (µg/mL)**	**Tmax (h)**	**AUC(TAU) (µg.h/mL)**
45	Gestation	IV	1279	0.05	15009
200	Gestation	IV	4154	0.05	49281
45	Lactation	IV	891	0.05	14983
200	Lactation	IV	3870	0.05	54646

Additional Information:

- Exposure increased in a dose-related manner

Table 2.6.5.7B: Pharmacokinetics: Studies in Pregnant or Nursing Animals (Study DN02003)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	010920-112 (Process D)
Compliance:	GLP
Study Number:	DN02003
Document Control Number:	930002722
Location in Dossier:	Section 4.2.3.5.2

Species	**Rabbits**
Sex (M/F) / Number of Animals	F/27 (9 mated rabbits per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	25 mM sodium phosphate, 50 mM sodium chloride, pH 7.5
Method of Administration	IV
Dose (mg/kg)	10, 45, and 200
Dosing schedule	Once every 3 days from day 7 through 19 of presumed gestation
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Serum Concentration		
Dose (mg/kg)	**Route**	**Maternal Sera (µg/mL)**	**Fetal Sera (µg/mL)**	**Fetal:Maternal Serum Ratio**
10	IV	200.7	0.6	0.003
45	IV	989.7	1.1	0.001
200	IV	7261.2	4.3	< 0.001

Additional Information:

- Abatacept was present in both maternal and fetal sera at all doses tested indicating that abatacept is transferred from dam to fetus.

Table 2.6.5.7C: Pharmacokinetics: Studies in Pregnant or Nursing Animals (Study DN03069)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	3A64965 (Process E)
Compliance:	GLP
Study Number:	DN03069
Document Control Number:	930006449
Location in Dossier:	Section 4.2.3.5.4

Species	**Rabbit**
Sex (M/F) / Number of Animals	F/5
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	200
Dosing schedule	Once every 3 days on gestation days 7, 10, 13, 16, and 19
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

Dose (mg/kg)	**Route**	**Cmax (µg/mL)**	**Tmax (h)**	**AUC(TAU)[a] (µg.h/mL)**
200	IV	6330.4	0.05	145680.6

[a] TAU = 3 days

Table 2.6.5.8: Pharmacokinetics: Distribution - Placental Transfer (Study 95024)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C95201 (Process A)
Compliance:	GLP
Study Number:	95024
Document Control Number:	910054198
Location in Dossier:	Section 4.2.3.5.2

Species	**Rat**
Sex (M/F) / Number of Animals	F/75 (25 presumed pregnant rats per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	10, 45, and 200
Dosing schedule	Daily on days 6 through 15 of presumed gestation
Sample(s)	Serum
Analyte(s)	Abatacept
Assay(s)	ELISA

		Verification of Exposure on Day 20 (Mean Serum Concentration)	
Dose (mg/kg)	**Route**	**Maternal Sera (µg/mL)**	**Fetal Sera (µg/mL)**
10	IV	8.4	5.0
45	IV	26.7	14.7
200	IV	81.0	33.1

Additional Information:

- Dose related increases in exposure in dams and fetuses
- Presence of abatacept in fetuses indicate that abatacept crosses the placental barrier

Table 2.6.5.13: Pharmacokinetics: Excretion - Excretion in Milk (Study DN01060)

Test Article:	**Abatacept (BMS-188667)**
Lot #:	C00196 (Process D)
Compliance:	GLP
Study Number:	DN01060
Document Control Number:	930002920
Location in Dossier:	Section 4.2.3.5.3

Species	**Rat**
Sex (M/F) / Number of Animals	F/30 (10 presumed pregnant rats per dose group)
Feeding Conditions	Non-fasted
Vehicle/Formulation	Lyophile
Method of Administration	IV
Dose (mg/kg)	10, 45, and 200
Dosing schedule	Once every 3 days from day 6 of gestation through day 21 of lactation
Sample(s)	Serum and milk
Analyte(s)	Abatacept
Assay(s)	ELISA

		Mean Serum Concentration				
Dose (mg/kg)	**Route**	**Maternal Sera (µg/mL)**	**Maternal milk (µg/mL)**	**Milk:Serum Ratio**	**Female Pup Sera (µg/mL)**	**Male Pup Sera (µg/mL)**
10	IV	69.6	6.2	0.09	2.1	1.9
45	IV	299	28.1	0.09	7.4	8.2
200	IV	1726	135	0.08	30.6	21.7

Additional Information:

- Abatacept was present in maternal sera and milk on Day 12 of lactation and in pup sera on Day 21 postpartum for all treated groups.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology:

Genetic toxicology:

Carcinogenicity:

Reproductive toxicology:

Special toxicology:

2.6.6.2 Single-dose toxicity

2.6.6.3 Repeat-dose toxicity

Histopathology inventory (optional)

2.6.6.4 Genetic toxicology

Study title:

Key findings:

Study no.:

Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Strains/species/cell line:

Doses used in definitive study:

Basis of dose selection:

Negative controls:

Positive controls:

Incubation and sampling times:

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

Study outcome:

2.6.6.5 Carcinogenicity

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality:

Clinical signs:

Body weight:

Food consumption:

Toxicokinetics:

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Embryofetal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

Mortality (dams):

Clinical signs (dams):

Body weight (dams):

Food consumption (dams):

Toxicokinetics:

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.):

Offspring (malformations, variations, etc.):

Prenatal and postnatal development

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:

Methods

Doses:
Species/strain:
Number/sex/group:
Route, formulation, volume, and infusion rate:
Satellite groups used for toxicokinetics:
Study design:
Parameters and endpoints evaluated:

Results

F_0 in-life:

F_0 necropsy:

F_1 physical development:

F_1 behavioral evaluation:

F_1 reproduction:

F_2 findings:

2.6.6.7 Local tolerance

2.6.6.8 Special toxicology studies

Study title:

Key study findings:

Study no.:
Volume #, and page #:
Conducting laboratory and location:
Date of study initiation:
GLP compliance:
QA reports: yes ( ) no ( )
Drug, lot #, and % purity:
Formulation/vehicle:

Methods

Doses:

Study design:

Results:

2.6.6.9 Discussion and Conclusions

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

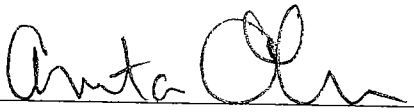
Conclusions:

Unresolved toxicology issues (if any):

Recommendations:

Suggested labeling:

Signatures (optional):

Reviewer Signature 

Supervisor Signature Marten Green Concurrence Yes ✓ No ___

APPENDIX/ATTACHMENTS

APPEARS THIS WAY
ON ORIGINAL

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

BLA NUMBER:	125118
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	11/16/04
PRODUCT:	Abatacept (BMS-188667)
INTENDED CLINICAL POPULATION:	Patients with moderate to sever rheumatic arthritis
SPONSOR:	Bristol-Myers Squibb
DOCUMENTS REVIEWED:	Electronic Submission
REVIEW DIVISION:	Division of Therapeutic Biologic Internal Medicine Products (HFD-108)
PHARM/TOX REVIEWER:	Hanan Ghantous PhD, DABT
PHARM/TOX SUPERVISOR:	Martin Green, PhD
DIVISION DIRECTOR:	Marc Walton, MD
PROJECT MANAGER:	Beverly Conner Pharm D

Date of review submission to Division File System (DFS):

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

It is recommended that abatacept (BMS-188667) be approved.

B. Recommendation for nonclinical studies

No additional nonclinical studies are recommended.

C. Recommendations on labeling

The text for labeling submitted by the sponsor is listed below. Reviewer's suggestions are in italics. ORENCIA is the sponsor's proposed trade name.

The reviewer agrees with the sponsor on the text concerning mutagenesis, the impairment of fertility, and nursing mothers. However, changes in immune function observed at a dose of 200 mg/kg in the F_1-generation females which consisted of an increase (9-fold) in the T-cell-dependent antibody response and inflammation of the thyroid gland of one rat should be added to the pregnancy category text. In addition, the text suggested by the sponsor for carcinogenicity might change after consulting with the executive carcinogenicity assessment committee (CAC).

Carcinogenesis, mutagenesis, and impairment of fertility

No mutagenic potential of abatacept was observed in the in vitro reverse Ames or Chinese hamster ovary/hypoxanthine guanine phosphoribosyl-transferase (CHO/HGPRT) forward point mutation (with or without S-9 activation) assays, and no chromosomal aberrations were observed in human lymphocytes (with or without metabolic activation) treated with abatacept. In rats, abatacept had no adverse effects on male or female fertility at doses up to [redacted] based on AUC.

In a mouse carcinogenicity study, weekly subcutaneous injections of 20, 65, or 200 mg/kg of abatacept administered each week for up to 84 weeks in males and 88 weeks in females were associated with increases in the incidence of malignant lymphomas (all doses) and mammary gland tumors (intermediate- and high-dose in females). [redacted]

[redacted]. The doses used in these studies were 0.8-, [redacted] and 3.0-fold, [redacted] the human exposure at 10 mg/kg based on AUC. The relevance of these findings to the clinical use of ORENCIA is unknown.

In a one-year toxicity study in cynomolgus monkeys, abatacept was administered intravenously once weekly at doses up to 50 mg/kg (9— fold the human exposure at 10 mg/kg based on AUC). Abatacept was not associated with any significant drug-related toxicity. Reversible pharmacological effects consisted of minimal transient decreases in serum IgG and minimal to severe lymphoid depletion of germinal centers in the spleen and/or lymph nodes. No evidence of lymphomas or preneoplastic morphologic changes was observed, despite the presence of a virus (lymphocryptovirus) known to cause these lesions in immunosuppressed monkeys within the time frame of this study. The relevance of these findings to the clinical use of ORENCIA is unknown.

[

]

Nursing Mothers

Abatacept has been shown to be present in rat milk. It is not known whether abatacept is excreted in human milk or absorbed systemically after ingestion. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants from ORENCIA, [

]

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The sponsor conducted general toxicity, genotoxicity, carcinogenicity, reproductive/developmental toxicity, local tolerance and immunotoxicology studies.

General toxicology studies were conducted in rats, mice and monkeys administered subcutaneously and/or intravenously. The duration of exposure in these studies ranged from a single dose to 1-year of weekly dosing. BMS-188667 was pharmacologically active in all of the toxicology species used (mice, rats,

monkeys) as determined by suppression of T-cell-dependent antibody responses or models of efficacy. The pharmacokinetic characteristics of BMS-188667 in mice, rats, rabbits and monkeys were comparable to humans. Systemic exposure was related to the dose with no apparent gender differences and only minimal accumulation upon repeat dosing. The terminal half-life in animals ranged from 3-6 days in mice and rats, 1-3 days in rabbits and 5-7 days in monkeys.

In a pivotal single-dose intravenous toxicity study performed in monkeys at doses ranging from 10 to 100 mg/kg. BMS-188667 was well tolerated at 100 mg/kg dose (x10 human dose) with no target organ toxicity identified. In the pivotal repeat-dose studies, reversible pharmacologic changes observed included minimal decreases in serum IgG in mice, rats, and monkeys, decreases in the percentage of splenic B cells and inhibition of ex vivo B- and T-cell mitogen activation in mice and mild to moderate decreases in the number and diameter of germinal centers, reflective of a decrease in germinal center activity, in the spleen and/or lymph nodes of monkeys. The NOEL and NOAEL in the 1-year monkey study were <10 and 50 mg/kg/weekly, respectively providing estimated human exposure multiples of <1.9 and 9.2, respectively. These changes in immune parameters were not associated with any clinical manifestation of an infection in any of these repeat-dose studies of up to 1 year in duration. In a repeat-dose study in mice, but not primates, an increase in the incidence and severity of karyomegaly in renal tubular epithelial cells occurred following chronic treatment. In this 6-month mouse study, the NOEL was 20 mg/kg (0.9-fold human exposure), but this finding was observed at all doses (≥20 mg/kg) following longer treatment in the mouse carcinogenicity study. This renal finding was not associated with any effects on renal function and was interpreted as a spontaneous, age-related renal change that occur in mice but has no known relevance to humans, therefore, the NOAEL was considered to be 200 mg/kg (human exposure multiple of 4.7).

BMS-188667 was not mutagenic in the Ames assay at concentrations up to 5000 µg/plate, in a Chinese hamster ovary/hypoxanthine guanine phosphoribosyl-transferase (CHO/HGPRT) assay at concentrations up to 3180 µg/ml, or clastogenic in *in vitro* chromosomal aberration test in primary human lymphocytes at concentrations up to 3110 µg/ml, with or without S-9 metabolic activation.

Since BMS-188667 is being developed for long-term use as a selective immunomodulator, and long-term immunosuppression has been associated with increased incidence of neoplasia in humans and rodents, a rodent carcinogenicity study was performed. BMS-188667 is biologically active and not immunogenic in rodents when maintained at biologically active levels. As suggested in the ICH guidelines for biologics, only a single rodent species was evaluated. The mouse was used since the drug has demonstrated activity in this species. In addition, the literature indicated that long-term immunosuppressed mice have an increased incidence of neoplasia (particularly lymphomas), and a previous chronic toxicity study in the mouse enabled dose selection.

BMS-188667 was administered subcutaneously once weekly at doses of 20, 65, or 200 mg/kg. Systemic exposures to BMS-188667 after the 53rd weekly dose were dose related with corresponding exposure multiples of 0.8, 1.9, and 3.0 times, respectively, that of humans administered 10 mg/kg monthly. At weeks 84 and 88, 25% survivability was reached in the male and female low-dose groups, respectively, and after consultation with the FDA, all remaining animals of that sex were sacrificed.

The survival rate was lower in BMS-188667-treated mice relative to controls. In general, the incidence of death/morbidity was similar in all treated groups. Lymphoma was the apparent cause of death for approximately 50% of the drug-treated mice. Statistically significant increases in lymphoma ($p<0.0001$) were observed microscopically in all treated groups, but group incidences were not dose-related. Incidence of lymphoma in CD-1 mice administered BMS-188667 was higher than reported for prior carcinogenicity studies at BSM and in the published literature. The incidence of mammary gland adenocarcinomas was statistically significantly increased in females at 65 and 200 mg/kg/week. The incidences of mammary gland adenomas alone were not statistically increased when compared with controls, although they occurred at a greater percentage than those noted in previous mouse studies. The incidence of adenocarcinomas in high-dose females was greater than the highest control range of 12% reported by Charles River Laboratory in 1995 and the incidence in both intermediate- and high-dose groups were well above BMS in-house historical control levels. The incidence in the vehicle-control group, although higher than previously seen in controls in BMS laboratory, was not significantly different from the saline-control group. Thus, the saline- and vehicle-control groups were combined (as per protocol) for statistical comparison to treated groups. Based on the significance of $P <0.0001$ in the Peto and Pike trend test, which adjusts for mortality, and the fact that the incidences were above the in-house historical controls (0-1%), the increased incidence of mammary gland carcinomas at 65 and 200 mg/kg/week were considered to be drug related.

Drug-related non-neoplastic findings were limited to increases in the incidence and severity of karyomegaly in renal tubular epithelial cells associated with chronic inflammation and tubular degeneration at all doses. These renal findings were not associated with any detectable functional renal changes and are believed to be of no relevance to humans.

In mice, retroviruses (MLV and 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 verbal communication with Charles River Laboratories personnel indicated that CD-1 mice are not retrovirus free. Results from transmission electron microscopic evaluation of mammary tumors from this study identified large numbers of virions in the cytoplasm, budding from the plasma membrane, and in the extracellular space. Ultrastructural characteristics of the viral particles were consistent with those of murine mammary tumor viruses and immuno-

histochemistry with an anti-MMTV antibody confirmed the presence of this oncovirus in mammary tumors from both control- and abatacept-treated mice. Significant immunosuppression was observed at every dose level in this study as demonstrated by the ability of BMS-188667 to suppress antibody response. Immunosuppression was also confirmed at these doses in a separate pharmacokinetic/pharmacodynamic study, using the same route and dosing regimen as the carcinogenicity study, that demonstrated strong suppression of the KLH antibody response and anti-drug antibody response at ≥20 mg/kg. These findings support the conclusion by the sponsor that the increased malignancies in this study were not a direct drug effect but likely secondary to long-term induced immuno-suppression and the control of these specific oncoviruses. In addition, this conclusion is further strengthened by the absence of any genotoxicity observed in a battery of tests with BMS-188667.

A complete battery of reproductive toxicity studies was conducted with BMS-188667. Fetal exposure was demonstrated following the administration of BMS-188667 to pregnant rats and rabbits (not determined in mice), and secretion of abatacept into milk was demonstrated in lactating rats. BMS-188667 did not show any adverse effect on fertility, reproductive function, gestation, parturition, or lactation in the F_0-generation rats. It also did not affect embryonic and fetal development in mice, rats and rabbit, and growth, development, and reproductive performance of F_1-generation rats. BMS-188667 crossed the placenta and fetuses were exposed during organogenesis. The immune function in the offspring rats was generally unaffected. Drug-related changes in the F_1-generation were limited to females and consisted of an increase (9-fold) in the T-cell-dependent antibody response and inflammation of the thyroid gland of one rat. These findings were considered to represent the lower threshold limit for effects of BMS-188667 on immune parameters in F_1-generation rats as these changes were either limited to only one sex or one animal, and no other immune parameters were affected (splenic-lymphocyte and natural killer-cell phenotypes, serum Ig levels, and presence of anti-nuclear antibodies). BMS-188667 was also detected in the milk of rats.

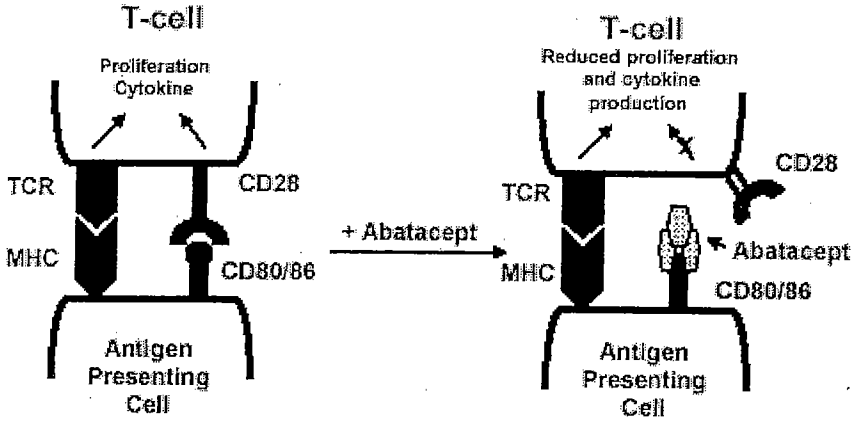
The local tolerance of BMS-188667 intended for marketing (Process E) was assessed after single IV, intra-arterial, and paravenous injections at concentrations similar to or greater than those to be used in humans in New Zealand White rabbits. No injection-site irritation was observed with any route of parenteral administration. In addition, no adverse effects at the injection site were seen in repeat-dose studies up to 1 year in monkeys. The subcutaneous route was also evaluated for clinical use, therefore, local tolerance of the product was assessed after single- and repeat-dose subcutaneous administration in rats. No significant injection-site irritation was observed.

BMS-188667 specific antibodies, were evaluated in all pivotal toxicity studies, as well as a number of exploratory/investigative studies. BMS-188667, a fully human fusion protein, was immunogenic in mice, rats, dogs, and monkeys. However, BMS-188667-specific antibodies were detected mostly during the

recovery period, after drug serum concentrations had dropped below immunosuppressive levels. Thus, in each species tested, BMS-188667 suppressed the antibody response against itself for the duration of treatment. When BMS-188667–specific antibodies were present, clearance of drug from the vascular compartment was often accelerated. The appearance of abatacept-specific antibodies was not associated with any acute or target-organ toxicity in any species when drug exposure was continuous. In mice and dogs, when drug levels fell below immunomodulatory levels and the animals were subsequently administered an IV challenge dose of abatacept, the presence of circulating abatacept-specific antibodies was associated with clinical signs of hypersensitivity reactions.

B. Pharmacologic activity

BMS-188667 is a human fusion protein that selectively modulates T-cell co-stimulation via CD28 by binding to CD80 and CD86 surface-receptors on antigen presenting cells, thus blocking its interaction with CD28 on T cells. The following figure summarizes the binding and activity of BMS-188667.



