CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761034Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

Tecentriq (atezolizumab)

Date: April 27, 2016
To: File for BLA 761034
From: John K. Leighton, PhD, DABT
Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review and labeling for Tecentriq conducted by Dr. Ricks and secondary memorandum and labeling provided by Dr. Palmby. In his memorandum, Dr. Palmby recommends a Post-Marketing Commitment (PMC) to address the effect of atezolizumab on the magnitude of primary and recall response to antigen challenge. I concur with Dr. Palmby's conclusion that Tecentriq may be approved for the proposed indication and the recommendation for a PMC.

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/s/

JOHN K LEIGHTON
04/27/2016

MEMORANDUM

Date: April 26, 2016
From: Todd R. Palmby, PhD
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for BLA 761034 Tecentriq (atezolizumab)
Re: Approvability for Pharmacology and Toxicology
Indication: Treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy

Non-clinical pharmacology and toxicology literature and original reports for studies to support BLA 761034 for Tecentriq (atezolizumab) indicated for the treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy or have disease progression within 12 months of neoadjuvant or adjuvant treatment with platinum-containing chemotherapy were reviewed by Tiffany Ricks, PhD. Studies conducted by the Applicant with atezolizumab, or with murine chimeric anti-PD-L1 antibodies, for which reports were submitted to this BLA include pharmacology, pharmacokinetics and general toxicology. A detailed evaluation of the nonclinical data submitted to the BLA can be found in Dr. Ricks' review.

Atezolizumab is a humanized IgG1 monoclonal antibody that binds to human PD-L1, thereby inhibiting the interaction of PD-L1 with PD-1 and B7-1 receptors. In order to decrease antibody-dependent cell-mediated cytotoxicity (ADCC), the Applicant modified amino acid 298 in the heavy chain from asparagine to alanine. This modification results in a non-glycosylated antibody, demonstrated to have no Fc-effector function (Fcγ), but retains binding to the neonatal Fc receptor (FcRn). The Applicant generated mouse IgG2a/human chimeric antibodies for murine in vivo studies in order to reduce immunogenicity, which was observed in prior mouse studies. Based on the submitted pharmacology data, the Established Pharmacologic Class (EPC) of "programmed death-ligand 1 (PD-L1) blocking antibody" was determined to be both clinically meaningful and scientifically valid for atezolizumab. Atezolizumab would be the first product in this class to be approved in the US.

Based on its mechanism of action, atezolizumab treatment may increase the inflammatory response and enhance the severity of some infections in patients.

General toxicology studies evaluated atezolizumab in mice for two weeks and in Cynomolgus monkeys for up to 26 weeks. Atezolizumab was immunogenic in

mice, resulting in significantly reduced exposures by Week 3. The major toxicological finding in mice was minimal sciatic neuropathy (vacuolation and lymphocytic infiltration). In Cynomolgus monkeys, IV administration of atezolizumab caused multi-organ arteritis/periarteritis and irregular menstruation, including an increase in mean menstrual cycle length and a corresponding lack of newly formed corpora lutea. The immune-mediated findings were consistent with the role of PD-L1 in regulating and maintaining peripheral tolerance. The mechanism of the effects on menstruation in monkeys is not clear, but these effects were reversible. Safety pharmacology assessments were incorporated into the repeat-dose toxicology study in monkeys, but no effects were observed on the respiratory, neurological or cardiovascular systems. Administration of atezolizumab to patients at the recommended dose of 1200 mg resulted in immune-related adverse events.

Since it is a monoclonal antibody and is not expected to interact with DNA, genetic toxicology studies were not conducted with atezolizumab.

The Applicant submitted a non-product specific literature-based assessment to characterize the potential risk of reproductive and developmental toxicity. Data in the scientific literature demonstrate that interference with PD-L1 leads to a loss of fetal tolerance and an increased risk of immune-mediated abortion. This assessment provides evidence that atezolizumab can cause fetal harm if administered to a pregnant woman.

