

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125377Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Ipilimumab (Yervoy)

Date: March 4, 2011

To: File for BLA 1253777/000

From: John K. Leighton, PhD, DABT

Associate Director for Pharmacology/Toxicology

Office of Oncology Drug Products

John K. Leighton

3/4/11

I have examined pharmacology/toxicology supporting review by Dr. McDougal and supervisory memorandum provided by Dr. Pilaro. I concur with their conclusions that Yervoy may be approved for the proposed indication and that the requested reports should be submitted. I also concur with the proposed labeling.

MEMORANDUM

TO: The file
CC: Patricia Keegan, M.D., Director, Division of Biologic Oncology Products,
Office of Oncology Drug Products (OODP), Center for Drug Evaluation
and Research (CDER)
FROM: Anne M. Pilaro, Ph.D, Supervisory Toxicologist, Pharmacology/Toxicology
Branch, Division of Biologic Oncology Products, OODP, CDER

AP
3/2/2011

BLA #: 125377/000
SPONSOR: Bristol Myers-Squibb Corp.
PRODUCT: Yervoy™ (ipilimumab; BMS-734016, MDX-010)
SUBMISSION TYPE: original BLA application
DATE: March 2, 2011

SYNOPSIS:

Bristol Myers-Squibb Corp. (BMS) has submitted an original biologics licensing application (BLA) for their fully human, anti-cytotoxic T lymphocyte antigen-4 (CTLA-4)-directed monoclonal antibody, ipilimumab (Yervoy™). Yervoy™ is indicated "for the treatment of patients with unresectable or metastatic melanoma."¹ Nonclinical studies investigating the pharmacology, pharmacokinetics and toxicology of ipilimumab in tumor-bearing, immune-deficient mice, transgenic mice expressing the human CTLA-4 antigen, and cynomolgus monkeys were submitted with the BLA in support of the safety of Yervoy™. In the Highlights section of the label, Yervoy™ is defined as a "human cytotoxic T lymphocyte antigen-4 (CTLA-4)-blocking antibody" for its pharmacologic class. Yervoy™ binds to CTLA-4 expressed on a subset of activated T lymphocytes, and blocks the interaction of CTLA-4 with its counter-ligands B7.1 (CD80) and B7.2 (CD86) on professional antigen-presenting cells. Interference with the CTLA-4 and CD80/86 interactions results in prolonged stimulation of activated T lymphocyte function secondary to blockade of the inhibitory modulation mediated through this pathway, thereby breaking tolerance to "self" antigens.

The nonclinical data in support of Yervoy™ for the proposed indication were reviewed by the primary reviewer, Andrew J. McDougal, Ph.D. D.A.B.T., and are briefly summarized in the "Executive Summary" and "Integrated Summary and Safety Evaluation" sections of his review. Initial nonclinical studies conducted by the sponsor identified the non-human primate as the only pharmacologically responsive test animal species, and similar distribution of binding of ipilimumab to tissue samples from human and cynomolgus monkey (lymphocytes, tonsil, spleen) confirmed that the macaque was the relevant species in which to conduct further safety evaluations of ipilimumab. However, the affinity for ipilimumab binding to cynomolgus monkey CTLA-4 was approximately 3-fold less than the affinity for its binding to human CTLA-4, which should be taken in to consideration when comparing the doses tested across the two species.

Pharmacology, safety pharmacology, pharmacokinetic evaluations and toxicology studies supporting the BLA for Yervoy™ were conducted *in vitro*, in *in vivo* tumor

¹ from the indication statement in the current, draft labeling language for Yervoy™

inhibition studies using SA1/N mouse fibrosarcoma, MC-38 or CT-26 murine colon adenocarcinoma models in transgenic mice expressing the human CTLA-4 target antigen, and in 1-month (Q weekly dosing x 4) or 6-month (Q 28 day dosing x 5) repeat-dose toxicity studies in cynomolgus monkeys. The *in vitro* and *in vivo* pharmacodynamic, anti-tumor effects and pharmacokinetic/toxicokinetic profiles of ipilimumab were consistent with those observed with other monoclonal antibodies, including an apparent, saturable receptor-mediated clearance resulting in prolonged elimination half-life and increased area under the concentration-time (exposure, AUC) curve following repeated dosing. Evaluation of cardiac safety pharmacology during selected repeat-dose toxicology studies revealed no remarkable effects on heart rate, mean arterial and systolic blood pressures, or electrocardiogram evaluation in cynomolgus monkeys treated with 3.3 to 10 times the recommended human dose of ipilimumab (on a mg/kg basis, unadjusted for affinity differences).

In general, the toxicities observed in the single- and repeat-dose toxicity studies of ipilimumab in cynomolgus monkeys did not reflect the clinical toxicities observed in the phase 2 and phase 3 trials, namely, severe and fatal autoimmune-mediated enterocolitis, hepatitis, dermatitis (including toxic epidermal necrolysis), neuropathy, and endocrinopathies. Patients in these studies were treated with either 3 or 10 mg/kg of ipilimumab every 3 weeks for up to 2 years; it is likely that the differences in toxicity profiles reported between the human subjects and the test animals were related to either the difference in affinity of ipilimumab binding to human versus monkey CTLA-4, the duration of treatment on the clinical versus the repeat-dose monkey toxicity studies, or both. Toxicities associated with ipilimumab treatment of cynomolgus monkeys were minimal, and included slight increases in circulating T cells, slight-to-moderate lymphocyte infiltration of multiple organs, lymph node hyperplasia, and some evidence of decreased spleen weight. Additionally, increased antibody titers against target antigens were present in monkeys co-administered ipilimumab and either peptide or DNA vaccines, suggesting augmented T cell-mediated activation of plasma cell responses. These findings were considered related to an exaggerated pharmacologic response to ipilimumab, and are expected based on the purported mechanism of action, i.e. dysregulation of T lymphocyte immunomodulatory function.

There were no nonclinical genotoxicity or carcinogenicity studies performed with ipilimumab, as per the guidance provided in ICH S6 "*Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals*" and ICH S9 "*Nonclinical Safety Evaluation for Anticancer Pharmaceuticals*." A combined, enhanced pre- and post-natal developmental toxicity study treating pregnant cynomolgus monkeys with vehicle, 10, or 30 mg/kg ipilimumab beginning at the start of organogenesis on gestation day 20 and continuing through parturition is currently ongoing. Two interim reports were provided to the BLA submission, the later one containing in-life gestational and delivery data for all pregnant female monkeys on study and preliminary findings from their offspring in the first several weeks of life. According to Dr. McDougal's review, no treatment-related effects were apparent in the maternal animals during the first two trimesters of pregnancy. Beginning in the third trimester, the two groups of pregnant monkeys receiving ipilimumab experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower neonate body weights), and higher incidences of

infant mortality, as compared to control animals. The 30 mg/kg/dose level was associated with increased incidence and severity of these effects; this dose level corresponds to approximately 7-fold greater than the recommended human dose when dose comparisons between species are scaled by AUC, or 10-fold greater than the recommended human dose when the dose comparisons are scaled by body weight (unadjusted for the difference in affinity for human versus monkey CTLA-4). These results were incorporated into the labeling for Yervoy™, and the product was designated Pregnancy Category C. As a post-marketing requirement BMS will be requested to provide the final report for this study which includes the in-life results from the maternal animals and offspring out to 6 month post-natal, as well as specialized assessments for immune function and histopathology in the offspring, with a due date of December 30, 2011.

Comment: Typically, dose comparisons between test animal species and humans for monoclonal antibody products are scaled on a body weight (i.e. mg/kg) basis, since most monoclonal antibodies are administered intravenously, and their volume of distribution at steady state approximates the plasma space, which also scales to body weight (e.g. 40 to 70 ml plasma/kg). Scaling by comparative exposure (i.e. AUC) is not usually feasible, due to the presence of antibodies in most test animal species directed against the monoclonal antibody of interest (anti-drug antibodies, ADA). However, with ipilimumab, the incidence of ADA over all nonclinical toxicology studies in cynomolgus monkeys was only 8%, and ADA were not reported in the combined, enhanced pre-and post-natal developmental toxicity study. Therefore, I concur with Dr. McDougal's decision to utilize the comparative exposures in reporting the dose comparisons between the pregnant cynomolgus monkeys and the recommended human dose of ipilimumab in the product labeling.

Comment: The decrease in the incidence of ADA in ipilimumab-treated cynomolgus monkeys compared to the incidences of ADA reported historically with other monoclonal antibodies may be due, in part to the immunomodulatory effects of ipilimumab via inhibition of CD80/86 signalling by professional antigen presenting cells, or to the fact that ipilimumab contains a fully human, (b) (4) sequence for IgG₁k, and is therefore less likely to be perceived as "foreign" by the monkey antigen presenting cells.

Recommendation: In summary, I concur with Dr. McDougal's conclusions regarding the nonclinical findings for Yervoy™, the current recommendation that the licensing application be approved for marketing, and the proposed nonclinical language for Section 8.1 (Special Populations: Use in Pregnancy) in the product labeling to convey the risks of ipilimumab use during pregnancy to patients and prescribing physicians. A copy of Dr. McDougal's review, with supervisory sign-off, has been conveyed to the regulatory project manager for inclusion in the final action package.

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: STN 125377/0

Supporting document/s with nonclinical information: eCTD link: \\cber-fs3\m\eCTD Submissions\STN125377\125377.enx
#0000 (submitted 6/25/2010), #0016 (submitted 9/20/2010), #0020 (submitted 9/30/2010), #0028 (submitted 10/19/2010), and #0045 (submitted 1/19/2011)

Applicant's letter date: 6/25/2010

CDER stamp date: 6/25/2010

Product: Ipilimumab (BMS-734016, MDX010)

Indication: Advanced melanoma pretreated

Applicant: Bristol-Myers Squibb Company (BMS)

Review Division: Division of Biologic Oncology Products (DBOP),
Office of Oncology Drug Products (OODP),
Office of New Drugs (OND), Center for Drug
Evaluation and Research (CDER). HFD-107

Reviewer: Andrew J. McDougal, Ph.D., D.A.B.T. *Andrew J. McDougal 2/24/2011*

Supervisor/Team Leader: Anne M. Pilaro, Ph.D. *Anne M. Pilaro Feb 24, 2011*

Division Director: Patricia Keegan, M.D.

Project Manager: Erik Laughner

Disclaimer: Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125377 are presented in the BLA as either owned by BMS or are data for which BMS has obtained a written right of reference.

Any information or data necessary for review of BLA 125377 that BMS does not own or have a written right to reference are obtained from 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 BMS does not own are for descriptive purposes only and are not relied upon for approval of BLA 125377.

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1 Executive Summary

1.1 Recommendations

1.1.1 Approvability

Nonclinical Pharmacology/Toxicology recommends approval of ipilimumab for treatment of patients with unresectable or metastatic melanoma. The nonclinical data submitted in the BLA are adequate to support approval for this indication.

1.1.2 Additional Non Clinical Recommendations

This reviewer recommends a postmarketing requirement (PMR), to ensure submission of the results of nonclinical study # DN10020, "Ipilimumab (BMS-734016): intravenous study of pre- and postnatal development in cynomolgus monkeys with a 6-month postnatal evaluation". This study is ongoing and is being conducted in compliance with United States Code of Federal Regulations part 58 (21CFR58), Good Laboratory Practices (GLP) for Nonclinical Laboratory Studies. On January 18, 2010, the applicant provided draft wording regarding the nonclinical PMR for submission of the results of nonclinical study #DN10020

- Applicant notes that the protocol was submitted March 26, 2010, and that the study initiated May 19, 2011
- The applicant proposes to submit the final study report in December 2011

Negotiation of the PMR language is ongoing at the time of finalization of this review. This reviewer recommends a milestone of January 31, 2012 for submission of the final GLP study report

1.1.3 Labeling

Draft labeling was conveyed to the sponsor on December 10, 2010; the sponsor's response was sent February 18, 2011. At the time of finalization of this review, the final labeling is not yet complete. If appropriate, this review will be updated by an amendment to present any changes in the nonclinical sections that were incorporated in the final labeling.

1.2 Brief Discussion of Nonclinical Findings

Ipilimumab is a fully human monoclonal antibody (mAb) of the immunoglobulin (Ig) G subclass 1, kappa light-chain, specific for human cytotoxic T lymphocyte-associated antigen-4 (CTLA-4, CD152, CD 152, CD152).

T cell activation in response to antigen presentation is a multi-step process. Antigen presenting cells (APC) express B7.1 (CD 80) and B7.2 (CD86); these two proteins bind CD28 expressed on T cells, which leads to T cell activation and proliferation. As part of a negative feedback loop, a subset of activated T cells express CTLA-4. CTLA-4 binds B7.1 and B7.2, preventing their binding to CD28. Ipilimumab binds to CTLA-4 and prevents the interaction of CTLA-4 with its B7.1 and B7.2 (CD86), resulting in increased T cell activation and proliferation. Ipilimumab does not act directly upon tumor cells. The putative mechanism of action of ipilimumab is to break tolerance against tumor antigens, to enhance the anti-tumor activity of the adaptive immune system. Putatively, the auto-immune toxicities caused by ipilimumab are directly related to primary pharmacology and the activation of T cells.

The applicant provided data showing that ipilimumab binds human CTLA-4 and blocks binding of human CTLA-4 with human B7.1 and B7.2. As a proof-of-concept, ipilimumab exhibited anti-tumor activity in tumor-bearing transgenic mice (expressing human CTLA-4, but not expressing mouse CTLA-4). As further proof-of-concept, anti-mouse CTLA-4 was active against several mouse tumor types implanted into normal mice.

The cynomolgus monkey appears to be a pharmacologically relevant nonclinical model. Ipilimumab binds monkey CTLA-4, approximately 2 to 4-fold less strongly than ipilimumab binds human CTLA-4. In the general toxicology studies, monkeys receiving 3 or 10 mg/kg of ipilimumab (not in combination with other drugs) did not exhibit clear signs of toxicity. Ipilimumab treatment was associated with slight increases in circulating T cells, slight-to-moderate lymphocyte infiltration of multiple organs (consistent with the mechanism of action), increased antibody titers against co-administered vaccines and antigens (suggesting T cell-mediated activation of plasma cells), lymph node hyperplasia, and some evidence of decreased spleen weight.

For ipilimumab, clear treatment-related toxicity was limited to the ongoing ePPND study in cynomolgus monkeys (study # DN10020). Pregnant monkeys received ipilimumab every 21 days from the beginning of organogenesis through delivery, at 10 or 30 milligrams of ipilimumab per kilogram of body weight (mg/kg). No treatment-related effects were apparent in the first two trimesters of pregnancy. Beginning in the third trimester, the groups receiving ipilimumab experienced higher incidences of abortion, stillbirth, premature delivery (with corresponding lower body weight), and higher incidences of infant mortality compared to controls. The higher dose level was associated with increased incidences and severity of these effects.

The applicant submitted several monkey general toxicology studies that investigated the combination of ipilimumab with other putative immunostimulatory agents. When observed, the toxicities were attributable to the effects of the combinations, rather than ipilimumab alone. Only two monkeys exhibited severely toxic effects: one monkey exhibited an infusion-like reaction (signs of shock, requiring supportive care) and one death (caused by auto-immune gastrointestinal toxicity).

2 Drug Information

2.1 Drug

2.1.1 CAS Registry Number

477202-00-9

2.1.2 Generic Name

Ipilimumab

2.1.3 Code Names

- BMS-734016-01
- BMS-734016
- 5022
- MDX-010
- MDX010
- MDX-CTLA-4
- 10D1
- mAb10D1

2.1.4 Chemical Name¹

Immunoglobulin G1, anti-(human CTLA-4 [antigen]) (human γ -chain), disulfide with human κ -chain, dimer

2.1.5 Molecular Formula/Molecular Weight

The predominant product has a molecular formula of (b) (4)
The predominant form has a predicted molecular weight of 147,991 Daltons.

2.1.6 Structure

"Ipilimumab is a fully human immunoglobulin (IgG1 κ) (b) (4)
." (BLA module 2.3.S.1)

¹ **NOTE:** CTLA-4 is an abbreviation for cytotoxic T-lymphocyte antigen 4. The Greek symbol " γ " is gamma. The Greek symbol " κ " is kappa.

2.1.7 Pharmacologic class

Ipilimumab is a human cytotoxic T-lymphocyte antigen-4 (CTLA-4) blocking antibody

2.2 Relevant INDs

- IND 9186. Active, DBOP. Ipilimumab, treatment of melanoma. Sponsor is BMS
- [REDACTED] (b) (4)
- IND [REDACTED] (b) (4), [REDACTED] (b) (4)
- IND [REDACTED] (b) (4), [REDACTED] (b) (4)
- IND [REDACTED] (b) (4), [REDACTED] (b) (4)

2.3 Clinical Formulation

Ipilimumab is formulated in single use vials, containing pH-buffered saline. Ipilimumab is diluted with either 0.9% Sodium Chloride Injection, USP, or 5% Dextrose Injection, USP and administered by intravenous (IV) infusion.

No nonclinical concerns regarding the drug formulation were identified.

NOTE: Ipilimumab from three manufacturing methods was used in the nonclinical studies to support clinical development:

- Process A (mAb 10D1 or MDX-010-HYB), produced using a hybridoma
- Process B material, produced using a CHO cell line (at [REDACTED] (b) (4) scales)
- Process C material, produced using a CHO cell line at [REDACTED] (b) (4) scale

2.4 Proposed Clinical Population and Dosing Regimen

- Patients with unresectable or metastatic melanoma (unresectable Stage III or Stage IV melanoma) who have received prior therapy
- 3 mg/kg administered intravenously over 90 minutes once every 3 weeks, for a total of 4 doses
 - Patients are to receive the entire regimen (4 doses) as tolerated, regardless of the appearance of new lesions or growth of existing lesions

2.5 Regulatory Background

Medarex Inc. submitted three INDs to FDA: IND (b) (4) on March 3, 2000; IND 9186 on July 10, 2000; and IND (b) (4) on September 29, 2000. BMS, the applicant, acquired Medarex, and these INDs were transferred to BMS on September 2, 2009. IND 9186 may be considered the primary IND that resulted in this BLA.

Table 1: Nonclinical Regulatory Background for BLA 125377:

November 28, 2006	Fast track granted
March 4, 2010	Pre-BLA meeting between DBOP and the applicant
June 25, 2010	BLA received by FDA
July 16, 2010	Applicant orientation meeting and technical navigation meeting, between DBOP and the applicant
October 19, 2010	Applicant submitted an interim study report (358 pages) for the nonclinical ePPND study # DN10020
January 19, 2011	Applicant submitted an interim study report (7 pages) for the nonclinical ePPND study # DN10020, and proposed PMR language

3 Studies Submitted

3.1 Studies Reviewed

NOTE: BMS assigned a document tracking code (study #) to each study report; many of the reports already had study numbers. The BMS codes begin with "9300").

Table 2: *In vitro* nonclinical primary pharmacodynamic study reports

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
MDX-010-008-R (930020099)	Human anti-CTLA-4 monoclonal antibody cell line development	1/31/2007	No	4.3	4.1.2.1
MDX-010-011-R (930020111)	Physiochemical characterization of MDX-010	1/18/2006	No	4.3	4.1.2.2
MDX-010-013-R (930020126)	Cross-reactivity of MDX-010 to CTLA-4 from multiple species	12/22/2006	No	4.2.1.1	4.1.2.3

930019518	Summary of MDX-010 SPR experiments: cross-reactivity with rat CTLA-4-Ig	2/14/2006	No	4.2.1.1	4.1.2.4
930019505	Biophysical characterization of protein reagents used in MDX-010 SPR experiments	2/14/2006	No	4.3	4.1.2.5
930021444	Ipilimumab (BMS-734016): exploratory <i>in vitro</i> activation and cell-surface binding using mouse, rat, rabbit, monkey, and human tissues	4/05/2007	No	4.2.1.1	4.1.2.6
9300159520	Preliminary analysis of dose response MDX-010 binding to human CTLA4-Ig and mouse CTLA4-Fc surfaces by Biacore [®, sic]	2/27/2006	No	4.2.1.1	4.1.2.7
MDX-1106/010-001-R (930036348)	Effect of ipilimumab and MDX-1106 on T cell activation during an allogeneic mixed lymphocyte reaction (MLR)	2/04/2009	No	4.2.1.1	4.1.2.8
2005/0201994A1	Human CTLA-4 antibodies and their uses	9/15/2005	No	4.3	4.1.2.9

Table 3: *In vivo* nonclinical primary pharmacodynamic study reports

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
Treatment with ipilimumab					
TIB-006-001 (930041502; related to study # TIB-06-001)	Peripheral blood long term immunophenotyping in cynomolgus monkeys after treatment with ipilimumab (BMS-734016), BMS-663513, alone or in combination, during concurrent treatment with Simian Immunodeficiency Virus (SIV) DNA test antigens	1/15/2010	No	4.2.1.1	4.1.3.1
930031729	Monoclonal antibody adjuvants for the improvement of DNA vaccines	10/2009	No	4.2.1.1	4.1.3.2
MDX-010-005-R (930020107)	Effects of human anti-CTLA-4 administration on unstaged MC38 tumors in CTLA-4 transgenic mice	12/22/2006	No	4.2.1.1	4.1.3.3
Treatment with anti-mouse CTLA-4 (surrogate antibody)					
MDX-010-001-R (030020103)	Effects of anti-CTLA-4 and dexamethasone in a therapeutic SA1/N tumor model	1/09/2007	No	4.2.1.1	4.1.3.4
MDX-1106-010-002-R (930036349)	PD-1 and CTLA-4 blockade in murine MC38 colon adenocarcinoma tumor model	7/22/2009	No	4.2.1.1	4.1.3.5
MDX-1106-010-003-R (930036351)	PD-1 and CTLA-4 blockade in the murine CT26 colon carcinoma model	7/07/2009	No	4.2.1.1	4.1.3.6
MDX-1106-010-004-R (930036352)	PD-1 and CTLA-4 blockade in the murine SA1/N fibrosarcoma model	7/14/2009	No	4.2.1.1	4.1.3.7

MDX-1106-010-005-R (930036353)	Tumor response to PD-1 and CTLA-4 blockade in murine B16-F10 melanoma and J558 myeloma models	7/07/2009	No	4.2.1.1	4.1.3.8
MDX-1106-010-006-R (930036354)	Effects of combined PD-1 and CTLA-4 blockade in the murine FcγRIIb -/- autoimmune model	7/15/2009	No	4.2.1.1	4.1.3.9

Table 4: Study reports of ADCC and CDC

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
930023602	The effect of ipilimumab (MDX-010) on antibody-dependent cellular cytotoxicity	12/04/2007	No	4.2.1.1	4.2.1
930025694	Effect of ipilimumab on complement-dependent cytotoxicity	12/17/2007	No	4.2.1.1	4.2.2
MDX-010-006-R (930020459)	MDX-010-mediated effector function <i>in vitro</i>	12/20/2006	No	4.2.1.1	4.2.3
MDX-010-015-SR (930026820)	Activity of MDX-010 antibodies derived from CHO transfectoma and hybridoma cell lines in cytotoxicity assays	11/27/2007	No	4.2.1.1	4.2.4
STR-131 (930022419)	Binding of ipilimumab (MDX-010) to human FcγRI/CD64, FcγRIIA/CD32A and FcγRIII/CD16 determined by ELISA	7/16/2007	No	4.2.1.1	4.2.5
MDX-010-016-R	Comparison of MDX-010 (ipilimumab) manufacturing processes to demonstrate antibody equivalency for <i>in vitro</i> ADCC and CDC activity	8/19/2009	No	4.2.1.1	4.2.6

Table 5: Study report of the evaluation of toxicity for a surrogate antibody (anti-mouse CTLA-4) in a disease model

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
930031040	Activity of antibodies to CTLA-4, CD137 and their combination in murine models of colitis	10/15/2008	No	4.2.1.1	6.1.1
MDX-1106-010-007-R (930036359)	Effects of combined anti-PD-1 and anti-CTLA-4 monoclonal antibodies in the murine NOD autoimmune model	7/16/2009	No	4.2.1.1	6.1.2
930031045	Antitumor activity of antibodies to CTLA-4, CD137 or their combination in murine tumor models	10/23/2008	No	4.2.1.1	6.1.3

NOTE: For the three reports listed in Table 5, the applicant provided the reports in Module 4.2.1.1 (Primary Pharmacodynamics) of the BLA; this reviewer considers these studies more relevant to understanding toxicology, and therefore the reviews of these studies are grouped with the review of the general toxicology study reports.

Table 6: Nonclinical general toxicology studies

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
DS07167 (930032302)	Ipilimumab (BMS-734016). Single-dose intravenous exploratory pharmacokinetic comparability study in monkeys	11/25/2008	No	4.2.3.1.1	6.2.1
126-002 (930009967)	Repeated dose toxicity study of anti-CTLA4/10D1 administered via intravenous injection to cynomolgus monkeys	3/02/2000	No	4.2.3.2	6.3.1

TIB-06-001 (930022368)	Effect of BMS-734016, BMS-663513 or their combination, on the immune responses to Simian Immunodeficiency Virus DNA test antigens. Interim report	11/21/2007	No	4.2.1.1	6.3.5
SUV00006 (9300021733)	An investigative repeat-dose toxicity and efficacy study of MDX-010, 4C5, and 5H1 in combination with HBsAg, DNP-Ficoll and SKMel immunostimulants following three monthly administrations	6/21/2007	No	4.2.3.2	6.3.10
7114-100 (930009970)	14-day intravenous toxicity study with mAb 10D1 in cynomolgus monkeys	6/29/2000	Yes	4.2.3.2	6.3.2
(b) (4)-0919-128 (930009982)	A 2-week toxicity study of human anti-CTLA-4 administered by intravenous injection to cynomolgus monkeys	6/22/2000	Yes	4.2.3.2	6.3.3
DS06064 (930021064)	BMS-663513 and BMS-734016. One month intravenous combination toxicity study in monkeys	8/08/2007	Yes	4.2.3.2	6.3.4
(b) (4)-I-1416-128 (930009973)	A repeated dose toxicity and efficacy study of MDX-010 or MDX-010-HYB in combination with HBsAg and SKMel vaccines in cynomolgus monkeys	8/02/2003	Yes	4.2.3.2	6.3.5
(b) (4)-0992-128 (930009968)	An immunogenic study following combined intravenous/intramuscular administration of MAb10D1/HBsAg, respectively, to cynomolgus monkeys	4/16/2001	Yes	4.2.3.2	6.3.6

SUV00106 (930036346)	A 4-week combination toxicity study of MDX-010 and MDX-1106 administered by intravenous injection to cynomolgus monkeys, with a 1-month recovery period	6/09/2009	Yes	4.2.3.7	6.3.8
01-3460 (930009980)	MDX-CTLA4: a 6-month intravenous toxicity study in cynomolgus monkeys	2/22/2002	Yes	4.2.3.2	6.3.9

Table 7: Other toxicology studies

Study #	Title	Report date	GLP	e-BLA Module Location	Location in this review
IM578 (930009993)	Cross-reactivity of fluoresceinated, human monoclonal antibody 10D1 with normal human tissues	2/28/2000	Yes	4.2.3.7.7	10.1
IM993 (930010006)	Cross-reactivity study of fluoresceinated MDX-010 with limited normal human tissues	3/2003	Yes	4.2.3.7.7	10.2
DSO05067 (930021297)	BMS-734016 (MDX-010). Comparative tissue-binding study with mouse, rat, rabbit, monkey, and human tissues	5/15/2007	Yes	4.2.3.7.7	10.3
930034490	BMS-663513 and Ipilimumab. Exploratory <i>in vitro</i> proliferation and cytokine release assessment using human peripheral-blood mononuclear cells	11/06/2009	No	4.2.3.7.7	10.4
MDX-1106/010-008-R (930036361)	Ipilimumab and MDX-1106 (anti-PD-1 antibody). Effect of combined MDX-1106 and ipilimumab treatment <i>ex vivo</i> cytokine release in human peripheral blood cells	6/14/2009	No	4.2.3.7.7	10.5

3.2 Studies Not Reviewed

- Module 4.2.3 (Nonclinical Study Reports - Literature References) provides approximately 108 papers (published and unpublished); these were not all fully reviewed
- Several of the nonclinical studies submitted to the BLA compare ipilimumab against other therapeutic agents. These studies were fully reviewed for potential ipilimumab toxicity and other potential ipilimumab activity; however, the data for other agents were not necessarily fully reviewed
- This reviewer fully reviewed the nonclinical information presented in study # MDX-010-011-R, but did not fully review all of the physiochemical characterization data presented in this study report.

3.3 Previous Reviews Referenced

- No previous nonclinical reviews are referenced; documentation is made herein of the review or re-review of the essential nonclinical data.
- For IND 9186 and IND (b) (4)7, the regulatory decisions and review conclusions, as documented in CDER's Document Archiving, Reporting & Regulatory Tracking System (the DARRTS database) were considered for this review and are referenced.
- As a matter of record, DARRTS does not fully record the assigned nonclinical reviewers for IND 9186. For IND 9186, DARRTS does note that Dr. Alexandra Worobec was the nonclinical reviewer in 2006, succeeded by Dr. Anne M. Pilaro in 2007, and Dr. Andrew McDougal was assigned to the file 7/15/2008-present.