TCR = T-cell receptor; MHC = major histocompatibility complex

BMS-188667 modulates CD28-mediated co-stimulation resulting in decreased antigen-specific T-cell proliferation and decreased pro-inflammatory cytokine production by human naive T cells in vitro. It also partially inhibits antigen-specific circulating human memory T-cell proliferation and cytokine production in vitro. BMS-188667 does not appear to affect CD8+ T-cell cytolytic activity and the production of TNF-α from LPS-activated monocytes in vitro. In vivo, selective co-stimulation modulation by BMS-188667 resulted in decreased antigen-specific antibody production to T cell-dependent antigens in rodents and nonhuman primates.

C. Nonclinical safety issues relevant to clinical use

The main concern identified during nonclinical testing was an increase in the incidence of malignant lymphomas and mammary gland tumors (in females) in the mouse carcinogenicity study. The increased incidence of lymphomas and mammary tumors observed in mice treated with abatacept (BMS-188667) was associated with the decreased control of murine leukemia virus and mouse mammary tumor virus, respectively, in the presence of long-term immunomodulation. No mutagenic potential of abatacept and no chromosomal aberrations in human lymphocytes with abatacept were observed in a battery of in vitro genotoxicity studies. These findings support the conclusion by the sponsor that the increased malignancies in this study were secondary to long-term induced immunosuppression and the control of these specific oncoviruses. These concerns have been discussed with the clinical review staff and are being addressed through labeling and/or post-marketing commitments.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

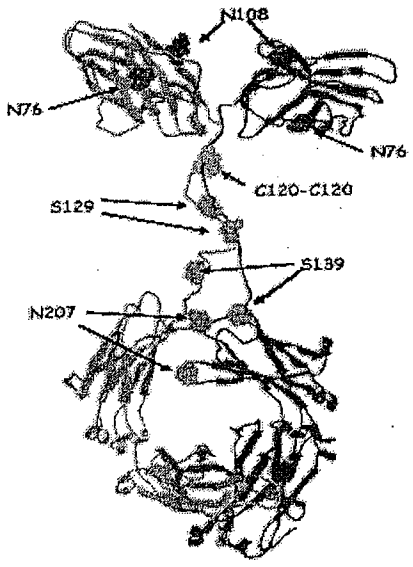
BLA number: 125118
Review number: 1
Sequence number/date/type of submission: 0/Nov.16, 2004/BLA
Information to sponsor: Yes (x) No ( )
Sponsor and/or agent: Bristol-Myers Squibb

Manufacturer for drug substance: Bristol-Myer Squibb Company, East Syracuse, NY

Reviewer name: Hanan Ghantous, Ph.D., DABT
Division name: Division of Therapeutic Biologic Internal Medicine Products
HFD #:108
Review completion date: April 26, 2005

Drug:

Trade name: Orencia™ (proposed by the sponsor)
Generic name: Abatacept
Code name: BMS-188667, CTLA4Ig
Chemical name: 1-25-oncostatin M (human precursor) fusion protein with CTLA-4 (antigen) (human) fusion protein with immunoglobulin G1 (human heavy chain fragment)
CAS registry number: 332348-12-6
Molecular formula/molecular weight: ~92300 Daltons
Structure: A model of abatacept is shown below with the N-linked glycosylation sites (N76, N108 and N207), O-linked glycosylation sites (S129 and S139), and the C120-C120 disulfide bond.



Relevant INDs/NDAs/DMFs: IND-9391

Drug class: Recombinant, fusion protein consisting of extracellular domain of human CTLA-4 and a fragment (hinge-CH_2-CH_3 domains) of Fc domain of human IgG1.

Intended clinical population: Patients with moderate to sever rheumatic arthritis who have failed at least 1 disease-modifying anti-rheumatic drug (DMARD), including tumor necrosis factor (TNF) blocking agents.

Clinical formulation: Abatacept for injection, 250 mg vial, is a sterile non-pyrogenic lyophylic for IV injection. Each vial contains the following ingredients:

Component	Quality Standard	Function	mg per Vial[a]
Abatacept	BMS Specification	Active Ingredient	
Maltose	BMS Specification		
Sodium Phosphate, Monobasic,	USP		
Sodium Chloride	USP		
	NF		
	NF		

[a] Each vial.

[b] These components are present in the abatacept drug substance solution

(Table was copied from the submission)

Route of administration: Intravenously

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:
Single-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys (Study # 94703)
Repeat-Dose Subcutaneous/Intravenous Toxicity and Toxicokinetic Study in Rats (Study # 95676)
Six-Month Intermittent-Dose (QWx26) Subcutaneous Toxicity Study in Mice (Study # 96633)
One-Month Intermittent-Dose Intravenous Toxicity Study in Monkeys (Study # 94704)
Six-Month Intermittent-Dose Intravenous Toxicity and Toxicokinetics Study in Monkeys (Study # 99655)
One-Year Intermittent-Dose Intravenous Toxicity and Toxicokinetic Study in Monkeys (Study # DS0228)
Subcutaneous Carcinogenicity Study in Mice (Study # 97610)
Ames Reverse-Mutation study in Salmonella and *Escherichia* (Study # 96616)
CHO/HGPRT Mammalian-Cell Forward Gene-Mutation Study (Study # 95713)

Cytogenetics Study in Primary Human Lymphocytes (Study # 94729)
Intravenous Study of Fertility and Early Embryonic Development in Rats (Study # DN01093)
Study of Embryo-Fetal Development in Rats (Study # 95024)
Study of Embryo-Fetal Development in Mice (Study # 95019)
Intravenous Study of Embryo-Fetal Development in Rabbits (Study # DN02003)
BMS-188667: Intravenous Study of Pre- and Postnatal Development in Rats (Study # DN01060)

The following studies have been reviewed and tabulated summaries as presented by the sponsor are listed in sections (2.6.6.7 Local tolerance and 2.6.6.8 Special toxicology studies).
Intravenous Toxicokinetics Study in Pregnant and Lactating Rats (Study # DN03068)
Thirteen-Day Intravenous Toxicokinetics Study in Pregnant Rabbits (Study # DN03069)
Single-Dose Intravenous, Intraarterial, and Paravenous Tolerance Study in Rabbits (Study # DS03238)
Two-Week Intermittent-Dose Subcutaneous Irritation and Comparative Toxicokinetic Study in Rats (Study # 96615)
Single-Dose Subcutaneous Exploratory Comparative Irritation Study in Rats (Study # DS03019)
Single-Dose Intravenous Immunomodulatory Study in Mice (Study # 92643)
Five-Day Intravenous Toxicity and *Ex Vivo* Immunotoxic Assessment in Mice (Study # 92675)

Studies not reviewed within this submission: Pharmacology and Pharmacokinetic studies were reviewed by Anita O'Connor Ph.D.

2.6.2 PHARMACOLOGY

(See Pharmacology review by Anita O'Connor, Ph.D.)

2.6.2.1 Brief summary
2.6.2.2 Primary pharmacodynamics
2.6.2.3 Secondary pharmacodynamics
2.6.2.4 Safety pharmacology
2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

(See Review by Anita O'Conner, Ph.D.)

2.6.4.1 Brief summary
2.6.4.2 Methods of Analysis
2.6.4.3 Absorption
2.6.4.4 Distribution
2.6.4.5 Metabolism
2.6.4.6 Excretion
2.6.4.7 Pharmacokinetic drug interactions
2.6.4.8 Other Pharmacokinetic Studies
2.6.4.9 Discussion and Conclusions
2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: General toxicology studies were conducted in rats, mice and monkeys administered subcutaneously and/or intravenously. The duration of exposure employed in these studies ranged from a single dose to 1-year of weekly dosing. BMS-188667 was pharmacologically active in all of the toxicology species used (mice, rats, monkeys) as determined by suppression of T-cell-dependent antibody responses or models of efficacy. The pharmacokinetic characteristics of BMS-188667 in mice, rats, rabbits and monkeys were comparable to humans. Systemic exposure was related to the dose with no apparent gender differences and only minimal accumulation upon repeat dosing. The terminal half-life in animals ranged from 3-6 days in mice and rats, 1-3 days in rabbits and 5-7 days in monkeys.

In a pivotal single-dose intravenous toxicity study performed in monkeys at doses ranging from 10 to 100 mg/kg. BMS-188667 was well tolerated at 100 mg/kg dose (x10 human dose) with no target organ toxicity identified. This study sufficiently characterized the acute toxicity of intravenously administered BMS-188667 and provided relevant information with regard to potential clinical effects following accidental overdose to humans.

Repeat-dose studies were conducted in rats, mice and monkeys. In rats, a 2-week intravenous/subcutaneous GLP study was conducted to support subcutaneous dosing in the clinic. In mice, a 6-month subcutaneous GLP study was conducted prior to the carcinogenicity study to aid in selection of doses for the mouse carcinogenicity study and to support long-term treatment in clinical trials. In monkeys, 1-month and 1-year GLP studies were conducted with BMS-188667 to support 1-month and long-term treatment, respectively. A 6-month GLP monkey study conducted with BMS-224818, the second generation molecule, is also included to support long-term treatment.
Systemic exposure (mean combined-gender, steady-state, 168-hr AUC values) in the pivotal repeat-dose toxicity studies compared to the therapeutic dose in humans (10 mg/kg administered once monthly) are presented below.

Species	Study	Dose (mg/kg)	AUC (TAU) (µg•h/mL)	AUC (30 days) (µg•h/mL)	Multiple of Human Exposure (x)
Human[a]	Multiple dose once monthly, IV	10	50,102	50,102	--
Mouse	6-month, once weekly, subcutaneous	20	10,033	43,142[b]	0.9
		65	29,581	127,198[b]	2.5
		200	54,478	234,255[b]	4.7
Monkey	1-year, once weekly, IV	10	22,092	94,996[b]	1.9
		22	47,253	203,188[b]	4.1
		50	107,402	461,829[b]	9.2

[a] Source of human AUC data - Study IM101-100; exposures determined at steady state

[b] AUC (TAU), where TAU = 7 days, was multiplied by 4.3 to normalize for 1 month of exposure; exposures determined after the 26th dose in mice and the 52nd dose in monkeys

(Table was copied from the submission)

In the pivotal repeat-dose studies, reversible pharmacologic changes observed included minimal decreases in serum IgG in mice, rats, and monkeys, decreases in the percentage of splenic B cells and inhibition of ex vivo B- and T-cell mitogen activation in mice and mild to moderate decreases in the number and diameter of germinal centers, reflective of a decrease in germinal center activity, in the spleen and/or lymph nodes of monkeys. The NOEL and NOAEL in the 1-year monkey study were <10 and 50 mg/kg/weekly, respectively, providing estimated human exposure multiples of <1.9 and 9.2, respectively. These changes in immune parameters were not associated with any clinical manifestation of an infection in any of these repeat-dose studies of up to 1 year in duration. In repeat-dose study in mice, but not primates, an increase in the incidence and severity of karyomegaly in renal tubular epithelial cells occurred following chronic treatment. In this 6-month mouse study, the NOEL was 20 mg/kg (0.9-fold human exposure), but this finding was observed at all doses (≥20 mg/kg) following longer treatment in the mouse carcinogenicity study. However, this renal finding was not associated with any effects on renal function and was interpreted by the sponsor as spontaneous, age-related renal changes that occur in mice but has no known relevance to humans, therefore the NOAEL was considered to be 200 mg/kg (human exposure multiple of 4.7).

Genetic toxicology: Genotoxicity testing is usually not required for biotechnology-derived pharmaceuticals for it is not expected that these molecules interact directly with DNA or other chromosomal material. However, the sponsor evaluated the genotoxic potential of BMS-188667 in a battery of in vitro test systems with and without metabolic activation. BMS-188667 was not mutagenic in the Ames assay at concentrations up to 5000 µg/plate, in a CHO/HGPRT assay at concentrations up to 3180 µg/ml, or clastogenic in *in vitro* chromosomal aberration assay in primary human lymphocytes at concentrations up to 3110 µg/ml, with or without S-9 metabolic activation.

Carcinogenicity: BMS-188667 is being developed for long-term use as a selective immunomodulator, and long-term immunosuppression has been associated with

increased incidence of neoplasia in humans and rodents. In addition, BMS-188667 was biologically active and not immunogenic in rodents when maintained at biologically active levels. Therefore, a rodent carcinogenicity study was performed and as suggested in the ICH guidelines for biologics, only a single rodent species was evaluated. The mouse was the species selected as the drug has demonstrated activity in mice, the literature indicated that long-term immunosuppressed mice have an increased incidence of neoplasia (particularly lymphomas), and a previous chronic toxicity study in the mouse enabled dose selection.

BMS-188667 was administered subcutaneously once weekly at doses of 20, 65, or 200 mg/kg. Systemic exposures to BMS-188667 after the 53rd weekly dose were dose related with corresponding exposure multiples of 0.8, 1.9, and 3.0 times, respectively, that of humans administered 10 mg/kg monthly (table below). At weeks 84 and 88, 25% survivability was reached in the male and female low-dose groups, respectively, and, after consultation with the FDA, all remaining animals of that sex were sacrificed.

Species	Study	Dose (mg/kg)	AUC (TAU) (µg•h/mL)	AUC (30 days) (µg•h/mL)	Multiple of Human Exposure (x)
Human[a]	Multiple dose once monthly, IV	10	50,102	50,102	--
Mouse	Once weekly, subcutaneous	20	8,812	37,889[b]	0.8
		65	22,600	97,178[b]	1.9
		200	34,925	150,178[b]	3.0

[a] Source of human AUC data - Study IM101-100; exposure at steady state

[b] AUC, interval = 168 h (7 days), multiplied by 4.3 to normalize for 1 month of exposure; exposures determined after the 53rd dose

(Table was copied from the submission)

The survival rate was lower in BMS-188667-treated mice relative to controls. In general, the incidence of death/morbidity was similar in all treated groups. Lymphoma was the apparent cause of death for approximately 50% of the drug-treated mice. Statistically significant increases in lymphoma ($p<0.0001$) were observed microscopically in all treated groups, but group incidences were not dose-related. Incidence of lymphoma in CD-1 mice administered BMS-188667 was higher than reported for prior carcinogenicity studies at BSM and in the published literature. The incidences of mammary gland adenocarcinomas were statistically significantly increased in females at 65 and 200 mg/kg/week. The incidences of mammary gland adenomas alone were not statistically increased when compared with controls, although they occurred at a greater percentage than those noted in previous mouse studies. The incidence of adenocarcinomas in high-dose females was greater than the highest control range of 12% reported by Charles River Laboratory in 1995 and the incidence in both intermediate- and high-dose groups were well above BMS in-house historical control levels. The incidence in the vehicle-control group, although higher than previously seen in controls in BMS laboratory, was not significantly different from the saline-control group. Thus, the saline- and vehicle-control groups were combined (as per protocol) for

statistical comparison to treated groups. Based on the significance of P <0.0001 in the Peto and Pike trend test, which adjusts for mortality, and the fact that the incidences were above the in-house historical controls (0-1%), the increased incidence of mammary gland carcinomas at 65 and 200 mg/kg/week were considered to be drug related.
Drug-related non-neoplastic findings were limited to increases in the incidence and severity of karyomegaly in renal tubular epithelial cells associated with chronic inflammation and tubular degeneration at all doses. These renal findings were not associated with any detectable functional renal changes and are believed to be of no relevance to humans.

In mice, retroviruses (MLV and 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 verbal communication with Charles River Laboratories personnel indicated that CD-1 mice are not retrovirus free. Results from transmission electron microscopic evaluation of mammary tumors from this study identified large numbers of virions in the cytoplasm, budding from the plasma membrane, and in the extracellular space. Ultrastructural characteristics of the viral particles were consistent with those of murine mammary tumor viruses and immunohistochemistry with an anti-MMTV antibody confirmed the presence of this oncovirus in mammary tumors from both control- and abatacept-treated mice. Significant immunosuppression was observed at every dose level in this study as demonstrated by the ability of BMS-188667to suppress antibody responses. Immunosuppression was also confirmed at these doses in a separate pharmacokinetic/pharmacodynamic study, using the same route and dosing regimen as the carcinogenicity study, that demonstrated strong suppression of the KLH antibody response and anti-drug antibody response at ≥20 mg/kg. These findings support the conclusion by the sponsor that the increased malignancies in this study were not a direct effect of the drug but likely secondary to long-term induced immunosuppression and the control of these specific oncoviruses. In addition, this conclusion is further strengthened by the absence of any genotoxicity observed in a battery of assays with BMS-188667.

Reproductive toxicology: A complete battery of reproductive toxicity studies was conducted with BMS-188667. Systemic exposures to BMS-188667 in support of the reproductive studies were measured from separate TK studies in pregnant rats or rabbits. TK profiles were not established in pregnant mice. Fetal exposure was demonstrated following the administration of BMS-188667 to pregnant rats and rabbits (not determined in mice), and secretion of abatacept into milk was demonstrated in lactating rats. Multiples of BMS-188667 exposures in the reproductive studies in rats and rabbits compared to humans are listed below.

Species	Study	Dose (mg/kg)	AUC (TAU) (µg•h/mL)	AUC (30 days) (µg•h/mL)	Multiple of Human Exposure (x)
Human[a]	Multiple dose once monthly, IV	10	50,102	50,102	--
Rat	Segment I and III every 3 days, intravenously	45	14,983	149,830[b]	3
		200	54,646	546,460[b]	11
Rat	Segment II daily, intravenously	45	15,009	450,270[c]	9
		200	49,281	1,478,430[c]	30
Rabbit	Segment I every 3 days, intravenously	200	145,681	1,456,810[b]	29

Segment I: IV study of fertility and embryonic development
Segment II: IV study of embryo-fetal development
Segment III: IV study of pre- and postnatal development
[a] Source of human AUC data - Study IM101-100; exposures obtained at steady state
[b] AUC (TAU) where TAU = 3 days, multiplied by 10 to normalize for 1 month of exposure
[c] AUC (TAU) where TAU =1 day, multiplied by 30 to normalize for 1 month of exposure

(Table was copied from the submission)

BMS-188667 did not show any adverse effects on fertility, reproductive function, gestation, parturition, or lactation in the F_0-generation rats. It also did not affect embryonic and fetal development in mice, rats and rabbit, and growth, development, and reproductive performance of F_1-generation rats. BMS-188667 crossed the placenta and fetuses were exposed during organogenesis. The immune function in the offspring rats was generally unaffected. Drug-related changes in the F_1-generation were limited to females and consisted of an increase (9-fold) in the T-cell-dependent antibody response and inflammation of the thyroid gland of 1 rat. These findings were considered to represent the lower threshold limit for effects of BMS-188667 on immune parameters in F_1-generation rats as these changes were either limited to only one sex or one animal, and no other immune parameters were affected (splenic-lymphocyte and natural killer-cell phenotypes, serum Ig levels, and presence of anti-nuclear antibodies). BMS-188667 was also detected in the milk of rats.

Local Tolerance: The local tolerance of BMS-188667 intended for marketing (Process E) was assessed after single IV, intra-arterial, and paravenous injections at concentrations similar to or greater than those to be used in humans in New Zealand White rabbits. No injection-site irritation was observed with any route of parenteral administration. In addition, no adverse effects at the injection site were seen in repeat-dose studies up to 1 year in monkeys. The subcutaneous route was also evaluated for clinical use, therefore, local tolerance of the product was assessed after single- and repeat-dose subcutaneous administration in rats. No significant injection-site irritation was observed.

Immunogenicity: BMS-188667 specific antibodies, were evaluated in all pivotal toxicity studies, as well as a number of exploratory/investigative studies. BMS-188667, a fully human fusion protein, was immunogenic in mice, rats, dogs, and monkeys. However, BMS-188667-specific antibodies were detected mostly during the recovery period, after drug serum concentrations had dropped below immunosuppressive levels. Thus, in each

species tested, BMS-188667 suppressed the antibody response against itself for the duration of treatment. When BMS-188667–specific antibodies were present, clearance of drug from the vascular compartment was often accelerated. The appearance of abatacept-specific antibodies was not associated with any acute or target-organ toxicity in any species when drug exposure was continuous. In mice and dogs, when drug levels fell below immunomodulatory levels and the animals were subsequently administered an IV challenge dose of abatacept, the presence of circulating abatacept-specific antibodies was associated with clinical signs of hypersensitivity reactions.