Atezolizumab maintains binding to the FcRn receptor, so fetal exposure may occur if a patient is treated during pregnancy, although it is not recommended. It is unclear whether fetal exposure to atezolizumab would occur at levels sufficient to cause adverse effects on the developing immune system. Nevertheless, if a pregnant patient receives treatment with atezolizumab that does not result in loss of the fetus, there is a potential risk of developing immune-mediated disorders or altering the normal immune response in the offspring due to the mechanism of action.

Recommendation: I concur with Dr. Ricks' conclusion that submitted pharmacology and toxicology data support the approval of BLA 761034 for Tecentriq (atezolizumab) for the proposed indication. The Pharmacology/Toxicology team recommends that the Applicant conduct a pharmacology study to further characterize the effect of atezolizumab on the immune response as a Post-Marketing Commitment (PMC). We recommend that the Applicant conduct an animal study that will measure the effect of PD-L1 inhibition on the magnitude of the primary (1st vaccination) and recall (2nd vaccination) antibody responses to antigen challenge (e.g. KLH). This PMC is currently being discussed with the Applicant. There are no outstanding non-clinical issues that would preclude the approval of Tecentriq for the proposed indication.

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/s/

TODD R PALMBY
04/26/2016

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:	761034
Supporting document/s:	1, 53
Applicant's letter date:	January 12, 2016
CDER stamp date:	January 12, 2016
Product:	Tecentriq (atezolizumab)
Indication:	Adult patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy (b) (4) or have disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen
Applicant:	Genentech, Inc. 1 DNA Way South San Francisco, CA
Review Division:	Division of Hematology Oncology Toxicology (Division of Oncology Product 1)
Reviewer:	Tiffany K. Ricks, PhD
Supervisor/Team Leader:	Todd Palmby, PhD
Division Director:	John Leighton, PhD, DABT (DHOT) Geoffrey Kim, MD (DOP1)
Project Manager:	Kim Robertson

Disclaimer

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Any information or data necessary for approval of BLA 761034 that Genentech Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 761034.

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1 Executive Summary

1.1 Introduction

Tecentriq (atezolizumab) is a humanized, Fc-engineered, IgG1 monoclonal antibody targeting programmed death-ligand 1 (PD-L1). The Applicant submitted nonclinical study reports and relevant literature evaluating the pharmacology and toxicology of atezolizumab to support BLA 761034. The proposed indication is for treatment of patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy (b) (4) or have disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy regimen.

1.2 Brief Discussion of Nonclinical Findings

PD-L1 is a transmembrane protein that negatively regulates immune responses through its interactions with PD-1 and B7-1 receptors (Francisco et al. 2010, Keir et al. 2008). PD-L1 is constitutively expressed on B cells, dendritic cells, macrophages, bone marrow-derived mast cells, and T cells. PD-L1 is also expressed on a wide range of non-hematopoietic cells and can be upregulated on many cell types upon stimulation. Binding of PD-L1 to its receptors attenuates lymphocyte activation to maintain immune tolerance and prevent immune-mediated tissue damage. In the tumor microenvironment, tumor cells and tumor infiltrating immune cells can express PD-L1 and inhibit T-cell proliferation and function. Blocking PD-L1 releases inhibition of the immune response, resulting in enhanced immune surveillance and responses, including immune-mediated anti-tumor activity.

In vitro pharmacology studies demonstrated that atezolizumab binds to human, murine, and Cynomolgus monkey PD-L1 and inhibits the interaction of PD-L1 with PD-1 and B7-1 receptors. Atezolizumab contains (b) (4) mutation in the Fc domain to prevent glycosylation at that site, thereby limiting binding to human Fcγ receptors and preventing antibody-dependent cell-mediated cytotoxicity (ADCC). Atezolizumab retains binding to neonatal Fc receptors, despite the mutation in the Fc domain. In vitro, soluble or plate-bound atezolizumab did not stimulate cytokine release from unstimulated human peripheral blood mononuclear cells (PBMCs). For in vivo pharmacology studies, the Applicant generated murine/human chimeric antibodies of atezolizumab to minimize immunogenicity in mice and evaluated PD-L1 blockade in syngeneic mouse models of melanoma and colorectal cancer. Anti-PD-L1 antibody reduced tumor growth by ≥ 80%. Based on the submitted pharmacology data, the Established Pharmacologic Class (EPC) of “programmed death-ligand 1 (PD-L1) blocking antibody” was determined to be both clinically meaningful and scientifically valid for atezolizumab.