4 Pharmacology

4.1 Primary Pharmacology

4.1.1 Summary of the putative mechanism of action of ipilimumab

The applicant provided literature references² to explain the physiology of CTLA-4 (cytotoxic T-lymphocyte antigen 4, CTLA4, cluster of differentiation [CD] 125, CD 125, CD125), in support of the putative mechanism of action of ipilimumab. Briefly:

- Ipilimumab is a monoclonal antibody (mAb) specific for human CTLA-4.
- CTLA-4 is an endogenous cell-surface protein expressed on activated T cells
- B7.1 (CD80) and B7.2 (CD86) are expressed on antigen-presenting cells (APCs)
- In the absence ipilimumab, B7.1 and B7.2 bind to and activate CTLA-4, resulting in the inhibition of T-cells

² For example: Teft et al. 2006. A molecular perspective of CTLA-4 function. *Annual Rev. Immunol.* 24:65-97.

- **Ipilimumab binds CTLA-4 and prevents the interaction of CTLA-4 with B7.1 and B7.2. This blockade potentiates T-cell responses.**

- This is the intended mechanism of action

- The putative anti-tumor activity is secondary to the increase in activated T cells

- This mechanism of action suggests:

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-

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(b) (4)

- CD8+ cytotoxic T cells (CTLs) recognize tumor cells via major histocompatibility complex (MHC) restriction, and killing is perforin-mediated

- CD4+ helper T cells help active CTLs and also stimulate B cells to produce anti-tumor antibodies

- **Another putative mechanism is**

(b) (4)

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-

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(b) (4)

NOTE: CTLA-4 has one extracellular domain. The applicant provided information on the epitope specificity in a study report (study # MDX-010-011-R) and in a BLA amendment (dated September 22, 2010, sequence # 0017).

(b) (4)

(b) (4)

4.1.2 Primary review of *in vitro* primary pharmacodynamic studies

The applicant submitted *in vitro* primary pharmacodynamic studies; they support the proposed mechanism of action.

Note: Tissue cross-reactivity studies are reviewed below, as special toxicology studies (section 10 of this review). Studies on ADCC and complement dependent cytotoxicity (CDC) are reviewed below, as secondary pharmacology studies (section 4.2 of this review).

4.1.2.1**Study title: Human anti-CTLA-4 monoclonal antibody cell development**

Study no:	<ul style="list-style-type: none"> MDX-010-008-R 930020099 Halk, 2004 (Note: this is not a literature reference)
Study report location:	Module 4.3 (Literature References)
Report length:	37 pages
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	January 31, 2007
Study period:	August 1995 through October 2005
GLP compliance:	No
Drug:	Ipilimumab (antibody 10D1 (b) (4))

Key Study Findings:

- Based on the results summarized in this report, ipilimumab (antibody 10D1 (b) (4)) was selected as the lead anti-CTLA-4 candidate for further preclinical development
- This report documents efforts to produce a fully human mAb that binds human CTLA-4, blocks binding of CTLA-4 to B7.1 and B7.2, (b) (4)
- Ipilimumab binding of human CTLA-4 inhibited the binding of CTLA-4 to B7.1 and B7.2:
 - IC₅₀ for CTLA-4 binding to B7.1 (CD80) (b) (4)
 - Maximal inhibition of B7.1 binding was observed in the range of (b) (4)
 - IC₅₀ for CTLA-4 binding to B7.2 (CD86) = (b) (4)
 - Maximal inhibition of B7.2 binding was observed in the range of (b) (4)

Methods notes:

- A cell line was engineered to express recombinant human CTLA-4
 - The cell line was designated α - β -CTLA4-CD3 ζ (alpha-beta-CTLA4-CD3 zeta), and was also designed BW-huCTLA-4CD3 ζ (b) (4)
- NOTE: The location of this study report in the electronic BLA submission appears to be an error; placement under module 4.2.1 (Pharmacology) would have been appropriate. Module 2.6.3 (Pharmacology Tabulated Summary) incorrectly reports that the location is 4.2.1.1

Results notes:

- In enzyme-linked immunosorbent assays (ELISA assays), ipilimumab:
 - bound to a (b) (4) protein (human CTLA-4-mouse Ig)
 - blocked binding of human B7.1 to the human CTLA-4-mouse Ig (b) (4)
 - IC₅₀ approximately (b) (4) (across several assays)
 - blocked binding of human B7.2 to the human CTLA-4 mouse Ig (b) (4)
 - IC₅₀ = (b) (4) (across several assays)
 - competed with other anti-CTLA-4 mAb for binding to cells expressing recombinant human CTLA-4
- **NOTE:** The IC₅₀ values noted in 'Key Study Findings' (above) appear to have been calculated by the applicant; the values do not appear in the study report but are presented in module 2.6.2 (Pharmacology Written Summary)

4.1.2.2**Study title: Physiochemical characterization of MDX-010**

Study no:	• MDX-010-011-R
	• 9300200111
	• Srinivasan 2006 (Note: this is not a literature reference)
Study report location:	EDR Module 4.3 (Literature References)
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	January 18, 2006
Study period:	November 28, 2001 to January 10, 2007
GLP compliance:	No
Drug:	Ipilimumab (MDX-010)

Key Study Findings:

- Ipilimumab exhibited specificity for human CTLA-4 and monkey CTLA-4
 - Human CTLA-4 bound ipilimumab 1.8 to 4.2-fold more strongly than monkey CTLA-4 bound ipilimumab, suggesting the cynomolgus monkey may underpredict the pharmacology and toxicology of ipilimumab in humans
- Ipilimumab blocked binding of human B7.1 to human CLTA-4, and the binding of human B7.1 to monkey CTLA-4

Methods notes:

- Ipilimumab binding was evaluated by surface plasmon resonance (SPR) using (b) (4), in several configurations
 - (b) (4)
 - (b) (4)

o [REDACTED] (b) (4)

- Cynomolgus monkey CTLA-4 was prepared “in-house” (report page 9, method not reported)
- NOTE: The location of this study report in the electronic BLA submission appears to be an error; placement under module 4.2.1 Pharmacology would have been appropriate.
- NOTE: Module 2.6.3 (Pharmacology Tabulated Summary) summarizes the results of this study (pages 3 and 10), but cites BLA module 3.2.S.3 (Biological Activity) rather than the study report

Results notes:

- Using ipilimumab affixed to the Biacore® chip, and exposed to human CTLA-4, the binding affinity K_D ranged from [REDACTED] (b) (4)
- Using human CTLA-4 affixed to the Biacore® chip, and exposed to ipilimumab, the binding affinity K_D ranged from [REDACTED] (b) (4)
- Human B7.1 bound to human CTLA-4 with 83% higher affinity than human B7.1 bound to cynomolgus monkey CTLA-4
- Ipilimumab bound human CTLA-4 more strongly than ipilimumab bound cynomolgus monkey CTLA-4. From page 16 of the study report:

Table 8: Ipilimumab bound human CTLA-4 1.8 to 4.2-fold more strongly than ipilimumab bound monkey CTLA-4 (study # MDX-010-001-R)

Test	Units	Human	Cynomolgus monkey	Human:monkey (fold difference)
Ipilimumab binding to human or monkey CTLA-4 (ipilimumab fixed on chip)	nM	8.24	4.51	1.83
Ipilimumab binding to human or monkey CTLA-4 (CTLA-4 fixed on chip)	nM	20.1	4.79	4.20

- Epitope mapping of the binding between ipilimumab and human CTLA-4 was analyzed by enzymatic digestion and mass spectrometry. The study authors

[REDACTED] (b) (4)

4.1.2.3**Study title: Cross-reactivity of MDX-010 to CTLA-4 from multiple species**

Study no:	<ul style="list-style-type: none">• MDX-010-013-R• 930020126
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	15 pages
Conducting laboratory and location:	Medarex Inc., Milpitas, CA
Report date:	February 9, 2007
Study period:	August 2000 through August 2006
GLP compliance:	No
Drug, lot #:	<ul style="list-style-type: none">• Ipilimumab, 10D1 (used for the rhesus study)• Ipilimumab, lot # M31A-05-03FC, CHO-derived product (used for the rodent studies)

Key Study Findings: Under the conditions tested, ipilimumab bound rhesus monkey CTLA-4, but not rat or mouse CTLA-4

Methods notes:

- Three different cell lines were transfected to express recombinant CTLA-4 from one of three species: rhesus monkey, rat, or mouse
 - Mouse CTLA-4 was expressed in (b) (4)
 - Rat CTLA-4 was expressed in (b) (4)
 - Rhesus monkey CTLA-4 was expressed in (b) (4)
- Ipilimumab binding was evaluated using by fluorescence-activated cell sorter (FACS)
 - Positive and negative control antibodies were used for the rodent CTLA-4 experiments (i.e. an anti-mouse CTLA-4 and its isotypic control, an anti-rat CTLA-4 and its isotypic control)
 - A negative isotypic control (but no positive control) was used for the monkey CTLA-4 experiment

4.1.2.4

Study title: Summary of MDX-010 SPR experiments: cross reactivity with rat CTLA4-Ig

Study no:	930019518
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics) Cross referenced in 4.2.3.7.7 (Other Toxicity Studies – Other)
Report length:	11 pages
Conducting laboratory and location:	(b) (4) (location not reported)
Report date:	February 14, 2006
Date of study initiation:	Not reported
GLP compliance:	No
Drug, lot #:	Ipilimumab (MDX-010, BMS-734016), lot # 901004

Key Study Finding: Under the conditions tested *in vitro*, ipilimumab bound to a chimeric protein expressing human CTLA-4, but not to chimeric proteins expressing mouse or rat CTLA-4.

Method notes:

- Fusion proteins that included rat, mouse, and human CTLA-4, and including human immunoglobulin sequences (CTLA-4-Ig) were used
- As positive controls, two anti-rat CTLA-4 antibodies, an anti-mouse CTLA-4 antibody, and a different anti-human CTLA-4 antibody were used

4.1.2.5

Study title: Biophysical characterization of protein reagents used in MDX-010 SPR experiments

Study no:	• 930019505 • (b) (4) (Note: not published literature)
Report length	6 pages
Study report location:	Module 4.3 (Literature References)
Conducting laboratory and location:	(b) (4) (location not reported)
Report date	February 14, 2006
Date of study initiation:	Not reported
GLP compliance:	No

Synopsis: A 6-page memorandum provided details regarding characterization of the materials used in study # 930019518. The electronic BLA location, in module 4.3 (Literature References), appears to be a formatting error. This document was reviewed.

4.1.2.6**Study title: Ipilimumab (BMS-734016): exploratory in vitro activation and cell-surface binding using mouse, rat, rabbit, monkey, and human tissues**

Study no:	930021444
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics) Cross referenced in module 4.2.3.7.7 (Other Toxicity Studies – Other)
Report length:	6 pages
Conducting laboratory and location:	Bristol-Myers Squibb Research and Development, Syracuse NY
Report date:	May 4, 2007
Date of study initiation:	Not reported
GLP compliance:	No
Drug:	Ipilimumab (BMS-734016) (lot # not reported)

Key Study Findings:

- Ipilimumab bound specifically to a small proportion of rat lymphocytes stimulated to express rat CTLA-4
- The applicant did not consider these results to be evidence of “specific target binding of ipilimumab” (Module 2.6.6 Toxicology Written Summary, pages 12-13, 46) because the binding of ipilimumab to simulated rat lymphocytes was “minimal” (up to 1.8%) compared to ipilimumab binding to stimulated human T cells (~ 15 to 35%).

Method notes:

- Ipilimumab and a negative control (isotypic antibody) were conjugated to a fluorescent label
- Primary blood lymphocytes were isolated from rat, mouse, rabbit, monkey and human peripheral blood (strains and types of animals not reported)
- Cells were cultured (b) (4)

(b) (4), with the goal of inducing CTLA-4 expression. Stimulants used:

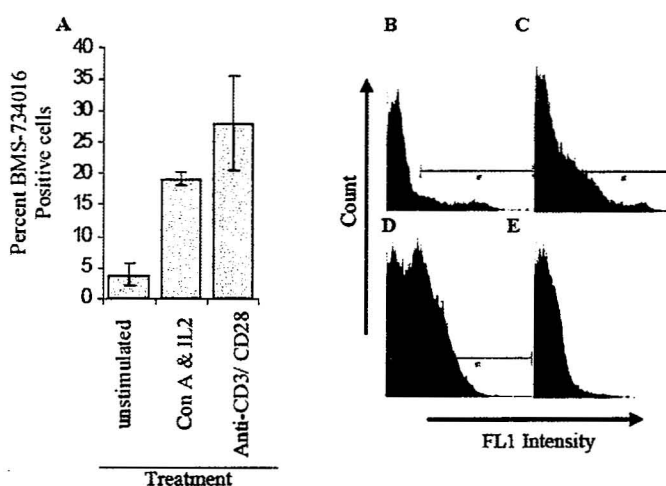
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- After incubation, cells were pelleted, resuspended with either ipilimumab or the negative control for 30 to 60 minutes, then evaluated by FACS

Results notes:

- Ipilimumab bound more strongly to stimulated human lymphocytes, compared to unstimulated human lymphocytes
- From report page 4:

Figure 1: Ipilimumab binds more strongly to human lymphocytes stimulated to express CTLA-4 (study # 930021444)



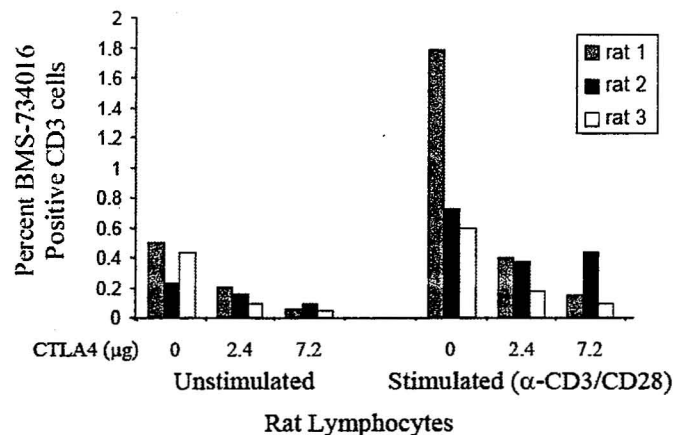
Ipilimumab (BMS-734016) binds to human lymphocytes stimulated in vitro. Purified human lymphocytes were incubated in the presence or absence of combinations of Con A and IL-2 or anti-CD3 and anti-CD28 antibodies for approximately 85 hours. Following incubation, the cells were stained with Ipilimumab conjugated with AlexaFluor® 488 or human IgG isotype conjugated with AlexaFluor® 488. Immuno-stained cells were analyzed on a BD FACScalibur. (A) Graphical representation of percent cells positive for BMS-734016 binding (y-axis) in the presence of indicated treatment (x-axis). (B-E) Histograms from Cell Quest Pro software analysis of flow cytometric data graphed in Figure 1A. (B) Unstimulated cells stained with BMS-734016. (C) Con A and IL-2 treated cells stained with BMS-734016. (D) Anti-CD3 and anti-CD28 treated cells stained with BMS-734016 and (E) isotype control.

Note: The text in the Figure 1 (above) is from the study report

- Ipilimumab bound to monkey lymphocytes stimulated by either anti-CD3 in combination with anti-CD28, or PMA in combination with Ion (data not shown in the study report)
- No binding of ipilimumab to mouse or rabbit lymphocytes was detected
- Ipilimumab bound to approximately 0.3 to 1.8% of stimulated rat CD3+ lymphocytes
 - The authors reported that the results were inconsistent
 - To reduce background, competition assays with recombinant human CTLA-4 were performed, resulting in a dose-dependent inhibition of

- ipilimumab binding to stimulated rat T cells. The authors considered this evidence of specific binding of ipilimumab to rat CTLA-4.
- From report page 5:

Figure 2: Ipilimumab bound stimulated rat T lymphocytes (study # 930021444)



Ipilimumab (BMS-734016) binds to rat lymphocytes and the binding is inhibited by addition of recombinant CTLA4. Purified rat lymphocytes were incubated in the presence or absence of anti-CD3 and anti-CD28 antibodies for approximately 85 hours. Following incubation, the cells were stained with ipilimumab conjugated with AlexaFluor® 488, human IgG isotype conjugated with AlexaFluor® 488 (data not shown), phycoerythrin-conjugated anti-rat CD3 and/or its corresponding isotype control. Phycoerythrin-conjugated anti-rat CD152 was also used as a positive control for CTLA4 expression (data not shown). Three and 9 times microgram quantities of human CTLA4 recombinant protein were used as a competitor for ipilimumab binding. Immuno-stained cells were analyzed on a BD FACScalibur.

Note: The text in Figure 2 (above) is from the study report

4.1.2.7

Study title: Preliminary analysis of dose response MDX-010 binding to human CTLA4-Ig and mouse CTLA4-Fc surfaces by Biacore [®, sic]

Study no:	930019520
Report length:	3 pages
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics) Cross referenced in module 4.2.3.7.7 (Other Toxicity Studies – Other)
Conducting laboratory and location:	Not reported
Report date:	February 27, 2006
Date of study initiation:	Not reported
GLP compliance:	No
Drug:	Ipilimumab (lot # not reported)

Key Study Findings:

- Ipilimumab bound to human CTLA-4 approximately 450-fold more strongly than ipilumab bound to mouse CTLA-4
- These data suggest that the naïve mouse is not a pharmacologically relevant model for nonclinical evaluation of ipilimumab

Study notes:

- Ipilimumab binding to chimeric proteins, CTLA-4 from human or mouse and human immunoglobulin, were evaluated by Biacore® analysis
- Ipilimumab exhibited specific binding to human CTLA-4, avidity $K_D = 1 \times 10^{-7}$ (b) (4)
- Ipilimumab binding to mouse CLTA-4 was not saturated, the avidity K_D ranged from 1×10^{-7} to 1×10^{-6} (b) (4)

4.1.2.8

Study title: Effect of ipilimumab and MDX-1106 on T cell activation during an allogenic mixed lymphocyte reaction (MLR)

Study no:	• MDX-1106/010-001-R • 930036348
Report length:	15 pages
Study report location:	125377/0.16 (amendment received 9/20/2010), Module 4.2.1.1
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	May 25, 2009
Study dates:	October 24, 2008 to February 4, 2009
GLP compliance:	No
Drug, lot #:	Ipilimumab, lot # M31A-04-03Fc

Key Study Findings:

- Under the conditions tested, ipilimumab alone did not induce production of IFN- γ by co-cultures of T cells and dendritic cells

- In combination with another immunostimulatory therapeutic agent, MDX-1106, 5 or 50 µg/m of ipilimumab slightly increased IFN-γ production, compared to that induced by MDX-1106 alone
- The authors conclude that these data indicate that ipilimumab can enhance T cell responses, and that CTLA-4 limits the response of T cells
- Because the T cells were not stimulated to express CTLA-4, the relevance of these results to humans is unclear

Method notes:

- Dendritic cells (DC) were cultured from fresh whole human blood samples
- CD4⁺ T cells were isolated from human blood samples
- MDX-1106 is a monoclonal IgG4 antibody against the programmed death-1 receptor (PD-1)
- To attempt to evaluate MLR, dendritic cells and CD4⁺ T cells were Cells were cultured together with varying concentrations of MDX-1106 and 0, 5 or 50 µg/ml of ipilimumab for 5 days. Culture supernatant was analyzed by ELISA for IFN-γ
- NOTE: The study authors attribute the production of IFN-γ to the T cells in culture; theoretically, the dendritic cells may have produced IFN-γ

4.1.2.9

Study titles:

- **Human CTLA-4 antibodies and their uses**

- Korman et al. United States Patent Application Publication

Study no: • US 20050201994A1
 • US 2005/0201994 A1

Study report location: Module 4.3 (Literature References)

Document date: September 15, 2005

Report length: 86 pages

Synopsis: The applicant provided, as supporting information, a patent application assigned to Medarex, Inc. This reviewer verified that the nonclinical data summarized in the presented in this document was also presented in other study reports submitted to this BLA, and review of these data are documented in this review.

4.1.3 Primary review of *in vivo* primary pharmacodynamic studies

The applicant submitted three *in vitro* primary pharmacodynamic studies evaluating ipilimumab, and an additional six *in vitro* studies evaluating anti-mouse CTLA-4.

4.1.3.1

Study title: Peripheral blood long term immunophenotyping in cynomolgus monkeys after treatment with ipilimumab (BMS-734016), BMS-663513, alone or in combination, during concurrent treatment with Simian Immunodeficiency Virus (SIV) DNA test antigens

Study no.:	<ul style="list-style-type: none">• TIB-06-001• TIB-006-001• 930041502
Study report location:	<ul style="list-style-type: none">• Module 4.2.1.1 (Pharmacology)• Cross-referenced in modules:<ul style="list-style-type: none">◦ 4.2.1.3 (Safety Pharmacology)◦ 4.2.3.2 (Repeat-Dose Toxicity)◦ 4.2.3.7.2 (Immunotoxicology)
Conducting laboratory and location:	Bristol-Myers Squibb, Pharmaceutical Research and Development, Lawrenceville, NJ
Date of first dosing	September 11, 2006
Report date:	November 21, 2007
Report length:	60 pages
GLP compliance:	No
Drug, lot #:	Ipilimumab (BMS-734016, MDX-010), lot # 6G19359

Key Study Findings

- In monkeys treated with 10 mg/kg of ipilimumab for 88 days, treatment had no clear effect on T cells, as measured by flow cytometry
- This reviewer disagrees with the study authors, who concluded that the combination of ipilimumab plus an BMS-663513 (an anti-CD137 antibody) increased mean lymphocyte counts and affected T cell subpopulations
- **NOTE:** The toxicity data for this study were reported in the interim study report (# TIB-06-001 [note the extra leading zero in the final report number]; # 930031729) but not in the final study report reviewed here. The interim study report is reviewed below (under Repeat-Dose Toxicity, section 6.3). Briefly, two animals co-treated with ipilimumab plus BMS-663513 exhibited toxicity and recovered after cessation of treatment.

Methods

Route of administration: Intramuscular (IM) or intravenous (IV)

Doses: 5 dose groups:

- IM doses on D1, 2, 29, 30, 56, 58, 85, 85
- Group 5 (control) received IM and IV doses of vehicle
- Groups 1-4 received IM doses of 2 mg/monkey each of three SIV test antigens (DNA vaccines: SIV gag, SIV pol, and SIV

- env)
- Group 1 received IV doses of saline
- Group 2 received 10 mg/kg IV of BMS-663513 (fully human IgG4 against human CD137)
- Group 3 received 10 mg/kg IV of ipilimumab
- Group 4 received 10 mg/kg of BMS-663513 plus 10 mg/kg of ipilimumab
- Frequency of dosing
 - IM: D1, 2, 29, 30, 57, 58, 85, 86
 - IV: D4, 9, 30, 32, 58, 60, 86, 88
- Dose volume:
 - IM: 2 ml/day
 - IV: 2 to 5 ml/kg
- Formulation/Vehicle:
 - IM: 0.15 citrate buffer, pH 6.7 with 0.25% bupivacaine in water
 - IV: 0.9% sodium chloride for injection, USP
- Species/Strain: Treatment-naïve cynomolgus monkeys
- Number/Sex/Group:
 - 3/sex for the control group (group 5)
 - 4 males and 2 females for groups 1-4
- Age: 2 to 7 years
- Weight: 2.3 to 6.1 kg

Methods notes:

- The putative mechanism of action for BMS-663513 is binding to and activation of CD137, resulting in T cell activation. The published literature indicates that CD137 is expressed on several types of immune cells (including activated T cells, dendritic cells, and NK cells).
- The IM dosing of the SIV DNA vaccines was on two consecutive days, 4 weeks apart, x 4.
- The study report did not provide a rationale for the timing of administration of the antibodies.
- Blood was collected for flow cytometry-based immunophenotyping: pre-dose, D16, 44, 72, 116, 185, 212, and 275. The markers assayed were: PD-1, CCR5, CD4, CD8, CD3, CD69, CD25, CD45RA, CCR7, IFN- γ , and 4-1BB. The authors note (pages 18-19):
 - PD-1 was evaluated as an inhibitory receptor
 - CCR5 was evaluated as a putative homing receptor
 - 4-1BB (CD137) was evaluated as a putative co-stimulatory receptor
 - CD25 and CD69 were evaluated to assess T cell activation status
 - CD45RA and CCR7 were evaluated to assess T cell memory status
 - IFN- γ was evaluated to assess functional status

Results notes:

- Numerous statistically significant differences were detected; the relationship to treatment was generally unclear
- The authors concluded that none of the treatments affected CD3⁺CD4⁺ or CD3⁺CD8⁺ counts, and this reviewer concurs
- The authors concluded that the addition of ipilimumab to the SIV-DNA vaccine (with or without BMS-663513) increased the frequency of PD-1⁺ cells, CD25⁺ cells, CCR7⁺CD4⁺ cells, and CCR7⁺CD45RA⁺ cells compared to the SIV-DNA alone group. The authors also concluded that antibody co-treatment was associated with higher mean absolute lymphocyte counts (ALC). This reviewer disagrees, and concludes that no clear differences in flow cytometry endpoints were detected
 - Dramatic variability among time points suggest that the assay was not calibrated consistently
 - In the context of the magnitudes of the pre-dose differences, the variability among time points, the within-group variability, and the relative small magnitudes of differences, no treatment-effect is apparent
 - The study authors did not attempt to quantify the differences
 - The data were presented graphically (means and error bars), but line-listed data were not provided

4.1.3.2**Study title: Monoclonal antibody adjuvants for the improvement of DNA vaccines**

Study no:	930031729
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	Not reported; data were analyzed in October, 2009
GLP compliance:	No
Drug:	Ipilimumab (lot # not reported)

Key Study Findings:

- The results suggest that ipilimumab treatment (dose not reported) may have transiently increased proliferation of CD4⁺ T cells in cynomolgus monkeys
- Due to lack of detail, this study is of limited usefulness for evaluating the potential pharmacology of ipilimumab

Method notes:

- Groups of 6 cynomolgus monkeys (genders, age, and weights not reported) were immunized four times with DNA constructs (as vaccines for simian immunodeficiency virus [SIV]), on D15, 43, 71, and 115

- After each immunization, monkeys received ipilimumab (dose level not reported), BMS-663513 (anti-CD137 antibody), or ipilimumab plus BMS-663513
- Blood samples were collected on D0, 15, 43, 71, 115 and 397, and PBMCs cultured.
- Supernatant from cultured PMBCs was analyzed for IFN- γ and IL-4 production
- Additionally, PBMCs were stimulated to proliferate for 5 days with (unspecified) antigenic peptides, and the percentages of CD8 $^{+}$ cells and CD4 $^{+}$ cells were measured by flow cytometry
- Statistical analyses were performed by Bristol-Myers Squibb Research and Development (and documented in three memoranda submitted with the main report)
- The study is submitted to module 4.2.1.1 as four .pdf documents
 - The main document is nine pages, titled "Monoclonal antibody adjuvants for the improvement of DNA vaccines"
 - The second document is a nine page memorandum, subject line "statistical analysis of %CD4 $^{+}$ T cell response"
 - The third document is an eight page memorandum, subject line "statistical analysis of %CD8 $^{+}$ T cell response"
 - The fourth document is an seven page memorandum, subject line "statistical analysis of the data from IFN- γ ELISpot assays"

Results notes:

- The authors at the (b) (4) report that 10 to 12 months after monkeys were dosed, ipilimumab treatment was associated with inhibition of IFN- γ , and increased proliferation of CD8 $^{+}$ cells
- However, the authors at BMS (who did statistical analysis) concluded that:
 - On D43 only, ipilimumab treatment was associated with increased percentages of CD4 $^{+}$ T cells by approximately 9-fold, compared to control-vaccinated animals (i.e. monkeys that did not receive ipilimumab or BMS-663413)
 - No treatment-related effects on CD8 $^{+}$ T cells were detected
 - No treatment-related effects on IFN- γ secretion were detected

4.1.3.3

Study title: Effects of human anti-CTLA-4 administration on unstaged MC38 tumors in hCTLA-4 transgenic mice

Study no:	<ul style="list-style-type: none"> • MDX-010-005-R • 930020107
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	38 pages
Conducting laboratory and location:	Medarex, Milipitas, CA
Report date:	December 22, 2006
Study period:	April 22, 2005 to April 30, 2006
GLP compliance:	No
Drug:	Ipilimumab (lot # not reported)

Key Study Findings:

- Ipilimumab exhibited clear anti-tumor activity, slowing growth of implanted MC38 (murine) tumors and prolonging survival in transgenic mice, genetically engineered to express human CTLA-4, but not mouse CTLA-4
 - Because MC38 cells do not express human CTLA-4, this data provide a proof-of-concept that the mechanism of action for ipilimumab is indirect
- In the transgenic mice, 9D9 (anti-mouse CTLA-4) did not inhibit growth of the implanted murine tumor. This finding is consistent with expected pharmacology (because the transgenic mice express human CTLA-4 but not mouse CTLA-4)

Methods notes:

- A transgenic mouse strain was developed by (b) (4) to be human CTLA-4^{+/+} and mouse CTLA-4^{-/-}, on a C57Bl/6 background
- MC38 cells, a murine colon adenocarcinoma, were implanted into the right flank of each animal
- Animals were checked daily (mortality, posture, grooming, respiration, lethargy) and weighed twice weekly. Animals were euthanized if weight loss reached or exceeded -15% of baseline.
- Tumors were measured at 3 to 5 day intervals in three dimensions.
 - For experiment 1, animals with tumors $\geq 4000 \text{ mm}^3$ were euthanized
 - For experiments 2-4, animals with tumors $\geq 1500 \text{ mm}^3$ were euthanized
- For all 4 experiments, groups of 10 mice received saline (negative control), 10 mg/kg of ipilimumab, 10 mg/kg of a human IgG1 (hulgG1, isotypic control)
- For experiments 3 and 4 only, groups of 10 mice received 10 mg/kg of 9D9, an anti-mouse CTLA-4 antibody

Experiment 1 notes:

- Mice were implanted with 2×10^6 MC38 cells on D0, and then received intraperitoneal (IP) injections (saline, ipilimumab, or hulgG1) on D0, 3, 6, 10 and 13
- The experiment was terminated on D24
- Although a treatment-effect was not initially apparent (D0 to D17), 8/10 mice treated with ipilimumab had smaller tumor sizes on D20 (approximately half the tumor size of controls) and D24 (smaller than half the tumor size of controls)

Experiment 2 notes:

- Mice were implanted with 1×10^6 , 2×10^6 , or 3×10^6 MC38 cells on D0, and then received IP injections (saline, ipilimumab, or hulgG1) on D3, 6, and 10
- The experiment was terminated on D41 or D49
- The inhibition of tumor growth by ipilimumab was apparent by approximately D30
- Ipilimumab treatment was clearly associated with longer survival
 - For the groups inoculated with 1×10^6 MC38 tumor cells, 8/9 of the hulgG1-treated control mice did not survive to D24, while all ipilimumab-treated mice survived to D41 (these groups were euthanized on D41)
 - For the groups inoculated with 2×10^6 tumor cells, 5/10 of the hulgG1-treated mice did not survive to D21, while all ipilimumab-treated mice survived to D34 and 4 ipilimumab-treated mice survived to D49

- For the groups inoculated with 3×10^6 MC38 cells, 7/10 of the hulgG1-treated mice did not survive to D24, while all ipilimumab-treated mice survived to D34, and 8/10 survived to D49

Experiment 3 notes:

- The number of MC38 cells implanted was not reported. Animals received IP injections (saline, ipilimumab, hulgG1, or anti-mouse CTLA-4) on D3, 6 and 10
- The experiment was terminated on D52
- Ipilimumab treatment delayed tumor growth and prolonged survival compared to the other groups
 - In the saline control group, 6/10 mice did not survive to D24
 - In the hulgG1 control group, 10/10 mice survived to D24, but 8/10 did not survive to D31
 - In the ipilimumab-treated group, 10/10 animals survived to D31 and 3/10 survived to D52
- Treatment with anti-mouse CLTA4 did not exhibit an anti-tumor effect

Experiment 4 notes:

- Mice were implanted with 2×10^6 MC38 cells on D0.
- Animals were received IP injections (saline, ipilimumab, hulgG1, or anti-mouse CTLA-4) on D3 and D6 only
- The experiment was terminated on D21
- No treatment-related effect was apparent for ipilimumab. The authors concluded that the reduced dosing schedule was not sufficient.