2.6.6.2 Single-dose toxicity

BMS-188667, (aqueous solution in 25 mM sodium phosphate and 50 mM sodium chloride), was administered to cynomolgus monkeys (2/sex/group) at single intravenous doses of 10 and 33 mg/kg to assess toxicokinetics and immunogenicity for up to 42 days postdose, and 100 mg/kg to assess toxicity for 14 days post dose. A control group was given vehicle. Systemic exposure (AUC and C_{max}) was dose-related and increased with increasing the dose, with no sex-related differences. The $T_{1/2}$ was similar between groups before BMS-188667 specific antibody formation. However, in the presence of BMS-188667-specific antibodies, the rate of elimination increased. BMS-188667-specific antibodies were seen on day 42 in 2 of 4 monkeys given 10 mg/kg and in 3 of 4 monkeys given 33 mg/kg. At 100 mg/kg, no drug-related changes occurred and no BMS-188667-specific antibodies were seen prior to the day 14 necropsy. A single dose of 100 mg/kg was well tolerated in monkeys.

2.6.6.3 Repeat-dose toxicity

Study title: Repeat-Dose Subcutaneous/Intravenous Toxicity and Toxicokinetic Study in Rats

Key study findings: The toxicity and toxicokinetics of repeat-dose subcutaneous and intravenous administration of BMS-188667 once every 2 days for 7 days (q2dx7) was investigated in rats. BMS-188667 was well tolerated at all doses with no target organ toxicity. Changes at the subcutaneous injection sites were seen but considered tolerable for this route of administration. BMS-188667 was immunogenic by both route of administration (but more so by the subcutaneous route), immunogenicity occurred when serum-drug concentrations decreased below immunosuppressive levels. Bioavailability following subcutaneous administration was between 41% (200 mg/kg) and 63% (10 mg/kg).

Study no.: 95676
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Department of Pathology, Biologics Evaluation, and Metabolism and Pharmacokinetics, Syracuse. New York, USA
Date of study initiation: August 16, 1995
GLP compliance: Yes
QA report: Yes

Drug, lot #, and % purity: BMS-188667, Lot # C95201, purity NA

Methods

Doses: 0, 80 and 200 mg/kg SC, 0 and 200 mg/kg IV, administered once every other day for 7 days (q2dx7) over 13 days.
Species/strain: Rats / [J Sprague-Dawley obtained from [J
Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: SQ (20% maltose, 100mM sodium phosphate, and 200mM sodium chloride) and IV (5% maltose, 25mM sodium phosphate, and 50mM sodium chloride). Vehicle contained maltose, sodium phosphate and sodium chloride. Dilutions were performed with sterile water for injection.
Satellite groups used for toxicokinetics: 3/sex/group dosed with 0, 10, 80 and 200 mg/kg SQ or IV as a single dose and 10 mg/kg SQ or IV every other day for 7 days.
Age: 8 weeks for toxicity portion, 9-10 weeks for toxicokinetics portion
Weight: 181-308 g for toxicity portion, 187-345 g for toxicokinetics portion (to facilitate jugular vein cannulation).
Sampling times: Toxicokinetics: for single dose groups, on day 1: pre-dose, 1, 2, 4, 8, 10, 12, 24, 48 and 96 hours post-dose and on days 9, 13, 21, 28, 35 and 42. For multiple dose groups, on day 13: 1, 2, 4, 8, 10, 12, 24, 48 and 96 hours post-dose and days 21, 28, 35, 42, 49, 56 and 63.
Unique study design or methodology (if any):
Serum Immunoglobulin: Total serum Ig levels of IgM, IgG and IgA were assessed pre-dose and on day 14 from rats on the toxicology portion of the study using an ELISA method.
Antigenicity: Serum levels of antibodies for BMS-188667 were assessed on serum samples obtained from the toxicokinetic animals pre-dose and on days 9, 12, 21, 28, 35, 42, 29, 56 and 63 by an ELISA method.

Observations and times:

Mortality: Daily
Clinical signs: Daily
Body weights: Daily
Food consumption: Weekly
Ophthalmoscopy: Twice, pre-start of study and before necropsy.
EKG: Not measured
Hematology: Day 14
Clinical chemistry: Day 14
Urinalysis: Collected over night for 18 hours prior to necropsy.
Gross pathology: Necropsy was performed on all animals in the toxicology portion of the study on day 15. Limited necropsy was performed on animals from the toxicokinetic portion that died or were sacrificed moribund.
Organ weights: See "Histopathology Inventory" table.

Histopathology: Adequate Battery: Yes
Peer review: Yes
Toxicokinetics: Blood samples (~ 0.2 ml) were taken from the jugular vein of cannulated rats or tail vein when withdrawal from jugular vein was not possible. The concentration of BMS-188667 was determined by using a validated ELISA procedure.

Results

Mortality: Death occurred in a non-dose-related manner in 5/48 toxicokinetic rats (3 received 10 mg/kg and 2 received 80 mg/kg either IV or SC) between Days 17 and 44. Death was attributed due to long-term cannulation. No death occurred in the toxicology rats.
Clinical signs: No drug-related observations were observed.
Body weights: No drug-related changes were observed.
Food consumption: No drug-related changes were observed.
Ophthalmoscopy: Results were not mentioned in the report.
EKG: Not measured.
Hematology: Minimal decreases in hematocrit (males at 200 mg/kg, SC) and increases in fibrinogen (females at 200 mg/kg, SC).
Clinical Chemistry: Minimal changes noted in animals given 80 or 200 mg/kg subcutaneously and 200 mg/kg intravenously included increases in serum cholesterol, chloride, and sodium, and decreases in serum aspartate aminotransferase (AST) and creatine kinase, decrease in these enzymes might indicate a reduction in protein synthesis. Minimal decrease in serum albumin was also seen at 80 and 200 mg/kg, SC.
Urinalysis: No drug-related changes were observed.
Gross pathology: No drug-related gross findings were observed.
Organ weights: No drug-related organ weight changes were observed.
Histopathology: Minimal to mild subcutaneous hemorrhage and minimal to moderate inflammation at the injection sites were observed microscopically in animals given subcutaneous injections, including controls. The chronic inflammatory infiltrate was minimally greater among drug-treated rats than control.
Toxicokinetics: systemic exposures (C_{max} and AUC) increased with increasing the dose following single intravenous doses of BMS-188667. $T_{1/2}$ values increased as the administered dose increased. After a single subcutaneous dose, increases in C_{max} and AUC values were also dose proportional while T_{max} was prolonged (36 to 48 h). Mean TK parameters for a single dose, are listed in the table below:

Dose mg/kg	Route	C_{max} µg/ml	T_{max} hours	$AUC_{0-\alpha}$ µg.h/ml	MRT hours	$T_{1/2}$ hours	CLT ml/h/kg	VSS mg/kg
10	SC	26	48	5536	165	74	-	-
80	SC	133	48	35153	232	132	-	-
200	SC	262	36	56900	223	167	-	-
10	IV	243	0.05	8857	131	64	1.2	147
80	IV	2162	0.05	63167	152	108	1.3	193
200	IV	4610	0.05	138608	167	170	1.5	243

- value not calculated

Mean TK parameters for multiple doses (q2dx7) of BMS-188667 at 10 mg/kg dose level are listed in the table below.

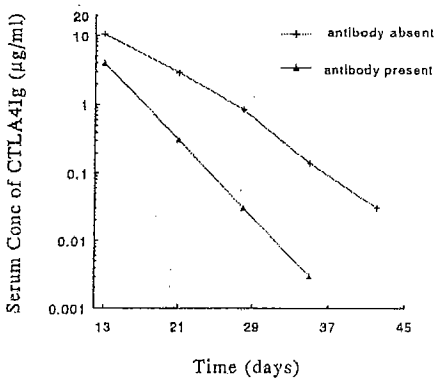
Route	C_{max} µg/ml	$AUC_{0-\alpha}$ µg.h/ml	$T_{1/2}$ hours
Sc	99	4340	96
IV	414	9412	115

Three to five fold accumulations occurred after multiple intravenous or subcutaneous doses. Bioavailability following subcutaneous administration was between 63% (10 mg/kg) and 41% (200 mg/kg).

Route	AUC(TAU) (h. ug/ml)		Ratio of AUC(TAU) last dose / first dose
	First dose	Last (7th) dose	
Subcutaneous	934.5	4340	4.7
Intravenous	3041	9412	3.1

(Table was copied from the submission)

The decline in serum concentrations of BMS-188667 was faster in samples that showed significant anti BMS-188667 antibody formation. An example of serum level of BMS-188667 in rats in the presence and absence of antibodies is shown in the graph below:



(Graph was copied from the submission)

Serum Immunoglobulin: A decrease in serum IgG and IgA (but not IgM) levels was seen on day 14 in rats receiving multiple 200 mg/kg intravenous or subcutaneous doses. This decrease was similar in both subcutaneously and intravenously treated animals and was considered a pharmacologic effect. Summary of serum IgG, IgM and IgA levels is listed in the table below.

			IgG µg/ml .		IgM µg/ml		IgA µg/ml	
Dose mg/kg	**Route**	**Sex**	**Pre-dose**	**Day 14**	**Pre-dose**	**Day 14**	**Pre-dose**	**Day 14**
control	SC	M	1305	1776	354	502	15	23
		F	2266	4834	555	811	27	51
control	IV	M	1186	2024	343	471	20	30
		F	1437	3978	450	710	24	56
80 q2dx7	SC	M	1152	1061	403	472	19	20
		F	2594	3147	660	617	46	40
200 q2dx7	SC	M	1213	890	387	418	17	11
		F	1774	1210	558	525	24	18
200 q2dx7	IV	M	1181	529	337	333	20	7
		F	1793	1306	484	539	37	18

Antigenicity: BMS-188667-specific antibodies were seen after single or repeated doses in the 10 mg/kg groups treated subcutaneously and intravenously. The onset of the BMS-188667-specific antibody response in rats receiving repeat doses was delayed relative to rats receiving single doses. This delay is likely due to the length of dosing and accumulation of BMS-188667 following repeat-dose administration. There was a greater incidence and magnitude of drug-specific antibody formation in the rats receiving subcutaneous doses compared to rats receiving intravenous doses which is probably due to lower serum concentrations of BMS-188667 following subcutaneous administration or that antigen presentation is more efficient by the subcutaneous route. This data suggest that BMS-188667 is immunogenic in the rat when serum-drug levels decrease below immunosuppressive levels. Incidence of anti- BMS-188667 antibody formation is listed in the table below.

Dose mg/kg	**Route**	**Sex**	**Day 9**	**Day 13**	**Day 21**	**Day 28**	**Day 35**	**Day 42**	**Day 49**	**Day 56**	**Day 63**
10 q2dx7	SC	M	0/3	0/3	0/3	0/3	0/3	2/3	2/3	2/3	2/3
		F	0/3	0/3	0/3	0/3	0/3	0/3	2/3	2/3	3/3
10 q2dx1	SC	M	0/3	0/3	0/3	3/3	3/3	3/3	NA	NA	NA
		F	0/3	0/3	0/2	1/2	2/2	2/2	NA	NA	NA
80 q2dx1	SC	M	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
		F	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
200 q2dx1	SC	M	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
		F	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
10 q2dx7	IV	M	0/3	0/3	0/3	0/3	1/3	0/3	0/2	1/2	1/2
		F	0/3	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
10 q2dx1	IV	M	0/3	0/3	0/3	0/3	1/3	1/3	NA	NA	NA
		F	0/3	0/3	1/3	1/2	2/2	2/2	NA	NA	NA
80 q2dx1	IV	M	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
		F	0/3	0/3	0/3	0/3	0/2	0/2	NA	NA	NA
200 q2dx1	IV	M	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA
		F	0/3	0/3	0/3	0/3	0/3	0/3	NA	NA	NA

NA – non-applicable, animals were sacrificed on day 42.
An individual animal was considered to have positive anti BMS-188667 antibody response when its titer increased by two or more serial solutions compared to its predose value.

Study title: Six-Month Intermittent-Dose (QWx26) Subcutaneous Toxicity Study in Mice

Key study findings: The toxicity and toxicokinetics of BMS-188667 was studied in mice after subcutaneous administration of 20, 65, or 200 mg/kg once weekly for 6 months followed by 4-month recovery period. Systemic exposure increased with increasing the dose, but was less than dose-proportional. BMS-188667 was well tolerated at all dose levels. At doses of 65 and 200 mg/kg, decreases in the percentages of splenic B-cells (~ 60 to 85%) and inhibition of ex vivo B- and T-cell activation in males and a decrease in serum IgG was observed at the end of the 6-month dosing period, but recovered by the end of the 4-month recovery period. Organ weight changes in mice given 65 or 200 mg/kg were reversible increases in splenic weights and reversible decreases in thymic weights. Drug-related histopathology findings at these doses were generally reversible increases in the incidence and severity of karyomegaly in renal tubular epithelial cells, accompanied by mild, multifocal, chronic inflammation, lymphocytic infiltration, and tubular cell degeneration. These renal changes were not associated with any effect on renal function, therefore they were considered spontaneous and age-related. Based on these results, the NOAEL was considered 200 mg/kg, the highest dose level tested.

Study no.: 96633
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Department of Pathology, Biologics Evaluation, and Metabolism and Pharmacokinetics, Syracuse. New York, USA
Date of study initiation: June 11, 1996
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: BMS-188667, Lot # C96118, purity, NA

Methods

Doses: 0, 20, 65 and 200 mg/kg SC, administered once per week for 26 weeks (6 months) followed by 4 months recovery period.
Species/strain: CD-1 [ ] CD-1 [ICR] BR VAF+) mice, [ ]
Number/sex/group or time point (main study): 20/sex/group
Route, formulation, volume, and infusion rate: SQ (430 mg maltose, 15.2 mg sodium phosphate, and 12.6 mg sodium chloride). Maltose 5% was used for dilution. Vehicle contained 4% maltose, 10 mM sodium phosphate, and 20 mM sodium chloride.
Satellite groups used for toxicokinetics: 9/sex/group dosed with 20, 65 and 200 mg/kg SQ once per week for 26 weeks.
Age: 6 weeks
Weight: 27.6 – 34.1 g and 19.9 – 25.8 g for males and females respectively.

Sampling times: Predose, and 6, 12, 24, 36, 48, 72, 120 and 168 hours after the first and 13th dose. Additional blood samples were taken predose and 12, 24, 48, and 72 hours and 1, 2, 3, 4, 5, 6, and 8 weeks after the last dose.
Unique study design or methodology: The following tests, except for serum immunoglobulin levels and immunogenicity analyses were performed on single-cell suspensions obtained from individual spleens from 3 of the last 5 toxicology mice/sex/group at the scheduled necropsy. These assays were not conducted according to GLPs.
Lymphocyte activation Ex vivo: At necropsy, the ability of splenocytes to proliferate following stimulation with the T-cell mitogen, anti-CD3, or the B-cell mitogen, lipopolysaccharide (LPS), was assessed by measuring [^{3}H]thymidine incorporation into DNA.
Lymphocyte phenotype analysis: The percentages of splenocytes expressing CD3 (pan-T-cells), CD4 (T-helper-cells), CD8 (T-cytotoxic-cells), and CD22 (pan-B-cells) were determined by using flow cytometry.
Serum Immunoglobulin: Serum levels of IgM, IgG and IgA were assessed pre-dose and at 14 and 27 weeks on test, and on weeks 29, 33, 36 and 44 during recovery using an ELISA method.
Immunogenicity: Total antibody formation against BMS-188667 were assessed using an ELISA assay on serum samples obtained pre-dose and 14 and 27 weeks on test, and on weeks 29, 33, 36 and 44 during recovery.

Observations and times:

Mortality: Daily
Clinical signs: Daily
Body weights: Prior to each dose and weekly during the 4-month recovery period.
Food consumption: Weekly
Water intake: Recorded over 24 hours pre-dose and after 3 and 6 months on the study and at the end of the recovery period.
Ophthalmoscopy: NA
EKG: NA
Hematology: At 1, 3, and 6 months and at the end of the 4-month recovery.
Clinical chemistry: At 1, 3, and 6 months and at the end of the 4-month recovery.
Urinalysis: Not collected
Gross pathology: Necropsy was performed on the first 15 mice/sex/group of the toxicology groups approximately one week following the end of the 6-month treatment period, and on the remaining mice at the end of the 4-months recovery period. No necropsy was performed on the toxicokinetic mice.
Organ weights: See "Histopathology Inventory" table.
Histopathology: Adequate Battery: Yes
Peer review: Yes
Toxicokinetics: Blood samples (~ 0.2 ml) were collected from 3 mice/sex for each time point for each dose level. Blood samples were obtained under anesthesia from the retro orbital sinus. The concentration of BMS-188667 was determined by using a validated ELISA procedure.

Results

Mortality: No drug related deaths occurred during the study. Seven mice died or were sacrificed during the 6-month dosing period. These animals died from head trauma (1M/control) or under anesthesia during blood sampling (2M/20 mg/kg, 1M/65 mg/kg) or as a result of spontaneous conditions (1F/20 mg/kg on day 169, 1F/65 mg/kg on day 122, 1F/200 mg/kg on day 157). Five male mice died or were sacrificed in moribund condition during the recovery period. These mice died either under anesthesia while blood sampling (1/control) or of poor condition (1/20 mg/kg on day 232, 1/200mg/kg on day 196, 1/control on day 228 and 1/65 mg/kg on day 266). Incidence of mortality is listed in the table below:

Group No.	Weekly Dose of BMS-188667 (mg/kg)	Incidence of Mortality			
		During the 6-Month Dosing Period (n/sex/group = 20)		During the Recovery Period (n/sex/group = 5)	
		Males	Females	Males	Females
1	0	1	0	2	0
2	20	2	1	1	0
3	65	1	1	1	0
4	200	0	1	1	0

(Table was copied from the submission)

Clinical signs: There were no significant drug-related observations.
Body weights: There were no significant drug-related effects.
Food consumption: There were no significant drug-related effects.
Water consumption: There was a decrease in group water consumption over time during treatment and recovery in all male groups.
Ophthalmoscopy: NA
EKG: NA
Hematology: There were no drug-related effects.
Clinical Chemistry: There were no drug-related effects.
Urinalysis: Was not collected.
Gross pathology: There were no drug-related gross findings.
Organ weights: After 6 months drug administration, drug related, statistically significant increases in absolute and relative splenic weights were observed in intermediate and high dose males and females. The mean relative splenic weight at the intermediate and high dose males and females was 24% greater than in control males and 34% greater than control females, respectively. Also after 6-month drug administration, a drug related, statistically significant decrease in absolute and relative thymic weights were observed in intermediate (30% relative) and high dose (35% relative) females, relative to control. No drug related statistically significant changes in organ weights were seen after the 4-month recovery period. Mean absolute and relative weights of the spleen and thymus are listed below.

Dose (mg/kg)	Organ	Mean absolute weight (g) Males	Mean % body weight (g) Males	Mean % brain weight (g) Males	Mean absolute weight (g) Females	Mean % body weight (g) Females	Mean % brain weight (g) Females
0	**Spleen**	0.0871	0.2408	16.4236	0.1077	0.3890	19.8398
20		0.0973	0.2582	18.4023	0.1292	0.4817	24.5333
65		0.1061*	0.2986**	20.2274*	0.1255	0.4655	22.8622
200		0.1045*	0.2982**	19.9843*	0.1392	0.5202*	26.7930*
0	**Thymus**	0.0220	0.0614	4.1719	0.0282	0.1008	5.2650
20		0.0248	0.0660	4.7279	0.0246	0.0916	4.6645
65		0.0224	0.0624	4.2777	0.0189**	0.0708**	3.4636**
200		0.0215	0.0612	4.1008	0.0173**	0.0656**	3.3099**

* = $p<0.05$, ** = $p<0.01$

Histopathology: drug-related increase in incidence and severity of karyomegaly in renal tubular epithelial cells were seen in mice sacrificed after 6- month administration. The incidence of renal karyomegaly is listed in the table below.