The Applicant also evaluated murine/human chimeric antibodies of atezolizumab and an independently derived anti-mouse PD-L1 antibody in a mouse model of chronic lymphocytic choriomeningitis virus (LCMV) CL-13. Administration of anti-PD-L1 before and during the peak of viral infection and acute T-cell response resulted in ~40 to 100% mortality. Anti-PD-L1 was not lethal when administered at later time points or in three

other mouse models of acute viral infection. As reported in the literature, PD-1$^{-/-}$ mice succumb to *M. tuberculosis* infection with marked lung inflammation and bacterial loads and an increase in circulating pro-inflammatory cytokines (Lazar-Molnar et al. 2010). Based on its mechanism of action, atezolizumab may increase the inflammatory response and enhance the severity of some infections.

General toxicology studies evaluated atezolizumab in mice for two weeks and in Cynomolgus monkeys for up to 26 weeks. Atezolizumab was immunogenic in mice, resulting in significantly reduced exposures by Week 3. The major toxicological finding was minimal sciatic neuropathy (vacuolation and lymphocytic infiltration), observed at the end of dosing and recovery period. In Cynomolgus monkeys, atezolizumab caused irregular menstruation and multi-organ arteritis/periarteritis. The immune-mediated findings were consistent with the role of PD-L1 in regulating and maintaining peripheral tolerance. In humans, administration of atezolizumab at the recommended dose of 1200 mg resulted in immune-related adverse events. In the 26-week monkey study, females administered 50 mg/kg IV atezolizumab experienced irregular menstruation during the dosing period, including an increase in mean menstrual cycle length compared to controls and a corresponding lack of newly formed corpora lutea. These effects were reversible.

The Applicant submitted a non-product specific literature-based assessment to characterize the potential risk of reproductive and developmental toxicity. PD-L1 is expressed in the human placenta at the fetal/maternal interface throughout pregnancy (Holets et al. 2006, Petroff et al. 2003). In mouse models of allogenic pregnancy, loss of PD-L1 activity increased the rate of fetal resorption and reduced litter size (D'Addio et al. 2011, Guleria et al. 2005). Anti-PD-L1 antibody reduced fetal antigen-specific regulatory T cells (Tregs) and increased accumulation of fetal antigen-specific effector T cells in maternal lymphoid organs (D'Addio et al. 2011, Taglauer et al. 2009). Collectively, the scientific literature demonstrates that PD-L1 blockade leads to a loss of fetal tolerance and an increased risk of immune-mediated abortion.

It is unclear whether atezolizumab crosses the placental barrier at levels that would cause adverse effects on developing offspring. The allogenic pregnancy models did not evaluate offspring for teratogenicity or adverse developmental effects. Data from knockout mice demonstrate that syngeneic PD-1$^{-/-}$ and PD-L1$^{-/-}$ fetuses develop normally with no reported malformations (Keir et al. 2008, Okazaki and Honjo 2006, Okazaki and Honjo 2007). Depending on the genetic background, PD-1$^{-/-}$ and PD-L1$^{-/-}$ mice develop late onset autoimmune phenotypes. Inhibition of the PD-L1/PD-1 pathway can exacerbate disease in mouse models of autoimmunity. Additionally, single nucleotide polymorphisms (SNPs) in the gene encoding human PD-1 are associated with several autoimmune diseases. It is unknown whether exposure to atezolizumab during pregnancy may increase the risk of offspring developing immune-mediated disorders or experiencing adverse effects on the normal immune response; however, this potential risk cannot be dismissed based on its mechanism of action.