4.1.3.4

Study title: Effects of anti-CTLA4 and dexamethansone in a therapeutic SAI/N tumor model

Study no:	<ul style="list-style-type: none"> • MDX-010-001-R • 930020103
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	53 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
Date of study report:	January 9, 2007
Study period:	May 16, 2006 to November 3, 2006
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA-4 monoclonal antibody), lot # 1-2-05 and lot 4-27-06

Key Study Findings:

- Anti-mouse CTLA-4 exhibited anti-tumor activity against SA1/N cells implanted into A/J mice
- Co-treatment with anti-mouse CTLA-4 and dexamethasone was less active in inhibiting tumor growth, as compared to anti-mouse CTLA-4 treatment alone
 - Dexamethasone treatment alone did not affect tumor growth

Notes:

- SA1/N cells are mouse fibrosarcoma cells
- Dexamethasone is a corticosteroid; the purpose of co-treatment was to support the clinical adjuvant use of corticosteroids to treat adverse events related to ipilimumab
- Three different experiments were performed. The authors combined the results for Day 18:
 - Anti-CTLA alone resulted in 43% of treated mice being tumor-free
 - Anti-CTLA with 1 mg/kg of dexamethasone resulted in 25% of mice being tumor-free
 - Anti-CTLA with 10 mg/kg of dexamethasone resulted in 18% of mice being tumor free
- Colon lengths were measured as an indicator of inflammation, and histopathology was performed. No treatment-related inflammation, or increases in inflammatory cell infiltration, were observed

4.1.3.5

Study title: PD-1 and CTLA-4 blockade in the murine MC38 colon adenocarcinoma model

Study no:	<ul style="list-style-type: none"> • MDX-1106/010-002-R • 930036349
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	69 pages
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	July 22, 2009
Study period:	October 14, 2009 to July 27, 2006
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA-4 monoclonal antibody), lot # 01.07.05 and lot # 03.08.06

Key Study Findings:

- Anti-mouse CTLA-4 exhibited anti-tumor activity against MC38 cells implanted in C57Bl/6 mice
- Co-treatment with MDC-1106, an anti-mouse PD-1 antibody, increased the anti-tumor effect

Notes

- MC38 cells are mouse colon adenocarcinoma cells
- Three different experiments were conducted, to test different doses and time courses
- Anti-mouse CTLA-4 treatment delayed tumor growth and prolonged survival, and both parameters were improved by co-treatment with the anti-PD-1 antibody

- The authors report that treatment did not affect mouse body weights (data not provided in the study report)

4.1.3.6**Study title: PD-1 and CTLA-4 blockade in the murine CT26 colon adenocarcinoma model**

Study no:	• MDX-1106/010-003-R • 930036351
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	20 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
Report date:	February 16, 2006
Study period:	January 23, 2006 to February 16, 2006
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA-4 monoclonal antibody), lot # PC CTLA4 40

Key Study Finding:

- Anti-mouse CTLA-4 exhibited anti-tumor activity against CT36 cells implanted in female BALB/c mice
- Co-treatment of mice with anti-mouse CTLA4 in combination with anti-mouse PD1 resulted in increased anti-tumor activity, compared to anti-mouse CTLA-4 alone

Notes:

- CT36 cells are murine colon adenocarcinoma
- Tumors were implanted subcutaneously (D0)
- Groups of 10 mice were dosed on D10, 14, 17 and 21 with saline (negative control), 9D9, anti-mouse PD1, both 9D9 and anti-mouse PD1 together, or an isotypic control (mouse IgG1). All mice survived to necropsy (D24)
- Anti-mouse CTLA-4 slowed tumor growth by approximately 38% compared to controls on D21
- Anti-PD1 alone was not active. Anti-mouse CTLA-4 in combination with anti-mouse PD1 slowed tumor growth by approximately 66% compared to controls on D21
- Body weight was measured on D10 and D17 only; no treatment-related effects were apparent

4.1.3.7**Study title: PD-1 and CLTA-4 blockade in the murine SA1/N fibrosarcoma model**

Study no:	• MDX-1106/010-004-R • 930036352
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	24 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
Report date:	July 14, 2009
Study period:	April 21, 2005 to May 31, 2005
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA4), lot # PC CTLA4 40

Key Study Findings:

- Anti-mouse CTLA-4 exhibited anti-tumor activity against SA1/N tumor cells implanted into A/J mice at doses of 10 mg/kg x4 (delayed tumor growth, more mice tumor-free by D40), but not at doses of 0.2 mg/kg x4
- Results suggested that combining a sub-active dose of anti-CTLA-4 (0.2 mg/kg) with another therapeutic agent might enhance anti-tumor activity
 - Anti-mouse PD-1 exhibited anti-tumor activity in this model, and the combination of anti-mouse PD-1 and 0.2 mg/kg of anti-mouse CTLA-4 exhibited stronger anti-tumor activity than either agent alone

Notes:

- SA1/N tumors are a murine fibrosarcoma cell line
- Groups of 10 female A/J mice were dosed with saline (negative control), an isotypic control (mouse IgG1), 0.2 or 10 mg/kg of 9D9, 10 mg/kg of anti-mouse PD-1, or 10 mg/kg of anti-mouse PD-1 in combination with 0.2 mg/kg of 9D9 on D1, 4, 7 and 11
- Mice were euthanized when tumors either reached $\geq 1500 \text{ mm}^3$ or ulcerated
- Dosing with the combination of 10 mg/kg of anti-mouse CTLA-4 and 10 mg/kg of anti-mouse PD-1 antibodies delayed tumor growth
- The combination of 10 mg/kg of anti-mouse PD-1 and 0.2 mg/kg of anti-mouse CTLA-4 appeared more active for delaying tumor growth
- Tumor growth and survival were measured; the Day 40 (D40) data are representative. At D40:
 - None of the control mice were tumor-free (0/9 for saline, 0/10 for the mouse IgG1), and none of the mice receiving 0.2 mg/kg of anti-mouse CTLA-4 were tumor free (0/10)
For the mice receiving 10 mg/kg of anti-mouse CTLA-4, 4/10 were tumor free
 - For the mice receiving 10 mg/kg of anti-mouse PD-1, 4/10 were tumor free
 - For the mice receiving 10 mg/kg of anti-mouse CTLA-4 in combination with 0.2 mg/kg of 9D9, 8/10 were tumor free

- Body weights were measured pre-dose, D1, D7 and D11 only; no treatment-related effects were apparent

4.1.3.8

Study title: Tumor response to PD-1 and CTLA-4 blockade in murine B16-F10 melanoma and J558 myeloma models

Study no:	<ul style="list-style-type: none">• MDX-1106/010-005-R• 930036353
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	26 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
Report date:	May 18, 2006
Study period:	January 9, 2006 to May 18, 2006
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA4), lot # PC CTLA4 40

Key Study Findings:

- Treatment with anti-mouse CTLA-4 antibody did not inhibit tumor growth of murine melanoma or myeloma tumor cells implanted into female mice, either alone or in combination with anti-PD-1 antibody treatment

Notes:

- B16-F10 tumor cells were implanted into female C57 mice (D0)
 - Mice received IP injections on D8, 11, 14 and 17
- J558 tumor cells were implanted into BALB/c mice (D0)
 - Mice received IP injections on D10, 13 and 17
- 10 mice per group were used
- Dose groups: saline (negative control), mouse IgG1 (inactive antibody control), 10 mg/kg of anti-mouse CTLA-4, 10 mg/kg of anti-mouse PD-1, or 10 mg/kg of anti-mouse CTLA-4 combined with 10 mg/kg of anti-mouse PD-1 antibodies
- No body weight information was presented in the study report
- The study authors concluded, and this reviewer agrees, that neither monotherapy with either anti-mouse CTLA-4 and anti-mouse PD-1, nor the combination of anti-mouse CTLA-4 and anti-mouse PD-1, showed anti-tumor activity under the conditions tested

4.1.3.9**Study title: Antitumor activity of antibodies to CTLA4, CD137 or their combination in murine tumor models**

Study no:	• 930031045 • SA1N#23 • P815#26 • EMT-6#15 • B16F10luc#1 • Renca#4
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	20 pages
Conducting laboratory and location:	Bristol-Myers Squibb Company, Research and Development, Lawrenceville, NJ
Report date:	October 23, 2008
Study period:	Not reported
GLP compliance:	No
Drug:	BMS-863019 (hamster IgG anti-mouse CTLA-4 antibodies (b) (4))

Key Study Findings:

- Treatment with the anti-mouse CTLA-4 antibody alone exhibited anti-tumor activity against the three of the five murine tumor cell lines implanted into female mice
- The anti-tumor activity of the anti-mouse CTLA-4 was enhanced by co-treatment with another immunostimulatory therapeutic agent in the three lines for which anti-mouse CTLA-4 alone had exhibited activity

Notes:

- Cell lines (implanted on D0):
 - P815 cells are murine mastocytoma, and were implanted into female DBA/2 mice
 - SA1/N cells are murine fibrosarcoma, and were implanted into female A/J mice
 - EMT-6 are murine mammary tumors, and were implanted into female BALB/c mice
 - B16-F10-Lu are murine melanoma, and were implanted into female C57/BL6 mice
 - Renca are murine renal tumors, and were implanted into BALB/c mice
- Groups of 9 mice were used, and received 3 doses (once every 3 days, the starting time relative to tumor implantation varied from D9 to D12)
- Treatment groups were inactive IgG; 10 mg/kg of BMS-863019; 5 mg/kg of an anti-mouse CD137 antibody, or 10 mg/kg of BMS-863019 in combination with the anti-CD137 antibody (0.04, 0.2, or 1 mg/kg/dose)

- The anti-mouse CTLA-4 antibody treatment slowed tumor growth and increased the number of tumor-free mice relative to the control group for the P815, SA1/N and EMT-6 tumors, but not the B16-F10-Luc or the Renca tumors
- The combination therapy increased the anti-tumor responses against the P815, SA1/N and EMT-6 tumors, but no activity was observed with the combination treatment in either B16-F10-Luc or Renca tumor-bearing animals

4.2 Secondary Pharmacology

NOTE: The sponsor reports that no stand-alone experiments were conducted to evaluate secondary pharmacology (Module 2.6.2, section 3 "Secondary Pharmacodynamics"). However, because the proposed mechanism of action of ipilimumab is CLTA4-blockade, the investigations into antibody dependent cellular cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC) may properly be considered relevant to secondary pharmacology rather than primary pharmacology. Therefore, this reviewer documented review of these studies here.

4.2.1

Study title: The effect of ipilimumab (MDX-010) on antibody-dependent cellular cytotoxicity

Study no:	930023602
Study report location:	Module 4 .2.1.1. (Primary Pharmacodynamics)
Report length:	13 pages
Conducting laboratory and location:	Bristol-Myers Squibb Research and Development. LVL site (it is not clear to this reviewer if this is the Lawrenceville, NJ site)
Report date:	December 4, 2007
GLP compliance:	No
Drug, lot #:	<ul style="list-style-type: none">• Ipilimumab (lot # 902530)• Biotinylated MDX-010 (i.e. biotinylated ipilimumab; lot # not reported)

Key Study Finding

Ipilimumab induced ADCC against stimulated T-cells from normal donors, under the conditions tested

Methods notes:

- Ipilimumab is a fully human IgG1 monoclonal antibody. IgG1 antibodies bind with high affinity to CD64 (FcγRI) and with lower affinity to CD32 (FcγRII) and CD16 (FcγRIII). CD16 is expressed on natural killer (NK) cells, and CD64 is

expressed on macrophages and neutrophils. Binding of IgG1 antibodies to these CD proteins results in antibody-dependent cellular cytotoxicity (ADCC)

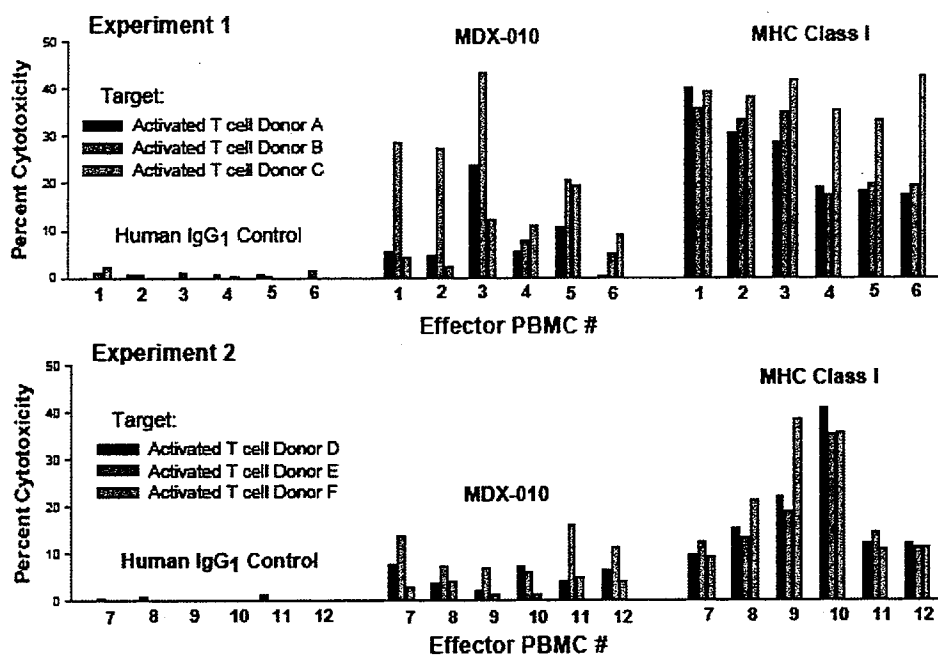
- Target T-cells were isolated from blood samples from six healthy donors, by rosetting with sheep red blood cells
- Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of 12 healthy donors
- The T-cells were stimulated to express CTLA-4: they were grown in (b) (4) and treated for 3 days with an anti-CD28 antibody and recombinant interleukin-2 (rIL2).
 - Expression of CTLA-4 was verified by flow cytometric analysis, using both biotinylated ipilimumab and biotinylated anti-CTLA-4 (murine anti-human CD152, obtained commercially from (b) (4)). As a positive control for the flow cytometric assay, MHC Class 1 antibodies were used.
- For the ADCC assay (b) (4)

Results notes:

- Ipilimumab co-culture with the CTLA-4 expressing T cells induced ADCC against the target PBMC by 9.4 to 42.5%, as compared to target PBMC incubated with effector cells alone. The induction of cytotoxicity was markedly greater with ipilimumab co-culture than the response with the negative control antibody, but was generally less robust than the positive control antibody response
- The authors considered the induction of ADCC variable

- From the report (p 11):

Figure 3: Ipilimumab induced ADCC in human T-cells stimulated to express CTLA-4 (study # 930023602)



4.2.2

Study title: Effect of ipilimumab on complement-dependent cytotoxicity

Study no: 930025694
 Study report location: Module 4.2.1.1 (Primary Pharmacodynamics)
 Report length: 13 pages
 Conducting laboratory and location: Bristol-Myers Squibb, Research & Development, Lawrenceville, NJ
 Report date: December 17, 2007
 GLP compliance: No
 Drug, lot #: Ipilimumab, lot # 902530

Key Study Findings: Ipilimumab did not induce CDC under the conditions tested. The usefulness of the study is limited by the design, and the results may not be relevant for humans.

Methods notes:

- Human T cells were isolated from the blood of seven healthy volunteers, and were stimulated to express CLTA-4, (b) (4)

- Stimulated T cells were exposed for 30 minutes to ipilimumab or isotypic control (human IgG1), or to positive control antibodies (anti-CD45RO)
- After antibody exposure, T cells were incubated with (b) (4). Uptake of propidium iodide was determined by flow cytometry as a measure of viability
- To verify expression of CTLA-4, flow cytometry was performed on stimulated T cells, using another anti-CTLA-4 antibody

Results notes:

- Ipilimumab was not associated with increased CDC
- However, the positive control antibody gave muted responses (2- to 3.5-fold over the negative control antibody)
- The report does not explain why (b) (4) was used; nor does the applicant provide any justification for the use of (b) (4)

4.2.3

Study title: MDX-101-mediated effector function *in vitro*

Study no:	• MDX-010-006-R • 930020459
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	34 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
Report date:	February 22, 2007
Study period:	October 18, 2005 to December 20, 2006
GLP compliance:	No
Drug:	Ipilimumab (lot # not reported)

Key Study Finding:

- Ipilimumab bound stimulated human T cells, but not resting human T cells
- Ipilimumab induced ADCC in stimulated human T cells, but not resting human T cells
- Blockade of human FcγIII (CD16) by anti-CD16 antibodies inhibited ipilimumab-induced ADCC, suggesting that binding of ipilimumab by CD16 is necessary for induction of ADCC
- No treatment-related CDC detected

Methods notes:

- CD4⁺ T cells were isolated from human blood and activated by exposure to anti- (b) (4)
- Expression of CTLA-4 and MHC class 1 was evaluated by flow cytometry
- For the ADCC assay (b) (4)

- For the CDC assay, [REDACTED]

(b) (4)

Results note:

- A dose-response was observed for ipilimumab-induced ADCC in stimulated T cells
- No treatment-related CDC was apparent. This reviewer considers these CDC results to be more relevant to humans than the CDC results reported in study # 930025694 (because this study used human complement and tested activated T cells)

4.2.4

Study title: Activity of MDX-010 antibodies derived from CHO transfectoma and hybridoma cell lines in cytotoxicity assays

Study no:	• MDX-010-015-SR • 930026820
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	5 pages
Report date:	November 25, 2007
Study period:	January 2003
Conducting laboratory and location:	Medarex, Bloomsbury, NJ
Report date:	January 2003
Study period:	January 2003
GLP compliance:	No
Drug:	Ipilimumab (no lot # reported; both CHO and hybridoma-derived products were tested)

Key Study Findings:

- Stimulation of human T cells with phytohemagglutinin (PHA) induced CTLA-4 expression
- Ipilimumab induced ADCC in PHA-stimulated human T cells, but the results were not consistent
- CDC was not detected

Methods notes:

- Human mononuclear cells were isolated from human blood, and exposed to PHA for 3 days
- For the ADCC assay, details are not provided
- For the CDC assay, [REDACTED] (b) (4) was used
- Flow cytometry was used to verify expression of CTLA-4, CD69 and HLA-DR by the stimulated cells

Results notes:

- The authors conclude that ADCC was not induced by ipilimumab. This reviewer disagrees – based on the summary data provided in the study report, ADCC was induced, although less strongly than the positive control antibody (anti-CD3)
- The study authors concluded, and this reviewer agrees, that no treatment-related CDC was apparent. Although activated T cells were evaluated, the use of (b) (4) is a study limitation. The relevance of the CDC results from this experiment to humans is unclear.

4.2.5

Study title: Binding of ipilimumab (MDX-010) to human FCyRI/CD64, FCyRIIA/CD32A and FCyRIII/CD16 determined by ELISA

Study no:	• STR-131 • 930022419
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	17 pages
Conducting laboratory and location:	Bristol-Myers Squibb Company, Protein Therapeutics Development – Bioanalytical Sciences, Hopewell, NJ
Report date:	July 2007
GLP compliance:	No
Drug, lot #:	Ipilimumab, lot #s: 41969, 43357, 43964

Key Study Findings: As expected for an IgG1, ipilimumab bound human FcyRI (CD64) with high affinity, and also bound human FcyRIIA (CD32a) and human FcyRIII (CD16)

Methods notes:

- Ipilimumab is a human IgG1 monoclonal antibody. FcyRI is a high affinity receptor for IgG proteins (binding IgG1 and IgG3 with comparable affinity, and IgG4 with relatively lower affinity)
- Three different lots of ipilimumab were compared
- The assay method was ELISA; commercially available, recombinant human FcyRI, FcyRIIA and FcyRIII were used
- As negative controls, human IgG1 kappa, IgG3 kappa, and IgG4 kappa monoclonal antibodies were used as controls

Results notes:

- EC₅₀ (b) (4) for ipilimumab binding to FcyRI
- EC₅₀ values could not be calculated for either FcyRIIA and FcyRIII (the lower affinity binding prevented identification of the upper asymptote of the binding curve)

4.2.6**Study title: Comparison of MDX-010 (ipilimumab) manufacturing processes to demonstrate antibody equivalency for *in vitro* ADCC and CDC activity**

Study no:	<ul style="list-style-type: none">• MDX-010-016-R• 930046913
Study report location:	BLA 125377, amendment submitted 9/30/2010, Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	<ul style="list-style-type: none">• Main report: 26 pages• Amendment: 7 pages
Conducting laboratory and location:	Medarex, Sunnyvale, CA
GLP compliance and QA statement:	No
Report date:	August 19, 2009
Study period:	June 15, 2009 to June 19, 2009
Drug, lot #:	Ipilimumab process B material (lot # 7J27973) and process C material (lot # 8K256)

Key Study Finding: Ipilimumab induced ADCC (but not CDC) against stimulated human T cells

Notes:

- Blood from 7 donors was obtained and PBMCs isolated
- T cells were isolated from the PBMCs; T cells were stimulated with CD3 and CD28 (b) (4)
- Anti-MHC Class 1 antibody was tested as a positive control for ADCC and CDC
- Two isotype control antibodies were tested as negative controls
- For the ADCC experiment, ipilimumab induced specific lysis of stimulated T cells, ranging from approximately 10% to approximately 50%
- NOTE: The amendment was reviewed

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted (Module 2.6.2, section 4 "Safety Pharmacology"). Safety pharmacology endpoints were included in several of the GLP-toxicology studies (reviewed in Section 6, below). Overall, the results did not raise concerns for effects of ipilimumab on safety pharmacology parameters (i.e. neuromotor, cardiac or respiratory function).

The GLP one-month study (study # DS06064) did not observe signs of toxicity. Endpoints measured included clinical signs, nervous system evaluation (behavior, coordination and balance, movement, muscle tone, proprioception, spinal reflexes, peripheral and cranial nerve function), body temperature (recorded 1, 4 and 24 hours

after the first dose), cardiovascular evaluation (thoracic auscultation, heart rate, femoral pulse), respiratory evaluation (lung sounds by thoracic auscultation, respiratory rate, arterial oxygen saturation), and electrocardiogram.

The GLP 6-month study (study # 01-3460) administered ipilimumab (10 mg/kg) alone or in combination with another agent on D0, 28, 56, 84 and 140. No changes noted at cage-side observations (mortality, general condition, appearance, behavior, activity, respiration).

Notably, signs consistent with acute infusion reaction were observed in one monkey (study # TIB-006-001): approximately 5 minutes after ipilimumab treatment (and 2 minutes after the administration of a test antigen) the animal exhibited difficulty breathing, cyanosis, thready pulse, and muffled heart sounds. The animal was re-challenged 5 months later without toxicity.

5 Pharmacokinetics/ADME/Toxicokinetics

Regarding ADME (absorption, distribution, metabolism, and excretion), the *in vivo* studies in monkeys administered ipilimumab IV, formulated in saline.

The pharmacokinetic (PK) and toxicokinetic (TK) data are reviewed below in section 6 of this review (General Toxicology). Notable findings are summarized here:

- Elimination half-life ($t_{1/2}$):
 - $t_{1/2} = 203 \pm 63$ hours (following a single 10 mg/kg IV dose, data collected for 28 days; study # 1416-128)
 - $t_{1/2} = 339 \pm 112$ hours (data collected for 9 weeks after the final dose, study # DS07167)
- Steady-state volume of distribution (V_{ss}) was variable, but indicates that ipilimumab was confined to the plasma space (approximately 40 to 70 ml/kg):
 - $V_{ss} = 44 \pm 6$ ml/kg (study # 1416-128)
 - $V_{ss} = 81 \pm 14$ ml/kg (study # DS07167)
- Generally, exposure was dose-proportional or greater than dose-proportional
- Accumulation was observed with frequent dosing (e.g. weekly or more often than weekly dosing)

The PK portions of the toxicology studies were GLP-compliant only for:

- The 79-day study (# 1416-128)
- The one-month studies (#DS06064 and SUV00106)

ELISA was used to detect circulating levels of ipilimumab, and the PK detection methods were improved during product development. The current ELISA method is standard test method (STM) 1477-00; it has a lower limit of detection of 1.2 µg/ml of ipilimumab.

6 General Toxicology

The sponsor identified two pharmacologically relevant non-human animal models for testing ipilimumab: the primate and the transgenic mouse model (expressing human CTLA-4 under the mouse CTLA-4 promoter). Toxicology studies with ipilimumab were performed only in the cynomolgus monkey, and only by the IV route of administration. Additionally, three primary pharmacology studies performed with anti-mouse CTLA-4 are relevant to the evaluation of toxicity for ipilimumab (studies reviewed below in section 6.1 of this review)

6.1 Disease models

The applicant submitted three non-GLP studies (studies #93003104, # MDX-106-010-006-R, and # MDX-1106/010-007-R), investigating anti-mouse CTLA-4 in disease models, under Primary Pharmacodynamics. This reviewer considers the results to be more relevant to toxicity than to pharmacology.

6.1.1

Study title: Activity of antibodies to CTLA-4, CD137 and their combination in murine models of colitis

Study no:	• 930031040
	• "Colitis # 2, 3, 4, 7, 8, 10"
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Conducting laboratory and location:	Bristol-Myers Squibb Company Research & Development, Lawrenceville, NJ
Report date:	October 15, 2008
GLP compliance:	No
Drugs:	BMS-863019, an anti-mouse CTLA-4 antibody. Purity < 95%

Key Study Finding: In two models of auto-immune related colitis, the surrogate anti-CTLA-4 antibody "exacerbated the onset and severity of the disease" (report p 7). The treatment-related toxicity was comparable to the gastrointestinal toxicity observed in patients receiving ipilimumab.