Incidence of Renal Karyomegaly in Study Mice Necropsied at the End of the 6-Month Treatment Period (n = 15 mice/sex/group)

Dose Level	Males	Females
Control	0	1 (Minimal = 1)*
Low	1 (Minimal = 1)	0
Intermediate	5 (Minimal = 1, Mild = 1, Moderate = 3)	1 (Moderate = 1)
High	7 (Minimal = 1, Mild = 2, Moderate = 4)	1 (Moderate = 1)

* The incidence (number of affected mice/sex/group) for each severity (minimal, mild, moderate, or marked) of karyomegaly is listed in parentheses.

(Table was copied from the submission)

After 4 months recovery period, the incidence of renal tubular epithelial cell karyomegaly was as follows:

Incidence of Renal Karyomegaly in Study Mice Necropsied at the End of the 4-Month Drug-Free Observation Period (n = 5 mice/sex/group)

Dose Level	Males	Females
Control	3 (Minimal = 1, Mild = 2)*	1 (Mild)
Low	0	1 (Mild)
Intermediate	1 (Mild)	1 (Minimal)
High	3 (Minimal = 2, Mild = 1)	3 (Minimal = 2, Mild = 1)

* The incidence (number of affected mice/sex/group) for each severity (minimal, mild, moderate, or marked) of karyomegaly is listed in parentheses.

(Table was copied from the submission)

The incidence and/or the severity of the renal tubular epithelial cell karyomegaly was not significantly different from the control. Karyomegaly in tubular renal epithelial cells was accompanied by mild, mutifocal, chronic inflammation, lymphocytic infiltiration and tubular cell degeneration. The incidence of karyomegaly increased mildly in males and significantly in females, while the severity decreased during the 4-month recovery period. This suggests a limited degree of reversibility in regard to severity. The sponsor stated that morphologically, the karyomegaly observed in the kidneys of controls and treated mice was consistent with spontaneous, age-related changes observed in control mice of other studies conducted in the same laboratory. In addition, these renal findings were not associated with any clinical or clinical chemistry changes. The impact of the increased incidence of renal tubular epithelial cell karyomegaly observed in this study will probably be limited, based on the lack of clinical nephrotoxicity, absence of drug-related mortality and the absence of increased severity of the changes during the recovery period.

Toxicokinetics: There were no sex-related differences in the TK parameters of BMS-188667. Exposure to BMS-1887667 in mice increased in less than dose proportional manner. Regardless of the dose-level, BMS-188667 accumulated to a small degree (1.5 to 2 folds) after repeated once a week dosing. The T_{max} ranged between 6 to 24 hours. TK parameters are listed in the table below.

Dose (mg/kg)	Schedule, Gender	CMAX (μg/ml)	TMAX (h)	T-HALF (h)	AUC(TAU) (h.μg/ml)
20	Week 1, M	110.1	12.0	82.6	6735
	Week 1, F	91.0	12.0	71.5	6851
	Week 26, M	115.2	24.0	117.6	11878
	Week 26, F	99.6	24.0	154.9	8187
65	Week 1, M	171.1	6.0	111.1	14964
	Week 1, F	188.6	6.0	91.8	15217
	Week 26, M	264.7	24.0	179.1	31102
	Week 26, F	242.2	24.0	64.1	28060
200	Week 1, M	316.2	12.0	127.9	34658
	Week 1, F	412.8	6.0	137.5	40479
	Week 26, M	463.1	24.0	181.6	51624
	Week 26, F	676.0	24.0	165.4	57332

AUC (TAU) = 168 h (7 days)
(Table was copied from the submission)

Lymphocyte activation *Ex vivo*: A decrease (98% and 83%) in mean peak day 2 proliferation to B-cell mitogen relative to control was observed in male mice in the intermediate and high dose groups at the end of the 5 month dosing period. Mean peak day 2 proliferation to T-cell mitogen, anti-CD3 was reduced by approximately 97% and 84% relative to control in male mice in the intermediate and high dose groups respectively. Recovery of B-cell and T-cell activation was demonstrated at the end of the 4-month recovery period.

Lymphocyte phenotype analysis: A decrease in the mean percentage of splenic B-cells (CD22+) relative to control was observed in male mice at the intermediate (85%) and high (61%) dose levels at the end of the 6 month dosing period. Increase in mean percentage of splenic T-cells (CD3+) relative to control was also observed in male mice at the intermediate (27%) and high (33%) dose levels. The level of T-cells and B-cells in male mice returned to levels similar to the control at the end of 4-month recovery period.

Serum Immunoglobulin: A decrease in group mean serum IgG levels was observed in the intermediate (week 29) and high dose (week 14) level mice. Mean nadirs of 72% at week 29 and 78% at week 14 in serum IgG levels relative to controls were observed at the intermediate and high dose level mice, respectively. Levels of IgG were similar to control levels at week 44.

Immunogenicity: Female mice in the low dose group developed significant anti-BMS-188667-antibody responses reaching antibody titer up to 196830 by week 14 during the 6-month dosing period. Male mice in the low dose group generally did not develop BMS-188667-antibody response until week 33. Overall, the mean BMS-188667-antibody response in the low dose group became positive at week 14 and peaked at week 36 with a geometric mean titer of 4209. Detectable anti-BMS-188667 antibody titer were also observed in control animals which was explained by the sponsor as the possibility that mice spontaneously developed antibodies that reacted with BMS-188667 presumably due to cross reactivity between endogenous mouse "rheumatoid factor-like" activity (endogenous anti-mouse IgG) and the human immunoglobulin portion of CTLA4IG. To test this hypothesis, the sponsor analyzed serum taken from CD-1 female sentinel mice of similar age housed in the same facility but in different rooms than those used in this study and serum obtained from [ ] from male and female mice (6-9 months of age) for the presence of BMS-188667-antibody response. In addition, serum samples collected at different time points were analyzed for the presence of BMS-188667 to determine if the control group was inadvertently misdosed. The results suggested that the control animals were not misdosed and detectable anti BMS-188667-antibodies were observed in the serum of the sentinel mice. Naturally occurring anti-BMS-188667 antibody titers of CD-1 mice are listed below.

Animal No.	Sex	Anti-BMS-188667 Antibody Titer
1	Male	90
2	Male	90
3	Male	270
4	Female	810
5	Female	30
6	Female	30
	Geometric Mean	108

* Results are expressed as end-point titer defined as the reciprocal of the greatest dilution that gives an absorbance 405/550 that is five-times greater than plate background.

(Table was copied from the submission)

Amendment No. 1: A report on Ki67 nuclear antigen staining of sections of mammary glands was added to study no. 96633 in May 2004. A drug related increase in the incidence of mammary neoplasia was observed in female mice in the carcinogenicity study performed with BMS-188667 (study no. 97610) at the intermediate and high dose levels (65 and 200 mg/kg/week). The purpose of this amendment was to detect potential proliferation which might pre-stage carcinogenicity at a later time in mammary glands of mice in this study (96633). The earlier staining (H&E staining) and histological examination of mammary tissues from mice in this study did not reveal pre-neoplastic

proliferative changes such as hyperplastic alveolar nodules. It was hoped that the staining with Ki67 nuclear antigen, a marker specific to proliferation, might be more sensitive in detecting stages of mammary neoplasia in mice treated for 6 months. However, no increase in Ki67 was apparent in mammary epithelial cells of mammary tissue in control or treated animals. An early indication of carcinogenicity was not detected using this technology.

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

Key study findings: No significant drug-related toxicity was observed in monkeys given 10, 22.4 or 50 mg/kg by IV administration every other day for 1 month. Reversible, minimal decreases in serum IgG were observed which are probably related to the pharmacology of the drug and not clinically significant. BMS-188667-specific antibodies developed 6 to 9 weeks after completion of dosing when the rate of drug elimination from serum was increased.

Study no.: 94704
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb, Pharmaceutical Research Institute, Department of Pathology, Biologics Evaluation, and Metabolism and Pharmacokinetics, Syracuse. New York, USA
Date of study initiation: November 15, 1994
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: BMS-188667, Lot # 940922-J, purity, NA
Methods

Doses: 0, 10, 22.4 and 50 mg/kg IV, administered once every other day such as 15 doses were administered in 29 days, followed by 6-week recovery period for the control and high dose monkeys and 11-week recovery period for the low and intermediate dose monkeys.
Species/strain: Cynomolgus (M. fascicularis) monkeys.
Number/sex/group or time point (main study): 3/sex/group
Route, formulation, volume, and infusion rate: IV once every other day, BMS-188667 ready to use solution. Vehicle contained 25 mM sodium phosphate, and 50 mM sodium chloride (pH 7.5).
Satellite groups used for toxicokinetics and antigenicity: NA
Age: Not mentioned
Weight: 2.0 – 2.9 kg and 2.3 – 2.9 kg for males and females respectively.
Sampling times: Pre-dose, 3 min. after dosing on the first day and the last day. Additional blood samples were collected from all remaining treated-animals during the 6-week recovery period on Day 29 at 0.75, 2, 6, 24, 48 hours and on days 35, 44, 52, 60, 66 and 72. Blood samples were also obtained from the remaining animals during the 11-week recovery period on days 79, 86, 93, 100 and 105.

Unique study design or methodology (if any): Lymphocyte activation and phenotype analyses were performed on single-cell suspensions obtained from peripheral blood samples.
Lymphocyte activation Ex vivo: Pre dose and at necropsy. Lymphocyte activation was assessed by measuring cellular proliferation as incorporation of [^{3}H]thymidine into cellular DNA.
Lymphocyte phenotype analysis: Pre dose, on days 15, 28 and in recovery monkeys on days 44, 71/72 and 105/106. The percentages of peripheral blood lymphocytes expressing CD2 (B-cells), CD4 (T-helper-cells), CD8 (T-cytotoxic-cells), and CD20 (B-cells) were determined by using flow cytometry.
Serum Immunoglobulin: Serum levels of IgM, IgG and IgA were assessed pre-dose and on day 28 and in recovery monkeys on days 44, 71/72 and 105 using an ELISA method.
Immunogenicity: Total antibody formation against BMS-188667 were assessed using an ELISA assay on serum samples obtained pre-dose and on day 28 and in recovery monkeys on days 44, 71/72, 79, 86, 93, 100 and 105.

Observations and times:

Mortality: Daily
Clinical signs: Daily
Body weights: Twice weekly during the dosing period and once weekly during recovery and prior to scheduled necropsy.
Food consumption: Daily
Body temperature: Twice weekly before and 1-hr after dosing.
Ophthalmoscopy: Prior to start of study and prior to necropsy.
EKG: Prior to start of study and prior to necropsy.
Hematology: Pre-dose and on days 15 and 28 for animals sacrificed on day 30 and on days 15, 28, 44 and 71/72 for all 6-week recovery animals. The low and intermediate dose 11-week recovery animals were also assessed on day 105/106.
Clinical chemistry: Pre-dose and on day 28 for animals sacrificed on day 30 and on days 28, 44 and 71/72 for all 6-week recovery animals. The low and intermediate dose 11-week recovery animals were also assessed on day 105/106.
Urinalysis: Urine samples were collected over 18 hours pre-dose and on day 28 for animals sacrificed on day 30, and on days 28, and 71/72 for all 6-week recovery animals. The low and intermediate dose 11-week recovery animals were also assessed on day 106/107.
Gross pathology: Necropsy was performed on 2 monkeys/sex/group at the end of 1-month treatment period, and on 1 monkey/sex/control and high dose at the end of 6-weeks recovery period. Necropsy was performed on the remaining animals (1/sex) from the low and intermediate dose groups after 11-week recovery period to assess toxicokinetics and immunogenicity.
Organ weights: See "Histopathology Inventory" table.
Histopathology: Adequate Battery: Yes
Peer review: Yes
Tissues from control, and all dose level animals sacrificed on day 30 and 72/73 were examined. Only select tissues were examined from the low and intermediate dose animals

sacrificed on days 106/107 since no drug related changes were noted in the high dose animals.
Toxicokinetics: Blood samples (~ 2 ml) were collected from femoral vein of unanesthetized monkeys. Serum samples were analyzed for levels of BMS-188667 by using a validated ELISA method.

Results

Mortality: All animals survived to the scheduled necropsy.
Clinical signs: There were no drug-related observations.
Body weights: There were no drug-related changes.
Food consumption: 3 male monkeys (1/22.4 mg/kg and 2/50 mg/kg) showed minimal to moderately lower food intake throughout the study including the recovery period. Lower food intake was not associated with any effects on body weight.
Body temperature: There were no drug-related changes.
Ophthalmoscopy: There were no drug-related changes.
EKG: There were no drug-related cardiac alterations.
Hematology: There were no drug-related effects.
Clinical Chemistry: There was a decrease in albumin and total protein on day 28 in intermediate- and high-dose monkeys. The decrease in total protein was statistically significant only in males at the high dose. No effects were seen after day 28. All groups including control had decreased average protein and albumin (except low dose group) on day 28 compared to pre-dose.
Urinalysis: There were no drug-related effects.
Gross pathology: There were no drug-related gross findings.
Organ weights: There were no drug-related effects.
Histopathology: There were no drug-related effects.
Toxicokinetics: Serum concentrations of BMS-188667 increased as the dose increased. C_{max} values at the end of treatment (day 29) were 1.6 to 2.0 fold greater than on day 1, indicating drug accumulation over the treatment period. Half-life values decreased as the administered dose decreased which might be due to the formation of anti-BMS-188667 antibody formation. No sex differences were seen any TK parameters. Mean male and female (together) TK parameters are listed in the table below:

Dose (mg/kg)	C_{max} (day 1)	C_{max} (day29)	AUC_{0-24} (day 29)	$T_{1/2}$ (day 29)
10	258	483	7104	44 (50M, 38F)
22.4	586	1164	16828	135 (158M, 112F)
50	1290	2078	29688	281 (243M, 320F)

Lymphocyte activation Ex vivo: There were no significant effects on the ability of T-cells or B-cells to be activated.
Lymphocyte phenotype analysis: There were no drug-related changes in the percentage of lymphocytes expressing CD2, CD4, CD8 or CD20.
Serum Immunoglobulin: There were no drug-related effects on levels of serum IgA or IgM. However, there was a dose-dependent decrease (8% low-, 18% intermediate- and 26% high-dose level) in serum IgG relative to pretreatment levels, at day 28. No decrease in IgG levels was seen in the low- and intermediate-dose groups at other time points. In

the high dose recovery monkeys, the level of IgG appeared to be increasing on days 71/72. This effect was considered as a pharmacological effect of BMS-188667. Mean serum IgG levels (mg/ml) are listed in the table below:

Dose (mg/kg)	Pre-dose	Day 28	Day 44	Day 71/72	Day 105
0	6.38	7.09	7.06	6.08	ND
10	5.72	5.24	5.76	6.28	7.11
22.4	6.85	5.63	7.78	7.26	9.10
50	7.40	5.47	4.73	5.67	ND

ND = not determined due to previous necropsy of animal

Immunogenicity: No significant anti-BMS-188667 antibody titers were developed during the treatment period in any group. Monkeys in the high dose group did not develop any significant anti-BMS-188667 antibody titers on day 72/73 (6-week recovery), therefore the recovery period was extended to day 106/107 (11-week) for one male and one female in each the intermediate- and the low-dose groups. Anti-BMS-188667 antibody titers were observed in 1 low- and 2 intermediate-dose monkeys 6 to 9 weeks after completion of treatment, after BMS-188667 serum levels had dropped below immunosuppressive levels.

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

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 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

Methods

Doses: 0, 10, 22 and 50 mg/kg IV, administered once per week for 6 months.
Species/strain: Cynomolgus (M. fascicularis) monkeys

Number/sex/group or time point (main study): 5/sex/group
Route, formulation, volume, and infusion rate: IV once every week, BMS-188667 as ready to use solution. Vehicle was saline.
Satellite groups used for toxicokinetics and antigenicity: The first 3 monkeys/sex/group were sacrificed at the end of 6-month treatment period. The remaining two monkeys from each sex/group were immunized with IM injection of keyhole limpet hemocyanin (KLH) at a dose of 10 mg/animal at approximately 2 months of the 3-month recovery period. Antibody titers to KLH were measured weekly for 3 weeks following immunization. Necropsy was performed on these monkeys at the end of the 3-month recovery period.
Age: Not mentioned
Weight: 2.9 – 4.5 kg and 2.6 – 3.5 kg for males and females respectively.
Sampling times: For pharmacokinetic analysis, blood samples were taken at 3 minutes, 2, 6 and 24 hours and 3, 5, and 7 days after the first dose, during week 12 and following the last dose. Additional samples were collected from the remaining monkeys 14, 28 and 42 days after the last dose.
Unique study design or methodology (if any): Some of the assays used below were validated, others were not fully validated but were considered scientifically accurate and followed SOP's of the laboratory were they were performed.
Lymphocyte phenotype analysis: Phenotype analyses were performed on single-cell suspensions obtained from peripheral blood samples and spleen. Blood samples were taken pre dose, during weeks 3, 7, 14 and 24 and in recovery monkeys during week 37. Spleen lymphocyte phenotypic analysis was performed at necropsy in week 27 and 38. The percentages of peripheral blood lymphocytes and splenic lymphocytes expressing CD2 (pan-T-cells), CD4 (T-helper-cells), CD8 (T-cytotoxic-cells), and CD20 (B-cells) were determined by using flow cytometry.
Serum Immunoglobulin: serum levels of IgM, IgG and IgA were assessed pre-dose and on weeks 3, 7, 15 and 24 and in recovery monkeys during week 37 using an ELISA method.
Immunogenicity: BMS-224818-specific and Chinese hamster ovary (CHO) protein-specific antibodies were assessed on serum obtained pre-dose and during weeks 3, 7, 15, 20, 24 and 38 by an ELISA. KLH-specific antibody formation was evaluated by ELISA in recovery monkeys before immunization in week 35 and in weeks 36, 37 and 38 after immunization.
C3a, Histamine, TNF-α, and IL-6 analysis: C3a, Histamine, TNF-α, and IL-6 were evaluated by ELISA.

Observations and times:

Mortality: Daily
Clinical signs: Daily
Body weights: Weekly
Food consumption: Daily
Body temperature: Twice weekly before and 1-hr after dosing.
Ophthalmoscopy: Prior the start of study and prior to necropsy.

EKG: Prior the start of study, 4 hour post-dose, after 3 months of dosing and before necropsy.
Hematology: Blood samples collected pre-study and during week 3, 7, 15 and 24 and from recovery animals before necropsy.
Clinical chemistry: Blood samples collected pre-study and during week 3, 7, 15 and 24 and from recovery animals before necropsy.
Urinalysis: Pre-study and before necropsy.
Gross pathology: Necropsy was performed on the first 3 monkeys/sex/group at the end of the 6-month treatment period, and on the remaining two monkeys from each sex/group following the 3 months recovery period.
Organ weights: See "Histopathology Inventory" table.
Histopathology: Adequate Battery: Yes
Peer review: Yes
Toxicokinetics: Blood samples (~1. 2 ml) were collected from femoral vein of un-anesthetized monkeys. Serum samples were analyzed for levels of BMS-188667 by using a validated ELISA method.

Results

Mortality: All animals survived to the scheduled necropsy.
Clinical signs: There were no drug-related observations.
Body weights: There were no drug-related changes.
Food consumption: There were no drug-related changes.
Body temperature: There were no drug-related changes.
Ophthalmoscopy: There were no drug-related changes.
EKG: There were no drug-related cardiac alterations.
Hematology: There were no drug-related changes.
Clinical Chemistry: There were no drug-related changes.
Urinalysis: There were no drug-related changes.
Gross pathology: There were no drug-related gross findings.
Organ weights: There were no drug-related effects.
Histopathology: At the end of dosing, BMS-244818-related depletion of lymphoid germinal centers was observed in the spleen and lymph nodes at all dose level. The lesion was minimal to mild and characterized by the absence of clearly defined germinal centers or by germinal centers that contained fewer immature lymphocytes. At the end of the recovery period and after 1 month of immunization with keyhole limpet hemocyanin, the lymphoid germinal centers of dosed animals were reconstituted and comparable to those of the control animals.
Toxicokinetics: Systemic exposure to BMS-224818 was dose related with no apparent sex-related differences. Steady state was reached by day 78 following repeated weekly dosing. The mean pharmacokinetic parameters are listed in the table below.