Methods notes:

- BMS-863019 is hamster IgG anti-mouse CTLA-4. The clone used to produce BMS-863019 for this study was commercially-available (b) (4)
- Another antibody, an anti-mouse CD137 (BMS-469492) was tested alone and in combination with the anti-mouse CTLA-4
- Six-week old male SJL/J mice (18-22 g) were used for this study
- Oxazolone-induced colitis model
 - Mice received a dermal dose of 150 µg of 3% oxazolone (4-ethoxymethylene-2-phenyl-2-oxazolin-5-one) in ethanol vehicle,

- administered to shaved skin (2 x 2 cm patch) on day 0, to sensitize the mice
- Five days later, mice were anesthetized and re-challenged with an intra-rectal dose of 100 µl of either 0 or 0.75 % oxazoline in 50% ethanol vehicle. The catheter was inserted 4 cm proximal to the anal verge
 - Mice received varying concentrations of vehicle (negative control), anti-mouse CTLA-4, anti-mouse CD137, or a combination of both agents repeatedly (various schedules)
 - Clinical signs and body weight were evaluated daily
 - At necropsy, colons were excised and colon length was measured.
 - The authors report that this model is “a mixed T helper (Th)1/Th2 colitis model” (report page 7) and that mainly the distal half of the colon becomes inflamed (report page 17)
- Trinitrobenzene sulfonic acid (TNBS)-induced colitis model
 - Mice received an intra-rectal doses of 0 or 2 mg of TNBS in 35% ethanol (volume of 100 µl/mouse) on D0. The catheter was inserted 4 cm proximal to the anal verge
 - Mice received varying concentrations of vehicle (negative control), vehicle (negative control), anti-mouse CTLA-4, anti-mouse CD137, or a combination of both agents repeatedly (various schedules)
 - repeatedly (various schedules)
 - Clinical signs and body weight were evaluated daily
 - The authors report that this model is “mainly driven by a Th1 response” (page 7) and that it causes inflammation in “most of the colon” (page 17)

Results notes:

- The surrogate anti-mouse CTLA-4 antibody, BMS-863019, caused weight-loss (transient, approximately 10% decrease at the nadir) and shortened colon length in both models, compared to mice that did not receive oxazolone/trinitrobenzene. In the oxazolone-induced colitis model, the anti-CTLA-4 antibody caused earlier onset of mortality, and histopathologic changes in the colon were detected, “high infiltration of monocytic cells and tissue injury” (p 12), compared to oxalone-treated mice that did not receive anti-mouse CTLA-4 (i.e. receiving either vehicle or anti-mouse CD137 without anti-mouse-CTLA-4)
- The anti-mouse CD137 antibody improved survival (compared to oxazolone alone) when given alone, and when given in combination with anti-mouse CTLA-4 (compared to oxazolone + anti-mouse CTLA-4)
- From the report (page numbers 12 and 14):

Table 9: anti-mouse CTLA-4 antibody increased mortality in a mouse colitis model (study # 930031040)

Group #	D0 skin treatment	Treatment	Dose (mg/kg)	Days of dosing	D5 intra-rectal challenge	Dead mice/total
1	3% oxazolone	None	0	0	Ethanol	0/7
2	3% oxazolone	PBS (control vehicle)	0	0, 3, 7	0.75% oxazolone	2/8
3	3% oxazolone	Hamster IgG (isotypic control)	20	0, 3, 7	0.75% oxazolone	1/8
4	3% oxazolone	Anti-CTLA-4 mAb	20	0, 3, 7	0.75% oxazolone	4/8
5	3% oxazolone	Anti-CTLA-4 mAb	40	0, 3, 7	0.75% oxazolone	6/8

Figure 4: Anti-mouse CTLA caused earlier mortality in a mouse colitis model (study # 930031040)

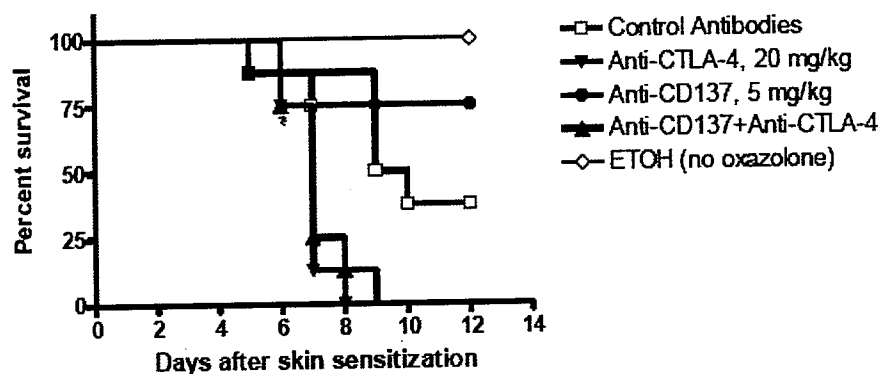


Figure 2. Effect of simultaneous treatment with BMS-469492 and CTLA-4 mAb in the oxazolone-induced colitis model.

CD137 mAb (BMS-469492, 5 mg/kg, q3dx3) and CTLA-4 mAb (UC10, 20 mg/kg, q3dx3) were administered intraperitoneally on days 0, 3, and 6 after epicutaneous challenge with oxazolone (day 0) alone or in combination. On day 5, animals were re-challenged with 0.75% oxazolone intrarectally. ETOH (ethanol) -treated group did not receive oxazolone. Survival was monitored daily.

Figure 4 (study report p 14) is representative of the results reported from several experiments

Table 10: anti-mouse CTLA-4 decreased colon lengths in two mouse colitis models (study # 930031040)

Treatment	Dose	Mean colon length (mm)
Oxazolone colitis model		Measured D9
Untreated control (no oxazolone)	0	71.3 \pm 6.7
Control vehicle (oxazolone pre-treated)	0	59.5 \pm 3.1
CTLA-4 mAb (oxazolone pre-treated)	10 mg/kg on D0, 3, 6	51.0 \pm 3.6
TNBS colitis model		Measured D4
Untreated control (no TNBS)	0	70.0 \pm 2.2
Control vehicle (TNBS pre-treated)	0	64 \pm 6.6
CTLA-4 mAb (TNBS pre-treated)	20 mg/kg on D0, 3	61.0 \pm 8.4

Data presented as means \pm standard deviation**6.1.2****Study title: Effect of combined PD-1 and CTLA-4 blockade in the murine FcγRIIb^{-/-} autoimmune model**

Study no:	• MDX-1106-010-006-R • 930036354
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Report length:	44 pages
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	May 30, 2007
Study period:	January 22, 2007 to June 5, 2007
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA-4), lot # PC CTLA4 76

Key Study Findings:

- Anti-mouse CTLA-4 treatment (twice weekly dosing x12) increased antinuclear antibodies (ANA) in both normal female BALB/c mice and FcγRIIb-deficient female BALB/c mice (no apparent difference)
- Several FcγRIIb-deficient mice receiving anti-mouse CTLA-4 exhibited weight loss and signs of renal impairment (elevated BUN and elevated albumin in the urine)
- The limited reporting of methods and results limits the usefulness of this study

Notes:

- The study authors report that FcγRIIb exhibits an inhibitory effect on the immune system in mice, and FcγRIIb –deficient “mice develop an autoimmune disorder characterized by kidney pathologies such as hydronephrosis” (page 7)
- FcγRIIb-deficient mice were obtained from a commercial source (b) (4)
- Groups of 10 normal or FcγRIIb-deficient mice were dosed twice weekly for 6 weeks (12 doses total) IP with: an isotypic control antibody (mouse IgG1), 0.5 mg/mouse of 9D9, 0.5 mg/mouse of anti-mouse PD-1, or 0.5 mg/kg of 9D9 in combination with 0.5 mg/mouse of anti-mouse PD-1 antibodies
- ANA were measured by ELISA pre-dose and during weeks 3, 6, 9, 12, 15 and 18
- Urine was measured weekly for proteinuria
- Body weight was measured once or twice weekly
- It is not clear from the study report when blood was collected for serum chemistry (i.e. reported only for mouse # 124592)
- The study report indicates that no renal changes were apparent by histology (but the report does not indicate which animals were evaluated)
- No difference in ANA was apparent until 9 weeks. By 9 weeks, the mice receiving 9D9 (either alone or in combination with anti-mouse PD-1 antibody) exhibited elevated ANA (less than 2-fold) compared to the control groups
 - ANA levels were slightly higher in FcγRIIb-deficient mice compared to healthy mice
- Results were summarized for several experiments (data not shown), confounding interpretation of the weight loss, proteinuria, and histopathology

6.1.3

Study title: Effect of combined anti-PD-1 and anti-CTLA-4 monoclonal antibodies in the murine NOD autoimmune model

Study no:	• MDX-1106/010-007-R
	• 930036359
Study report location:	Module 4.2.1.1 (Primary Pharmacodynamics)
Conducting laboratory and location:	Medarex, Milpitas, CA
Report date:	July 25, 2007
Study period:	January 19, 2007 to July 25, 2007
GLP compliance:	No
Drug, lot #:	9D9 (anti-mouse CTLA-4), lot #01.05.07

Key Study Findings:

- Male non-obese diabetic (NOD) mice receiving anti-mouse CTLA-4 antibody alone did not develop diabetes more quickly than NOD mice receiving a negative control antibody
- Anti-mouse PD-1 antibody treatment caused diabetes in all mice by D34
- In mice treated with both anti-mouse PD-1 and anti-mouse CTLA-4 antibodies, diabetes developed sooner (all mice by D23)

Notes

- NOD mice are an established inbred model for type 1 diabetes; they spontaneously develop an autoimmune, insulin-dependent diabetes mellitus
 - Onset is more rapid in females than males
- Blood glucose levels were monitored twice weekly. Mice with two consecutive readings above 250 mg/dl were deemed diabetic. Mice were euthanized after 3 consecutive readings above 250 mg/dl
- Body weights were measured once or twice weekly
- 9D9 did not affect body weight compared to isotypic control (mouse IgG)
- Control and 9D9-treated mice survived to the end of the study (D180)

6.2 Single-Dose Toxicity

One single-dose PK study was conducted, for comparability (study # DS07167); no signs of toxicity were observed. For reference, one repeat-dose comparability study was also conducted (study # 1416-128).

6.2.1

Study title: Single-dose intravenous exploratory pharmacokinetics comparability study in monkeys

Study no.:	<ul style="list-style-type: none">• DS07167• 930032302
Study report location:	Module 4.2.3.1.1 (Single Dose Toxicity) <ul style="list-style-type: none">• 194 page report• 2 page amendment (as a separate pdf file)
Conducting laboratory and location:	<ul style="list-style-type: none">• In-life portion: Bristol-Myers Squibb, Drug Safety Evaluation, Syracuse, New York• PK evaluation: (b) (4)
Report date:	November 25, 2008
Date of study initiation:	November 15, 2007
GLP compliance:	No
Drug, lot #, and % purity:	Ipilimumab: <ul style="list-style-type: none">• Process C material (b) (4), manufactured by Bristol-Myers Squibb, Syracuse NY). Lot # SYR PS 29OCT07 [HPW04SEP07MS1]. > 99% pure• Process B material (b) (4), manufactured by (b) (4). Lot # 6M144887. ≥ 99% pure

Key Study Findings

- No toxicity noted in the limited battery of endpoints evaluated
- Weak, treatment-related immunogenic responses noted

Methods

Doses: 10 mg/kg of Process B or Process C materials
(no vehicle control)

Frequency of dosing: Single dose

Route of administration: IV injection into the saphenous vein, at an approximate rate of 0.1 ml/second

Dose volume: 2 to 2.1 ml/kg

Formulation/Vehicle:

- Provided as ready-to-use product in vials
- Filtered (0.2 µm) prior to injection

Species/Strain: Cynomolgus monkeys

NOTE: monkeys were not treatment naïve, but were naïve to previous protein (previous treatments not reported)

Number/Sex/Group: 4 females/group

Age: 3-4 years of age

Weight: 3.9 to 3.8 kg

Methods notes:

- Animals were allowed to recover 41 days, then returned to the colony
- PK blood samples were collected pre-dose, approximately 5 to 10 minutes post-dose, then 0.5, 1, 2, 4, 8, 24, 48 and 72 hours post-dose, then 7, 11, 15, 22, 29, 36, and 42 days post-dose
- Blood samples for immunogenicity collected
- Endpoints measured: viability (daily), clinical observations (daily), feeding behavior (at least daily), body weight (weekly), body temperature (pre-dose and D42), heart rate (pre-dose and D42), respiratory rate (pre-dose and D42)

Results

- All animals survived, no treatment-related effects noted
- PK summary: The mean values were comparable, except for V_{ss} (the process B value was 79.5% of the process C value). Please see Table 11 of this review.

Table 11: Mean PK parameters in the single-dose comparability study (# DS07167)

Process material	C_{max} (µg/ml)	AUC _(0-1008 h) (µg·h/ml)	AUC _(INF) (µg·h/ml)	$T_{1/2}$ (h)	MRT (h)	CLT (ml/h/kg)	V_{ss} (L/kg)
B	252	521,000	57,500	307	410	0.175	0.0717
C	234	467,000	55,200	370	507	0.187	0.0902

MRT: mean residence time

CLT: total serum clearance

 V_{ss} : volume of distribution at steady state

- Anti-drug antibody (ADA) summary:
 - 1/4 animals receiving process B material exhibited a weak positive response on D42
 - 1/4 animals receiving process C material exhibited weak positive responses pre-dose and on D29, 36 and 42. The authors speculate that the animal may have had a pre-existing response to human antibody
 - The authors concluded that the magnitude of the ADA responses were small and unlikely to have affected the PK results

6.3 Repeat-Dose Toxicity

The repeat-dose toxicity of ipilimumab was evaluated in cynomolgus monkeys.

6.3.1

Study title: Repeated dose toxicity study of anti-CTLA4/10D1 administered via intravenous injection to cynomolgus monkeys

Study no.:	<ul style="list-style-type: none"> • 126-002 • 930009967
Study report location:	Module 4.2.3.2 (Repeat Dose Toxicity)
Page length:	Cross-referenced in module 4.2.3.6 (Local Tolerance)
Report page length:	38 pages
Conducting laboratory and location:	(b) (4)
Date of study initiation:	November 2, 1999
Date of first dosing:	November 2, 1999
End of in-life:	November 15, 1999
GLP compliance:	No
Drug, lot #, and % purity:	Anti-CTLA4/10D1, lot # 784-16, purity not reported

Key Study Finding

- 3 mg/kg of ipilimumab x3 was a NOAEL under the conditions tested (dosing D1, 4, 7; necropsy on D14)

Methods

Dose:	3 mg/kg (no negative control group)
Number/Sex/Group/Dosing:	Two female cynomolgus monkeys received 3 mg/kg on D1, D4 and D7 (3 doses total)
Route of administration:	IV bolus injection
Dose volume:	0.6 ml/kg
Formulation/Vehicle:	<ul style="list-style-type: none"> • 5.1 mg/ml • Dosed as received from Medarex (the formulation was not otherwise reported, and was not evaluated by the conducting

laboratory)
Species/Strain: Cynomolgus monkey
Weight: Approximately 3.5 kg

Method notes:


- Clinical observations and body weights were monitored
- The two monkeys were necropsied on D14: gross pathology, organ weights (adrenal, brain, kidneys, liver, ovaries, spleen), and histopathology (bone marrow, brain, heart, kidneys, liver, lymph nodes, lung, spleen, thyroid)

Results notes:

No treatment-related effects were apparent

6.3.2

Study title: 14-Day intravenous toxicity study with mAb 10D1 in cynomolgus monkeys

Study no.:	• 7114-100 • 9300009970
Study report location:	Module 4.2.3.2 (Toxicology) Cross-referenced in e-BLA modules: <ul style="list-style-type: none">• 4.2.1.1 (Primary Pharmacodynamics)• 4.2.1.3 (Safety Pharmacology)• 4.2.1.4 (Pharmacodynamic Drug Interactions)• 4.2.2.2 (Pharmacokinetics)• 4.2.3.6 (Local Tolerance)• 4.2.3.7 (Antigenicity)• 4.2.3.7.2 (Immunotoxicology)
Report date:	June 29, 2000
Report length:	130 pages
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 24, 2000
Date of first-dose:	February 1, 2000
End of in-life:	February 14, 2000
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	Ipilimumab (mAb 10D1), lot # 010 99 01 F. Purity not reported.

Key Study Findings

- 30 mg/kg of ipilimumab x3 was well-tolerated under the conditions tested (dosing D1, D4, D7, necropsy D14)
- Authors attributed slight hematological and clinical chemistry changes to treatment; however, this reviewer concludes that this study did not detect clear treatment-related changes.

Methods

Doses: 3 or 30 mg/kg of ipilimumab (no negative control group)
Frequency of dosing: D1, 4, 7
Route of administration: Bolus IV injection (saphenous vein)
Dose volume:

- 0.6 ml/kg for the 3 mg/kg dose
- 6.0 ml/kg for the 30 mg/kg dose

Formulation/Vehicle: Phosphate buffered saline
Species/Strain: Cynomolgus monkeys
NOTE: The monkeys were not treatment-naïve (previous treatments not reported); they had not been on study within 4 weeks prior to use
Number/Sex/Group:

- 2 males received 3 mg/kg
- 2 males and 2 females received 30 mg/kg

Age: 2 to 8 years
Weight: Males 2.5 to 5.6 kg
Females 2.8 to 3.0 kg

Methods notes:

- Animals were dosed on D1, 4, and 7, then necropsied on D14.
- Endpoints included:
 - clinical observations, physical examination, ophthalmic examination (pre-dose and D11), body weight (pre-dose, D1, D14)
 - hematology, coagulation, clinical chemistry (pre-dose and D14)
 - Flow cytometric evaluation of blood cells (pre-dose and D14)
 - PK and ADA evaluation (pre-dose and D14)
 - Necropsy (organ weights, gross pathology, histopathology)

Results

- No mortalities
- The authors noted the following hematological findings:
 - At 30 mg/kg: increased total leukocyte and lymphocyte counts, basophil counts at D14 compared to pre-dose
 - At 30 mg/kg: slightly decreased red blood cell count, hemoglobin, and hematocrit

- Higher serum globulin, glucose, and blood urea nitrogen in all monkeys at D14 compared to pre-dose
 - This reviewer did not consider the hematology effects to be clearly-treatment related, based on the individual variability
- The authors noted the following signals:
 - At 30 mg/kg: slight increase in the percentage of CD3⁺ cells at D7 (+29%) and D14 (+20%), with corresponding decreases in the percentage of CD20⁺ cells (-50%) compared to pre-dose
 - At 30 mg/kg: slight increase in the percentage of CD3⁺CD4⁺ cells at D14 (+38%), with a corresponding decrease in the percentage of CD3⁺CD8⁺ cells at D7 (-18%) and D14 (-33%) compared to pre-dose
 - At 30 mg/kg: slight increase in the percentage of CD3⁺CD29⁺ cells at D14 (+23%)
 - This reviewer did not consider the flow cytometry differences to be clearly-treatment related, based on the individual variability
- Weak ADA responses were detected in 3/4 of the monkeys receiving 30 mg/kg, but not in either monkey receiving 3 mg/kg of ipilimumab.
- No other treatment-related findings were apparent
- PK:
 - PK parameters were not calculated
 - Blood was collected pre-dose, and prior to dosing on D4, 7 and 14, and serum concentrations of ipilimumab were reported
 - For the 3 mg/kg group, the mean concentrations were 0, 20, 49, and 55 µg/ml, for the four time points respectively
 - For the 30 mg/kg group, the mean concentrations were 0, 370, 682, and 526 µg/ml, for the four time points respectively
 - These results suggest accumulation after 2 doses but not after 3 doses
- Histology:
 - Chronic inflammation was observed in multiple tissues. No background incidence of inflammatory infiltrates was reported.
 - The lack of concurrent negative controls, and the small number of treated animals precludes identifying clear treatment-related effects. Overall, the results suggest possible lymph node hyperplasia

Table 12: Selected pathology findings (study # 7114-100)

Finding	3 mg/kg males (N=2)	30 mg/kg males (N = 2)	30 mg/kg females (N = 2)
Gross pathology			
Enlarged mandibular lymph node	0/2	Not evaluated	2/2
Histopathology			
Ileum: lymphoid hyperplasia	0/2	1/2	0/2

Colon: lymphoid hyperplasia	2/2	1/2	1/2
Mandibular lymph node: lymphoid hyperplasia	0/2	0/2	1/2

6.3.3**Study title: A 2-week toxicity study of human anti-CTLA4 administered by intravenous injection to cynomolgus monkeys**

Study no.: • (b) (4)-0919-128
• 930009982

Study report location: Module 4.2.3.2 (Repeat Dose Toxicity)

Cross-referenced in modules:

- 4.2.1.1 (Primary Pharmacodynamics)
- 4.2.1.3 (Safety Pharmacology)
- 4.2.1.4 (Pharmacodynamic Drug Interactions)
- 4.2.2.2 (Pharmacokinetics)
- 4.2.3.6 (Local Tolerance)
- 4.2.3.7 (Antigenicity)
- 4.2.3.7 (Immunotoxicology)

Conducting laboratory and location:

(b) (4)

Report date: June 22, 2000
 Report length: 204 pages
 Date of study initiation: January 18, 2000
 Date of first dosing: January 20, 2000
 GLP compliance: Yes, signed
 QA statement: Yes, signed
 Drug, lot #, and % purity: Ipilimumab (human anti-CTLA-4), lot # 010-99-01F. Purity was between 92% (activity by ELISA) and 100% (assayed by HPLC-GPC)

Key Study Findings

- 10 mg/kg of ipilimumab x3 was well-tolerated under the conditions tested (dosing D1, D3, D7, necropsy D14)
- Ipilimumab associated with flow-cytometry changes (increases in the percentages of CD3+ cells expressing CD4, CD29 and HLA-DR)
- ADA developed in 2/6 monkeys

Methods

Doses: 3 or 10 mg/kg of ipilimumab (no negative control group)
Frequency of dosing: D1, 4, 7
Route of administration: IV injection (slow push bolus) via the saphenous vein
Dose volume: 0.6 ml/kg for the 3 mg/kg dose
2.0 ml/kg for the 10 mg/kg dose
Formulation/Vehicle: Phosphate buffered saline
Species/Strain: Cynomolgus monkeys
NOTE: The monkeys were not treatment-naïve; they had not been on study within 4 weeks prior to use
Number/Sex/Group: • 2 males for the 3 mg/kg group
• 2 males and 2 females for the 10 mg/kg group
Age: 4 to 7 years
Weight: Males 2.4 to 3.8 kg
Females 3.3 kg

Methods:

- Animals were dosed on D1, 4, and 7, then necropsied on D14.
- Endpoints included:
 - clinical observations, physical examination, body weight (pre-dose, D1, D7, D14), food consumption (qualitative), ophthalmic examination (pre-dose and D11)
 - hematology, coagulation, clinical chemistry (pre-dose, D1, D4, D7 and D14)
 - Urinalysis (pre-dose and D13)
 - PK and ADA evaluation (pre-dose, prior to each dose [D1, D4, D7] and D14)
 - Flow cytometry for markers of T-cell activation (pre-dose, D1, D4, D7, D14)
 - Necropsy (organ weights, gross pathology, histopathology)

Results

- Flow cytometry evaluated detected:
 - Increased percentage of CD3+ cells expressing CD4+ (+12% and +25% for the low- and high-dose groups on D7; +27% and +41% for the low- and high-dose groups on D14, respectively)
 - Increased percentage of CD3+ cells expressing CD29+ (+7% and +36% for the low- and high-dose groups on D7; +81% and 2-fold increase for the low- and high-dose groups on D14, respectively)

- Dramatically increased percentage of CD3+ cells expressing HLA-DR (2.3-fold and 3.1-fold increases for the low- and high-dose groups on D7; 6.5-fold and 8.3-fold increases for the low- and high-dose groups on D14, respectively)
- ADA was detected in 2/6 monkeys at D14: one low-dose male and one high-dose female
- PK:
 - PK parameters were not calculated; however, trough levels were measured prior to dosing on study Days 1, 4, 7 and 14
 - For the 3 mg/kg group, the mean concentrations of ipilimumab prior to dosing on D1, 4 and 7 and on D14 were: 0, 38, 84 and 79 µg/ml, respectively
 - For the 10 mg/kg group, the mean concentrations of ipilimumab prior to dosing on D1, 4 and 7 and on D14 were: 0, 123, 240 and 254 µg/ml, respectively
 - Thrice-weekly dosing caused accumulation
- No treatment-related histopathology changes were detected
 - Congestion was observed for some organs. No background incidence of infiltration was reported
- No other treatment-related effects were detected

6.3.4

Study title: BMS-663513 and BMS-734016. One-month intravenous combination toxicity study in monkeys

Study no.:	<ul style="list-style-type: none">• DS06064• 930021064
Study report location:	Module 4.2.3.2 (Repeat Dose Toxicity) Cross-referenced in e-BLA modules: <ul style="list-style-type: none">• 4.2.1.1 (Primary Pharmacodynamics)• 4.2.1.3 (Safety Pharmacology)• 4.2.1.4 (Pharmacodynamic Drug Interactions)• 4.2.2.2 (Pharmacokinetics)• 4.2.3.6 (Local Tolerance)• 4.2.3.7.1 (Antigenicity)• 4.2.3.7.2 (Immunotoxicity)• 4.2.3.7.7 (Other)
Report length:	945 pages
Conducting laboratory and location:	Bristol-Myers Squibb Drug Safety Evaluation, Syracuse NY
Report date:	August 8, 2007
Start of dosing:	September 19, 2006
End of in-life:	December 19, 2006
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #, and % purity:	Ipilimumab (BMS-734016), batch 5J06544. The report notes this batch is the equivalent of batch 901004. Purities reported as 100% (by size exclusion HPLC), 88% (activity by ELISA) and 108% (potency by ELISA)

Key Study Findings:

- 10 mg/kg/week x4 of ipilimumab, alone or in combination with BMS-664513 (anti-CD137 mAb), was well tolerated
 - The authors and applicant consider 10 mg/kg of ipilimumab to be a NOAEL, under the conditions tested in this study. This reviewer disagrees
- Treatment with ipilimumab was associated with increased incidence and severity of multi-organ infiltration (described as "infiltration", "lymphocyte infiltration", and "lymphohistiocytic infiltration")
 - Although infiltration is a common background finding, overall the histology results are clearly treatment-related
 - the effect remained apparent in animals evaluated at the end of recovery
 - This reviewer considers this effect to be related to the mechanism of action of ipilimumab

- Treatment with ipilimumab increased the antibody titer to KLH (response was not apparent 5 days after KLH exposure, but was evident 13 days after KLH exposure)
- Treatment with 10 mg/kg of ipilimumab was weakly associated with decreased spleen weight at D28
 - Spleen weights were not increased in the ipilimumab + BMS663513 group, and no corresponding histopathologic changes were detected, suggesting that the increase may be incidental

Methods

Doses:	<ul style="list-style-type: none">• Group 1: 0 (negative control)• Group 2: 10 mg/kg of ipilimumab (BMS-734016) + 100 mg/kg of BMS-663513• Group 3: 10 mg/kg of ipilimumab
Frequency of dosing:	Once weekly x 4 (D1, 8, 15, 22)
Route of administration:	IV infusion into the saphenous vein, at approximately 0.1 ml/second
Dose volume:	<ul style="list-style-type: none">• Group 1 and Group 2: 11.7 ml/kg• Group 3: 1.9 ml/kg
Formulation/Vehicle:	<ul style="list-style-type: none">• 0.9% sodium chloride for injection (for ipilimumab)• 2 mg/ml pluronic F68 prill surfactant in 5mM sodium succinate buffer (for BMS-663513)
Species/Strain:	Treatment-naïve cynomolgus monkeys
Number/Sex/Group:	5/sex <ul style="list-style-type: none">• 3/sex were necropsied 2 days after the last dose (D30)• 2/sex were necropsied after a 9-week recovery period (D90 or D91)
Age:	2 to 3 years
Weight:	Males 2.3 to 2.9 kg Females 2.0 to 2.8 kg

NOTES:

- BMS-663513 is a fully human anti-CD137 monoclonal antibody of the IgG4 kappa subtype.
- On D10 (2 days after the second dose), all animals were immunized IM with 10 mg/animal of KLH antigen.

Observations and Results

Mortality

- No unscheduled deaths occurred
- Observations for moribundity/mortality were made once daily

Clinical Signs

- One treated female (#2204, group 2 [co-treated with both agents], allowed to recover prior to necropsy) exhibited reduced food consumption, thin appearance, and increased incidence of diarrhea. Diagnostic fecal testing performed on Day 23 detected *Clostridium difficile*. [This reviewer notes that diarrhea caused by *Clostridium difficile* has been associated with antibiotic use that eliminates commensal bacteria. The study report makes no mention of antibiotic use during quarantine or other times]. Due to apparent thinness and diarrhea, this animal received supportive nutritional care from D45 to necropsy
- No other treatment-related effects apparent
- Each animal was observed 1-4 hours after dosing
- Each animal was observed once daily for clinical signs
- Each animal received a veterinary physical examination, including arterial oxygen saturation (measured by pulse oximetry by rectal probe) and neurological evaluation by a veterinarian pre-dose, after dosing on D22, and either D90 (recovery females) or D91 (recovery males)
- Body temperature (rectal thermometer) was measured at each physical examination, and on D1 (1, 4, and 24 hours post-dose)

Body Weights

- No treatment-related effect apparent
 - Notably, female #2204 did not exhibit weight loss (and actually gained weight during the study)
- Each animal was weighed pre-dose, weekly, and at necropsy

Feed Consumption

- Food consumption was evaluated daily
- Female # 2204 (in group 2) frequently did not eat, or exhibited reduced food consumption compared to the other animals. The authors report that she did eat enrichment food. The relationship of this change in food consumption with treatment is unclear.

Ophthalmoscopy

- No treatment-related effect apparent
- Evaluated pre-dose, after dosing on D22, and either D90 (recovery females) or D91 (recovery males)

ECG

- No treatment-related effect apparent

- Evaluated pre-dose, after dosing on D15, and either D90 (recovery females) or D91 (recovery males). Endpoints: pulse rate³, R-R interval, heart rate, P wave duration, PR interval, QRS complex duration, QT interval and corrected Qt (QTcF), change in QTcF (δ QTcF), P wave amplitude, R wave amplitude, T wave height, T wave height negative, ST segment evaluation, and qualitative assessment for arrhythmias and other waveform abnormalities

Hematology

- No treatment-related effect apparent
- Blood samples were collected pre-dose, D23, and on either D90 (recovery males) or D91 (recovery females)
- Hematology endpoints were: red blood cell count, hemoglobin, hematocrit, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet count, percent and absolute reticulocyte count, absolute total and differential leukocyte counts, and evaluation of cell morphology
- Coagulation endpoints were: prothrombin time, activated partial thromboplastin time, and plasma fibrinogen

Clinical Chemistry

- No treatment-related effect apparent
- Blood samples were collected pre-dose, D23, and on either D90 (recovery males) or D91 (recovery females)
- Endpoints were: aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, alkaline phosphatase, total bilirubin, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, triglycerides, glucose, urea nitrogen, creatinine, calcium, phosphorus, sodium, potassium, and chloride

Urinalysis

- No treatment-related effect apparent
- Urine samples were collected over approximately 18 hours pre-dose, during the last week of dosing, and during the last week of recovery.
- Endpoints were: volume, color and appearance, pH, specific gravity, qualitative determinations (for glucose, ketones, bilirubin, occult blood, urobilinogen), urine total protein, urine total protein output/18 hours, and microscopic evaluation of urinary sediment

³ The study report lists pulse rate as a distinct endpoint from heart rate, but the difference is not clearly explained in the study report. The means are very similar for the two endpoints, but the standard deviation for pulse rate is smaller. This reviewer infers that pulse rate is measured over a longer period of time than heart rate.