Dose [mg/kg/week]	Study Day	CMAX [µg/ml] (n=5)		AUC(TAU) [µg·h/ml] (n=5)		T-HALF [h] (n=2)	
		Male	Female	Male	Female	Male	Female
10	1	233	253	7770	8462	-a	-a
	78	319	278	14304	14416	-a	-a
	176	314	254	14548	13448	140	156
22	1	556	575	19260	20291	-a	-a
	78	704	764	35733	34825	-a	-a
	176	692	622	36481	29217	217	134
50	1	1222	1253	44239	41004	-a	-a
	78	1678	1854	72436	67718	-a	-a
	176	1527	1518	76539	63094	211	156

[a] not reported because sampling interval was not adequate to characterize the T-HALF

(Table was copied from the submission)

BMS-224818 blood level after 2 months recovery period (before immunization with KLH) at days 238 or 239 were either below the limits of detection or significantly less than blood levels at day 176 (see table below).

Dose (mg/kg/week)	Study Day	Concentration (ng/ml)	
		Male	Female
10	238	--	5, <LLQ
	239	<LLQ, <LLQ	--
22	238	--	<LLQ, <LLQ
	239	383, 527	--
50	238	--	344, <LLQ
	239	2982, 136	--

Values represent BMS-224818 levels in each recovery monkey

<LLQ = Below lowest limit of quantitation (3.0 ng/ml)

(Table was copied from the submission)

Lymphocyte phenotype analysis: There were no drug-related changes in the percentage of lymphocytes expressing CD2, CD4, CD8 or CD20 of peripheral blood or spleen.
Serum Immunoglobulin: Minimal drug-related decreases were observed in the mean serum IgG levels in the low- (13% M, 22% F), intermediate- (23% M, 34% F), and high-dose (34% M, 31% F) for males and females respectively compared to mean pre-treatment levels. However, IgG values did not fall below the normal historical values, therefore these changes were not considered clinically significant. The effect on IgG was reversible by the end of the recovery period.

Serum IgG levels in males are listed below.

	IgG (mg/ml)					
	Prestudy*	Week 3*	Week 7*	Week 15*	Week 24*	Week 38**
1 - Vehicle	4.31 ± 0.80	4.27 ± 1.85	4.41 ± 1.36	4.69 ± 0.98	4.37 ± 0.76	4.36 ± ND
2 - 10 mg/kg BMS-224818	4.08 ± 0.76	4.03 ± 1.08	4.08 ± 1.33	3.80 ± 1.34	3.55 ± 1.04	4.45 ± ND
3 - 22 mg/kg BMS-224818	4.47 ± 0.71	4.17 ± 0.60	4.19 ± 0.90	3.45 ± 0.69	3.70 ± 1.06	4.53 ± ND
4 - 50 mg/kg BMS-224818	3.44 ± 0.85	2.61 ± 1.24	2.67 ± 1.08	2.28 ± 0.62	2.30 ± 0.85	4.21 ± ND

(Table was copied from the submission)

Serum IgG levels in females are listed below.

	IgG (mg/ml)					
	Prestudy*	Week 3*	Week 7*	Week 15*	Week 24*	Week 38**
1 - Vehicle	3.60 ± 1.56	4.06 ± 1.80	4.26 ± 1.12	3.88 ± 1.19	4.04 ± 1.41	2.75 ± ND
2 - 10 mg/kg BMS-224818	3.73 ± 0.81	3.97 ± 0.95	3.59 ± 0.83	2.94 ± 0.72	3.01 ± 0.49	3.11 ± ND
3 - 22 mg/kg BMS-224818	5.71 ± 1.61	4.25 ± 0.97	4.02 ± 0.66	3.79 ± 0.95	4.13 ± 0.90	5.30 ± ND
4 - 50 mg/kg BMS-224818	5.81 ± 1.47	4.89 ± 1.96	4.14 ± 1.18	3.99 ± 1.04	4.05 ± 1.12	5.11 ± ND

** values represent the mean ± standard deviation of five animals
*** values represent the mean of 2 animals, standard deviation was not calculated
(Table was copied from the submission)

Immunogenicity: No antibody responses to BMS-224818 were detected during the 6-month dosing period of this study. However, after 3-month recovery period (week 38), minimal BMS-224818-specific antibody responses were detected in all dose groups. The antibody response was seen in 4/6 males and 3/6 females in the recovery group with titers of 90 and 270 compared to pre-dose titers of 10 or 30 for both males and females. The antibody response noted in the recovery animals showed functional recovery of immune system. No antibody response to host-cell (CHO) proteins were detected during dosing or recovery periods. Following KLH immunization after an 8-week recovery period (week 35), KLH-specific antibodies were detected in all dose groups, indicating functional recovery of the immune system. KLH-specific antibody response is shown in the table below.

			Titer*			
Group	Animal No.	Sex	Week 35**	Week 36	Week 37	Week 38
1	104	M	90	810	2430	2430
DPBS	105	M	30	2430	7290	7290
(control)	124	F	30	2430	2430	2430
	125	F	90	2430	7290	7290
Geometric Mean			52	1846	4209	4209
% Change from Control				0	0	0
% Change from Predose				3453	8000	8000
2	109	M	30	2430	2430	2430
BMS-224818	110	M	270	810	2430	2430
(10 mg/kg)	129	F	90	2430	2430	2430
	130	F	30	7290	7290	7290
Geometric Mean			68	2430	3198	3198
% Change from Control				32	-24	-24
% Change from Predose				3453	4577	4577
3	114	M	30	810	2430	2430
BMS-224818	115	M	90	2430	7290	7290
(22 mg/kg)	134	F	90	7290	7290	2430
	135	F	90	2430	7290	7290
Geometric Mean			68	2430	5539	4209
% Change from Control				32	32	0
% Change from Predose				3453	8000	6055
4	119	M	90	270	810	810
BMS-224818	120	M	10	270	810	810
(50 mg/kg)	139	F	90	2430	7290	7290
	140	F	90	7290	7290	7290
Geometric Mean			52	1066	2430	2430
% Change from Control				-42	-42	-42
% Change from Predose				1952	4577	4577

* Results are expressed as endpoint titer, defined as the reciprocal of the greatest dilution with an absorbance greater than or equal to five times the mean plate background.
** Pre-KLH immunization.
Bold value indicates seroconversion, defined as an endpoint titer that is two serial dilutions or greater than that individual animal's predose titer.

(Table was copied from the submission)

C3a, Histamine, TNF-α, and IL-6 analysis: No BMS-224818-related changes in plasma histamine or C3a levels, serum TNF-α or IL-6 levels were observed.
Amendment to the protocol: In a recent publication, (Hutto, D. et al, B cell hyperplasia associated with immunosuppression in cynomolgus monkeys. Vet Pathol 2003;40(5):624), B-cell hyperplasia was observed in a number of monkeys on a T-cell depleting biotherapeutic. One of the 24 monkeys in the published study developed lymphoma which was concluded to be mediated by lymphocryptovirus (LCV). Therefore, the slides of the spleen and lymph nodes from all animals in this study were re-examined by the pathologist and peer review pathologist to ensure that no lymphoid lesions were present in the lymphoid tissues of this study. There was no evidence of drug-related lymphoid hyperplasia in these lymphoid tissues.

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

Key study findings: BMS-188667 administered weekly at doses of 10, 22, or 50 mg/kg/dose for 52 weeks was well tolerated. Drug-related changes consisted of minimal decreases in serum IgG levels in males at 50 mg/kg and mild to moderate decreases in the number and diameter of germinal centers of the spleen and mandibular lymph nodes, showing decreased germinal center activity, at all doses. BMS-188667 was not immunogenic, except for 1 low-dose male monkey that had specific antibodies by week 39 and remained positive during recovery up to 8 weeks after the last dose. At the end of treatment, an antibody response to the neoantigen KLH following immunization 9 weeks into the recovery period was demonstrated at all doses showing a functional immune system. All monkeys in the 1-year study had evidence of previous exposure to one or more viruses (LCV, Herpes B, rhesus cytomegalovirus, and simian papovavirus) based on pre-study viral screening, however, BMS-188667 treatment did not result in any clinical effects associated with these viral infections. Based on these results the NOAEL was considered 50 mg/kg, the high dose tested.

Study no.: DS02008
Volume #, and page #: Electronic submission
Conducting laboratory and location: [redacted]
Date of study initiation: July 31, 2002
GLP compliance: Yes
QA report: Yes
Drug, lot #, and % purity: BMS-188667, Lot # MQJ611, purity, NA

Methods

Doses: 0, 10, 22 and 50 mg/kg IV, administered once per week for 1 year (52 weeks) followed by 13-week recovery period.
Species/strain: Cynomolgus (Macaca fascicularis) monkeys.[redacted]
Number/sex/group or time point (main study): 5/sex/group

Route, formulation, volume, and infusion rate: IV once every week for 1 year. The volume of injection was 2, 0.4, 0.88 and 2 mL/kg for 0, 10, 22 and 50 mg/kg respectively. BMS-188667 was supplied in vials containing maltose, sodium phosphate, sodium chloride. Vehicle was 0.9 % sodium chloride for injection, USP.

Satellite groups used for antigenicity: Necropsy was performed on the first 3 monkeys/sex/group at the end of 6-month treatment period. The remaining two monkeys from each sex/group were immunized with IM injection of keyhole limpet hemocyanin (KLH) at a dose of 10 mg/animal at approximately 2 months of the 3-month recovery period. Antibody titers to KLH were measured weekly for 3 weeks following immunization. These monkeys were sacrificed at the end of the 3-month recovery period.

Age: 4-8 years

Weight: 3 – 5 kg and 2.5 – 3.4 kg for males and females respectively.

Sampling times: For pharmacokinetic analysis, blood samples were taken on day 1, 78, 267 and 358 before dosing on the day of dosing and at 3 minutes, 1, 4, 8, 24, 72, 120 and 168 hours post dose. Blood samples were also obtained from recovery animals on days 372, 381, 386, 395, 400 and 409

Unique study design or methodology (if any):

Viral Screening: The supplier screened all monkeys for Simian Retrovirus (SRV) and Simian Immunodeficiency Virus (SIV). Prior to study initiation one serum sample/animal was tested for Herpes B and STLV-1. At the end of the in-life portion of the study, serum samples from all animals were tested for rhesus cytomegalovirus (RhCMV), simian papovavirus (SV40) and parvovirus (Parvo). During weeks 4 (pre-study), 12, 24, 39, 53 and 65, serum samples were collected for future lymphocryptovirus (LCV) screening, however, only 4 weeks prior to study initiation samples were analyzed.

Lymphocyte phenotype analysis: Phenotype analyses were performed on peripheral blood lymphocytes by flow cytometric analysis. Blood samples (1 mL) were taken pre-dose, prior to dosing during weeks 4, 16, 32, 51 and during week 65. The percentages of peripheral blood lymphocytes expressing cell-surface markers indicative of recovery and purity (CD14-, CD45+), mature T cells (CD3), helper/inducer T cells (CD3+, CD4+), suppressor/cytotoxic T cells (CD3+, CD8+), B cells (CD20+), B cells bearing CD40 (CD20+, CD40+), and natural killer cells (CD3-, CD16+).

Serum Immunoglobulin: Serum immunoglobulins levels of IgM, IgG and IgA were evaluated pre-dose and on days of dosing during weeks 4, 16, 32, 51 and 65.

Immunogenicity: BMS-188667-specific antibodies were assessed on serum obtained pre-dose and on days 78, 267, 354 (last day of dosing), 372, 381, 386, 395, 400 and 409 from treated animals only. Recovery of T-cell dependent antibody response was evaluated in all recovery animals during week 61 and weekly for 4 weeks following the administration of KLH, a T-cell dependent antigen that requires the interaction of B-cells and macrophages for the animal to develop a humeral KLH-specific antibody response.

C3a, Histamine, TNF-α, and IL-6 analysis: C3a, and Histamine were evaluated in all monkeys prior to dosing and at 3 and 30 minutes post dose on day 1 and on the day of dosing during weeks 16, 32 and 52. TNF-α, and IL-6 were evaluated

also from all monkeys prior to dosing and at 1, 2 and 4 hours post dose on day 1 and on day of dosing during weeks 16, 32 and 52.

Observations and times:

Mortality: Twice daily
Clinical signs: Once weekly
Body weights: Once weekly
Food consumption: Daily
Body temperature: Prior to start of study, 1 hour post-dose on day 1 and approximately 1 hour post dose during weeks 4, 12, 24, 39, 52 and 65
Ophthalmoscopy: Prior to start of study and weeks 4, 12, 24, 39, 52 and 65
EKG and blood pressure: Prior to start of study and 3 hour post-dose on day 1, and at approximately 3 hours post-dose during weeks 4, 12, 24, 39, 52 and 65
Hematology and clinical chemistry: Blood samples were collected pre-study and pre-dose on dosing days during weeks 4, 12, 24, 39, 52 and 65 from all fasted monkeys
Urinalysis: Urine was collected over 18 hours from fasted animals pre-study, pre-dose and on dosing days during weeks 4, 12, 24, 39, 52 and 65
Gross pathology: Necropsy was performed on 3 monkeys/sex/group after 52 weeks of treatment, and on the remaining two monkeys from each sex/group following 13 weeks recovery period.
Organ weights: See "Histopathology Inventory" table.
Histopathology: Adequate Battery: Yes
Peer review: Yes
Toxicokinetics: Blood samples (1 ml) were collected from the femoral vein of un-anesthetized monkeys. Serum samples were analyzed for levels of BMS-188667 by using a validated ELISA method.

Results

Mortality: All animals survived to the scheduled necropsy except for one male in the high dose group who was sacrificed on day 52 for humane reasons due to fracture of the distal femur.
Clinical signs: Clinical observations were noted in different animals at different intervals but were not considered drug-related.
Body weights: There were no drug-related changes.
Food consumption: There were no drug-related changes.
Body temperature: There were no drug-related changes.
Ophthalmoscopy: There were no drug-related changes. One female from the low dose group had bilateral diffuse maculopathy at week 24 and retinopathy described as unilateral or bilateral and either focal or mild for weeks 39, 52 and 65.
EKG and blood pressure: There were no drug-related cardiac alterations.
Hematology: There were no drug-related changes among the groups at each interval. One female (I54581) from the high dose had a low lymphocyte count (1100/μL) which correlated with decreased white pulp activity of the lymphoid germinal centers seen histologically in spleen and thymus of this female. This female was dehydrated, thin, hunched, and hypoactive and had diarrhea before sacrifice.

Clinical Chemistry: There were no drug-related changes among the groups at each interval. Female (I54581) had elevated triglyceride and urea nitrogen concentrations at week 52, slightly lower total protein, albumin and Ca values and low alanine aminotransferase and cholesterol values.
Urinalysis: There were no drug-related changes among the groups at each interval. Female (I54581) had a trace of hyaline casts at week 52, however there was no microscopic correlate to this finding.
Gross pathology: There were no drug-related gross findings.
Organ weights: There were no drug-related effects seen. A significant increase in mean absolute heart weight at the high dose and in mean absolute liver weight at the low and high dose was seen in male monkeys at week 52. However, these increases were not significant when analyzed relative to body weight or brain weight except for the analysis of liver weight relative to brain weight at the high dose which was significant. The effects are probably due to higher body weights in this group and low number of animals/group. Male organ weights were unaffected after the recovery period. Female organ weights were unaffected at all time. Changes in absolute and relative mean organ weights of male heart and liver are listed in the table below.

Organ	Control	10 mg/kg	22 mg/kg	50 mg/kg
Heart (g)	16.2	19.6	17.4	22.5*
% body weight	0.31	0.33	0.36	0.34
% brain weight	0.25	0.29	0.25	0.36
Liver (g)	72	85*	78	95*
% body weight	1.4	1.5	1.6	1.4
% brain weight	1.1	1.3	1.1	1.5*

* = P≤0.05

Histopathology: Changes were seen in the spleen and mandibular lymph node at all dose levels. Mild to moderate decreases in the number and diameter of germinal centers, containing fewer centrally located blast cells (centrocytes) and peripherally located small lymphocytes, were observed reflecting decreased germinal center activity. These findings were seen at all doses and were present to a very slight degree in the controls. Complete recovery occurred during the 3-month recovery period. Incidence of BMS-188667-related microscopic findings at terminal sacrifice are listed in the table below.

Dose (mg/kg/dose):	0	10 mg/kg/dose	22 mg/kg/dose	50 mg/kg/dose
No. of monkeys (M/F):	3/3	3/3	3/3	2/3
Sex:	M/F	M/F	M/F	M/F
Lesion: Spleen—Decreased White Pulp Activity				
Number examined:	3/3	3/3	3/3	2/3
Minimal severity	-/1	-/-	-/-	-/-
Mild severity	-/-	2/3	2/3	2/2
Moderate severity	-/-	1/-	1/-	-/1
Marked severity	-/-	-/-	-/-	-/-
Lesion: Mandibular Lymph Node-Decreased Germinal Center Activity				
Number examined:	3/3	3/3	3/3	1/3
Minimal severity	1/-	-/-	-/-	-/-
Mild severity	-/1	2/2	3/3	1/3
Moderate severity	-/-	-/-	-/-	-/-
Marked severity	-/-	-/-	-/-	-/-

- Indicates absence of finding in group

(Table was copied from the submission)

Toxicokinetics: Systemic exposure to BMS-224818 increased with increasing the dose with no apparent sex-related differences. Minimal to moderate accumulation was observed following multiple dosing. The mean half-life values ranged between 7-9 days in males and 4-7 days in females. The mean pharmacokinetic parameters are listed in the table below.

Dose (mg/kg)	Day	Cmax (µg/mL)		AUC(TAU)[a] (µg.h/mL)		Accumulation Ratio[b]	
		Male n=5	Female n=5	Male n=5	Female n=5	Male n=5	Female n=5
10	1	311.35 (43.26)	279.66 (16.03)	10645.43 (2524.73)	8803.59 (1451.27)	NA	NA
22		738.93 (99.17)	607.95 (79.72)	21190.68 (2319.49)	21354.51 (2425.29)	NA	NA
50		1330.40 (141.39)	1456.62 (208.39)	44449.27 (5363.33)	47854.81 (7906.27)	NA	NA
10	78	354.04 (85.30)	300.71 (26.64)	16366.19 (4638.96)	14321.92 (2423.78)	1.53 (0.19)	1.64 (0.24)
22		871.33 (187.16)	679.36 (69.24)	35769.97 (7710.98)	27133.25 (3701.51)	1.68 (0.19)	1.29 (0.23)
50		1860.82[c] (140.57)	2213.47 (551.54)	79200.81[c] (6159.97)	74225.06 (22626.94)	1.78[c] (0.23)	1.54 (0.27)
10	267	409.61 (175.99)	299.66 (43.52)	20703.07 (5776.25)	14693.20 (3116.38)	1.93 (0.18)	1.68 (0.33)
22		862.51 (131.24)	789.83 (42.97)	42818.26 (10456.88)	36362.94 (3199.81)	2.00 (0.26)	1.72 (0.24)
50		1953.89[c] (327.87)	1990.42 (562.53)	99178.58[c] (8339.58)	76285.80 (20635.13)	2.25[c] (0.46)	1.61 (0.38)
10	358	494.50 (81.73)	511.28 (197.73)	24781.83 (6877.33)	19401.57 (5281.40)	2.32 (0.28)	2.22 (0.59)
22		1041.95 (208.94)	1073.82 (98.15)	49864.92 (9633.29)	44641.79 (5409.34)	2.35 (0.29)	2.12 (0.36)
50		2488.19[c] (357.47)	2173.88 (735.25)	134648.66[c] (38716.16)	85605.52 (30002.42)	3.06[c] (1.10)	1.81 (0.64)

a: Calculated from time zero to 168 h (dosing interval = 168 h)

b: Accumulation ratio: $= \frac{AUC(0-168h)_{DayX}}{AUC(0-168h)_{Day1}}$ [where Day X is Day 78, Day 267, or Day 358, respectively]

c: n=4 in 50 mg/kg-males-group

NA: Not applicable

Nominal collection times were used

(Table was copied from the submission)

Lymphocyte phenotype analysis: There were no remarkable changes in percentages or absolute number of lymphocytes expressing cell-surface markers indicative of recovery and purity (CD14-, CD45+), mature T cells (CD3), helper/inducer T cells (CD3+, CD4+), suppressor/cytotoxic T cells (CD3+, CD8+), B cells (CD20+), B cells bearing CD40 (CD20+, CD40+), and natural killer cells (CD3-, CD16+).

Serum immunoglobulin: A transient decrease in mean serum IgG concentration (58% relative to control, 51% relative to mean pre-treatment levels) was observed in males at 50 mg/kg at week 32 but returned to pre-study levels by week 51 during continued BMS-188667 treatment. There were no effects on mean serum IgA or IgM levels in these males at 50 mg/kg. No effects were seen in females at any dose level or in males at the 10 and 22 mg/kg dose levels.

Immunogenicity: One male from the 10 mg/kg group showed BMS-188667-specific antibodies by week 39 and remained positive during recovery up to 8 weeks after the last dose. Clearance of BMS-188667 was accelerated during recovery in this animal compared to the others. Recovery of T-cell-dependent

antibody response was tested following KLH administration at 9 weeks into the recovery period (week 61). KLH-specific antibody response was detected in all monkeys, indicating functional activity of the immune system.

Histamine, Complement C3a, TNF-α, and IL-6 analysis: Sporadic and transient fluctuations in levels were observed in all groups which were attributed by the sponsor to a stress response during sample collection and assay variability, and not drug-related.

Viral screening: Incidence of positive pre-study viral status is listed in the table below. Treatment with BMS-188667 did not result in any clinical manifestation associated with a viral infection.

Group	1	2	3	4
Dose (mg/kg/week)	0	10	22	50
No. of Monkeys (M/F)	5/5	5/5	5/5	5/5
Sex	M/F	M/F	M/F	M/F
Virus[a]:				
Simian Retrovirus	0/0	0/0	0/0	0/0
Simian Immunodeficiency Virus	0/0	0/0	0/0	0/0
Simian T-cell Leukemia Virus-1	0/0	0/0	0/0	0/0
Herpesvirus Simiae (B virus),	4/3	3/3	3/3	4/2
Rhesus Cytomegalovirus	3/1	2/4	3/1	2/2
Simian Papovavirus (SV40)	1/2	3/3	3/4	2/1
Parvovirus	0/0	0/0	0/0	0/0
Lymphocryptovirus (LCV)	5/5	5/4	5/5	4/5

[a] All evaluations were based on serology with the exception of lymphocryptovirus, which was determined by DNA analysis.

(Table was copied from the submission)

Histopathology inventory:

Study	95676	96633	94704	99655	DS02008	97610
Species	Rats 1 week	Mice 6 months	Monkeys 1 month	Monkeys 6 months	Monkeys 1 year	Mice Carcinogenicity
Adrenals	X*	X*	X*	X*	X*	X
Aorta	X	X	X	X	X	X
Bone Marrow smear					X	
Bone (femur)	X	X	Rib	Rib	X	X
Brain	X*	X*	X*	X*	X*	X
Cecum	X	X	X	X	X	X
Cervix	X*	X	X	X	X	X
Colon	X	X	X	X	X	X
Duodenum	X	X	X	X	X	X
Epididymis	X	X	X	X	X	X
Esophagus	X	X	X	X	X	X
Eye	X	X	X	X	X	X
Fallopian tube						

Gall bladder		X	X	X	X* (with liver)	X
Gross lesions						
Harderian gland						
Heart	X*	X*	X*	X*	X*	X
Ileum	X	X	X	X	X	X
Injection site	X	X	X	X	X	X
Jejunum	X	X	X	X	X	X
Kidneys	X*	X*	X*	X*	X*	X
Lacrimal gland	X	X	X	X	X	X
Larynx						
Liver	X*	X*	X*	X*	X*	X
Lungs	X	X	X	X	X	X
Lymph nodes, cervical				Iliac lymph node		
Lymph nodes mandibular	X	X	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X	X	X
Mammary Gland	X	X	X	X	X	X
Nasal cavity						
Optic nerves						
Ovaries	X	X*	X*	X*	X*	X
Pancreas	X	X	X	X	X	X
Parathyroid	X	X	X	X	X	X
Peripheral nerve						
Pharynx						
Pituitary	X*	X	X*	X*	X*	X
Prostate	X*	X*	X*	X*	X*	X
Rectum	X	X	X	X	X	X
Salivary gland	X	X	X	X	X	X
Sciatic nerve	X	X	X	X	X	X
Seminal vesicles	X*	X*	X*	X*	X*	X
Skeletal muscle	X	X	X	X	X	X
Skin	X	X	X	X	X	X
Spinal cord	X	X	X	X	X	X
Spleen	X*	X*	X*	X*	X*	X
Sternum	X	X	X	X	X	X
Stomach	X	X	X	X	X	X
Testes	X	X*	X*	X*	X*	X
Thymus	X*	X*	X	X	X	X
Thyroid	X*	X*	X*	X*	X*	X
Tongue	X	X	X	X	X	X
Trachea	X	X	X	X	X	X
Urinary bladder	X	X	X	X	X	X
Uterus	X*	X*	X	X	X	X
Vagina	X	X	X	X	X	X
Zymbal gland						
Other organ/tissue	Gonads*	Diaphragm	Diaphragm	Diaphragm		Diaphragm

X, histopathology performed
*, organ weight obtained

i92.6.6.4 Genetic toxicology

Study title: Ames Reverse-Mutation study in Salmonella and *Escherichia Coli*

Key findings: BMS-188667 was not mutagenic in the Ames reverse-mutation assay.

Study no.: 96616
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: February 6, 1996
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, Lot # 941219-J, purity, NA

Methods

Strains/species/cell line: *Salmonella typhimurium* strains TA98, TA100, TA1537, TA1535 and *Escherichia coli* WP2 uvrA.
Doses used in definitive study: 312.5, 625, 1250, 2500 and 5000 µg/plate for main study and the independent confirmatory assay in the presence and absence of S-9 metabolic activation harvested from livers of male Sprague-Dawley rats.
Basis of dose selection: BMS-188667 was not expected to be cytotoxic, based on previous in vitro studies. No range finding study was performed and a high dose of 5000 µg/plate was selected as the high dose for the main study. A dose of 5000 µg/plate is considered by ICH as the maximum dose required for testing in Ames assay. The experimental design is shown in the table below:

Test Article	Dosing Solution Concentration (mg/ml)	In Vitro Concentration (µg/plate)	Strain	S9	Code
buffer	0	500 µl/plate	A-E	+,-	NC
BMS-188667	0.625	312.5	A-E	+,-	1
	1.25	625	A-E	+,-	2
	2.5	1250	A-E	+,-	3
	5.0	2500	A-E	+,-	4
	10.0	5000	A-E	+,-	5
2-AA	.025	2.5	A-D	+	PC1
2-AA	.1	10	E	+	PC2
2-NF	.02	2	A	-	PC3
Sodium azide	.01	1	B,C	-	PC4
9-AA	1	100	D	-	PC5
MMS	25 µl/ml	2.5 µl/plate	E	-	PC6

Strains: A = TA98, B = TA100, C = TA1535, D = TA1537, E = *E. coli*
S9: + = with metabolic activation, - = without metabolic activation
Code: NC = Negative Control PC = Positive Control
2-AA = 2-aminoanthracene, 2-NF = 2-nitrofluorene
9-AA = 9-aminoacridine, MMS = methyl methane-sulfonate

(Table was copied from the submission)

Negative controls: salt phosphate buffer
Positive controls: see experimental design above.
Incubation and sampling times: 48 hours incubation at 37°C in darkness with and without S-9 metabolic activation followed by counting.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Criteria for validity were met.
Study outcome: No cytotoxicity was observed in any test strain and at any BMS-188667 concentration tested in the main and the confirmatory study. Under the conditions of this study, BMS-188667 at the maximum dose tested (5000 µg/plate), was not mutagenic.

Study title: CHO/HGPRT Mammalian-Cell Forward Gene-Mutation Study

Key findings: BMS-188667 was not mutagenic in CHO cells at the HGPRT locus.

Study no.: 95713
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: November 15, 1995
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, Lot # 941219-J, purity, NA

Methods

Strains/species/cell line: Chinese hamster ovary fibroblasts (CHO-K1).
Doses used in definitive study: 397.5, 795, 1590, and 3180 μg/ml for main study and the independent confirmatory assay in the presence and absence of S-9 metabolic activation harvested from livers of male Sprague-Dawley rats.
Basis of dose selection: The highest dose was selected on the basis of the concentration of the test article formulation and dose volume limitation. Previous in vitro studies showed that BMS-188667 was not cytotoxic at all achievable concentrations.
Negative controls: Sodium phosphate buffer.
Positive controls: Benzo(a)pyrene (with S-9) and ethyl methanesulfonate (without S-9).
Incubation and sampling times: 5 hours with and without S-9 metabolic activation followed by counting.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Criteria for validity was met.
Study outcome: There were no drug-related increases in mutant frequency relative to the negative controls observed with BMS-188667. The positive controls produced markedly elevated mutant frequencies when compared to the concurrent negative control cultures. In conclusion, the test was valid and BMS-188667 had no mutagenic potential in CHO cells at the HGPRT locus.

Study title: Cytogenetics Study in Primary Human Lymphocytes

Key findings: BMS-188667 was not clastogenic in *in vitro* human lymphocyte chromosome aberration assay.

Study no.: 94729
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: January 23, 1995
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, Lot # 940922-J, purity, NA

Methods

Strains/species/cell line: Human peripheral blood was obtained from 2 donors who were previously screened to determine suitability of donor lymphocytes for use in this study.
Doses used in definitive study: 390, 779, 1555, and 3110 μg/ml for the main study in the presence and absence of S-9 metabolic activation harvested from livers of male Sprague-Dawley rats.
Basis of dose selection: A range-finding study was performed using 25, 49, 97, 195, 390, 779, 1555 and 3110 μg/ml of BMS-188667 with and without S-9 metabolic activation.

Based on the maximum dosing volume and the lack of cytotoxicity in the range finding study, doses were selected for the main study.
Negative controls: Sodium phosphate buffer
Positive controls: Mitomycin C (without S-9) and cyclosphosphamide (with S-9).
Incubation and sampling times: 24-hour exposure without metabolic activation and-5 hour exposure with metabolic activation at 37C with 5% CO_2 in a humidified atmosphere.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): The positive controls caused significant elevation in the frequency of damaged metaphases, thus criteria for validity was met.
Study outcome: BMS-188667 was not clastogenic in mitogen-stimulated peripheral blood lymphocytes at concentrations up to 3110 µg/ml for 24-hour exposure in the absence of metabolic activation, or for 5-hour exposure in the presence of metabolic activation.

2.6.6.5 Carcinogenicity

Study title: Subcutaneous Carcinogenicity Study in Mice

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 Charles River Laboratories personnel informed the sponsor that CD-1 mice are not retrovirus free (verbal communication to the sponsor). 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 immunosupression 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 sponsor 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.

Adequacy of the carcinogenicity study and appropriateness of the test model: The protocol for this study was not submitted to CAC. The sponsor performed the

carcinogenicity study because the drug is being developed for long term use as a selective immunomodulator, and it is biologically active and not immunogenic in rodents when maintained at biologically active levels. The sponsor used mice for the carcinogenic study since BMS-1886678 has immunosuppressive activity in this species, and the mouse is one of the standard species for carcinogenicity bioassay and has a substantial historical tumor database. The subcutaneous route of administration was selected since bioavailability was ≥ 78% in mice and SC administration has suppressed primary T-cell-dependent response greater than 90% in mice. Weekly treatment was chosen since BMS-1886678 has a serum half life of 3-5 days in mice and weekly treatment would result in the maintenance of pharmacologically significant immunosuppressive levels of the drug. A high dose of 200 mg/kg was selected based on renal lesions (karyomegaly in renal tubular epithelial cells) observed microscopically in the 6-month study in mice (study # 96633) and was considered to have a possible impact on 2-year survivability. The low dose (20 mg/kg) and the high dose (200 mg/kg) were 2x and 20x respectively, the doses that resulted in pharmacologically significant immunosuppression in mice based on models of efficacy and inhibition of bioactivity. The intermediate dose is approximately the geometric mean of the low and high doses. The study was adequately designed, included evaluation of exposure at all dose level, and histopathological data from organs and tissues to evaluate neoplastic and non-neoplastic effects. At weeks 84-88, 25% of survivability was reached in male and female low-dose mice respectively and after consultation with FDA, all remaining animals were sacrificed.

Evaluation of tumor findings: Statistical analysis of mortality and histopathological data detected the following statistically significant findings:

- Mortality was significantly increased ($p < 0.0001$) for all drug-treated mice
- Incidences of lymphomas were significantly increased ($p < 0.0001$) in all drug-treated mice
- Incidences of hemangiomas were significantly increased ($p < 0.0007$) in high dose males
- Incidences of mammary gland adenocarcinomas were significantly increased in intermediate- ($p < 0.006$) and high-dose females ($p < 0.0001$)
- The increase in mammary gland adenocarcinomas caused the incidences of combined mammary gland adenomas and adenocarcinomas to be statistically significant when compared with control
- Hemangiomas and hemangiosarcomas, when combined and analyzed statistically, no significant difference was detected between control and treated groups

Study no.: 97610
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: 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

Methods

Doses: 0, 20, 65, and 200 mg/kg

Basis of dose selection (MTD, MFD, AUC etc.): A high dose of 200 mg/kg was selected based on renal lesions (karyomegaly in renal tubular epithelial cells) observed microscopically in the 6-month study in mice (study # 96633) and was considered to have a possible impact on the 2-year survival of the animals. The low dose (20 mg/kg) and the high dose (200 mg/kg) were 2x and 20x respectively, the doses that resulted in pharmacologically significant immuno-suppression in mice based on models of efficacy and inhibition of bioactivity. The intermediate dose was approximately the geometric mean of the low and high doses.

Species/strain: CD-1 out bred albino mice, [redacted]

Number/sex/group (main study): 65/sex/group

Route, formulation, volume: SC, formulation: 20 mg/kg solution of BMS-188667 containing 4% maltose, 10 mM sodium phosphate and 20 mM sodium chloride was prepared using 5% Dextrose Injection USP (D5W), vehicle control (lyophilized in vials) contained 4% maltose, 10 mM sodium phosphate and 20 mM sodium chloride was prepared using 5% Dextrose, second control was saline, dose volume was 10 mg/kg.

Frequency of dosing: Once weekly

Satellite groups used for toxicokinetics or special groups: The last 5 mice/sex/group were used to assess immunologic and kinetic parameters. These mice were sacrificed and disposed of without necropsy after the last blood sample was collected. An insufficient number of mice remained therefore the splenic lymphocyte ex vivo activation and phenotype analysis was not performed as originally intended.

Age: 4 weeks upon arrival and 6 weeks at the start of the study. The average body weight at the start of the study was 29.06 - 29.82 g for males and 23.35 – 23.91 g for females.

Animal housing: Individual

Restriction paradigm for dietary restriction studies: NA

Drug stability/homogeneity: NA

Dual controls employed: Yes, control vehicle and saline.

Interim sacrifices: NA

Deviations from original study protocol: Deviations are listed in the table below as presented by the sponsor.

Deviation	Study Day (s)	Mice Affected (No.)
30 minutes without light due to power outage; fire alarms	2 days power outages 2 days of fire alarms	All
Mice Outside of their Cages	22, 37-49; 239; 386; 419 (one or more days)	160, 354, 406, 546, 505, 510, 515, 496, 347, 198, 199, 430; 153
Pregnant	56	496
Mice Treated with 3% Hydrogen Peroxide on one or more days	On one or more days	597, 308, 223, 593, 78, 336, 570, 585, 121, 328, 287, 336, 605, 197, 175, 37, 146, 261, 102
No Feeder	128; 309; 393; 463; 470; 477; 498	486; 405; 49; 150, 384; 420; 454; 90, 95, 104
No Water	210; 506; 462	284; 119-210; 436
Temperature/Humidity Outside of Specified Range	One or more days	All

(Table was copied from the submission)

Other deviations were misdosing or not dosing few animals which occurred only once. The above deviations occurred occasionally or affected only a small percentage of animals/sex in any group, therefore did not have a big impact on the study.

Observation and times

Mortality: Twice daily
Clinical signs: Monthly for the first 12 months and every other week thereafter.
Body weights: Weekly
Food consumption: Weekly for the first 6 months and monthly thereafter.
Gross pathology: Animals found dead or sacrificed in a moribund condition were sacrificed as soon as possible. Remaining mice were sacrificed after survival in the low dose group reached approximately 25% (during week 84 for males and week 88 for females). Necropsy was performed on all sacrificed animals.
Histopathology: See "Histopathology Inventory" table.
Peer review: Yes
Toxicokinetics: BMS-188667 in serum levels was determined after the 53rd and the 79th doses using a validated ELISA method. Whole blood was collected from the retro orbital sinus under anesthesia at 3, 24, 72, 120 and 168 hrs.
Clinical Immunology: Antibodies were determined from blood samples collected after the 53rd and 79th doses. All assays including PCR analysis for exposure to murine leukemia virus were non-GLP, however, the methods were considered scientifically accurate and followed SOPs of immunotoxicology in the laboratories where these assays were performed.
Immunogenicity: Antibody formation against BMS-188667 was assayed using an ELISA method.

Murine Leukemia Virus: Chromosomal DNA isolated from the spleen of 2 CD-1 mice (one male mouse treated with 200 mg/kg BMS-188667 and sacrificed moribund and a male sentinel mouse sacrificed for the purpose of obtaining DNA) was analyzed for murine leukemia virus (MLV) using a polymerase-chain reaction (PCR) assay.

Results

Mortality: Mortality data of control-vehicle and saline-control mice was pooled for comparison to drug treated mice since no vehicle effects were evident. There were statistically significant ($p < 0.0001$) increases in mortality in drug-treated mice of either sex compared to the pooled control.
The total number of mice in each group that survived to necropsies at week 84 for males and week 88 for females, when survival in the low-dose group was 25% is shown in the table below.

Dose (mg/kg)	0 (Saline)	0 (Vehicle – Control)	20	65	200
Males	44/60	51/60	14/60	19/60	13/60
Females	38/60	35/60	15/60	10/60	7/60

The cause of death for found dead and moribund mice is listed in the table below.

Group No.	1		2		3		4		5	
Dose Mg/Kg/Week	Control		Vehicle		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
No. Animals/Group	60	60	60	60	60	60	60	60	60	60
Total Deaths/Moribundities	16	22	9	25	46	45	41	50	47	53
Cause of Deaths/Moribundities										
Not Determined	10	3	1	2	9	8	10	7	16	4
Malignant Lymphoma	1	3	0	5	18	25	20	29	17	33
Histiocytic Sarcoma	0	0	0	0	0	2	1	1	0	2
Adenocarcinoma/Mammary	0	0	0	4	0	1	0	4	0	5
Osteosarcoma	0	2	1	1	0	0	0	0	0	1
Hemangiosarcoma	0	0	0	0	0	3	0	0	0	1
Hepatocellular Carcinoma	1	0	0	0	0	0	1	0	1	0
Leiomyosarcoma	0	2	0	0	0	0	0	2	0	0
Adenocarcinoma/Lung	0	1	0	0	0	0	0	0	0	0
Adenocarcinoma/Pituitary	0	1	0	0	0	0	0	0	0	0
Granulocytic Leukemia	0	0	0	0	0	0	0	1	1	0
Fibrosarcoma	0	0	0	0	0	0	0	0	0	1
Rhabdomyosarcoma	0	0	0	1	0	0	0	0	0	0
Amyloidosis	0	8	2	4	1	5	0	3	0	2
Nephropathy	0	0	2	4	1	0	0	0	0	0
Inflammation	3	2	3	2	14	0	9	1	10	2
Prolapse/Penis	0	N	0	N	3	N	0	N	1	N
Prolapse/Uterus/Vagina	N	0	N	0	N	1	N	0	N	0
Atrial Thrombosis	0	0	0	1	0	0	0	0	1	0
Intestinal Obstruction	0	0	0	0	0	0	0	0	0	1
Hemorrhage	0	0	0	0	0	0	0	0	0	1
Accidental Death	1	0	0	1	0	0	0	2	0	0

N = not applicable
(Table was copied from the submission)

Clinical signs: Hunched posture, decreased activity, whole-body paleness, soiling, dehydration, abdominal bloating and dyspnea incidences were higher than the vehicle-control or saline-control at all dose levels. There were no vehicle-related effects. These findings were generally seen at the latter stages of life in mice bearing lymphomas. A higher incidence of convulsions without whole-body tremors occurred in saline- and vehicle control mice compared to the treated ones. Convulsions were acknowledged by the animal supplier to occur spontaneously in this strain of mice.