Gross Pathology

- Main-group animals were necropsied on D30 (2 days after the last dose)
- Recovery animals were necropsied on D (9 weeks after the last dose)
- Endpoints included gross examination of: adrenal glands, aorta, bone and bone marrow, brain, cervix, epididymides, esophagus, eyes with optic nerve, gallbladder, heart, skin of the injection site, kidneys, large intestine (cecum and colon), liver, lung, lymph node (mandibular and mesenteric), mammary gland, lacrimal gland, ovaries, pancreas, parathyroid glands, sciatic nerve, pituitary gland, mandibular salivary gland, seminal vesicles, skeletal muscle (quadriceps femoris, diaphragm), skin (dorsal thorax), small intestines (duodenum, jejunum, ileum), spinal cord (cervical and lumbar), spleen, stomach (cardia, fundus, pylorus), testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, and vagina

Organ Weights

- At necropsy, the following were weighed: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland (with seminal vesicles), spleen, testes, thyroid gland (with parathyroid)
- Ipilimumab-related changes in spleen weight after 4 weeks of treatment:
 - The male ipilimumab-only main group exhibited lower spleen weights (-20% absolute weight, -23% spleen:body weight), and the male ipilimumab-only recovery group appeared to have rebounded (+30%).
 - Notably, this effect was not apparent in the main-group males that received ipilimumab + BMS-663513
 - One female (#2203) in the ipilimumab + BMS-663513 main group exhibited a dramatically higher spleen weight (+2.2 fold compared to controls). This animal exhibited histological evidence of slight spleen congestion.
 - The study authors considered the differences to be incidental to treatment; this reviewer considers the effect to be potentially treatment-related, based on the putative mechanism of action of ipilimumab.
- From report pages 736-782):

Table 13: Ipilimumab-related spleen weight changes (study # DS06064)

	Males			Females		
	0	Ipilimumab + BMS-663513	Ipilimumab	0	Ipilimumab + BMS-663513	Ipilimumab
Main group (N=3/group)						
Body weights (kg)	2.45	2.55	2.85	2.34	2.28	2.36
	2.72	2.55	2.54	2.14	1.96	2.29
	2.75	2.83	2.81	2.41	2.52	2.26
Mean body weight (kg)	2.65	2.64	2.73	2.30	2.25	2.30
Spleen weights (g)	5.61	5.46	4.28	2.72	5.46	4.10

	5.45 5.36	5.38 5.98	4.31 4.52	5.16 5.23	5.72 12.1	6.21 3.76
Mean Spleen weights (g)	5.47	5.60	4.37**	4.37	7.76	4.67
Mean spleen:body wt	0.207	0.212	0.160**	0.191	0.337	0.204
Recovery group (N=2/group)						
Body weights (kg)	2.81 2.66	2.60 3.00	2.82 2.38	2.95 2.39	2.66 2.56	2.37 2.64
Mean body weight (kg)	2.74	2.80	2.60	2.67	2.61	2.51
Spleen weights (g)	5.06 2.62	3.02 5.34	5.31 4.67	4.35 3.56	4.80 2.72	5.85 5.14
Mean Spleen weights (g)	3.84	4.18	4.99	3.95	3.76	5.49
Mean spleen:body wt	0.139	0.147	0.192	0.148	0.143	0.221

Notes: Values rounded by this reviewer for readability. ** Means were statistically significantly different from control ≤ 0.01 (by Dunnett's test)

- One male in group 2 was sexually mature (#2103, mature testes with sperm), and this animal had higher testes and prostate weights (7.1 and 5.2 g respectively, final body weight 2.8 kg) compared to other animals. Histologic evaluation detected immature testes for each of the other males in this study. This incidental finding (higher testes weight) is noted for comparison to the 6-month study results (study # 01-3460).

Histopathology

- Adequate Battery: Yes. The tissues listed above (under gross pathology) were evaluated microscopically
- Peer Review: Yes, by a veterinary pathologist. The report has a signed pathology peer-review statement.
- Histological Findings: Ipilimumab treatment was associated with increases in the incidences and/or severities of infiltration, in multiple organs (described variously as "infiltration", "lymphocytic infiltration", and "lymphohistiocytic infiltration").
 - The authors noted "lymphocytic and lymphohistiocytic infiltration in a variety of tissues were considered to be incidental and unrelated to drug treatment, because minimal to slight mononuclear cell infiltrates in these tissues are commonly observed in cynomolgus monkeys" (report

page 50). This reviewer disagrees – overall, a treatment-effect is clearly apparent.

- Notably, only one main-group control animal exhibited mild infiltration (urinary bladder, lymphocyte infiltration)
- Notably, moderate infiltration was observed only in one monkey (heart lymphohistiocytic infiltration, in a female in the ipilimumab + BMS-663513 group)
- Despite the apparent treatment-related change in spleen weight, histopathologic evaluation noted a spleen lesion in only one animal (a group 2 female exhibited slight congestion of the spleen)
- From report pages 245-270 and 799-837):

Table 14: Selected main-group histopathology: ipilimumab-related lymphocyte infiltration after 4 weeks of treatment (study # DS06064)

Organ/finding	Severity	Males			Females		
		0	Ipilimumab + BMS- 663513	Ipilimumab	0	Ipilimumab + BMS- 663513	Ipilimumab
Main groups (N=3/dose)							
Brain, lymphohistiocytic infiltration	minimal	0	1	0	2	2	0
	slight	0	1	0	0	1	0
Cervix, infiltration	minimal				1	1	1
	slight				0	1	0
Epididymides, lymphocyte infiltration	minimal	0	0	1			
Eye, lymphocyte infiltration	minimal	0	1	1	0	2	0
Gallbladder, eosinophil infiltration	minimal	0	0	0	0	0	1
Gallbladder, lymphohistiocytic infiltration	minimal	2	1	0	0	0	1
	slight	0	1	0	0	0	0
	mild	0	0	0	0	1	0
Heart, lymphohistiocytic infiltration	minimal	1	2	3	2	2	2
	slight	1	1	0	1	0	1
	moderate	0	0	0	0	1	0
Injection site, lymphohistiocytic infiltration	minimal	0	0	0	0	0	1
	slight	0	0	0	0	1	2
Kidneys, leukocyte infiltration	minimal	0	1	0	0	1	1
Lacrimal glands,	minimal	1	0	3	0	1	3

lymphocyte infiltration	slight	0	1	0	0	0	0
Liver, lymphohistiocytic infiltration	minimal	2	1	2	3	0	1
	slight	1	1	1	0	3	1
	mild	0	1	0	0	0	0
Lung, lymphohistiocytic infiltration	minimal	0	1	2	1	3	3
Parathyroid gland, lymphocyte infiltration	minimal	0	0	0	0	1	0
Prostate gland, lymphocyte infiltration	minimal	0	3	2			
	slight	1	0	0			
Stomach, lymphohistiocytic infiltration	minimal	2	2	2	1	1	2
	slight	0	0	0	2	1	0
	mild	0	1	0	0	0	1
Thyroid, lymphocyte infiltration	minimal	0	0	2	1	0	1
Tongue, lymphocyte infiltration	minimal	1	2	2	1	1	2
Trachea, lymphohistiocytic infiltration	minimal	0	2	3	1	2	2
	slight	0	1	0	1	1	0
Urinary bladder, lymphocyte infiltration	minimal	1	2	2	1	1	2
	slight	0	0	0	0	1	0
	mild	0	0	1	1	0	0
Uterus, lymphohistiocytic infiltration	minimal				2	2	2
	slight				0	1	0
Vagina, lymphocyte infiltration	minimal				0	0	2
	slight				3	1	0
	mild				0	2	1

Note: In the main study animals, infiltrating leukocytes were also observed in the following organs/tissues; incidences were comparable for control versus treated: cecum, colon, esophagus, salivary gland, diaphragm muscle, skin/subcutis

Table 15: Selected recovery-group histopathology: ipilimumab-related lymphocyte infiltration after 9 weeks of recovery (study # DS06064)

Organ/finding	Severity	Males			Females		
		0	Ipilimumab + BMS-663513	Ipilimumab	0	Ipilimumab + BMS-663513	Ipilimumab
Recovery groups (N=2/dose)							

Brain, lymphohistiocytic infiltration	minimal	0	1	1	1	0	0
	slight	0	0	0	0	1	1
Esophagus, lymphohistiocytic infiltration	minimal	0	2	2	1	0	1
	slight	0	0	0	0	1	0
Eye, lymphocyte infiltration	minimal	0	0	1	0	0	0
Heart, lymphocyte infiltration	minimal	0	1	2	1	2	1
Heart, lymphohistiocytic infiltration	minimal	1	0	0	0	0	0
Lacrimal gland, lymphocyte infiltration	minimal	1	0	0	0	0	1
	slight	0	0	1	0	1	0
Liver, lymphohistiocytic infiltration	minimal	2	1	1	2	2	1
	slight	0	0	1	0	0	1
Lung, lymphohistiocytic infiltration	minimal	1	2	2	1	1	1
Pancreas, lymphocyte infiltration	minimal	0	0	0	0	0	1
Prostate gland, lymphocyte infiltration	minimal	1	2	0			
Diaphragm skeletal muscle, infiltration	minimal	0	0	0	0	0	2
Stomach, lymphohistiocytic infiltration	minimal	2	2	0	0	1	1
	slight	0	0	2	1	1	1
Tongue, lymphohistiocytic infiltration	minimal	0	0	0	1	1	1
	slight	1	0	2	0	0	0
Trachea, lymphohistiocytic infiltration	minimal	0	0	0	0	2	1
	slight	1	1	0	0	0	0
	mild	0	0	0	1	0	0
Vagina, lymphocyte infiltration	slight				2	0	1
	mild				0	2	1

Note: In the main study animals, inflammatory infiltrates were also observed in the following organs/tissues; incidences were comparable for control versus treated: cecum,

cervix, colon, injection site, kidneys, mammary gland, mandibular salivary gland, skin, thyroid gland, urinary bladder

Special Evaluation: T-Cell Dependent Antibody Response to KLH

- Ipilimumab-treatment was associated with an increased antibody titer in response to KLH compared to controls
- Each animal received an intramuscular injection of 10 mg/animal of keyhole limpet hemocyanin (KLH; diluted in water for injection at a concentration of 10 mg/ml) on D10
 - Note: Incomplete Freund's adjuvant was not used, in contrast with study #SUV00106 (reviewed below)
- Blood samples were collected pre-dose on D15, on D23, and from recovery animals on D29 and D36, and were evaluated for antibodies (IgG, IgM, or IgA isotypes combined) against KLH
 - The authors note that "KLH is a T-cell-dependent antigen that requires the interaction of B cells, T cells, and macrophages for the animal to develop a humoral KLH-specific antibody response" (report page 42)
- The control animals exhibited a robust response to KLH, for D23, D29, and D36 compared to D15
- Both antibody-treated groups exhibited a more robust antibody response to KLH than the control group on D23, D29 and D39
- From report pages 601-602:

Table 16: Ipilimumab induced antibody response to KLH (study # DS06064)

	Males			Females		
Titer (/1000)	0	Ipilimumab + BMS-663513	Ipilimumab	0	Ipilimumab + BMS-663513	Ipilimumab
Study Day						
D15	0.4	0.8	0.3	0.8	1.0	0.5
D23	15	102	57	22	137	102
D29	14	236	113	46	172	224
D36	14	240	124	65	172	239

Note: The results are antibody titer. Means only presented; values rounded by this reviewer for readability.

Special Evaluation: Peripheral Blood Lymphocyte Phenotyping

- No treatment-related effect apparent
- Blood samples were collected pre-dose, during the last week of treatment, and during the last week of recovery
- Whole blood samples were evaluated by flow cytometry. The endpoints were: CD2⁺CD20⁻ cells (T cells), CD4⁺CD8⁻ cells (T-helper cells), CD8⁺CD4⁻ cells (T-cytotoxic cells), CD20⁺CD2⁻ cells (B cells) and CD3⁺CD16⁺ cells (NK cells).

Toxicokinetics

- For group 2 animals, blood samples for TK were collected prior to dosing on D1, 8, 15, and D22, and after dosing on D1 and 22 only (approximately 5-10 minutes after the start of each antibody infusion, and 4 and 48 hours post-dose)
- For group 3 animals (i.e. two antibodies administered), blood samples for TK were collected from group 3 animals prior to dosing on D1, 8, 15, and 22, and after dosing on D1 and 22 only (approximately 5-10 minutes after the start of the ipilimumab infusion, and 4 and 48 hours post-dose)
- Blood concentrations of ipilimumab were higher on D22 compared to D1 (C_{max} +54%, AUC_{0-48hr} +66%), indicating accumulation with weekly dosing
- From report page 38:

Table 17: Ipilimumab PK following 4 weekly injections (study # DS06064)

Parameter	Day	# animals measured	Ipilimumab
C_{max} (µg/ml)	1	20	220 ± 26
	22	19	339 ± 47
$AUC_{0-48 hr}$ (µg*h/ml)	1	20	7160 ± 1080
	22	19	17,500 ± 2830
$AUC_{0-168 hr}$ (µg*h/ml)	1	20	18,000 ± 3900
$AUC_{0-1512 hr}$ (µg*h/ml)	22	7	90,600 ± 25,300
MRT (h)	22 to 85	7	432 ± 72
$T_{1/2}$ (h)	29 to 85	7	302 ± 90

Note: Values rounded by this reviewer for readability. These data are from both ipilimumab-treated groups combined. The study report presents these data, as well as the data segregated by group (i.e. group 2 is ipilimumab + BMS663513, group 3 is ipilimumab only; and by sex). MRT = mean residence time

- Blood samples for ADA were collected from groups 2 and 3 pre-dose on D1, 8, 15 and 22, and weekly during recovery
 - One animal (male # 2205, received ipilimumab + BMS-663413) exhibited ADA against ipilimumab, on D15 only
- Blood samples were collected pre-dose and during the last week of dosing for evaluation of anti-nuclear antibody levels
 - No treatment-related effect apparent on anti-nuclear antibody levels

Stability and Homogeneity

Certificates of analysis were provided for both antibodies. The dosing formulations were not analyzed (report pages 18-19); the authors reported that the stability of the test antibodies had previously been demonstrated and reported.

6.3.5

Study title: A repeated dose toxicity and efficacy study of MDX-010 or MDX010-HYB in combination with HBsAg and SKMel vaccines in cynomolgus monkeys

Study no.:	<ul style="list-style-type: none"> • (b) (4) 1416-128 • 930009973
Study report location:	Module 4.2.3.2 (Repeat dose toxicity) Cross-referenced in modules: <ul style="list-style-type: none"> • 4.2.1.1 (Primary Pharmacodynamics) • 4.2.1.3 (Safety Pharmacology) • 4.2.1.4 (Pharmacodynamic Drug Interactions) • 4.2.2.2 (Pharmacokinetics) • 4.2.3.6 (Local Tolerance) • 4.2.3.7.1 (Antigenicity) • 4.2.3.7.2 (Immunotoxicity) • 4.2.3.7.7 (Other)
Report length:	503 pages
Conducting laboratory and location:	(b) (4)
Report date:	August 5, 2003
Date of study initiation:	November 18, 2002
Start of dosing:	December 9, 2002
End of in-life:	February 26, 2003
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	<ul style="list-style-type: none"> • Ipilimumab produced in hybridoma cells (process A material, designated MDX-010-HYB in the study report) <ul style="list-style-type: none"> ○ Lot # MDI001 ○ Purity 94% by ELISA • Ipilimumab produced in Chinese hamster ovary (CHO) cells (process B material, designated MDX-010 in the study report) <ul style="list-style-type: none"> ○ Lot # 046-02-01F-TOX ○ Purity 100% by HPLC

Key Study Findings

- 10 mg/kg/month x3 (D1, D29, D57) of ipilimumab, administered in combination with other therapeutic agents, was well tolerated
 - Study authors conclude 10 mg/kg of ipilimumab was a NOAEL; this reviewer does not agree
- Compared to control, ipilimumab-treated animals exhibited: :

- higher incidences of mononuclear cell infiltration, across the panel of tissues evaluated microscopically.
- higher incidences of lymph node hyperplasia
- More severe ADA response to the vaccines
- Slightly more severe DTH response to the vaccines

Methods

Doses and frequency of dosing: • All animals received 10 µg/animal of HBsAg IM and SKMel cells subcutaneously (SC) on D1, 29 and 57

- Each animal also received 0.1 ml of HBsAg, SKMel, or saline by intradermal injection on D41/44 and D71, for the DTH evaluation

- Group 1: 0 mg/kg IV on D1, 29, 57
- Group 2: 10 mg/kg IV of MDX-010-HYB on D1, 29, 57
- Group 3: 0.1 mg/kg of MDX-010 on D1, 29, 57
- Group 4: 1 mg/kg of MDX-010 on D1, 29, 57
- Group 5: 10 mg/kg of MDX-010 on D1, 29, 57
- Group 6: 1 mg/kg of MDX-010 weekly x10 (on D1, 8, 15, 22, 29, 36, 43, 50, 57, 64)

Route of administration: • Ipilimumab by bolus IV injection (dose volumes 0.5 to 2 ml/kg)
 • HBsAg by IM (dose volume 0.5 ml/animal)
 • SKMel by SC (dose volume 0.5 ml)

Vehicle/formulation: 0.9% saline

Species/Strain: Experimentally naïve cynomolgus monkeys

Number/Sex/Group: 3/sex/dose

Age: Males 2.5 to 3.8 years

Females 1.8 to 3.5 years

Weight: Males 2.4 to 3.0 kg
 Females 2.0 to 2.6 kg

Notes:

- HBsAg is hepatitis B surface antigen vaccine; the applicant reports that the development of antibodies against HBsAg is T-cell mediated. Further details regarding HBsAg were not provided (either in the study report or the BLA Module 2.6 Nonclinical Written and Tabulated Summaries)
- SKMel is a human melanoma cell line transfected to express GM-CSF; SKMel is administered as a vaccine. Each animal received a nominal dose of 5×10^6 cells at

each dosing. It is not clear from the study report if, or how, consistency of the SKMel dose was maintained.

- All monkeys were necropsied on D79
- An evaluation of the PK data from this study was submitted to the BLA as a 25-page amendment to the main report (EDR module 4.2.2.2 [Repeat Dose Toxicity]), dated June 14, 2007. The amendment provides calculated PK parameters; the original report presented serum concentrations but not calculated PK parameters.
- No ECG monitoring was performed

Observations and Results

Mortality

- No premature mortality
- Cage side observations were made twice daily

Clinical Signs

- No treatment-related effects apparent
- One monkey in group 2 (10 mg/kg/month) exhibited watery stool on D40-44 and 72-79. This animal received supportive care from D72 to necropsy. This finding presumed treatment-related
- Cageside observations were made twice daily
- The vaccine injection sites were evaluated 24 and 48 hours after each dose, for erythema, eschar, and induration

Body Weights

- No treatment-related effects apparent
- Measured weekly

Feed Consumption

- No treatment-related effects apparent
- Measured qualitatively

Ophthalmoscopy

- No treatment-related effects apparent
- Evaluated pre-dose and on D74

Hematology

- No treatment-related effects apparent
- Blood collected pre-dose, D29 (prior to dosing), D58 and D74
- Endpoints measured: RBC and WBC counts, hemoglobin concentration, hematocrit, reticulocyte counts, MCH, MCV, MCHC, platelet counts, and blood cell morphology
- Coagulation markers were not evaluated

Clinical Chemistry

- No treatment-related effects apparent
- Blood collected pre-dose, D29 (prior to dosing), D58 and D74
- Endpoints measured: sodium, calcium, potassium, phosphorus, chloride, carbon dioxide, total bilirubin, ALP, LDH, AST, ALT, GGT, BUN, creatinine, total protein, albumin, globulin, albumin:globulin ratio, glucose, cholesterol, triglycerides

Urinalysis

- No treatment-related effects apparent
- Urine was collected during necropsy only, by bladder puncture

Organ Weights

- Organs/tissues weighed: adrenals, epididymides, kidneys, lungs, pituitary, testes, thyroid with parathyroid, brain, heart, liver, ovary, spleen, thymus

Gross Pathology

- All animals were necropsied on D79. Endpoints measured were: carcass and muscular/skeletal system; all external surfaces and orifices; cranial cavity and external surfaces of the brain; neck with associated organs and tissues; and thoracic, abdominal, and pelvic cavities with associated organs and tissues

Histopathology

- Adequate Battery: Yes. Organs/tissues evaluated were: cardiovascular (aorta, heart), digestive (mandibular salivary gland, tongue, esophagus, stomach, small intestines [duodenum, jejunum, ileum], large intestines [cecum, colon, rectum], pancreas, liver, gallbladder), respiratory (trachea, lungs), lymphoid/hematopoietic (sternum bone marrow, thymus, spleen, mesenteric and mediastinal lymph nodes), urogenital (kidneys, urinary bladder, testes, epididymis, prostate, seminal vesicles, ovaries, uterus, cervix, vagina), endocrine (adrenals, pituitary, thyroid/parathyroid),

skin/musculoskeletal (skin, mammary gland, femur head, rib, thigh muscle), nervous (eye with optic nerve, sciatic nerve, brain, spinal cord), gross lesions and injection site

- Peer Review: No
- Histological Findings:
 - The study authors concluded that no treatment-related effects were apparent. This reviewer disagrees: an apparent, treatment-related increase in the incidence of mononuclear cell infiltration is apparent. The severity of this effect was not graded in the study report. Ipilimumab treatment was also associated with increased incidence of lymph node hyperplasia.
 - From report pages 251-281:

Table 18: Ipilimumab induced multi-organ mononuclear cell infiltration and lymph node hyperplasia (study # (b) (4) 1416-128)

Organ	Males						Females					
Doses	1	2	3	4	5	6	1	2	3	4	5	6
Mononuclear cell infiltration (N=3/group)												
Brain, meinges	0	0	1	0	0	0	0	0	0	1	0	0
Brain, perivascular	0	0	0	0	1	0	0	0	0	0	0	0
Sciatic nerve	0	1	0	0	0	0	0	0	0	0	0	0
Parathyroid	0	0	0	1	0	0	0	0	0	0	1	0
Adrenal	0	0	0	0	0	0	0	1	0	0	0	0
Kidney	1	2	3	3	2	3	3	3	3	2	3	1
Prostate	2	2	2	2	3	3						
Seminal vesicle	2	0	0	0	0	1						
Lung	0	0	0	1	0	1	0	0	1	0	0	0
Heart	1	1	0	0	2	2	0	2	1	1	2	2
Tongue	0	0	0	1	0	0	0	0	0	0	0	0
Skeletal muscle	0	0	0	0	0	1	0	0	0	0	1	0
Skeletal muscle, perivascular	0	0	0	0	1	0	0	0	1	0	0	0
Pancreas	0	0	0	1	0	0	0	0	2	0	0	0
Liver	3	3	1	1	3	2	1	2	1	3	1	2
Lymph node hyperplasia (N = 3/group)												
Mediastinal lymph node hyperplasia	0	0	1	0	0	0	0	0	0	0	0	0

Mesenteric lymph node hyperplasia	0	1	0	0	1	1	0	2	1	0	1	0
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Note: Group 1 = control. Group 2 = 10 mg/kg/month of MDX-010-HYB. Group 3 = 0.1 mg/kg/month of MDX-010. Group 4 = 1 mg/kg/month of MDX-010. Group 5 = 10 mg/kg/month of MDX-010. Group 6 = 1 mg/kg/week of MDX-010

Special Evaluation: DTH Challenge

- Ipilimumab-treatment was associated with slight increases (up to 2-fold) in induration and erythema after both challenges, compared to the control group. No dose-response was apparent.
- All animals were dosed with both HBsAg and SKMel on D1, 29, and 57, and were challenged with both HBsAg and SKMel on D41 and 71, then evaluated 24 and 48 hours after each challenge.
- Erythema and eschar formation were evaluated on a qualitative 5-point scale (none, very slight, well defined, moderate-to-severe, severe)
- The area of induration was evaluated by measuring the diameter
- The overall DTH score was based on a formula that incorporated the induration and erythema scores

Special Evaluation: T-Cell Subsets and Activation Markers

- No treatment-related effects were apparent
 - The study authors concluded that ipilimumab treatment was associated with increased tumor necrosis factor-alpha (TNF-alpha, TNF- α) and interferon-gamma (IFN- γ) in some animals. This reviewer disagrees.
- Blood samples were collected pre-dose, D14, 43, 71 and 79
- Flow cytometry for CD3, CD4, CD8, CD25, CD69, and HLA-DR
- Blood samples were stimulated (with CD28 and CD49d, Brefeldin A) with or without HBsAg, and evaluated by immunohistochemistry for CD3, CD8, TNF- α alpha, and IFN- γ

Toxicokinetics

- Blood samples for TK were collected on D1 (1, 2, 4, 6, 8 and 24 hours post-dose), D4, 11, 14, D29 (pre-dose), D30, D57, D58 and D71
- For group 6 (weekly dosing), additional TK blood samples were collected pre-dose on D8 and D43

- From the study report amendment (page 18):

Table 19: PK parameters following single IV doses of 10 mg/kg of ipilimumab (study # (b) (4) 1416-128)

Parameter	MDX-010-HYB		MDX-010		Combined
	Male	Female	Male	Female	
C _{max} (µg/ml)	459	420	560	503	486
AUC _{0-T} (µg*h/ml)	44,900	45,300	51,600	49,600	47,800
AUC _{0-INF} (µg*h/ml)	48,900	48,200	59,500	53,900	52,600
V _{ss} (ml/kg)	38.3	43.1	50.0	44.9	44.1
T _{1/2} (hr)	169	173	275	194	203
MRT (hr)	184	207	298	241	233

- For assessment of ADA against ipilimumab, blood samples were collected pre-dose, D2, D30, 43, 58 and 79
 - One monkey in group 2 (1/6) and two group 6 monkeys (2/6) exhibited ADA against ipilimumab
- For assessment of ADA against HBsAg and SKMel, blood samples were collected pre-dose, D14, 43, 71 and 79
 - All of the animals exhibited ADA against both SKMel and HBsAg. Groups 2 and 5 (10 mg/kg/month of ipilimumab) exhibited stronger responses than the other groups.

Stability and Homogeneity

Certificates of analysis were provided for the ipilimumab lots used. The stability test data (report page 450) indicate that the MDX-010 process B material degraded (b) (4). No stability test results are presented for the MDX-010-HYB (process A material) in the study report.