Palpable tissues masses are listed in the table below.

Dose (mg/kg)	Abdomen	Thorax	Head	Neck	Eye	Lumbar	Perianal/ Perigenital	Fore/Hind Leg
Saline	12	1	0	1	1	1	3	0
Vehicle	16	3	1	0	0	0	1	0
20	17	0	0	0	0	0	2	1
65	19	4	1	4	0	1	2	1
200	12	4	0	6	0	0	0	1

(Table was copied from the submission)

Body weights: No biologically significant differences were seen. Mean body weights are listed in the table below.

Dose (mg/kg)	Mean Body Weights (gm) Saline Control		Vehicle Control		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
Day 1	29.6	23.9	29.8	23.9	29.5	23.7	29.1	23.4	29.1	23.7
Week 26	42.6	33.9	41.6	32.4	40.7*	33.1	40.8*	32.4	41.1	33.6
Week 51	42.6	35.1	42.2	33.9	40.6	34.6	40.8	33.2*	41.0	34.7
Week 82	43.5	37.7	44.2	37.0	41.6	35.6	41.6	35.4	42.3	37.2

* Significantly different from saline-control mice; $p < 0.05$.

(Table was copied from the submission)

Food consumption: No drug related consistent changes in food consumption were seen. There was a significant lower average food intake of high dose females compared to that of controls at week 82. This was not associated with any effects on body weight and the average food intake of high dose females at week 87 was comparable to that of control mice. Mean daily food intake is listed below.

Dose (mg/kg)	Mean Daily Food Intake (gm)** Saline Control		Vehicle Control		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
Week 1	5.9	5.3	5.7	5.4	5.7*	5.5*	5.6*	5.3	5.7	5.4
Week 26	5.4	5.4	5.2*	5.4	5.2*	5.4	5.1*	5.2*	5.2	5.3
Week 51	5.6	5.7	5.6	5.8	5.4	5.6	5.3*	5.4	5.3*	5.5
Week 82	5.2	5.4	5.3	5.3	5.2	5.4	5.0	5.2	5.1	4.7*

* Significantly different from saline-control mice; $p < 0.05$.
** Data rounded-off to the nearest decimal.

(Table was copied from the submission)

Gross pathology: Increased size of spleens, lymph nodes, thymuses and livers were seen in drug-treated mice (all dead and moribund sacrifices) compared to controls. Increased incidences of enlarged lymphoid tissues were not observed in drug-treated mice sacrificed at the end of dosing. The incidence of enlarged lymphoid tissue was similar in all treated groups and was not dose related. Lymphoma was the most common correlating microscopic finding for enlarged lymphoid tissues and also was the cause of death for approximately 50% of the drug treated mice. The total number of dead/moribund mice/group is listed in the table below.

Group No.	1		2		3		4		5	
Dose mg/kg/week	Saline		Vehicle		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
No. of Mice/Group	60	60	60	60	60	60	60	60	60	60
No. Deaths/Moribundities	16	22	9	25	46	45	41	50	47	53

(Table was copied from the submission)

Skin masses were seen in all groups. At necropsy, 8 and 9 skin masses were seen in intermediate- and high dose level females respectively, compared to 2 and 6 skin masses for saline-control and vehicle-control females respectively. Microscopically, mammary gland tumors were present in many of these skin masses.

Histopathology:

Non-neoplastic: Drug-related non-neoplastic findings were limited to increases in the incidence and severity of karyomegaly in renal tubular epithelial cells associated with chronic inflammation and tubular degeneration at all doses. The same findings were seen in the 6-month mice study and were considered to be consistent with a spontaneous background that is observed in mice. These renal findings were not associated with any detectable functional renal deficit and are believed by the sponsor to be of limited or of no relevance to humans. Incidence of karyomegaly in renal tubules is listed in the table below.

Group No.	1		2		3		4		5	
Dose mg/kg/week	Saline		Vehicle		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
No. of Mice/Group	60	60	60	60	60	60	60	60	60	60
Incidence of Karyomegaly	0	0	1	0	8	3	10	11	8	8

(Table was copied from the submission)

The incidences of minimal and mild subcutaneous inflammation at the injection sites of control and treated animals were similar indicating that the subcutaneous administration was not irritating.

Neoplastic: Statistically significant increases in lymphoma ($p<0.0001$) were observed microscopically in all treated groups, but group incidences were not dose-related. Incidences of lymphoma in CD-1 mice administered BMS-188667 were also higher than reported for prior carcinogenicity studies at BMS and in the published literature. Incidences of lymphomas are listed in the table below.

Group No.	1		2		3		4		5	
Dose mg/kg/week	Saline		Vehicle		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
No. of Mice/Group	60	60	60	60	60	60	60	60	60	60
No. with Lymphoma	1	4	1	7	18*	27*	22*	35*	17*	34*
% with Lymphoma	1.7	6.7	1.7	11.7	30	45	36.7	58.3	28.3	56.7

* p value for Peto and Pike (time adjusted) trend test was <0.0001) compared to pooled controls.

(Table was copied from the submission)

Incidences of lymphomas in CD-1 mice from prior studies at BMS and published reports are listed in the table below:

References	Bristol-Myers Squibb Study Numbers				Charles River Laboratories		Tox. Path.*
Study No./Year	90004	93601	96040	96651	1995	2000	1988
No. of Mice	100M 100F	100M 100F	120M 120F	120M 120F	423 M 425 F	2565 M 2822 F	891M 890F
Lymphoma	4%M 11%F	2% M 12% F	4.2% M 28% F	10% M 15% F	2-24% M 1-28% F	1-21% M 2-28% F	8.1% M 22% F

* Toxicologic Pathology

(Table was copied from the submission)

Incidences of mammary gland adenocarcinomas were statistically significantly increased in females at 65 and 200 mg/kg. The incidences of mammary gland adenomas alone were not statistically increased when compared with controls, although they occurred at a greater percentage than those noted in previous mouse studies. The incidence of adenocarcinomas in high-dose females was greater than the highest control range of 12% reported by Charles River Laboratory in 1995 and the incidence in both intermediate- and high-dose groups were well above the in-house historical control levels. The incidence in the vehicle-control group, although higher than previously seen in controls in BMS laboratory, was not significantly different from the saline-control group. Therefore, the sponsor combined the saline- and vehicle-control groups (as per protocol) for statistical comparison to treated groups. Based on the significance of $P < 0.0001$ in the Peto and Pike trend test, which adjusts for mortality, and the fact that the incidences were above the in-house historical controls (0-1%), the increased incidence of mammary gland carcinomas at 65 and 200 mg/kg/week were considered to be drug-related. The incidences of mammary gland adenomas and adenocarcinomas are listed in the table below.

Group No.	1	2	3	4	5
Dose mg/kg/week	Saline	Vehicle	20	65	200
Sex	F	F	F	F	F
No. of Females/Group	60	60	60	60	60
No. Mammary Glands Examined	60	57	55	58	58
Total Mice/Group with Mammary Tumors	2	4	3	7	10
No. Mice with Adenomas	1 (1.7%)	0 (0%)	2 (3.6%)	3 (5.2%)	2 (3.4%)
No. Mice with Adenocarcinomas	1 (1.7%)	4 (7%)	1 (1.8%)	6* (10.3%)	8** (13.8%)

* p value for Peto and Pike (time adjusted) trend test was = 0.006 compared to pooled controls. Mammary gland adenocarcinomas are common tumors in mice, and according to the protocol, increases are statistically significant only if p values are < 0.005.

** p value for Peto and Pike (time adjusted) trend test was < 0.0001 compared to pooled controls.

(Table was copied from the submission)

Incidences of mammary gland tumors in female CD-1 mice from previous studies at BMS and from published reports are listed below:

References	Bristol-Myers Squibb Study Nos.				Charles River Laboratories		Tox. Path.
Study No./Year	90004	93601	96040	96651	1995	2000	1988
No. of mammary glands examined	100	100	119	118	549	2573	890
Adenoma	1%	0%	0%	0%	0-2%	0-2.6%	1%
Adenocarcinoma	1%	3%	0.8%	2.5%	0-12%	0-8.3%	6.3%

(Table was copied from the submission)

Hemangiomas and hemangiosarcomas were evaluated as generalized tumors. The number of mice/group in this study with hemangiomas and hemangiosarcomas is listed below.

Group No.	1		2		3		4		5	
Dose mg/kg/week	Saline		Vehicle		20		65		200	
Sex	M	F	M	F	M	F	M	F	M	F
No. of Mice/Group	60	60	60	60	60	60	60	60	60	60
No. with Hemangiomas	0 0%	0 0%	0 0%	3 5%	0 0%	1 1.7%	1 1.7%	1 1.7%	3* 5%	2 3.3%
No. with Hemangiosarcomas	1 1.7%	1 1.7%	0 0%	0 0%	1 1.7%	3 5%	0 0%	0 0%	0 0%	2 3.3%

* p value for Peto and Pike (time adjusted) trend test was = 0.0007.

(Table was copied from the submission)

Incidences of liver (Liv) and generalized (Gen) hemangiomas and hemangiosarcomas in male CD-1 mice from previous studies at BMS and published reports are listed below.

References	Bristol-Myers Squibb Study Nos.				Charles River Laboratories		Tox. Path.
Study No./Year	90004	93601	96040	96651	1995	2000	1988
No. of Mice	100	100	120	120	549	2571	891
Hemangioma	Gen 5%	Gen 2%*	Gen 0.8%	Gen 3.3%	Liv 0.9%	Liv 0-4% Gen** 0.9%	Liv 1.5% Gen** 2.8%
Hemangiosarcoma	Gen 6%	Gen 6%	Gen 3.3%	Gen 1.6%	Liv 2.7%	Liv 1-5% Gen 1-12%	Liv 1.9% Gen 1.5%

* Incidences of generalized hemangiomas were based on the total hemangiomas in the liver and spleen
** Incidences of generalized hemangiomas were based on the sum of hemangiomas in individual tissues

(Table was copied from the submission)

Hemangiomas were seen in 3 high dose males, 2 in livers and one in a lymph node. No hemangiomas were seen in the controls, therefore the incidence was statistically significant in high dose males. However, the 3 hemangiomas (5%) in the high dose males were within the normal historical background range for hemangiomas in male CD-1 mice. Similar increases in endothelial tumors were not evident in females.

Electron microscopy: Ultra-thin sections of mammary tumors from 2 high-dose female mice were examined by electron microscopy. Mouse mammary tumor virus was present in the cytoplasm, budding from the plasma membranes and in the intercellular space of neoplastic mammary epithelial cells.

Toxicokinetics: Drug exposure increased with increasing the dose in a less than proportional manner. TK parameters were similar for the 53rd and 79th doses and there were no sex-related differences. PK parameters are listed in the table below.

Dose [mg/kg/week]	Dose		CMAX [µg/ml] Male	Female	TAUC(TAU) [µg.h/ml] Male	Female
20	53rd	Mean	75	81	8669	8954
		(N)	(3)	(2)	(3)	(2)
	79th	Mean	88	-[a]	12169	-[a]
		(N)	(1)		(1)	
65	53rd	Mean	177	230	21787	23412
		(N)	(5)	(4)	(5)	(4)
	79th	Mean	287	273	31818	-[b]
		(N)	(2)	(2)	(2)	
200	53rd	Mean	341	390	34343	35507
		(N)	(3)	(2)	(3)	(2)
	79th	Mean	424	-[a]	39872	-[a]
		(N)	(3)		(1)	

[a] No surviving toxicokinetics animals at week 79.
[b] TAUC(TAU) was not estimated because of an inadequate number of surviving animals.

(Table was copied from the submission)

Immunogenicity: Compared to the controls, detectable drug specific antibody titers were minimal in drug-treated mice with the exception of one mouse in the low dose group that had an end point titer of 65610. The geometric mean of BMS-188667 antibody response is listed below

Dose (mg/kg)	Week 53	Week 79
0 (Saline-Control)	3379 (8)	3669 (7)
0 (Vehicle-Control)	5539 (8)	3505 (6)
20	108 (6)	1403 (2)
65	27 (9)	17 (2)
200	16 (5)	30 (1)

( ) = no of animals

Detectable BMS-188667-specific antibodies were also seen in the control group in the 6-month study in mice (study no. 96633), and were presumed by the sponsor to be due to preexisting antibodies that are cross-reactive with BMS-188667. Data after the 79th week could not be assessed due to the low number of mice remaining. The BMS-188667 antibody titer in the drug treated mice is lower than the controls, likely due to the immunosuppression which is a pharmacological effect of the drug.

Murine leukemia virus (MLV): Endogeneous copies of MLV are a permanent and heritable part of the mouse genome and are therefore present in every cell. Activation of the virus in mice can result in neoplastic transformation and is thought to be behind the pathogenesis of malignant lymphoma in this study. There are 4 classes of endogenous MLVs and it is the ecotropic (emv) class that is responsible for development of an active viral infection and subsequent leukemia/lymphoma formation. There is evidence that CD-1 mice carry emv based on the relatively high rate of spontaneous lymphoma observed in older animals. Samples from the 2 CD-1 mice tested (a male mouse treated with 200 mg/kg BMS-188667 and sacrificed moribund and a male sentinel mouse) were emv positive. These results indicate that endogenous emv-specific DNA is present in the genome of CD-1 mice used in the carcinogenicity study. This supports the hypothesis by the sponsor that the increased incidence of lymphomas observed in the carcinogenicity study is due to activation of MLV in long-term immunosuppressed mice.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Intravenous Study of Fertility and Early Embryonic Development in Rats

Key study findings: No effects of BMS-188667 were observed on the reproductive function in either male or female rats and no effects on early embryonic development were observed at all doses tested.

Study no.: DN01093
Volume #, and page #: Electronic submission
Conducting laboratory and location: C
J
Date of study initiation: November 2, 2001
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, lot # C01237, % purity, NA

Methods

Doses: 0, 10, 45 and 200 mg/kg administered by IV route.
Species/strain: Rats/ CD®(SD)IGS VAF/Plus®
Number/sex/group: 25/sex/group
Route, formulation, volume, and infusion rate: IV, Vehicle was 5% Dextrose for injection, USP, volume was 10 ml/kg.
Satellite groups used for toxicokinetics: NA
Study design: Animals were dosed by the IV route once every 3 days for 2 weeks. Following the 2 week dosing period, male and female rats were placed in cohabitation for a maximum of 3 weeks. Dosing continued until day 7 of gestation for females and until scheduled termination for males after a total of 17 doses. Females were also dosed on days 0, 3 and 6 of gestation. Females were sacrificed and caesarean-sectioned on day 16 of gestation.
Parameters and endpoints evaluated: Daily clinical signs, body weights (twice weekly), food consumption (weekly) were measured. Gross necropsy of the thoracic, abdominal and pelvic viscera was performed for male rats. Gross lesions, prostate, seminal vesicles, testes of male rats were fixed for possible histopathology evaluation. Female rats were examined for gross lesions and pregnancy status after sacrificed and caesarean sectioned. The number of corpora lutea, implantation sites, fetuses (with vital status), and resorptions were recorded. Gross lesions, ovaries and uterus of each female rat, were fixed for possible histopathology evaluation. Blood samples were collected from male and female rats for possible evaluation. All other tissues were discarded.

Results

Mortality: All rats survived to the end of the study.
Clinical signs: No drug-related effects were observed.
Body weight: A statistically significant decrease in body weight gain was observed in males at the intermediate dose from days 43 to 47. This change was not dose-dependent and was not considered drug-related, no other changes in body weight or body weight gain were seen.
Food consumption: No drug-related changes were observed.
Toxicokinetics: Not measured
Necropsy: No drug-related effects were seen.
Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): No drug-related effects on estrous cycle, mating or fertility were observed at any dose level. No drug-related effects on early embryonic development were seen at any dose level. A statistically significant increase in the number of days males and females remained in cohabitation occurred at the low dose and a statistically significant decrease in litter size was also observed at the low dose. These effects were not considered drug-related since they were not dose-dependent.

Embryofetal development

Study title: Study of Embryo-Fetal Development in Rats

Key study findings: BMS-188667 was present in both maternal and fetal sera. Levels were dose-related and in higher concentrations in the dams than in the fetuses. No maternal or embryo-fetal toxicity was observed at any dose level. Under the conditions of this study, BMS-188667 was not teratogenic in rats.

Study no.: 95024
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Pathology, New Brunswick, New Jersey, USA
Date of study initiation: June 26, 1995
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, lot # C95201, purity, NA

Methods

Doses: 0, 10, 45, 200 mg/kg/day on days 6 to 15 of gestation.
Species/strain: Presumed pregnant female Sprague-Dawley, outbred albino rats , — .CD®(SD)BR VAF/Plus®, [ ] Virgin female rats were mated with males (one male/female) and rats observed with spermatozoa in vaginal smears or copulatory plugs *in situ* were considered to be at day 0 of gestation, (GD 0).
Number/sex/group: 25/females/group
Route, formulation, volume, and infusion rate: IV administration, vehicle was 5% Dextrose, volume administered was 20 ml/kg.
Satellite groups used for toxicokinetics: Maternal and fetal blood samples were collected from 10 dams and 10 litters in each group to determine serum levels of BMS-188667.
Study design: BMS-188667 was administered intravenously once daily to presumed-pregnant rats at doses of 0, 10, 45 and 200 mg/kg on days 6 through 15 of gestation. The control group was administered 5% Dextrose for injection, USP by the IV route. On day 20 of gestation, the dams were sacrificed and the litters were delivered by cesarean.
Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), abortion and premature delivery (daily), clinical observations (daily) body weight changes and food consumption (on GD 0 and daily on GD 6-20). On GD 20, rats were sacrificed, cesarean-sectioned and the intact gravid uterus (including ovaries) was weighed and corpora lutea, implantation sites, early and late resorptions and live and dead fetuses were counted. Fetuses were evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations. The thymus and spleen from one male and female/group were preserved for possible histopathology evaluation. At sacrifice, maternal and fetal blood samples were collected from 10 dams and 10 litters in each group to determine serum levels of BMS-188667.

Results

Mortality (dams): All rats survived to schedules sacrifice.
Clinical signs (dams): No drug-related clinical signs were observed.
Body weight (dams): No drug-related effects were observed.
Food consumption (dams): No drug-related effects were observed.
Toxicokinetics: Dose-related levels of BMS-188667 were seen in both maternal and fetal sera. This indicates that BMS-188667 crossed the placenta. BMS-188667 levels were higher in maternal sera than in fetal sera. Average BMS-188667 serum levels for dams and fetuses in each treated group is listed in the table below.

Group: Maternal Dose:		2 10 mg/kg	3 45 mg/kg	4 200 mg/kg
Maternal Sera µg/ml	Mean	8.4	26.7	81.0
	SD	7.4	7.9	38.9
Fetal Sera µg/ml	Mean	5.0	14.7	33.1
	SD	2.4	6.5	7.4

(Table was copied from the submission)

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No drug-related gross lesions were observed at maternal necropsy. A statistically significant decrease in the number of corpora lutea was observed in the high dose group (16.6) as compared to the control group (18.5). This value (16.6) was within the historical control range for this strain at this test facility, therefore this observation was not considered drug-related. There were no other significant maternal or fetal differences among groups.
Offspring (malformations, variations, etc.): No drug-related fetal malformations or variations occurred at any dose tested.

Study title: Study of Embryo-Fetal Development in Mice

Key study findings: BMS-188667 did not cause any maternal or embryo-fetal toxicity at the tested levels. Under the conditions of this study, BMS-188667 was not teratogenic in mice.

Study no.: 95019
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Pathology, New Brunswick, New Jersey, USA
Date of study initiation: May 18, 1995
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, lot # 940922-J, purity, NA

Methods

Doses: 0, 10, 55, 300 mg/kg/day on days 6 to 15 of gestation.
Species/strain: Presumed pregnant female albino mice, [ ]D®-1,[ ] Virgin female mice were mated with males (one male/female) and mice observed with copulatory plugs *in situ* were considered to be at day 0 of gestation, (GD 0).
Number/sex/group: 25/pregnant females/group.
Route, formulation, volume, and infusion rate: IV administration, vehicle was 0.9% sodium chloride, volume administered was 10 ml/kg.
Satellite groups used for toxicokinetics: Not measured.
Study design: BMS-188667 was administered intravenously once daily to presumed-pregnant mice at doses of 0, 10, 55 and 300 mg/kg on days 6 through 15 of gestation. The control group was administered 0.9% sodium chloride for injection, USP by the IV route. On day 18 of gestation, the dams were sacrificed and the litters were delivered by cesarean.
Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), abortion and premature delivery (daily), clinical observations (daily) body weight changes and food consumption (on GD 0 and daily on GD 6-18). On GD 18, rats were sacrificed, cesarean-sectioned and the intact gravid uterus (including ovaries) was weighed and corpora lutea, implantation sites, early and late resorptions and live and dead fetuses were counted. Placenta was examined grossly for alterations. Fetuses were evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations.

Results

Mortality (dams): All mice survived to schedules sacrifice.
Clinical signs (dams): No drug-related clinical signs were observed.
Body weight (dams): No drug-related effects were observed.
Food consumption (dams): No drug-related effects were observed.
Toxicokinetics: Not measured
Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No drug-related gross lesions were observed at maternal necropsy. One out of 22 pregnant dams from the low dose group and 3/24 pregnant dams from the high dose group had litters with early resorptions which were detected only by ammonium sulfide staining of the uterus. These effects were not statistically significant and were not considered drug-related based on the equivalence in resorption indices for all remaining dams/litters of the control and treated groups.
Offspring (malformations, variations, etc.): No drug-related fetal malformations or variations occurred at any dose tested. Bifurcated cervical vertebrae were significantly increased at 55 mg/kg dose level as compared to the control group. This incidence was not dose-dependent

Study title: Intravenous Study of Embryo-Fetal Development in Rabbits

Key study findings: BMS-188667 did not cause any maternal or embryo-fetal toxicity at the tested levels. Under the conditions of this study, BMS-188667 was not teratogenic in rabbits.

Study no.: DN02003
Volume #, and page #: Electronic submission
Conducting laboratory and location: Bristol-Myers Squibb Pharmaceutical Research Institute, Department of Reproductive Toxicology, New Brunswick, New Jersey, USA
Date of study initiation: February 4, 2002
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: BMS-188667, lot # 010920-112, purity, NA

Methods

Doses: 0, 10, 45, 200 mg/kg on days 7, 10, 13, 16, and 19 of gestation.
Species/strain: Nulliparous timed-mated — :(NZW)SPF rabbits. L
] Day of confirmed mating = GD 0.
Number/sex/group: 27/pregnant females/group
Route, formulation, volume, and infusion rate: IV administration, vehicle was 0.9% sodium chloride, volume administered was 4 ml/kg.
Satellite groups used for toxicokinetics: 5 rabbits from each dosing group were selected randomly and sacrificed on GD 19 to determine levels of BMS-188667 in maternal and fetal serum.
Study design: BMS-188667 was administered intravenously once daily to presumed-pregnant rabbits at doses of 0, 10, 45 and 200 mg/kg on gestation days 7, 10, 13, 16 and 19. The control group was administered 0.9% sodium chloride for injection, USP by the IV route. Five rabbits/group were sacrificed after dosing on GD 19 and blood samples were collected from the dams and the fetuses. On day 29 of gestation, the rest of the dams were sacrificed and the litters were delivered by cesarean.
Parameters and endpoints evaluated: The dams were evaluated for survival (twice/day), abortion and premature delivery (daily), clinical observations (daily) body weight changes and food consumption (on GD 0, GD 7 and daily for the rest of the study). On GD 29, rabbits were sacrificed, cesarean-sectioned and the intact gravid uterus (including ovaries) was weighed. Corpora lutea, implantation sites, early and late resorptions and live and dead fetuses were noted. Placenta was examined grossly for alterations. Fetuses were individually weighed, and evaluated for gender, body weight, gross external alterations and visceral or skeletal alterations. Thymus specimens from 2 treated fetuses, one from the low dose group and the other from the high dose group, and 3 control fetuses were evaluated microscopically.

Results

Mortality (dams): No drug-related deaths or abortions were observed.
Clinical signs (dams): No drug-related clinical signs were observed.
Body weight (dams): No drug-related effects were observed.
Food consumption (dams): No drug-related effects were observed.
Toxicokinetics: BMS-188667 was present in both maternal and fetal sera on GD 19 demonstrating that BMS-188667 is transferred from the does to the fetuses. However, the

exposure in does and fetuses were not proportional to dose. Values are listed in the table below.

Group Number	Daily Dose BMS-188667 (mg/kg/day)		BMS-188667 Concentration (µg/ml) Maternal Serum		Fetal Serum	
2	10	Mean (SD)	200.7	(27.8)	0.6	(0.7)
3	45	Mean (SD)	989.7	(162.7)	1.1	(0.7)
4	200	Mean (SD)	7261.2	(3699.7)	4.3	(1.7)

(Table was copied from the submission)

Terminal and necroscopic evaluations: C-section data (implantation sites, pre- and post-implantation loss, etc.): No drug-related gross lesions were observed at maternal necropsy.
Offspring (malformations, variations, etc.): No drug-related changes in the fetuses were noted at any dose level tested. Mottled red discoloration of the thymus was noted in 2 treated fetuses (one at 10 mg/kg and one at 200 mg/kg), however microscopical examination revealed mild, multifocal congestion/hemorrhage that was attributed to necropsy techniques or dissection artifact and not drug-related.

Prenatal and postnatal development

Study title: BMS-188667: Intraveneous Study of Pre-and Postnatal Development in Rats

Key study findings: There were no effects of BMS-188667 seen on the F_0-generation dams at any dose level tested or on the F_1-generation rats at the 10 and 45 mg/kg dose levels. Drug related inflammation of the thyroid in one rat and an increase of the T-cell-dependent antibody response were seen at the 200 mg/kg dose level in the F_1-generation female rats.

Study no.: DN01060
Volume #, and page #: Electronic submission
Conducting laboratory and location: [redacted]
Date of study initiation: May 29, 2001
GLP compliance: Yes except for the ANA assay, splenic lymphocyte and NK-cell phenotyping and antibody detection in milk.
QA reports: Yes
Drug, lot #, and % purity: Batch # C00196, Lot # J1A612, purity, NA

Methods

Doses: 0, 10, 45, and 200 mg/kg every 3 days from day 6 of gestation through day 21 of lactation.

Species/strain: Rats/ – CD®(SD)IGS VAF/Plus® [
]
Number/sex/group: 25/pregnant females/group
Route, formulation, volume, and infusion rate: Intravenous, Lyophilized BMS-188667 for injection reconstituted with 5% Dextrose for injection, USP. Vehicle was 5% Dextrose for Injection. Dose volume was 10 mg/kg.
Satellite groups used for toxicokinetics: 10 rats/group were assigned to the study for evaluation of BMS-188667 content and specific antibodies in maternal milk and serum on day 12 of lactation.
Study design: BMS-188667 was administered intravenously to pregnant rats (25/group) approximately every 3 days from GD day 6 through day 21 of lactation at doses of 0, 10, 45 and 200 mg/kg. Satellite groups of 10 rats/dose were assigned to the study for evaluation of BMS-188667 content and specific antibodies in maternal milk and serum on day 12 of lactation. All dams were allowed to deliver naturally. On day 21 of lactation, randomly selected F_1-generation pups from each litter were continued on the study (subset 1-5) while F_0-generation and the rest of F_1- generation were sacrificed.
Parameters and endpoints evaluated: All F_0-generation rats were observed for viability, clinical signs, abortions, premature deliveries, deaths, body weights, and food consumption. F_1-generation pups were observed for viability, clinical observations and body weight during lactation. F_1-genaration rats were observed post weaning for viability, clinical signs, body weights, and food consumption. F_1-generation rats assigned to Subset 1 were evaluated for drug and anti-drug antibody levels on postnatal day 21. Subset 2, 3, and 4 rats were evaluated for immunological parameters, as well as drug and anti-drug antibody levels on postnatal days 56, 63 or 112. Subset 5 rats were evaluated for sexual maturation, sensory perception, motor activity, learning memory, and reproduction. Immunological evaluation included T-cell dependent antibody responses on postnatal day 56 and splenic lymphocyte and NK-cell phenotype and serum immunoglobulin levels on postnatal day 63. In addition, the presence of anti-nuclear antibodies, serum immunoglobulin levels, clinical pathology, and histopathology of lymphoid organs (lymph nodes, spleen, thymus, and bone marrow) as well as the kidney, thyroid gland, pancreas, stomach, and testes/ovaries were evaluated on postnatal day 112.

Results

F_0 in-life: There were no drug-related maternal deaths or clinical signs at any dose level. There were no drug-related changes in maternal body weights, body weight gains or food consumption. There were no drug-related effects on any natural delivery endpoints.

F_0 necropsy: There were no drug-related necropsy observations at any dose level.

F_1 physical development: All F_1-generation rats survived to necropsy. There were no clinical signs or necropsy observations in males or females at any dose level. A statistically significant increase in the incidence of tail constrictions was only observed in males at 45 mg/kg therefore it was not considered drug related. No drug-related changes

in body weights, body weight gains or food consumption were seen. No changes in absolute and relative weights of the testes and epididymides in the F_1-generation male rats were seen.

F_0 and F_1 drug content in milk and serum: BMS-188667 was present in maternal serum and milk on day 12 of lactation. Dose-dependent levels of BMS-188667 were seen in pup serum of F_1-generation on postnatal day 21 but were not evident on day 63. However, there was no evidence of BMS-188667-specific antibodies in maternal milk or serum on day 12 of lactation or in pup serum on postnatal days 21, 62 or 112. Concentrations of BMS-188667 in maternal serum and milk are listed below.

Dose Group	10 mg/kg		45 mg/kg		200 mg/kg	
	Serum (µg/ml)	Milk (µg/ml)	Serum (µg/ml)	Milk (µg/ml)	Serum (µg/ml)	Milk (µg/ml)
Mean (SD)	69.6(9.3)	6.15(1.33)	299(39.9)	28(9.08)	1726(237)	135(28.8)

Concentrations of BMS-188667 in Pup serum on Day 21 postpartum are listed below.

Dose Group	10 mg/kg		45 mg/kg		200 mg/kg	
	Male (µg/ml)	Female (µg/ml)	Male (µg/ml)	Female (µg/ml)	Male (µg/ml)	Female (µg/ml)
Mean (SD)	1.86(1.56)	2.06(1.34)	8.20(5.97)	7.36(3.72)	21.7(14.2)	30.6(15.5)

F_1 behavioral evaluation: There were no drug-related changes in the F_1-generation rats for motor activity, auditory startle, and water maze learning and retention.

F_1 reproduction: There were no drug-related changes in the age of preputial separation in F_1-generation male rats or vaginal patency in female rats. Mating and fertility parameters were unaffected.

F_1-generation immune function endpoints: There were no drug-related changes in splenic-lymphocytes and NK-cell phenotype, serum IgM or IgG levels and antinuclear antibodies in F_1-generation pups. A drug-related increase in T-cell dependent antibody response to KLH was observed in F_1-generation female pups from high-dose dams. The increase was approximately 8.7 fold greater than the control females and 3 fold greater than the corresponding high-dose male pups.

F_1-generation histopathology and clinical chemistry endpoints: There were no drug-related changes in the clinical chemistry parameters measured. Diffuse chronic inflammation of the thyroid gland was seen in one F_1-generation female rat in the high dose group. This finding was described as multifocal with lymphoplasmacytic infiltrate of possible autoimmune origin and was considered drug related.

F_2 findings: There were no effect on caesarean-sectioning parameters in the F_1-generation dams or the F_2-generation litters. There were no drug-related fetal gross external alterations in the F_2-generation fetuses.

Other reproductive and developmental toxicity studies: Toxicokinetics

Studies are summarized below as presented by the sponsor.

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Dosing Period	Doses (mg/kg)	No. per Group	Noteworthy Findings	Study No./ Document Control Number
Rat/[redacted]CD (SD) IGS BR	Intravenous; BMS-188667 (lyophiled form reconstituted with Sterile Water for Injection, USP to produce a 25 mg/ml formulation	GD 6 - 15 TK assessed GD 15 GD 6, 9, 12, 15, 18, 21, LD 3, 6, 9, 12; TK assessed LD 12	45 and 200 mg/kg	16	Mean systemic exposure (AUC) during gestation at 45 or 200 mg/kg provided exposure multiples of 8.99 or 29.51, respectively, compared to the exposure in humans dosed monthly at the proposed clinical dose of 10 mg/kg. Mean systemic exposures (AUC) during lactation at 45 or 200 mg/kg provided exposure multiples of 2.99 or 10.91, respectively, compared to the exposure in humans dosed monthly at the proposed clinical dose of 10 mg/kg.	DN03068/ 930806845
Rabbit/ Hsd:NZW	Intravenous; BMS-188667 (lyophiled form reconstituted with Sterile Water for Injection, USP to produce a 25 mg/ml formulation	GD 7, 10, 13, 16, 19; TK assessed GD 19	200 mg/kg	5	The mean exposure (AUC) obtained in rabbits dosed every 3 days at 200 mg/kg provided an exposure multiple of 29.1 compared to the exposure in rheumatoid arthritis patients dosed monthly at a proposed clinical dose of 10 mg/kg.	DN03069/ 930806449

2.6.6.7 Local tolerance

The following local tolerance studies performed have been reviewed and are summarized below as presented by the sponsor.

Species/Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
BMS-188667: Single-Dose Intravenous, Intraarterial, and Paravenous Tolerance Study in Rabbits Rabbit/Hsd:NZW	Intravenous Intraarterial Paravenous	5 mg 5 mg 2 mg	F3	Paravenous injection of abatacept at 2 mg resulted in minimal irritation (minimal dermal hemorrhage and mononuclear-cell infiltration). Intravenous or intraarterial injection of abatacept at 5 mg did not cause significantly greater irritation than saline. No clinical signs of irritation were noted at any injection site.	DS032387 930006816
BMS-188667: Two-Week Intermittent-Dose Subcutaneous Irritation and Comparative Toxicokinetic Study in Rats Rat/Hsd:SD	Subcutaneous Once every 2 days for total of 7 doses	200 mg/kg	M10, F10	Minimal to moderate subcutaneous irritation at injection sites observed histopathologically. This inflammation was considered to be tolerable, and was similar to that observed in earlier study with the present clinical subcutaneous formulation.	96615/ 910056020

BMS-188667 and BMS-224818: Single-Dose Subcutaneous Exploratory Comparative Irritation Study in Rats Rat/SD	Subcutaneous BMS-188667 in four modified formulations BMS-224818 in one formulation	100 mg 40 mg	M6	Single subcutaneous injections of 100 mg of abatacept (BMS-188667) or 40 mg of BMS-224818 did not cause cutaneous irritation beyond that observed in vehicle-treated rats, and no significant differences were noted between formulations.	DS03019/ 930004100

2.6.6.8 Special toxicology studies

Species/ Strain	Method of Administration	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	Study No./ Document Control Number
Immunotoxicity						
Mouse/ [redacted]:B6C3f1	Intravenous	Single dose	0, 36[a]	F/10/group for toxicity and immunomodulation assessment; 5/group sacrificed on days 2 and 9 F/2/group/ timepoint for pharmacokinetics evaluation	36 mg/kg - No clinical signs of toxicity. Lower total leukocyte count (lymphocytes) on days 2 and 9 compared to controls. No significant drug-related changes in clinical-chemistry parameters, circulating IgG or IgM levels, or effects on relative number of lymphocyte sub- populations (T-cells, T-helper cells or T-cytotoxic/suppresser cells). No change in ability of splenic T- or B-cells to be activated or B-cells to differentiate to immunoglobulin production, *ex vivo*. Data suggest abatacept is not overtly toxic to lymphocytes or the immune system.	92643/ 910044004
Mouse/ [redacted]:B6C3f1	Intravenous	5 days and challenge on day 26	0, 7[b]	F5[c]	7 mg/kg - No drug-related deaths. Transient decreased activity and rapid breathing were observed in mice that received a challenge dose 3 weeks after completion of treatment, but symptoms resolved within 1 hr. These effects may have been the result of abatacept-specific antibodies that were observed by day 19. Minimal changes in *ex vivo* immune parameters (reversibly enhanced T-cell mitogenic response and decreased polyclonal IgG production).	92675/ 910044053
Immunotoxicity (Continued)						
Monkey/ cynomolgus	Intravenous	7 days	0, 5.7, 17.2[d]	M1 F1	5.7 and 17.2 mg/kg - No drug-related clinical signs, changes in body weight, food consumption, or hematology or serum chemistry parameters. No changes in peripheral lymphocyte subpopulations, or *ex vivo* lymphocyte activation. Abatacept-treated animals developed anti-abatacept antibodies by day 58 (magnitude of response lesser and peak response more delayed than typically observed with an immunogenic protein). Results consistent with abatacept-suppression of primary humoral immune response suggesting immunogenicity of abatacept may not be a significant issue in conducting longer term studies in monkeys.	93617/ 910043964

Dog/beagle	Intravenous injection	BMS-191352 (immunogen) once every 3 days for 5 doses and challenge on day 34	BMS-191352 0.12	M 2 or 4 F 2 or 4	By day 34, abatacept was most effective at suppressing the mean anti-BMS-191352 antibody response (mean titer suppressed 83%), followed by DSG given daily (suppressed 55%), while DSG given intermittently was not effective (suppressed 3%). Abatacept given at time of challenge was able to partially suppress recall response to BMS-191352 by 78%, while DSG was not. Deoxyspergualin and abatacept prevented the onset of BMS-191352-mediated hypersensitivity reactions (HSR) during the treatment period. However, upon challenge with BMS-191352, 10 of 14 dogs in groups treated initially with DSG had an HSR on day 34. Two out of four dogs treated with BMS-191352 and abatacept had an HSR on day 34 related to abatacept administration. Concomitant treatment with DSG or abatacept may be useful in reducing or delaying anti-BMS-191352 response, allowing for longer duration of treatment with greater exposure and reduced antibody-mediated toxicities.	95685/ 910055848
		Deoxy spergualin (DSG) daily 1.25 for 15 days or 1.88 mg/kg on day of immunogen and one day following each dose of immunogen	DSG 18.75 cumulative dose			
		Abatacept on same day of immunogen, once every 3 days for 5 doses	Abatacept 10			

Abbreviations: SRBC Sheep red blood cell, NZW New Zealand White, KLH Keyhole limpet hemocyanin, IL-6 Interleukin-6, PK pharmacokinetics.
All footnotes are available as table end notes.

2.6.7 TOXICOLOGY TABULATED SUMMARY

Toxicology tabulated summary of studies as provided by the sponsor are available at EDR.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The results of the nonclinical toxicology studies submitted by the sponsor adequately support the approval of abatacept (BMS-188667) for use in rheumatic arthritis patients. The effects observed in the nonclinical studies reflect the intended pharmacological effect of the product. The main concern identified during nonclinical testing was an increase in the incidence of malignant lymphomas and mammary gland tumors (in females) in the mouse carcinogenicity study. The increased incidence of lymphomas and mammary tumors observed in mice treated with abatacept (BMS-188667) was associated with the decreased control of murine leukemia virus and mouse mammary tumor virus, respectively, in the presence of long-term immunomodulation. No mutagenic potential of abatacept and no chromosomal aberrations in human lymphocytes with abatacept were observed in a battery of in vitro genotoxicity studies. These findings support the conclusion by the sponsor that the increased malignancies in this study were secondary to long-term induced immunosuppression and the control of these specific oncoviruses. These concerns have been discussed with the clinical review staff and are being addressed through labeling and/or post-marketing commitments.

Unresolved toxicology issues (if any): There were no unresolved toxicology issues.

Recommendations: None

Suggested labeling: See recommendations on labeling on page 5.

Signatures (optional):

Reviewer Signature H. Ghantous
Hanan Ghantous, PhD, DABT

Supervisor Signature Martin D. Green Concurrence Yes ✓ No ___
Martin D. Green, PhD

APPENDIX/ATTACHMENTS
None