6.3.6

Study title: An immunogenic study following combined intravenous/intramuscular administration of MAb10/D1/HBsAg, respectively, to cynomolgus monkeys

Study no.:	• (b) (4)-0992-128
	• 930009968
Study report location:	Module 4.2.3.2 (Repeat Dose Toxicity)
	Referenced in sections:
	• 4.2.1.1 (Primary Pharmacodynamics)
	• 4.2.1.3 (Safety Pharmacology)
	• 4.2.1.4 (Pharmacodynamic Drug Interactions)
	• 4.2.2.2 (Pharmacokinetics)
	• 4.2.3.7.2 (Immunotoxicity)
Report length:	157 pages
Conducting laboratory and location:	(b) (4)
Report date:	April 16, 2001
Date of study initiation:	August 18, 2000
Date of first-dosing:	September 18, 2000
End of in-life:	November 21, 2000
GLP compliance:	No
Drug, lot #, and % purity:	MAb10D1, lot # 00-00-01F, purity 99.6% (by HPLC-GPC)

Key Study Findings

- 10 mg/kg of ipilimumab x 2 (D1 and D37, necropsy D64), administered only in combination with other agents, was well-tolerated
 - No monkey received ipilimumab alone
- The combination of ipilimumab + HBsAg (Hepatitis B surface antigen vaccine), with or without oligoCpG (an oligonucleotide) was associated with changes in the immune system
 - Increased antibody response against HBsAg
 - Slight increases in lymphocyte counts (absolute and relative)

Methods

- Doses and frequency of dosing: 4 groups (2/sex/group)
- All animals received HBsAg IM on D2 and D30
 - Group 1 and group 3 received 10 mg/kg of MAbRSV (negative control antibody) IV on D1 and D29
 - Group 2 and group 4 received 10 mg/kg of ipilimumab IV on D1 and D29
 - Group 3 and Group 4 received oligo-CpG IM on D2 only
- Dose volume: Ipilimumab: 0.5 ml/animal
- Species/Strain: Cynomolgus monkeys
- NOTE:** The monkeys were not treatment-naïve; they had not been on study within 4 weeks prior to use
- Number/Sex/Group: 2/sex/group
- Age: Males 3.0 to 4.2 years
Females 3.3 to 5.3 years
- Weight: Males 3.6 to 5.6 kg
Females 2.3 to 3.1 kg

Methods notes

- A total of 16 monkeys were used; they were naïve to human antibodies but were not experimentally naïve (previous treatments not reported). A total of 8 monkeys received ipilimumab.
- The purpose of the study was to assess the immunizing effect of combining ipilimumab and a vaccine (HBsAg).
- Five different agents were administered:
 - HBsAg was Hepatitis B surface antigen vaccine
 - MAbRSV was an isotype-matched human Ig (specific for respiratory syncytial virus); it was administered as the negative control for ipilimumab
 - Oligo-CpG was an oligonucleotide, intended to be a "positive control immune-enhancer" (report page 7)
- Endpoints:
 - Cage side observations, qualitative food consumption, body weights
 - PK and immune cell assays. Blood collected pre-dose, D1, 15 and 29 (pre-dose), D36, D50
 - Hematology. Blood collected pre-dose, D1, 15 and 29 (pre-dose), D36, 50, 64
 - All animals were necropsied on D64
 - Gross pathologic and histopathologic examination of the stomach and colon, specifically for evidence of inflammation

Results notes

- No treatment-related toxicity was detected
 - Histopathology observed lymphoplasmacytic infiltration (stomach, colon), but the lack of concurrent negative controls and the small group sizes precludes identifying a treatment-related effect
- PK
 - No ADA detected against ipilimumab or the control antibody (MAbRSV)
 - Ipilimumab concentrations for groups 2 and 4 respectively:
 - D15 (14 days post-dose): 97 and 110 µg/ml
 - D29 (prior to the second dose): 40 and 33 µg/ml
 - D36 (7 days post-dose): 138 and 169 µg/ml
 - D51: 86 and 50 µg/ml
 - D64: 33 and 18 µg/ml
 - PK data indicate accumulation in monkeys with monthly dosing
- Increased anti-HBsAg antibodies
 - In group 1 (negative control antibody + HBsAg), the antibody response against HBsAg was highest on D36
 - Oligo-CpG co-treatment was associated with a strong response at the first time point (D15) onward (group 3 compared to group 1)
 - Adding ipilimumab to the oligo-CpG did not appear to enhance the effect of oligo-CpG (group 4 compared to group 3)
 - Ipilimumab enhanced the anti-HBsAg response (group 2 compared to group 1) beginning at D36 (not at D15 or D29)
 - From report page 130:

Table 20: Ipilimumab increased monkeys' antibody response to HBsAg vaccine (study # 0992-128)

Treatment	Group 1: Negative control (MAbRSV)	Group 2: 10 mg/kg of ipilimumab	Group 3: positive control (MAbRSV + oligoCpG)	Group 4: ipilimumab + positive control (ipilimumab + oligoCpG)
D1	0	0	0	0
D15	200 ± 170	110 ± 90	1940 ± 1380	2270 ± 1670
D29	110 ± 70	310 ± 250	310 ± 190	950 ± 620
D36	1670 ± 700	2260 ± 620	6140 ± 2130	3870 ± 1630
D51	820 ± 290	3520 ± 630	4240 ± 1350	1530 ± 640
D64	580 ± 240	2180 ± 480	2820 ± 920	1120 ± 690

Note: All animals received HBsAg. Means + standard error presented, both sexes combined. Values rounded by this reviewer for readability.

- CD3+CD8+ cells were evaluated for intracellular cytokines. Two monkeys treated with ipilimumab exhibited increased cytokine levels.
 - A male in group 2 exhibited detectable IL-2 and TNF-alpha on D51 and D654
 - A female in group 4 exhibited increased TNF-alpha (D36 only)

- The authors considered these findings to be evidence of T-cell activation

6.3.7

Study title: Effect of BMS-734016, BMS-663513 or their combination, on the immune responses to Simian Immunodeficiency Virus DNA test antigens. Interim report.

Study no.:	<ul style="list-style-type: none"> • TIB-06-001 • TIB-06-01 • 930022368
Study report location:	<ul style="list-style-type: none"> • Module 4.2.1.1 (Pharmacology) • Cross-referenced in modules: <ul style="list-style-type: none"> ○ 4.2.1.3.1 (Safety Pharmacology) ○ 4.2.2.2.1 (Pharmacokinetics) ○ 4.2.3.2 (Repeat-Dose Toxicity) ○ 4.2.3.7.2.1 (Immunotoxicology) ○ 4.2.3.7.7.1 (Other Toxicity)
Conducting laboratory and location:	Bristol-Myers Squibb, Pharmaceutical Research and Development, Lawrenceville, NJ
Date of first dosing	September 11, 2006
Report date:	November 21, 2007
Report length:	93 pages
GLP compliance:	No
Drug, lot #, and % purity:	Ipilimumab (BMS-734016, MDX-010), lot # 6G19359. Purity not reported.

Key Study Findings

- 10 mg/kg of ipilimumab (8 intermittent doses over 88 days, administered only with other agents), was well-tolerated
 - One monkey (female # 085-337, received ipilimumab in combination with SIV-DNA vaccines) exhibited a severe reaction (cyanosis, thready pulse, muffled heart sounds, vomiting) after receiving ipilimumab in combination with other therapies. The monkey received supportive care and recovered. This adverse effect is considered related potentially relevant to ipilimumab-treatment, but not clearly relevant to humans.
 - One monkey (female # 085-276, received ipilimumab in combination with SIV-DNA vaccines and BMS-663513) developed a rash (dermatitis) with lymphoedema, an apparent delayed hypersensitivity reaction, after having received ipilimumab in combination with other therapies. These effects appeared after cessation of treatment, worsened and then resolved within 2 months of appearance. The data indicate that ipilimumab-treatment contributed to the observed toxicities.
- Note: The toxicity data were presented in the interim report (dated November 21, 2007; report # 930022368) reviewed here. The final report (# TIB-006-001; #

930041502) did not address toxicity, and was reviewed above in section 4.1.3.1 of this review.

Methods

- Doses and frequency of dosing: 5 dose groups:
- All groups received IM doses, on D1, 2, 29, 30, 56, 58, 85, 85
 - Groups 1-4 received IV doses, on D4, 9, 30, 32, 58, 60, 86, 88
 - Groups 1-4 received IM doses of 2 mg/monkey each of three SIV test antigens (DNA vaccines: SIV gag, SIV pol, and SIV env) on D1, 2, 29, 30, 56, 58, 85, 85
 - Group 1 received IV doses of saline
 - Group 2 received 10 mg/kg IV of another antibody, BMS-663513 (fully human IgG4 against human CD137)
 - Group 3 received 10 mg/kg IV of ipilimumab
 - Group 4 received 10 mg/kg of BMS-663513 plus 10 mg/kg of ipilimumab
 - Group 5 (control) received IM and IV doses of vehicle
- Route of administration: IM or IV
- Dose volume:
- IM: 2 ml/day
 - IV: 2 to 5 ml/kg
- Formulation/Vehicle:
- IM: 0.15 M citrate buffer, pH 6.7 with 0.25% bupivacaine in water
 - IV: 0.9% sodium chloride for injection, USP
- Species/Strain: Treatment-naïve cynomolgus monkeys
- Number/Sex/Group:
- 3/sex for the control group (group 5)
 - 4 males and 2 females for groups 1-4
- Age: 2 to 7 years
- Weight: 2.3 to 6.1 kg

Methods notes:

- Physical examinations were conducted at time of dosing. Body weights were measured on D5, 16, 44, 57, 116, 185, and 211/212
- Blood samples were collected for hematology and serum chemistry prior to dosing on D16, 44, 72, 116, and 211/212
- Blood samples were collected periodically for PK and immunogenicity.

Results notes:

- Two monkeys exhibited adverse clinical signs:
 - A female (# 085-337) in group 3 (SIV-DNA vaccines plus ipilimumab) exhibited signs consistent with an acute infusion reaction on D58
 - On Day 30 (the first time both treatments were given together), this animal vomited after treatment
 - On D58 (the second time both treatments were given together), the animal was sedated (ketamine), received ipilimumab iv, and then the SIV vaccines were injected IM (four different sites) approximately 2-3 minutes later. Five minutes after the ipilimumab dose, the animal was cyanotic with thready pulse and muffled heart sounds.
 - The animal responded to supportive care (intubated for oxygen, IV fluids with diphenhydramine and dexamethasone). The animal stabilized ~45 minutes after dosing, vomited 4 hours after treatment, and was normal by the following day.
 - NOTE: The rate of infusion on D58 was not recorded or strictly monitored
 - Treatment for this animal was halted until D211, when this animal was challenged with ipilimumab alone. Additional endpoints were monitored (ECG, pulse oximetry, heart rate, blood pressure). No treatment effect was detected on D211.
 - This monkey exhibited ADA starting on D16. The ADA response on D30 and D32 was similar to the response on D16. Thereafter (D44, 72, 116, 211), the ADA response exceeded the assay limits (> 78000 titer).
 - The authors attribute the toxicity to the rapid administration of an antibody and also to anesthesia-induced hypotension (report page 25). This reviewer concurs with the authors' attribution, and also concludes that the adverse event is likely related to the strong ADA response in the cynomolgus monkey, and therefore may not be clinically relevant to patients.
 - A female (# 085-276) in group 4 (SIV-DNA vaccines plus ipilimumab and BMS-663513) developed dermatitis and lymphadenopathy
 - This monkey exhibited pre-dose alopecia
 - This monkey developed dermatitis in the axillary and inguinal areas, and peripheral lymphadenopathy on D133 (approximately one month after the last dose)
 - Antihistamine treatment appeared to transiently resolve the rash (rash returned by D140). On D140, the alopecia appeared to have spread
 - On D147, the dermatitis was more severe, having spread to the back of both knees. Prednisone was administered and three skin biopsies were collected on D147 (8 weeks post-dose): one from the left center of the inguinal area, a second from the right area of the

inguinal area, and a third from the periphery of the rash. Immunohistochemistry was performed on the biopsies for CD3, CD8, CD68 (marker for monocytes/macrophages), Ki-67 (marker for proliferating cells). The biopsies had:

- Mild epithelial and perivascular infiltration by CD8+ lymphocytes and CD68+ macrophages.
- Increased epithelial proliferation
- No evidence of infection
- Evaluation on D185 found that the rash had improved
- Evaluation on D212 found the rash had cleared
- This animal did not exhibit ADA against ipilimumab
- The authors considered these observations to be evidence of delayed type hypersensitivity (DTH), and noted that “rashes have been observed clinically with both BMS-663515 and BMS-734016” (report page 29)
- No apparent effects on body weight (measured D5, 16, 44, 57, 116, 185, 212), hematology or clinical chemistry (measured pre-dose, D16, D44, 72, 116, 212)
- ADA was only assessed against ipilimumab, and only in groups 3 and 4. In group 3 (SIV DNA vaccines + ipilimumab), 1/6 animals had ADA (detected beginning D16). None of the animals in group 4 (SIV DNA vaccines + ipilimumab + BMS-663513) had ADA against ipilimumab
- No necropsy was performed

6.3.8

Study title: A 4-week combination toxicity study of MDX-010 and MDX-1106 administered by intravenous injection to cynomolgus monkeys, with a 1-month recovery period

Study no.:	<ul style="list-style-type: none"> • SUV00106 • 930036346
Study report location:	Module 4.2.3.7.7 (Other) Cross referenced in: <ul style="list-style-type: none"> • 4.2.1.4 Pharmacodynamic Drug Interactions • 4.2.2.2 Pharmacokinetics • 4.2.3.6 Local Tolerance • 4.2.3.7.1 Antigenicity • 4.2.3.7.2 Immunotoxicity
Report length	991 pages
Conducting laboratory and location:	(b) (4)
Report date:	June 9, 2009
Date of study initiation:	February 5, 2008
Initiation of dosing:	February 19, 2008
End of in-life:	April 18, 2008
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #, and % purity:	MDX-010, lot # M31A-04-01FC. Purity was reported as 100% (size exclusion HPLC and antigen binding ELISA) and 105% (potency binding ELISA)

Key Study Findings

- The combination of ipilimumab with MDX-1106 (anti-PD-1 antibody) induced clear treatment-related effects on the spleen (increased spleen weight and spleen lymphoid follicle hypertrophy/marginal zone expansion), lymph nodes and thymus (decreased germinal centers, hypocellularity) and large intestine (mononuclear cell inflammation, associated with death in one animal)
- This study is of limited usefulness, because ipilimumab was administered only in combination with MDX-1106
 - The combination of 3 mg/kg/week x4 of ipilimumab with MDX-1106 was well tolerated
 - The combination of 10 mg/kg/week x4 of ipilimumab with MDX-1106 exceeded the highest non-severely toxic dose (HNSTD) based on the death of one high-dose monkey (1/10) on D23 (2 days after the 4th weekly dose) due to acute gastric dilation (bloat)

Methods

Doses: • 0 (negative control)
 • 3 mg/kg of ipilimumab (MDX-010) + 10 mg/kg of MDX-1106
 • 10 mg/kg of ipilimumab + 50 mg/kg of MDX-1106

Frequency of dosing: Weekly x4 (D1, D8, D15, D22)

Route of administration: Slow bolus IV injection, saphenous or cephalic vein

Dose volumes: 0.6 to 7 ml/kg

Formulation/Vehicle: Sterile saline for injection, USP

Species/Strain: Experimentally naïve cynomolgus monkeys

Number/Sex/Group: 5/sex/dose
 • 3/sex/dose main group (D30 necropsy)
 • 2/sex/dose recovery-group (D59 necropsy)

Age: Males 2.2 to 4.0 years
 Females 2.2 to 3.8 years

Weight: Males 2.2 to 3.0 kg
 Females 3.1 to 2.5 kg

Notes:

- Ipilimumab was not administered as mono-therapy in this study.
- On each dosing day, ipilimumab was administered prior to MDX-1106
- MDX-1106 is a monoclonal antibody against the human PD-1 receptor (putative mechanism of action is immunostimulatory)
- This study is summarized in the BLA module 2.6.6 (Toxicology Written Summary), and is listed twice in the reference section (as both reference #22 and reference #54). The double-listing appears to be an oversight; this reviewer verified that the same report is summarized (e.g. not different versions of the study report).

Observations and Results

Mortality

- A high dose male (#3003) was found dead on D23 (one day following the last dose).
 - This animal exhibited persistent diarrhea (from D9 onward); body weight loss (-13% from D14 to D21); reduced food consumption (D21 and D22); and decreased activity, dehydration, and hypothermia on D22
 - On D22, this animal received supportive care (subcutaneous fluids, supplemental fruit, buprenorphine for pain) and was placed in an incubator for warmth.
 - Cause of death was attributed to acute gastric dilatation (bloat). Gross pathology found marked stomach distension and moderate dilation of the duodenum, jejunum, ileum, cecum, and colon. The authors consider the

relationship of this death with treatment to be unclear (report pages 43, 741), and this reviewer agrees that the finding is not clearly related to ipilimumab treatment.

- Animals were observed for mortality and morbidity twice daily

Clinical Signs

- Treatment-related diarrhea was observed in both treated groups
- High-dose animals exhibited watery feces and low food consumption
- Cage-side observations were made once daily
 - Food consumption was noted qualitatively
- Physical examinations were conducted weekly, under ketamine sedation

Body Weights

- High-dose females exhibited slight weight loss (mean weight was -6% at week 4 compared to pre-dose), consistent with the observed clinical signs (watery feces and low food consumption)
- Measured weekly

Ophthalmoscopy

- No treatment-related effect apparent
- Evaluated pre-dose, week 4, and week 8 (for recovery animals)

ECG

- A slight increase in body temperature (+1°C) was observed in high-dose males on D1 (1-2 hours post-treatment)
 - The authors consider this finding to be incidental (report page 45)
 - This reviewer disagrees, and considers this finding potentially related to the bolus IV administration of human antibodies
- No other treatment-related effect apparent
- Measured pre-dose and 1-2 hours post-dose on D1 and D22
- Endpoints: ECG, body temperature, blood pressure, heart rate, and hemoglobin oxygen saturation by pulse oximetry

Hematology

- Slight treatment-related increases in neutrophil and eosinophil counts at D7, 28 and 58. The authors considered the changes secondary to GI inflammation
- Blood collected pre-dose, prior to dosing on D7 and D28, and from recovery animals on D58

- Endpoints measured: RBC, hemoglobin concentration, hematocrit, MCV, MCHC, MCH, red cell distribution width (RDW), reticulocyte count, platelet count, MPV, differential WBC count, PT, APTT, fibrinogen

Clinical Chemistry

- Slight dose-responses were apparent for decreased albumin and increased globulin at D7 and 28. As with the hematology parameters, the study authors considered these changes secondary to GI inflammation
- Blood samples were collected pre-dose, prior to dosing on D7 and D28, and from recovery animals on D58
- Endpoints measured: ALT, AST, ALP, GGT, LD, total bilirubin, BUN, creatinine, calcium, phosphorus, total protein, albumin, globulin, albumin/globulin ratio, glucose, cholesterol, triglycerides, sodium, potassium chloride, carbon dioxide

Urinalysis

- No treatment-related effects apparent
- Urine samples were collected during necropsy, by bladder puncture
- Endpoints measured: color/character, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, microscopic evaluation

Organ Weights

- Treatment-related increases in spleen weight (absolute weight, organ:body weight, and organ:brain weight) and decreases in thymus weight (absolute weight, organ:body weight, and organ:brain weight), were detected at D30 and D59.
- The study authors (report page 50) considered the changes in organ weight to be consistent with the histopathology findings
- Organs/tissues weighed: adrenals, epididymides, kidneys, lungs, pituitary, testes, thyroid with parathyroid glands, brain, heart, liver, ovaries, spleen, thymus

- From report pages 782-842):

Table 21: Ipilimumab + anti-PD-1 mAb increased spleen weight and decreased thymus weight (study # SUV00106)

Mean values	Males			Females		
	0	3 mg/kg ipilimumab + 10 mg/kg of MDX-1106	10 mg/kg of ipilimumab + 50 mg/kg of MDX-1106	0	3 mg/kg ipilimumab + 10 mg/kg of MDX-1106	10 mg/kg of ipilimumab + 50 mg/kg of MDX-1106
Day 30						
Body weight (kg)	2.9 ± 0.2	2.5 ± 0.3	2.6 ± 0.4	2.4 ± 0.2	2.4 ± 0.1	2.0 ± 0.3
Spleen weight (g)	3.9 ± 1.9	4.0 ± 0.3	6.1 ± 2.2	2.8 ± 0.9	3.6 ± 1.1	4.5 ± 1.7
Spleen:body weight	1.3 ± 0.6	1.6 ± 0.1	2.4 ± 0.5	1.2 ± 0.3	1.5 ± 0.4	2.2 ± 0.6
Thymus weight (g)	2.8 ± 0.5	2.1 ± 0.9	0.9 ± 0.2	2.5 ± 0.7	2.1 ± 0.7	1.4 ± 2.0
Thymus:body weight	0.99 ± 0.25	0.84 ± 0.34	0.37 ± 0.13	1.1 ± 0.3	0.58 ± 0.26	0.62 ± 0.85

Note: Results presented as means ± standard deviation; values rounded by this reviewer for readability.

Gross Pathology

- The high-dose male found dead (#3003) exhibited treatment-related gross-pathology:
 - Marked gas distension of the stomach and moderate gas dilatation of the duodenum, jejunum, ileum, cecum, and colon
 - Lung discoloration (mottled, dark red, purple, tan)
 - Decreased thymus size (correlated with marked, diffuse thymic atrophy)
- Main-group animals were necropsied on D30; recovery-group animals were necropsied on D59
 - Treatment-related findings were observed at D30 and D59: increased spleen size and decreased thymus size, increased lymph node size, and large intestine changes (fluid distension, black discoloration)
- Gross pathology endpoints were: evaluation of carcass and musculoskeletal system; external surfaces and orifices; cranial cavity and external surfaces of the brain; and thoracic, abdominal, and pelvic cavities with associated organs

Histopathology

- Adequate Battery: Yes. Organs/tissue systems evaluated were: cardiovascular (aorta, heart), digestive (mandibular salivary gland, tongue, esophagus, stomach, small intestines [duodenum, jejunum, ileum], large

intestine [cecum, colon, rectum], pancreas, liver, gallbladder), respiratory (trachea, lung), hematopoietic (sternum bone marrow, thymus, spleen, femoral bone, rib bone, skeletal muscle [psoas and diaphragm]), lymph nodes (inguinal, mandibular, mesenteric), urogenital (kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, cervix, vagina), endocrine (adrenals, pituitary, thyroid/parathyroid), nervous (eyes with optic nerve, sciatic nerve, brain, spinal cord), gross lesions, and injection sites

- Peer Review: Yes
- Histological Findings
 - The high-dose male found dead (#3003) exhibited treatment-related histopathology that correlated with gross pathologic findings:
 - Bone marrow marked myeloid hypercellularity, with corresponding erythroid hypocellularity
 - Thymus marked diffuse atrophy (lymphoid hypocellularity)
 - Moderate-to-marked lymphoid depletion/hypocellularity of the inguinal and mesenteric lymph nodes
 - Spleen depletion of the white pulp, characterized by marked decrease in germinal center size and cellularity
 - Findings attributable to stress/reduced food consumption:
 - Pancreatic acinar cell degranulation
 - Adrenal cortex depletion
 - Other treated animals exhibited treatment-related changes:
 - Large intestine (colon, cecum, rectum) inflammatory changes:
 - Infiltration by lymphocytes, histiocytes, eosinophils, and neutrophils
 - Mucosal epithelium minimal degeneration/regeneration
 - Spleen changes: increased size and number of lymphoid follicles with marginal zone expansion
 - Lymph node findings: decreased size and number of lymphoid follicles, decreased germinal center size and hypocellularity
 - Thymus involution/atrophy
 - Multi-organ mononuclear cell infiltrates: liver and kidney
 - Myeloid hypercellularity in the bone marrow: one low-dose animal (in addition to the high-dose early decedent)
 - Pancreas degranulation of acinar cells at the high-dose only, attributable to reduced food intake

Special Evaluation: Flow Cytometry

- T-cell counts were increased in high-dose males (+3.5-fold at D7, +4.6-fold at D28, compared to pre-dose)

- Correspondingly, high-dose males exhibited increased T-helper lymphocyte counts (+2-fold at D7 and D28 compared to pre-dose) and T-cytotoxic/suppressor cells counts (+67% at D7, +2.5-fold at D28 compared to pre-dose)
- T-cell counts were increased in high-dose females (+28% at D7, +14% at D28 compared to pre-dose). No changes in T-cell subsets were detected among the female groups.
- Evaluation was performed on blood collected as part of the clinical pathology blood draw (i.e. pre-dose, prior to dosing on D7 and D28, and from recovery animals on D58)
- Markers evaluated:
 - CD20⁺: B-lymphocytes
 - CD3⁺: T-lymphocytes
 - CD3⁺/CD4⁺: T-helper lymphocytes
 - CD3⁺/CD8⁺: T-cytotoxic/suppressor lymphocytes
 - CD3⁺/CD16⁺: natural killer cells
 - CD3⁺/CD14⁺: monocytes

Special Evaluation: Thyroid Hormone Analysis

- No treatment related effects apparent
- Evaluation was performed on blood collected as part of the clinical pathology blood draw (i.e. pre-dose, prior to dosing on D7 and D28, and from recovery animals on D58)
- Endpoints: T3, T4, thyroid stimulating hormone (TSH)

Special Evaluation: T-Cell Dependent Antibody Response to KLH

- Ipilimumab-treatment was associated with an increased antibody titer in response to KLH, approximately +1.5-fold to +2-fold compared to the control group. A dose-response was not apparent.
- Each animal received an intramuscular injection (left thigh) of 375 µg/animal of KLH in combination with 375 µg/animal of incomplete Freund's adjuvant (IFA) on D10
- Blood samples were collected pre-dose, prior to immunization on D10, prior to dosing on D15, D24, and from recovery animals on D33 and D40
 - The pre-dose, D10 and D24 samples were analyzed for anti-KLH IgM and IgG
 - The D15 sample was analyzed for anti-KLH IgM only
 - The D33 and D40 samples were analyzed for anti-KLH IgG only

Toxicokinetics

- Blood samples for TK analysis were collected on D1 and D22 (pre-dose, 1, 8 and 24 hours post-dose)

- Plasma concentrations were reported, but TK parameters were not calculated
 - The plasma concentrations after the first and last doses were dose-proportional, and accumulation (approximately +50%) was apparent at D22 compared to D1
- Blood samples for immunogenicity analysis were collected pre-dose, D28 and from recovery animals on D58
 - ADA against ipilimumab was detected in 2 mid-dose and 2 high-dose animals on D28, and in 2 different high-dose animals on D58

Stability and Homogeneity

- Certificates of analysis were provided. The concentrations of ipilimumab were tested at the end of the study, and reportedly fell with 90% of nominal (report pages 509-510), but no details or line-listed data of the testing results were provided.
- This reviewer does not consider these data omissions to affect the evaluation of this study

6.3.9**Study title: MDX-CTLA4: a 6-month intravenous toxicity study in cynomolgus monkeys**

Study no.:	<ul style="list-style-type: none">• 01-3460• 93009980
Study report location:	BLA section 4.2.3.2 (repeat-dose toxicology). Pdf file is 444 pages
Report status and report date:	Final report. February 22, 2002
Conducting laboratory and location:	(b) (4)
Date of study initiation:	<ul style="list-style-type: none">• Protocol signed April 23, 2001• Dosing initiated May 2, 2001
GLP compliance:	Yes. <ul style="list-style-type: none">• A signed statement of compliance with 21CFR58 was provided• The biopsy evaluation by (b) (4) was not GLP• The immunological evaluation was not GLP• The pharmacokinetic assays were not GLP
QA statement:	Yes, signed.
Drug, lot #, and % purity:	Ipilimumab (MDX-CTLA4), lot numbers 010-01-01PMB, MDI001. Purity \geq 95%

Key Study Findings

- The dose of 10 mg/kg/month x5 of ipilimumab was well-tolerated
 - Ipilimumab appeared to increase the antibody response to the SKMel vaccine in co-treated animals compared to animals only receiving the SKMel vaccine
 - Ipilimumab treatment appeared associated with decreased testes weight (absolute weight, organ:body weight, organ:brain weight); the observation is not clearly treatment-related

Methods

- Doses:
- Control
 - 10 mg/kg of ipilimumab
 - 5×10^6 SKMel cells (intended as a vaccine)
 - 10 mg/kg of ipilimumab with SK-mel
- Frequency of dosing: Monthly x5 (day 0, 28, 56, 84, and 140) for all four dose groups
- Route of administration:
- Ipilimumab and saline (negative control) by IV injection

- SK-mel by subcutaneous injection
- Dose volume: • The first 4 IV doses were 1.27 ml/kg; the 5th iv dose was 2 ml/kg
- The SC doses were 0.5 ml/animal
- Formulation/Vehicle: Saline for injection, USP
- Species/Strain: Cynomolgus monkey (*Macaca fascicularis*)
- Purpose-bred
- Supplied by (b) (4)
- Quarantined 4 weeks
- Acclimated 8 months prior to dosing
- Males were single-housed
- Females were pair-housed
- Number/Sex/Group: • 2/sex for the control and the ipilimumab alone groups
- 3/sex for the SK-mel alone and the ipilimumab + SK-mel groups
- Age: • 6 years 10 months to 7 years 5 months at receipt
- 7 years 5 months to 8 years at initiation of dosing
- Weight: • Males range 4.4 to 6.4 kg (mean 5.39 kg)
- Females range 2.4 to 3.2 kg (mean 2.96 kg)

Observations and Results

Ophthalmoscopy, ECG, and urinalysis endpoints were not evaluated

Mortality

No unscheduled mortalities

Clinical Signs

- Cage-side observations were made twice-daily; endpoints were: mortality, general condition, emesis, abnormal stool, other discharges, gross abnormalities in appearance/behavior/activity/respiration
- Physical examinations were made once weekly; endpoints were: general condition, skin, fur, eyes, nose, oral cavity, abdomen, external genitalia, evaluation of respiration, behavior, clinical signs
- No treatment-related clinical observations reported by the study authors.
- Slight dosing-site irritation was observed for 2/3 males the received both ipilimumab and SKMel
 - For each animal injected with SKMel, the injection site was marked at the day of administration and was examined pre-dose, 24, 48, 72, and 96 hours post-dose.

- One animal exhibited slight erythema and edema at the SKMeI injection site after the 4th dose (not detected 24 hours after dosing; observed from 48 hours to 5 days post-dose) with no sign of irritation after the 5th dose
- The other animal exhibited very slight edema after the fifth dose (noted 72- and 96-hours post-dose)

Body Weights

- No treatment-related changes were apparent
- Body weight was measured pre-dose, and weekly during treatment

Feed Consumption

- No treatment-related changes were apparent.
- Food consumption was assessed 4 times per week qualitatively, but not quantitatively.

Hematology

- No treatment-related effects were apparent
- Blood samples were collected pre-dose, and on D28, 56, 84, 112, 140 and 167/168 (necropsy)
- Hematology endpoints measured were: hemoglobin, hematocrit, red blood cell count, RBC morphology platelet count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total leukocyte count, differential leukocyte count, reticulocyte count
- Coagulation endpoints measured were: prothrombin time, activated partial prothrombin time

Clinical Chemistry

- No treatment-related effects were apparent
- Blood samples were collected pre-dose, and on D28, 56, 84, 112, 140 and 167/168 (necropsy)
- Endpoints measured/calculated were: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, creatinine, glucose, cholesterol, triglycerides, total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, sodium, potassium, chloride, calcium, inorganic phosphorus

Gross Pathology

- No treatment-related effects were apparent
- Postmortem evaluation included examination of: external surface, orifices, brain and spinal cord (external surface), organs of the cranial, thoracic, abdominal and pelvic cavities

Organ Weights

- Ipilimumab decreased testes weight approximately 27-50%, with or without SKMel (as shown in Table 22 of this review)
 - The study authors and the applicant considered these changes to be treatment-related. This reviewer disagrees, because the effects appear incidental to treatment. No testes histology findings were identified for any of the animals, and ages were not reported (preventing a determination of which males were sexually mature or immature). Based on the small number of animals used, this reviewer recommends against summarizing the testes weight change in the label (i.e. under section 13.1).
- Control animals had higher thyroid weights (44-50%) compared to the treated groups.
 - The study authors considered this effect to be treatment related. This reviewer disagrees; because the apparent difference in thyroid weight was also associated with SK-mel treatment (suggesting that the control results are incidentally high, and the other group results are normal)
- Organs weighed: adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary gland, prostate gland, spleen, thymus, thyroid/parathyroid glands, and uterus (body/cervix)

Table 22: Ipilimumab treatment associated with decreased testes weight in the 6-month study (study # 01-3460)

	MALE				FEMALE			
	0	Ipilim- umab	SKMel	Ipilim- umab plus SKMel	0	Ipilim- umab	SKMel	Ipilim- umab plus SKMel
N	2	2	3	3	2	2	3	3
Terminal body weight (kg)	5.40	5.70	5.57	5.33	2.60	2.95	2.93	3.10
Testes (g)	40.20	29.43	32.49	20.44	-	-	-	-
Testes:body wt	0.745	0.518	0.591	0.382	-	-	-	-
Testes:brain wt	62.5	44.7	51.9	31.7	-	-	-	-
Thyroid/parathyroid	0.986	0.557	0.682	0.430	0.583	0.328	0.400	0.459
Thyroid:body wt	0.018	0.010	0.012	0.008	0.022	0.011	0.014	0.015
Thyroid:brain wt	1.523	0.836	1.093	0.657	1.034	0.577	0.665	0.774

Histopathology

No treatment-related effects were apparent. Multi-organ lymphoid cell aggregates and lymphocytic infiltrates were detected, but the lack of a concurrent negative control and the small group sizes precludes identification of clear treatment-related effects.

- Adequate Battery: Yes

Tissues evaluated: adrenal gland, aorta, rib bone marrow smear, femur bone, brain (medulla/pons, cerebrum, and cerebellum), epididymides, esophagus, eye with optic nerve, gallbladder, heart, injection sites, kidneys, lacrimal glands, large intestine (cecum, colon, rectum), liver, lung, lymph nodes (mesenteric, mediastinal), mammary gland, sciatic nerve, ovaries, pancreas, pituitary, prostate, submandibular salivary glands, seminal vesicles, biceps muscle, skin, small intestines (duodenum, ileum, jejunum), spinal cord (cervical, thoracic, lumbar), spleen, stomach, testes, thymus, thyroid and parathyroid, trachea, urinary bladder, uterus (body and cervix), vagina, and gross lesions

- Peer Review: No. Only one veterinary pathologist is listed (report page 3)

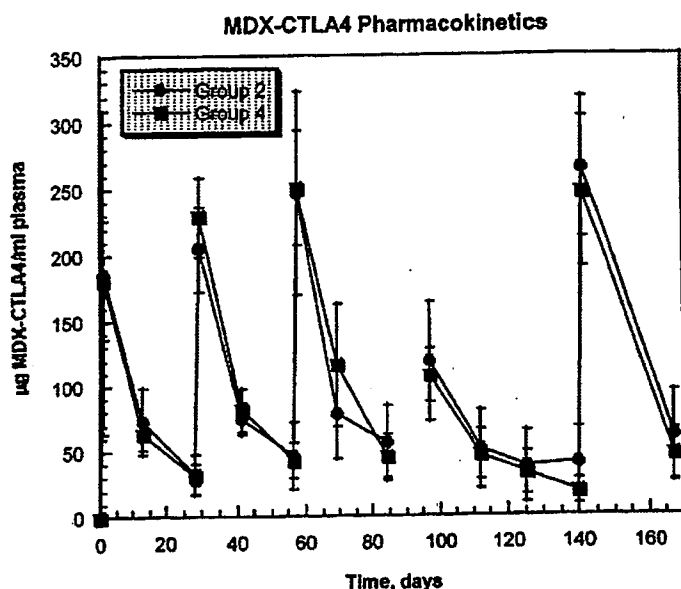
Special Evaluation: Delayed type hypersensitivity (DTH) assessment

- No apparent treatment-related effect associated with ipilimumab co-treatment
- For the two groups receiving SKMel (i.e. SKMel alone and ipilimumab plus SKMel), delayed type hypersensitivity assessment was assayed during week 16/17.
 - Each animals were injected intradermally (at different marked sites) with 0.1 ml of saline, SKMel, dendritic cells, or dendritic cells that had been "pulsed" with SKMel.
 - DTH was evaluated 24, 48 and 72 hours after injection. An area of induration ≥ 2 mm in diameter was considered positive. Positive responses were graded qualitatively.
- In the SKMel only groups, positive responses were observed in 3/3 males and 1/3 females. In the ipilimumab plus SKMel group, positive responses were observed in 2/3 males and 2/3 females.

Toxicokinetics

- Blood samples were collected pre-dose and on D1, 13, 28, 29, 41, 56, 57, 69, 84, 85, 97, 112, 125, 140, 141, and 167/168 (necropsy)
- Plasma levels were reported, but TK parameters were not calculated. After the first dose, the mean plasma levels were 185 $\mu\text{g/ml}$; plasma levels were detectable on the day prior to each subsequent dose, and the peak levels increased with repeated dosing.

Figure 5: TK data for 10 mg/kg of ipilimumab, IV dosing on D 0, 28, 56, 84 and 140 (study # 01-3460)



NOTE: Figure from page 389 of the study report. Group 2 = the group that received 10 mg/kg of ipilimumab. Group 4 = the group that received 10 mg/kg of ipilimumab plus SKMel.

Immunogenicity

- No anti-drug antibodies (ADA) against ipilimumab were detected
 - Blood samples were collected to measure antibodies against ipilimumab pre-dose, D112, and D141
- Co-treatment with ipilimumab and SKMel was associated with an increased antibody response against SKMel, beginning on D13 or D41
 - Blood samples were collected to measure antibodies against SKMel pre-dose, D13, 41, 69, 97, 125, and 167 (necropsy)
 - Two assays were used: flow cytometry (incubating fresh SK-mel-3 cells (SKMel) with plasma samples) and ELISA (coating plates with SK-mel cell lysate). Both assays were quantitative (arbitrary units of fluorescence intensity and optical density, respectively)
 - The authors reported that 1/6 animals in the SKMel group generated a strong antibody response to SKMel, while 5/6 animals in the ipilimumab plus SKMel group generated a strong antibody response to SKMel (report page 388).
 - NOTE: The definition of “strong” response was not provided in the study report
 - For both assays, the difference between the two groups was statistically significant

Table 23: Monkeys cotreatment with ipilimumab and SKMel exhibited a stronger anti-SK-mel response, compared to monkeys treated only with SKMel (study # 01-3460)

Day	Antibody to SK-mel (flow cytometry assay, mean fluorescence intensity)		Antibody to SK-mel (ELISA assay, mean optical density)	
	SKMel	Ipilimumab plus SKMel	SKMel	Ipilimumab plus SKMel
Pre-dose	61	67	0.12	0.12
D13	80	169	0.11	0.13
D41	325	1280	0.14	0.25
D69	337	798	0.15	0.31
D97	220	1029	0.13	0.45
D125	281	630	0.14	0.41
D167/168	148	454	0.13	0.29

NOTE: Mean values rounded by this author for clarity of presentation.

NOTE: The authors report that the differences were statistically significant (report page 36), but no details of the statistical analysis were provided in the study report (i.e. Appendix N).

Special Evaluation: T-cell subpopulations and activation markers

- No treatment-related effects were apparent
- Blood samples were collected pre-dose, D1, 29, 57, and 141
- Flow cytometry measured T cells (gating by CD3, CD25, CD29, and HLA-DR); the presentation of data focused on the comparison of CD4⁺ and CD8⁺ subsets

Stability and Homogeneity

- The test article was provided by the sponsor at each dose interval
- Based on an information request (sent August 5, 2010), the sponsor noted (September 20, 2010, sequence # 0016) that the certificate of analysis information is on report pages 402-406
- NOTE: No assessment of stability or homogeneity was included in the study report

6.3.10

Study title: An investigative repeat-dose toxicity and efficacy study of MDX-010, 4C5 and 5H1 in combination with HBsAg, DNP-Ficoll and SKMel immunostimulants following three monthly administrations

Study no.:	• SUV00006
	• 930021733
Study report location:	Module 4.2.3.2 (Repeat Dose Toxicity)
	Cross-referenced in:
	• 4.2.1.1 (Primary Pharmacodynamics)
	• 4.2.1.3 (Safety Pharmacology)
	• 4.2.1.4 (Pharmacodynamic Drug Interactions)
	• 4.2.2.2 (Pharmacokinetics)
	• 4.2.3.6 (Local Tolerance)
	• 4.2.3.7.2 (Immunotoxicology)
Report length:	321 pages
Conducting laboratory and location:	<div style="background-color: #cccccc; height: 40px; width: 100%;"></div> (b) (4)
Report date:	June 21, 2007
Date of study initiation:	February 15, 2005
Date of first dosing:	March 15, 2005
End of in-life:	August 16, 2005
GLP compliance:	No
Drug, lot #:	Ipilimumab (MDX-010, lot # M31A-03-05FC)

Key Study Findings

- In combination with three other agents (HBsAg, SKMel, and DNP-ficoll), 10 mg/kg/month of ipilimumab was not tolerated, based on one treatment-related death (1/6)
- This study is of limited usefulness for evaluating safety, because ipilimumab was not administered alone

Methods

- Doses and frequency of dosing:
- 0 or 10 mg/kg of ipilimumab IV on D1, 29, 57, 140
 - All animals also received:
 - 10 µg/animal of HBsAg IM on D1, 29 and 57
 - SKMel cells (5×10^6) SC on D1, 29 and 57
 - 100 µg/animal of DNP-Ficoll intradermally on D1 and 29
- Route of administration and dose volume: Ipilimumab by bolus IV injection (dose volume 2 ml/kg) in 0.9% saline USP, via the saphenous or cephalic vein
- Species/Strain: Experimentally naïve cynomolgus monkeys
- Number/Sex/Group: 3/sex/dose (12 monkeys total)
- Age: 3 to 5 years
- Weight: 2 to 4 kg

Observations and Results

Mortality

- One female in the ipilimumab-group (# 2503) was euthanized in moribund condition on D42
 - The study authors and the applicant consider this death related to ipilimumab treatment, and this reviewer concurs. The applicant notes (in Module 2.6.6 Toxicology Written Summary) that the lesions observed shared “similarities to adverse events related to ipilimumab in clinical trials”
 - From D32 onward, this animal exhibited persistent diarrhea, inappetence, and body weight loss (approximately -10% from D28 to D35, and an additional -10% from D35 to D42)
 - Agonal changes noted on D42 (shallow breathing, dehydration, low blood pressure, low heart rate, low body temperature)
 - Supportive care (IV and SC fluids) were administered on D42
 - Blood work changes were detected (decreased white blood cell counts, azotemia and electrolyte alterations, decreases in cholesterol, GGT, total protein, globulin, and albumin; increases in AST and BUN)
 - Gross evaluation noted:
 - Multifocal black discoloration of the mucosa in the colon, with green liquid stool in the large intestines
 - Decreased size of thymus and spleen
 - Enlargement and dark brown discoloration of the adrenals
 - Uniform red discoloration of the lungs
 - Red discoloration of the ipilimumab injection sites
 - Histologic evaluation noted:

- Colon inflammation, crypt abscesses, and erosion (extending to the rectum)
- Mixed cell infiltrates of the adrenals, liver, and kidney
- Adrenal hyperplasia (of the zona fasciculata)
- Lymphoid depletion of the thymus, spleen, and gut associated lymphoid tissues (GALT)
- The other 11 monkeys survived to scheduled necropsy (D154)
- Cageside observations were made twice daily for mortality, general appearance, and behavior

Clinical Signs

- One ipilimumab-treated male (#2003) exhibited transient rash on the lower abdomen and femoral areas on D42 (evaluated prior to dosing for the DTH challenge). Rash was also detected on this monkey from D44-98
- Cage side observations were made twice daily for mortality, general appearance, and behavior
- Injection sites were evaluated for irritation approximately 24 and 48 hours post-dose

Body Weights

- No treatment-related effects were apparent (excepting monkey # 2503)
- Measured weekly

Feed Consumption

- No treatment-related effects were apparent (excepting monkey # 2503)
- Evaluated qualitatively, daily

Hematology

- No treatment-related effect apparent
- Blood collection pre-dose, D14, D29 (prior to dosing), D43, 57 and 71
- Endpoints measured: RBC count, WBC count, hemoglobin, hematocrit, reticulocyte count, MCH, MCV, MCHC, platelet count, blood cell morphology

Clinical Chemistry

- No treatment-related effect apparent (excepting monkey # 2503)
- Blood collection pre-dose, D29 (prior to dosing) and D71
- Endpoints: sodium, potassium, chloride, carbon dioxide, total bilirubin, ALP, LD, AST, ALT, GGT, calcium, phosphorus, BUN, creatinine, total protein, albumin, globulin, albumin:globulin ratio, glucose, cholesterol, triglycerides

- Note: no urinalysis was performed

Gross Pathology

- No treatment-related effect was apparent (excepting monkey # 2503)
- Scheduled necropsy was D154.
- Endpoints: carcass and musculoskeletal system; external surfaces and orifices; cranial cavity and external surfaces of the brain; neck with associated organs and tissues; thoracic, abdominal and pelvic cavities with associated organs and tissues

Organ Weights

- No treatment-related effect was apparent
- Organs measured: adrenals, epididymides, kidneys, lungs, pituitary, testes, thyroid with parathyroids, brain, heart, liver, ovaries, spleen, thymus

Histopathology

- Adequate Battery: No. Organs/tissues evaluated were: eyes, heart, small intestine (duodenum, jejunum, ileum), large intestines (cecum, colon, rectum), pancreas, liver, lung, spleen, kidneys, adrenals, pituitary, thyroid/parathyroids, brain, and gross lesions.
- Peer Review: No
- Histological Findings: No treatment-related findings, excepting female #2503. Multi-organ inflammation and infiltration were observed frequently in all animals.

Special Evaluation – DHT Challenge

- Ipilimumab treatment was associated with a 2-fold stronger mean DTH challenge score against HBsAg, and weaker DTH challenge scores against SKMel
- No apparent effect on the response to DNP-Ficoll
- All monkeys received HBsAg and SKMel on D1, 29 and 57, and also DNP-Ficoll on D1 and D29.
- On D42, each monkey received 0.1 ml of HBsAg, SkMel, and saline by intradermal injection (three separate pre-shaved areas)
- Skin responses were assessed (erythema, eschar formation, induration) at 24 and 48 hours, and at 7 days after the DTH challenge

Special Evaluation – Flow cytometry and T-cell activation

- Although the study authors concluded that ipilimumab-treatment was associated with increased memory T cell populations; this reviewer disagrees. No difference is apparent at the time points measured.
- Spleen, inguinal lymph node, and colon cells were collected at necropsy for T-cell activation assays
- Blood was collected pre-dose, D2, 14, 30, 43, 58, 71, 85, 100, 140 (pre-dose) and D154
- Flow cytometry endpoints: CD20⁺ (B cells), CD3⁺ (total T cells), CD14⁺ (total monocytes), CD11c⁺ (dendritic cells), activated T cells (CD4⁺CD25⁺, CD8⁺CD25⁺, CD3⁺HLA⁺), memory T cells (CD3⁺CD45RO⁺, CD3⁺CD45RA⁻), effector memory T cells (CD4⁺CD28⁻CD95⁺), central memory T cells (CD4⁺CD28⁺CD95⁺), naïve T cells (CD4⁺CD28⁺CD95⁻), regulatory T cells (CD4⁺CD25⁺ and CD4⁺CD25⁺CTLA-4⁺)
- T-cell activation endpoints: intracellular IFN-gamma and TNF-alpha, intracellular CTLA-4 and FoxP3

Toxicokinetics

- Blood samples for PK and ADA collected prior to dosing, D1 (1 and 24 hours post-dose), D14, D29 (pre-dose and 24 hours post-dose), D43, D57 (pre-dose and 24 hours post-dose) and D71
 - The highest mean plasma concentration of ipilimumab was 335 µg/ml (measured 1 hour after the first dose) and the lowest mean plasma concentration of ipilimumab measured was 32 µg/ml (D29, pre-dose)
 - No ADA against ipilimumab was detected
- Blood for immunologic analyses (anti-HBsAg, anti-SKMel, anti-DNP-Ficoll) were collected pre-dose, D2, 14, 30, 43, 58, 71, 85, 100, 140 (pre-dose) and D154

Stability and Homogeneity

The study report provided no information regarding stability or homogeneity; no certificates of analysis were provided.

7 Genetic Toxicology

No experiments to evaluate potential genetic toxicity were submitted to the BLA. As noted by the applicant, short-term genotoxicity studies are not generally useful for evaluating biotechnology-derived pharmaceuticals such as ipilimumab. Based on ipilimumab being a human monoclonal antibody, and based on the current understanding of the mechanism of action of ipilimumab, direct toxicity to genetic material is not a regulatory concern.

8 Carcinogenicity

No experiments to assess carcinogenic potential of ipilimumab were submitted to the BLA. As noted by the applicant, ICH S1A does not recommend submission of carcinogenicity studies to support the safety evaluation of pharmaceuticals intended to treat advanced systemic disease. Moreover, because ipilimumab does not appear to be pharmacologically active in rodents, carcinogenicity testing of ipilimumab in rodents would not be useful.

NOTE: Nonclinical pharmacology experiments were performed with ipilimumab in a transgenic mouse model that expressed human CTLA-4 (study # MDX-010-005-r), and multiple nonclinical studies were performed in mice with 9C9, an anti-mouse CTLA-4 antibody. If carcinogenicity testing is needed to support future indications, these alternative models may warrant further investigation for feasibility.

9 Reproductive and Developmental Toxicology

No stand-alone nonclinical evaluation of fertility or early embryonic development has been performed with ipilimumab. The applicant notes (module 2.6.6 Toxicology Written Summary) that because the monkey is the only relevant nonhuman species identified, traditional nonclinical studies in the rodent with ipilimumab would not be useful for predicting safety.

9.1 Prenatal and Postnatal Development

One study, an expanded pre-/post-natal developmental (ePPND) study in cynomolgus monkeys, is ongoing. At the time of this review, all pregnancies in the treated monkeys had ended and newborns were being monitored. The timeline for submission of safety reports and interim reports is noted above (review section 2.5).

9.1.1

Study title: Ipilimumab (BMS-734016): intravenous study of pre- and post-natal development in cynomolgus monkeys with a 6-month postnatal evaluation

Study no: • DN10020
 • 930004258

Study report location: BLA 125377, amendments submitted
 October 19, 2010 and January 19, 2011

Conducting laboratory and location: • The interim reports list Bristol-Myers
 Squibb, Department of Drug Safety
 Evaluation, One Squibb Drive, New
 Brunswick, New Jersey
 • However, the draft protocol submitted
 for review (March 26, 2010, to active
 IND 9186) lists: (b) (4)

Report dates: Dosing initiated May 19, 2010
GLP compliance: Yes (not yet signed)
Drug: Ipilimumab (lot # not reported)

Key Study Findings:

- Ipilimumab treatment is associated with third-trimester abortion, stillbirths, early delivery (with corresponding decreased newborn body weight), and infant deaths.
- The applicant reports (1/19/2011 submission, page 5): "The clinical implications of infant hematology data, in particular, suggest that the concern for fatal lymphoproliferative conditions observed in knockout mouse pups is not borne out by data for viable cyno infants. (Knockout data may reflect an extreme state of unmitigated lymphoproliferation.) Presently, the cause(s) of adverse pregnancy outcome and infant mortality associated with ipilimumab administration are unknown; as such, the clinical implications of these findings are unclear."
- This reviewer disagrees; no regulatory conclusion regarding the cause of the infant mortality can be made, prior to submission of the data to FDA for review.

NOTE: In response to BMS's submission of the October 19, 2010 information amendment to the BLA, FDA requested available data regarding newborn body weight (e-mail sent November 4, 2010); this information has not yet been received, but the data omission is addressed in the January 19, 2011 interim study report from BMS (page 5).

Methods

Doses: 0, 10 or 30 mg/kg of ipilimumab
Frequency of dosing: Every 21 days (6 doses total), from the onset of
 organogenesis on gestation day (GD) 20, 21 or
 22 through parturition (approximately GD165)
Number/Sex/Group: 20 pregnant females/group

Protocol notes

- The applicant planned to monitor infants until birth day (BD) 180, and then perform full necropsy on newborns
 - X-ray evaluation of skeletal anomalies on BD28
 - Blood collection for TK, ADA, immunology, clinical pathology, and TDAR on BD42 and 120
- The applicant planned to return all surviving mothers to colony on post parturition day (PPD) 180
- Flow cytometry to evaluate T cells counts and T cell subsets was planned for both mothers and newborns
- **NOTE:** The knock-out mice data (reviewed below, in Section 9.2 of this review) raise a potential concern for ipilimumab-related cardiac toxicity following *in utero* exposure. No heart-related data have been submitted in the interim report. This reviewer notes that the protocol (submitted March 26, 2010 to active IND 9186) specifies:
 - Collection of fetal heart rates (as part of routine ultrasounds)
 - Birth day 1 evaluations (including heart rate and respiration rate)
 - Detailed gross heart evaluation of aborted fetuses, if feasible, for stillborn infants, and at scheduled necropsy for the newborns
 - Microscopic evaluation of the heart (but not weighing of the heart) at necropsy for aborted fetuses (GD100 and older), stillborn infants and at scheduled necropsy

Interim Reporting of Pregnancy Outcomes**Table 24: Interim reporting of pregnancy outcomes (study # DN10020)**

Dose	0 (control)	10 m/kg	30 mg/kg
Number of pregnancies:	20	20	20
# of 1 st trimester abortions:	2	2	0
# of 2 nd trimester abortions	2	0	2
# of 3 rd trimester abortions (i.e. \leq GD 140)	0	2	2
Stillbirths (i.e. \geq GD 141)	0	3	4
Infant death or infant euthanasia	2	3	4
Surviving infants (as of 1/19/2011)	14	10	8

Interim reporting of fetal/newborn anomalies

- None in the control group
- One occurrence in the low-dose group: a viable male was delivered with unilateral eye closure; massage failed to open the eye. The infant failed to thrive and was euthanized on BD14.
- One occurrence in the high-dose group: a viable male was delivered with non-patent prepuce and bilateral eye closure. This infant was euthanized on D2. Necropsy observed fluid in the abdomen, swollen and discolored (yellow) lining of the scrotal sac, and black testes.

Interim Reporting of Maternal effects:

- Increased incidence of emesis and watery/discolored feces for mothers in the high-dose group, as compared to controls
- Minimal changes in hematology (increased neutrophils, monocytes, basophils) and clinical chemistry (increased globulins, decreased albumin:globulin ratio)
- Decreased weight gain compared to controls during pregnancy (-8.3% for the 10 mg/kg group, -6.3% for the 30 mg/kg group), with recovery by PPD 28

Interim Reporting of Maternal PK:

From the 1/19/2011 interim report (page 2):

Table 25: Maternal blood levels of ipilimumab (study # DN10020)

Dose (mg/kg)	GD 20 AUC _[0-504 h] (µg*h/ml)	GD 125 AUC _[0-504 h] (µg*h/ml)	Safety margin from monkey to human	
			3 mg/kg	10 mg/kg
10	42,100	42,400	2.6	0.88
20	119,000	113,000	7.2	2.4

NOTE: This summary in this data could not be reviewed for accuracy (supporting data not provided). The applicant estimated the human PK values from data for trial # MDX-010-15 (13 patients) and trial # CA184007/C1848008 (16 patients), data after the 3rd or 4th dose of ipilimumab.

Interim Reporting of Maternal ADA

In the ipilimumab treated groups, 3/20 of the low-dose mothers and 4/10 of the high-dose mothers exhibited ADA. The applicant reports no apparent relationship between ADA and pregnancy outcome.

9.2 CTLA-4 Knock-Out Mouse Data

The original BLA submission (6/26/2010) briefly summarized publicly available information regarding CTLA-4 deficient mice in the context of the role of CTLA-4 in normal physiology (EDR Module 2.6.2 Pharmacology Written Summary), but omitted discussion of the CTLA-4 deficient mice in Module 2.6.6 Toxicology Written Summary (and specifically from sub-section 6 Reproductive and Developmental Toxicity).

The available literature raises a serious concern for infants exposed prenatally due to maternal use of ipilimumab, based on data regarding genetically engineered mice homozygous for deletion of CTLA-4. A summary of the information considered relevant to this review:

- Waterhouse et al. 1995⁴
 - Created “knock out” mice; a DNA construct designed to disrupt the murine *Ctla-4* gene was electroporated into embryonic stem cells, which were injected into C57BL/6 blastocytes to create chimeras. The resulting chimeras were bred to C57BL/6 females.
 - “Mice heterozygous for the *Ctla-4* mutation appeared normal.”
 - *Ctla-4*^{-/-} mice:
 - Born at the expected Mendelian frequency
 - Appeared healthy at birth
 - Clinical signs of sickness by 2 weeks of age
 - Moribund by 3 to 4 weeks of age
 - Prominent fresh and older myocardial infarctions observed at necropsy
 - Cause of death attributed to heart failure
 - Dramatic increase in activated T cells
 - Spleen and lymph nodes were 5 to 10-fold larger than normal
 - Dramatic increased in activated lymphocytes in the lymph nodes, thymus, and splenic white pulp
 - Diffuse and focal lymphocyte proliferation in the heart, lung, bone marrow, liver and pancreas
 - Expanded populations of activated B cells
- Tivol et al 1995⁵
 - Created “knockout” mice; a DNA construct to disrupt the murine *Ctla-4* gene by homologous recombination was introduced into embryonic stem

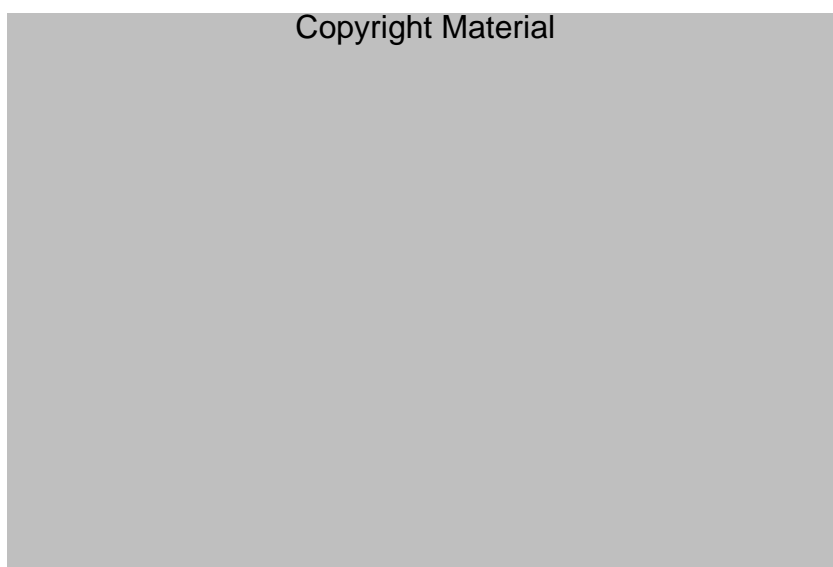
⁴ Waterhouse Paul, Penninger JM, Timms E, Wakeham A, Shainian A, Lee KP, Thompson CB, Griesser H, and Mak TW. 1999. Lymphoproliferative disorders with early lethality in mice deficient in *Ctla-4*. *Science*. 270:985-988.

⁵ Tivol Elizabeth, Borriello F, Schwitzer AN, Lynch WP, Bluestone JA, Sharpe AH. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multi-organ tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity*. 3:541-547.

cells, which were used to create germline-transmitting chimeric mice, bred to C57BL/6 or BALB/c mice.

- Heterozygotes survived and were fertile.
- CTLA-4^{-/-} mice:
 - Born at the expected Mendelian frequency
 - Died by 3-4 weeks of age
 - Multi-organ mononuclear infiltrates
 - Destructive myocarditis with massive interstitial infiltrates
 - Severe pancreatitis
 - Lungs, salivary glands, and liver also affected by abnormal aggregates of mononuclear cells
 - Some mice exhibited synovitis and vasculitis
- Araki et al. 1998⁶
 - All CTLA-4^{-/-} mice in the authors' colony die by D50 (deaths beginning at week 3 after birth). These mice exhibit "lymphoproliferative disease with massive mononuclear cell infiltration and tissue destruction of multiple organs, e.g., heart, lung, pancreas, and liver".
 - Notably, this paper mentions 4 isoforms of CTLA-4 in mice, including full-length CTLA-4 (flCTLA-4) and ligand-independent CTLA-4 (liCTLA-4)
 - Page 5154 of the paper provided a survival curve (the light grey line) for 39 CTLA-4^{-/-} mice (the black curve is not relevant to this safety review; it represents transgenic mice expressing the liCTLA-4 isoform):

Figure 6: Survival curve in CTLA-4^{-/-} mice (from Araki et al. 2009)



⁶ Araki Manabu, Chung D, Liu S, Rainbow DB, Chamberlain G, Garner V, Hunter KMD, Vijayakrishnan L, Peterson LB, Oukka M, Sharpe AH, Sobel R, Kuchroo VJ, and Wicker LS. 2009. Genetic evidence that the differential expression of ligand-independent isoform of CTLA-4 is the molecular basis of the Idd5.1 type 1 diabetes region in non-obese diabetic mice. *The Journal of Immunology*. 183:5146-5157.

NOTE: This reviewer concludes that the two papers (Tivol et al. 1995; Waterhouse et al. 1995) present the results of two independently created knock-out models, rather than only one model. The reported methods used to generate the knock-out mice are very similar - for example, both laboratories report using the neomycin cassette in the targeting construct. However, this reviewer noted that Tivol et al. cultured the recombinant embryonic stem cells in neomycin (CAS number 1404-04-2) and FIAU (fialuridine; CAS number 69123-98-4) whereas Waterhouse et al. cultured the recombinant embryonic stem cells in G418 (Geneticin®; CAS number 49863-47-0) and ganciclovir (CAS number 82410-32-0). This difference indicates that two different laboratories used similar, but not precisely the same methods, to create Ctla-4^{-/-} mice. The reporting of similar results from two different laboratories using (apparently) independent models, increases confidence in the validity of the findings. This reviewer was unable to determine the source of CTLA-4^{-/-} mice used by Araki et al.

9.3 Consideration of Ipilimumab-Related Endocrine Disorders

The clinical trials with ipilimumab observed auto-immune endocrine disorders: hypophysitis and hypopituitarism (both terms describe effects on the pituitary); adrenal insufficiency (Addison's disease), hyper- and hypo-thyroidism. This reviewer raised the theoretical question whether these adverse events, and their medical treatments, might affect patient's ability to become pregnant and maintain pregnancy.

This issue was discussed internally (personal communication McDougal/Shastri, 8/05/2010); the potential secondary toxicity of ipilimumab on pregnancy via endocrine disruption is not a regulatory concern for the clinical indication under consideration for this BLA. The potential reversibility of the effects is not known, and the clinical data are not yet mature.

9.4 Other Nonclinical Data Relevant to Reproductive and Developmental Toxicology

A tissue cross-reactivity study (study # DSO05067) detected ipilimumab binding to connective tissue in human and monkey placenta, and to connective tissue in monkey (but not human) ovary.

In the GLP 6 month toxicology study in monkeys (study # 01-3460), the study authors considered the decrease in testes weights (-27 to -50%) for male monkeys treated with ipilimumab compared to control monkeys, to be treatment-related. However, this reviewer disagrees, and considered the difference in testes weight to be incidental to treatment (and likely related to differences in maturity).

10 Special Toxicology Studies

The applicant submitted three tissue cross-reactivity studies (# IM578, # IM993, and # DSO5067) and two cytokine release studies (# 930034490 performed by BMS; # MDX-1106/010-008R performed by Medarex).

The *in vitro* primary pharmacodynamic data (i.e. study # 930021444) observed stronger binding of ipilimumab to stimulated lymphocytes; the applicant attributed this to higher CTLA-4 expression on activated T cells. The tissue cross-reactivity studies are more relevant to evaluation of constitutive expression of CTLA-4 (i.e. Treg cells and placenta) than to animals undergoing an active response of the adaptive immune system.

10.1

Study title: Cross-reactivity of fluoresceinated, human monoclonal antibody 10D1 with normal human tissues

Study no:	IM578 (document # 930009993)
Study report location:	Module 4.2.3.7.7 (Other Toxicity – Other) Cross-referenced in module 5.2.2.3 (Clinical Study Reports - Reports of Studies Using Other Human Biomaterials)
Conducting laboratory and location:	(b) (4)
Report date:	February 28, 2000
Date of study initiation:	February 7, 2000
GLP compliance:	Yes, signed. However, no certificate of analysis was provided in the original study report.
QA statement:	Yes, signed
Drug, lot #:	A fluoresceinated form of ipilimumab, 10D1-FITC. Lot # 10D1-011400-TK

Key Study Findings: Ipilimumab (10D1) bound human lymphocytes; off-target binding was not detected.

Methods notes:

- This study evaluated the tissue cross-reactivity of ipilimumab to a panel of human tissues, by immunohistochemistry
- 10D1-FITC was applied to cryosections of human tissues (obtained at biopsy or autopsy) at 2.5 or 10 µg/ml. Samples from three different donors were tested per tissue. Visualization was via an indirect immunoperoxidase method
- As positive control tissues for the expression of CTLA-4, human αβCTLA4CD38 cells and human tonsil lymphocytes were used
 - Although omitted from the study report, the BLA clarified that αβCTLA4CD38 cells are a human T-cell line that constitutively expresses

CTLA-4 (BLA section 2.6.6 Toxicology Written Summary, subsection 8.8.3.1)

- As a negative control tissue, lacking expressed CTLA-4, human cerebellum was used
- As a negative control antibody, an isotypic control (an IgG1-k-FITC) antibody was used (the target of this control antibody was not reported)
- The distribution of β_2 -microglobulin was evaluated on separate cryosections, as an assay control
- Tissues evaluated: tonsil (positive control), cerebellum (negative control), adrenal, blood (neutrophils, lymphocytes, eosinophils, monocytes, platelets), bone marrow, breast (mammary gland), eye, colon, esophagus, small intestine, stomach, heart, kidney (glomerulus and tubule), liver, lung, lymph node, ovary, fallopian tube, pancreas, parathyroid, peripheral nerve, pituitary, placenta, prostate, salivary gland, skin, spinal cord, spleen, striated muscle (skeletal muscle), testis, thymus, thyroid, ureter, urinary bladder, uterus (endometrium and cervix)

Results notes:

- The positive and negative control tissues yielded expected responses
 - Less than 2% of all tonsil lymphocytes stained positive
- Specific staining was of discrete, round granules at membrane and cytoplasm immediately below the membrane:
 - Tonsil lymphocytes (3/3 donors)
 - Lymphocytes in blood smears (2/3 donors)
 - Occasional follicular and interfollicular lymphocytes in the submucosal lymphoid nodules in the colon
- As expected, the tissue staining control (β_2 -microglobulin) was observed in vascular endothelia, platelets, and occasional intravascular cells
- **NOTE:** no binding was observed in the placenta (3 tissue samples) or ovary (3 tissue samples)
- **NOTE:** In response to an information request sent August 5, 2010, the applicant clarified (September 20, 2010, sequence # 0016) that the source lot 010-99-01F was fluoresceinated for this experiment. This source lot is the same as was used for studies #7114-100 and #0919-128. Information on the lot is provided in the BLA, table 3.2.S.4.4.1.T01.

10.2**Study title: Cross-reactivity study of fluoresceinated MDX-010 with limited normal human tissues**

Study no:	IM993 (document # 930010006)
Study report location:	Module 4.2.3.7.7 (Other Toxicity – Other)
Conducting laboratory and location:	(b) (4)
Report date:	March 2003
Date of study initiation:	January 7, 2003
GLP compliance:	Yes, signed
QA statement:	Yes, signed
Drug, lot #:	A fluoresceinated form of ipilimumab, MDX-010-FITC, lot # 046-02-03PMB-Tox

Key Study Findings: Ipilimumab bound human lymphocytes; off-target binding was not detected.

Methods notes:

- This study evaluated the tissue cross-reactivity of ipilimumab to a panel of human tissues, by immunohistochemistry
- Process B ipilimumab (derived from Chinese hamster ovary cells) was fluoresceinated, and tested at two concentrations: 2 and 10 µg/ml
- As a negative control antibody, an isotypic control (an IgG1-k-FITC) antibody was used (the target of this control antibody was not reported)
- A limited panel of 12 human tissues, cryosections from 3 donors, was evaluated: blood cells, brain, large intestine, esophagus, small intestine, stomach, heart, kidney, liver, lung, skin, tonsil
- As a positive control tissue, human tonsil with lymphocytes was used
- As a negative control tissue, human cerebellum was used
- The distribution of β_2 -microglobulin was evaluated on separate cryosections, as an assay control

Results notes:

- The positive and negative control tissues yielded expected responses
 - Less than 1-5% of tonsil lymphocytes stained positive
 - Specific binding was detected as discrete, round, fine granules at the membrane and in the cytoplasm immediately below the membrane
- Authors estimate that less than 5% of all peripheral blood leukocytes were reactive to ipilimumab.
- Specific staining of lymphocytes in:
 - tonsil (3/3 donors)
 - submucosal lymphoid nodules of the large intestine (3/3 donors), esophagus (1/3 donors), small intestine (1/2 donors) and stomach (2/3 donors)
 - Blood smears (3/3 donors)
 - lung (2/3 donors)

- kidney (2/3 donors)
- liver (1/3 donors)
- “No unanticipated cross-reactivities were observed” (report page 8)
- As expected, the tissue staining control (β_2 -microglobulin) was observed in vascular endothelia, platelets, and occasional intravascular cells, and various cells in all tissues examined
- **NOTE:** In response to an information request sent August 5, 2010, the applicant clarified (September 20, 2010, sequence # 0016) that the certificate of analysis is found in the BLA, table 3.2.S.4.4.1.T01 of section 3.2.

10.3

Study title: BMS-734016 (MDX-010). Comparative tissue-binding study with mouse, rat, rabbit, monkey, and human tissues

Study no:	<ul style="list-style-type: none"> • DSO05067 • 930021297
Study report location:	Module 4.2.3.7.7 (Other Toxicity - Other)
Conducting laboratory and location:	Bristol-Myers Squibb Pharmaceutical Research Institute, Departments of Immunotoxicology and Pathology, Syracuse, New York
Report date:	May 15, 2007
Date of study initiation:	Not reported
GLP compliance:	Yes
QA statement:	Yes, signed
Drug, lot #:	Ipilimumab (lot # 5J06544) was biotinylated

Key Study Findings:

- This study detected ipilimumab specific binding to human and monkey placenta, and to monkey ovary (connective tissue in the ovary)
 - NOTE: The apparent lack of consistency between this study and study # IM 578 is unclear. However, the applicant notes that expression of CTLA-4 on placenta has been previously reported⁷
 - NOTE: study # IM 993 did not examine ovary or placenta tissues
- Ipilimumab also bound with specificity to human and cynomolgus monkey lymphocytes
- Ipilimumab did not bind mouse, rat, or rabbit lymphoid tissues under the conditions tested.

⁷ Kaufman et al. 1999. The CTLA-4 gene is expressed in placental fibroblasts. Mol. Human Reprod. 5(1): 84-87.

Method notes:

- This tissue cross-reactivity study evaluated ipilimumab binding to human, cynomolgus monkey, CD-1 mouse, Sprague Dawley rat, and New Zealand White rabbit tissues
 - Tissues from at least 2 males and 2 females per species were evaluated
 - For each species: cerebellum, gastrointestinal tract (large intestines, esophagus, small intestine, stomach), heart, kidney, liver, lung, lymph node, ovary, pancreas, pituitary gland, placenta, skin, testes, thymus
 - Tonsil tissue was evaluated only for the rabbit, monkey, and human
- After preliminary assays to optimize the concentration of ipilimumab, two concentrations were selected: 1 or 10 µg/ml of ipilimumab
- As positive control tissues, human tonsillar lymphocytes and murine T-cell hybridomas (expressing human CTLA-4) were used
- As a negative control tissue lacking expressed CTLA-4, human cerebellum was used
- As a negative control antibody, an isotypic control (an IgG1-k-FITC) antibody was used (the target of this control antibody was not reported)
- The distribution of β_2 -microglobulin was evaluated on separate cryosections, as an assay control
- The immunohistochemistry data were evaluated and peer reviewed by veterinary pathologists.

Results notes:

- The positive and negative control tissues yielded expected responses
- In human tissues, specific binding was observed:
 - lymphocytes in the gastrointestinal tract, lymphoid system, and skin
 - Connective tissue in the placenta (slight binding in 2/2 donors at 1 µg/ml; moderate to strong binding in 2/2 donors at 10 µg/ml)
- In cynomolgus monkey tissues, specific binding was observed:
 - lymphocytes in the gastrointestinal tract, lymphoid system, and skin; results were consistent with the results for human tissues
 - Connective tissue in the placenta (slight binding in 1/2 samples at 1 µg/ml; slight to moderate binding in 2/2 samples at 10 µg/ml)
 - Connective tissue in the ovary (moderate binding in 1/2 samples at 1 µg/ml; slight to moderate binding in 2/2 samples at 10 µg/ml)
- No specific binding of ipilimumab to mouse, rat or rabbit tissues was detected
- **NOTE:** The biotinylated ipilimumab was not evaluated for concentration, content, or stability (report page 11). In response to an information request sent August 5, 2010, the applicant clarified (September 20, 2010, sequence # 0016) that the certificates of analysis (including for the pre-biotinylated ipilimumab lot) are in the study report, pages 28-40.

10.4**Study title: BMS-663513 and Ipilimumab. Exploratory *in vitro* proliferation and cytokine release assessment using human peripheral-blood mononuclear cells**

Study no:	930034490
Study report location:	Module 4.2.3.7.7 (Other Toxicity - Other)
Conducting laboratory and location:	Bristol-Myers Squibb Pharmaceutical Research Institute, Departments of Immunotoxicology and Pathology, Syracuse, New York
Report date:	November 6, 2009
Date of study initiation:	Not reported
GLP compliance:	No
Drug, lot #:	Ipilimumab, lot #s 6M13871 and 6M14407 (purity not reported)

Key Study Findings:

- In the context of available clinical data, the results of this study are not concerning
- The results of this study suggested a potential concern for ipilimumab-induced cytokine release
 - Under the conditions tested *in vitro*, ipilimumab induced cytokine release from human peripheral blood mononuclear cells (PBMCs)
 - The response to ipilimumab was weaker than the cytokine release caused by the positive control (an anti-CD28 mAb with agonist activity)

Study notes:

- PBMCs were obtained from 10 healthy human volunteers
- Exposure to 2 µg/ml of ipilimumab for 6 or 24 hours did not induce proliferation of PMBCs
- Exposure to 2 µg/ml of ipilimumab for 24 hours was associated with increases in IL-6 (9/10 samples), IL-8 (7/10 samples), IL-2 (4/10 samples), TNF-α (4/10 samples), IL-4 (1/10 samples) and IL-5 (1/10 samples). Ipilimumab did not induce IFN-γ or IL-12.
- The positive control antibody was 5.11A1, a mouse antibody against CD28. The authors note that TGN1412 is the IgG4 humanized version of 5.11A1. The positive control antibody gave the expected response, inducing PBMC proliferation and cytokine release.
- Ipilimumab was tested both alone and in combination with another monoclonal antibody (BMS-663513). The addition of BMS-663513 did not affect the cytokine release associated with ipilimumab alone.
- Because CTLA-4, the target of ipilimumab, is not constitutively expressed on T cells, the biological significance of these results to patients expressing CTLA-4 (e.g. in response to physiological stimulation of the immune system) is unknown

10.5

Study title: Effect of combined MDX-1106 and ipilimumab treatment on ex vivo cytokine release in human peripheral blood cells

Study no: • MDX-1106/010-008R
• 930036361

Study report location: Module 4.2.3.7.7 (Other Toxicity - Other)

Conducting laboratory and location: Medarex
Milpitas, CA

Report date: June 14, 2009

Date of study initiation: August 28, 2008

GLP compliance: No

Drug, lot #: Ipilimumab, lot # M31A-04-03Fc

Key Study Findings: Under the conditions tested *in vitro*, ipilimumab did not induce cytokine release from human PBMCs

Study notes:

- PMBCs were obtained from 10 healthy human volunteers
- PMBCs were incubated for 6 or 24 hours with 0, 10, or 100 µg/ml of ipilimumab, alone or in combination with another monoclonal antibody (MDX-1106)
- A positive control antibody (anti-CD3) and a negative control antibody were included
- The cytokines evaluated were: IFN-γ, TNF-α, IL-2, IL-4, IL-6, and IL-10
- Because CTLA-4, the target of ipilimumab, is not constitutively expressed on T cells, the biological significance of these results to patients expressing CTLA-4 (e.g. in response to physiological stimulation of the immune system) is unknown

11 Integrated Summary and Safety Evaluation

PD

The applicant provided pharmacodynamic data to support the proposed mechanism of action. Ipilimumab:

- Bound human CTL-4 ($K_D =$ (b) (4)) and cells expressing human CTLA-4
- Blocked binding of human CTLA-4 with human B7.1 ($EC_{50} =$ (b) (4)) and human B7.2 ($EC_{50} =$ (b) (4))
- Via the Fc portion of the antibody, bound human FcγRI ($EC_{50} =$ (b) (4)) and also bound (with lower affinity) to FcγRIIA and FcγRIII
- Did not induce CDC under the conditions tested
- Induced ADCC against resting human T cells; ADCC was enhanced against T cells stimulated to express CTLA-4
- Exhibited anti-tumor activity against human MC38 cells implanted into transgenic mice expressing human CTLA-4
- In monkeys, increased the antibody response to HBsAg (studies # 099-128, 1416-128, SUV00006) and Sk-mel cells (studies # 01-3460, 1416-128, SUV00006)
- In monkeys, increased the antibody response to KLH (study # DS06064)

The available nonclinical data do not raise an unusual concern for the safety pharmacology of ipilimumab. Consistent with other monoclonal antibodies, signs consistent with infusion reaction have been observed in monkeys receiving ipilimumab.

(b) (4)

PK/TK

- In monkeys, the elimination half-life of ipilimumab was approximately 8.5 to 14 days.
- The volume of distribution (approximately 40 to 80 ml/kg) suggests partial sequestration from circulation. This reviewer speculates this may involve binding of ipilimumab to the CTLA-4 target expressed by T cells residing in lymphoid tissues.
- Dosing with 10 m/kg of ipilimumab was associated with C_{max} values approximately 340 to 490 µg/ml and AUC values approximately 31,500 to 48,000 µg*hr/ml (from Module 2.6.7, Toxicology Tabulated Summary)

Toxicology

- In monkeys, the highest dose of ipilimumab tested was 30 mg/kg (study # 7114-100). However, this study is of limited usefulness, because of the short post-treatment observation period (animals were necropsied at D14, before potential auto-immune related toxicities might have manifested)

- Most of the monkey studies tested dose levels of 3 and 10 mg/kg, or only 10 mg/kg of ipilimumab

Table 26: Monkey toxicology summary (ipilimumab alone)

Study #	Dose information	Notes
Ipilimumab-only		
DS07167	10 mg/kg single dose	No toxicity observed
126-002	3 mg/kg x 3 (D1, 4, 7)	3 mg/kg = NOAEL
7114-100	3 or 30 mg/kg x3 (D1, 4, 7)	Weak evidence of changes: <ul style="list-style-type: none"> • in WBC counts (increased total leukocytes and lymphocytes) • anemia • T cell counts (elevated CD3⁺, CD3⁺CD4⁺ and CD3⁺CD29⁺ cells)
(b) (4)-0919-128	10 mg/kg x3 (D1, 4, 7)	Increased T cell subsets (elevated CD3 ⁺ , CD3 ⁺ CD4 ⁺ and CD3 ⁺ CD29 ⁺ cells)
DS06064	10 mg/kg/week x 4	<ul style="list-style-type: none"> • Multi-organ lymphocyte infiltration (minimal to moderate) • Suggestion of decreased spleen weight • Increased antibody titer against KLH
(b) (4)-1414-128	10 mg/kg/month x 3 (D1, 29, 57)	<ul style="list-style-type: none"> • Multi-organ mononuclear cell infiltration • Lymph node hyperplasia • Increased induration and erythema to DTH challenge

Table 27: Monkey toxicology summary (ipilimumab in combination with other immunostimulatory agents)

Study #	Ipilimumab dose information	Notes
DS06064	10 mg/kg/week x 4	No apparent difference versus ipilimumab alone
(b) (4)-0992-128	10 mg/kg x 2 (D1 and 37)	<ul style="list-style-type: none"> • Increased antibody responses • Slight increases in lymphocyte counts
TIB-06-001	10 mg/kg x 8 (over 88 days)	<ul style="list-style-type: none"> • Severe transient reaction in one animal • Rash with lymphoedema in one animal
SUV00106	3 or 10 mg/kg/week x 4	<ul style="list-style-type: none"> • Death in one animal due to gastric dilation (bloat), with corresponding inflammation and mononuclear cell infiltration of the large intestine • Spleen changes (increased weight, lymphoid hypertrophy and marginal zone expansion)

		<ul style="list-style-type: none"> • Lymph node and thymus changes (decreased germinal centers, hypocellularity) • Slight increase in body temperature (+1°C) post-dosing • Increased T-cell counts • Increased antibody response to KLH
01-3460	10 mg/kg/month x5	No apparent difference versus ipilimumab alone
SUV00006	10 mg/kg/month x 3 or 4	Death in one animal, apparently due to auto-immune related GI toxicity
DN10020	10 or 30 mg/kg every 21 days (ePPND)	Interim reporting of abortion, stillbirth, infant mortality
01-3460	10 mg/kg/month x5	<ul style="list-style-type: none"> • Increased antibody response to co-treatment • Weak suggestion of increased testes weight • Weak suggestion of decreased thyroid weight

NOTE: This reviewer identified clear increases in the incidence and severity of multi-organ lymphocyte infiltration in monkeys receiving ipilimumab, compared to controls. Infiltration is a common background finding, and neither the study authors nor the applicant identified the infiltration. For the studies listed in Table 26 and Table 27 above where infiltration was not noted, this reviewer attributes the lack of association to study design (e.g. short duration, no concurrent negative controls, small group sizes) rather than an absence of ipilimumab activity.

- The applicant submitted three reports of testing with anti-mouse CTLA-4 in mouse models of auto-immune diseases
 - A model of auto-immune colitis observed toxicity with anti-mouse CTLA-4, consistent with reported clinical SAEs for ipilimumab (study # 930031040)
 - Anti-mouse CTLA-4 increased anti-nuclear antibodies, raising a potential concern for auto-immune related renal toxicity (study MDX-1106-010-006-R)
 - Anti-mouse CTLA-4 alone did not induce an earlier onset of diabetes in a susceptible mouse strain, but the anti-mouse CTLA-4 was associated with a stronger response to another immunostimulatory agent (study # MDX-1106/010-007-R)
- Two special toxicity studies did not identify safety concerns for ipilimumab-induced cytokine release (studies # 930034490 and MDX-1106/010-008R)

NOTE: Two 14-day studies (dosing D1, 3 and 7, necropsy D14) were commissioned by the previous sponsor (Medarex): the first tested 3 and 30 mg/kg (study #7114-100). The second (study (b) (4)-0919-128) tested 3 and 10 mg/kg (dosing D1, 3, and 7). The studies were conducted at two different laboratories ((b) (4) and (b) (4), respectively) at essentially the same time (June-July, 2000). The overlapping timing, and the use of different laboratories, is perplexing. Presumably, a sponsor

would have wanted to incorporate the results of one study into the design of the other. Because no toxicity was observed in either study, and because additional study reports are available, this reviewer concludes that no investigation is warranted into the historical decisions/concerns of Medarex's nonclinical product development plan.

Discussion of ipilimumab-related spleen findings

Ipilimumab treatment was associated with variable effects on the spleen, indicating that the effect of ipilimumab is complex and is influenced by co-treatments with other immunostimulatory agents.

- Study #DS06064 detected evidence of decreased spleen weights at D28, in monkeys receiving 10 mg/kg/week x 4 of ipilimumab. One female (receiving a combination of 10 mg/kg of ipilimumab plus 100 mg/kg of BMS-663513, anti-CD137 mAb) exhibited increased spleen weight and histological evidence of slight spleen congestion
- Study # SUV00106 detected clear spleen-effects in monkeys receiving ipilimumab in combination with MDX-1106 (anti-PD-1 antibody): the combination was associated with increased spleen weight and microscopic evidence of spleen lymphoid follicle hypertrophy/marginal zone expansion. Moreover, monkeys receiving the combination also exhibited decreased thymus weight and microscopic changes in the thymus and lymph nodes (decreased germinal centers hypocellularity)
- Study # SUV00006 observed spleen and thymus effects in one monkey (1/6) that animal received 10 mg/kg/month of ipilimumab, in combination with HBsAg, SKMel and DNP-ficoll. This one monkey exhibited grossly-visible smaller thymus and spleen, as well as microscopically-visible lymphoid depletion of the thymus, spleen, and GALT
- Ctl α -4^{-/-} mice were born apparently normal but did not survive past 5 weeks; at necropsy, these mice exhibited dramatic enlargement of spleen and lymph node.

Discussion of reproductive and developmental toxicity results to date

- The ePPND study in cynomolgus monkeys (study # DN10020) is ongoing; the interim results raise concerns regarding the developmental toxicity of ipilimumab, including increased incidences of abortion, stillbirth, and infant death
 - FDA requested available newborn body weights; the applicant has not yet provided this data
 - FDA recommended that the applicant carefully evaluate available placenta tissues. The basis for this recommendation is the tissue cross-reactivity study detecting CTLA-4 in the placenta (study # DSO05067). It is unknown whether the observed toxicities are due to toxicity to the mother (e.g. immune-related rejection of the fetus) or due to direct toxicity to the fetus (e.g. transfer of ipilimumab to the fetus, and subsequent induction of ipilimumab with CTLA-4 expressed fetally)

- CTLA-4^{-/-} knock out mice are born normal, but die within 3-4 weeks due to a massive multi-organ infiltration and tissue damage by inflammatory leukocytes
- In the absence of additional reporting for the ongoing ePPND study, the CTLA-4^{-/-} knock out mice results appear consistent with the available monkey ePPND data, and are deemed relevant to patient safety

Discussion of the relevance of the cynomolgus monkey model

- Overall, the cynomolgus monkey model failed to predict the auto-immune related SAEs observed clinically. The applicant notes that ipilimumab induced some pharmacological responses (e.g. increased antibody responses to co-administered agents). Review of the studies also noted multi-organ lymphocyte infiltration. Possible explanation for the lack of observed toxicity include:
 - The clinical SAEs developed after 5 or more weeks, consistent with the putative mechanism of action (activation of T cells). Therefore, nonclinical studies that necropsied animals after only a short post-recovery observation period were not designed to detect subacute toxicity
 - The 2 to 4-fold lower affinity binding for ipilimumab to monkey CTLA-4 compared to human CTLA-4 (study # MDX-010-011-R). To the extent that reduced binding affinity is important, the results of the ePPND study, which tested higher doses (10 and 30 mg/kg/q21 days) may be more predictive.
 - Patients with cancer might have more CTLA-4 expression on their T cells than healthy monkeys (i.e. the monkey model is more predictive for healthy humans than for patients)
 - Tregs constitutively express CTLA-4, but other T cells require stimulation to express CTLA-4. Stimulation of lymphocytes to express CTLA-4 resulted in greater binding (studies # 930021444, 930031729, MDX-010-015-SR) and ADCC (studies # 930023602, MDX-010-006-R, MDX-010-016-R)
 - The tissue cross-reactivity studies noted binding of ipilimumab with approximately 1 to 5% of lymphocytes, and this number may represent the number of Tregs (studies # IM578, IM993)
 - In monkeys receiving ipilimumab alone, hypothetically, insufficient expression of CTLA-4 may have prevented the development of auto-immune toxicity. However, multiple monkey studies co-treated with both ipilimumab and other agents; it is therefore reasonable to expect that those other agents (i.e. KLH, SK-mel cells, HBsAg, or DNP-Ficoll) would have induced CTLA-4 expression. Therefore, the validity of this hypothesis is uncertain.
- Several polymorphisms in human CTLA-4 have been identified. Multiple studies have been published on genetic polymorphisms of CTLA-4, usually investigating potential associations with cancer, auto-immune disorders, and other diseases. For

example, a recent meta-analysis was performed by Zheng et al. 2010⁸. Hypothetically, differences between the CTLA-4 of the monkeys tested and the CTLA-4 against which ipilimumab was developed may partially explain the apparent lack of activity. Likewise, **polymorphic differences in CTLA-4 might be partially responsible for the apparent clinical differences in efficacy and toxicity among patients treated with ipilimumab.** [Confidential information before the Agency (b) (4)]

This hypothesis is reflected in this review's section 1.1.2 (Additional Non Clinical Recommendations), #3.]

Discussion of ipilimumab and abatacept

- Abatacept (Orencia®) is a soluble fusion protein that consists of the extracellular domain of CTLA-4 linked to the modified Fc (hinge, CH2, and CH3 domains) portion of human IgG1. The applicant, BMS, received initial license approval to market Abatacept on December 23, 2005, based on BLA 125118. The label⁹ and redacted primary reviews¹⁰ are publically available.
- Ipilimumab is a human IgG1 against human CTLA-4. Ipilimumab was generated (b) (4)

This reviewer did not determine whether ipilimumab recognizes (b) (4)

In accordance with the disclaimer on page 1 of this review notes, no information for abatacept (neither the label, public reviews, nor confidential information) was considered for this review. However, readers may find information regarding abatacept useful context for understanding ipilimumab.

⁸ Zheng, J., Yu X., Jiang L, Xiao M, Bai B., Lu J., and Y. Zhou. 2010. Association between the cytotoxic T-lymphocyte antigen 4 +49G > a polymorphism and cancer risk: a meta-analysis. BMC Cancer 10:552-563.

⁹ Label accessed online via:

http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/125118s0086lbl.pdf

¹⁰ Reviews accessed online via:

http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/125118_s0000_OrenciaTOC.cfm

12 No Appendix/Attachments

This review has no appendix or attachment.