The submitted nonclinical pharmacology and toxicology studies support approval of Tecentriq for the proposed indication. The Applicant provided data demonstrating that atezolizumab binds to PD-L1 and blocks its interaction with PD-1 and B7-1 receptors. Consequently, atezolizumab relieves inhibition of immune responses, including anti-tumor activity and peripheral tolerance. Atezolizumab had no effects on cardiovascular, respiratory, or neurological systems in monkeys. Toxicological effects of atezolizumab administration in animals were limited to irregular menstruation in female monkeys and immune-mediated effects, including tissue damage and multi-organ inflammation. Other effects of PD-L1 blockade observed in animals, which may be relevant to patients receiving atezolizumab, consisted of enhanced inflammatory response and severity of infections and a risk of embryo-fetal toxicity, primarily an increased risk of immune-mediated abortion.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval for the proposed indication.

1.3.2 Additional Non Clinical Recommendations

Treatment with atezolizumab may result in enhanced immune-mediated toxicity following vaccination and recall responses. To investigate this potential, the following post-marketing commitment (PMC) is recommended by the Pharmacology/Toxicology team.

“The PMC is to conduct an animal study that will measure the effect of PD-L1 inhibition on the magnitude of the primary (1st vaccination) and recall (2nd vaccination) antibody responses to antigen challenge (e.g. KLH). This study will evaluate the effect of PD-L1 inhibition on the primary immune response once steady state plasma levels have been achieved and will reassess the magnitude of the recall response after a suitable period in the presence or absence of continued dosing. The study should include, if possible, an evaluation of cytokine production by T cells at appropriate timepoints.”

(b) (4)

1.3.3 Labeling

This Pharmacology/Toxicology review contains the content for labeling Tecentriq (atezolizumab). Based on pharmacology data submitted in the BLA, the EPC of "programmed death-ligand 1 (PD-L1) blocking antibody" was determined to be both clinically meaningful and valid for atezolizumab.

In section 8.2 Lactation, the Division recommended that patients should not breastfeed during treatment and for 5 months after the last dose due to the potential for serious adverse reactions in breastfed infants from atezolizumab. This was included to be consistent with labels for US FDA-approved products inhibiting PD-1. In section 8.3 Females and Males of Reproductive Potential, the Division recommended that females of reproductive potential should use effective contraception during treatment and for 5 months after the last dose due to the potential risk to a fetus. The elimination half-life of atezolizumab in patients receiving the recommended dose is 27 days. The duration of 5 months was determined based on approximately 5 half-lives of atezolizumab, and rounded to the nearest whole month.

The following pharmacokinetic data was used to calculate the animal to human exposure ratios for atezolizumab. Based on population analysis of a dose escalation clinical trial in patients with advanced solid tumors, the steady-state AUC is 6409 day*µg/mL at the recommended human dose of 1200 mg every 3 weeks. The elimination half-life in patients is 27 days. In repeat-dose toxicology studies, Cynomolgus monkeys received doses up to 50 mg/kg weekly, or 150 mg/kg over a 3-week period. Following single and repeat dosing in monkeys, the AUC was calculated from data collected over a 3-day period. Due to the long half-life of 12 to 24 days in monkeys, the measured AUC underestimated the total exposure of atezolizumab in monkeys over the period of three weeks between repeat-dosing. Using data from a pharmacokinetic study in monkeys, the Applicant estimated the AUC in monkeys to be 40,500 day*µg/mL based on a clearance of 3.7 mL/day/kg and a dose of 150 mg/kg over a 3-week interval (Study No. 08-0598).

2 Drug Information

2.1 Drug

CAS Registry Number	1380723-44-3
Generic Name	Atezolizumab
Code Names	MPDL3280A, RO5541267, PRO#303280
Chemical Name	Humanized anti-PD-L1 IgG1 monoclonal antibody
Molecular Formula	$C_{6434}H_{9878}O_{1996}N_{1702}S_{42}$
Molecular Weight	144.356 kDa
Pharmacological Class	programmed death-ligand 1 (PD-L1) blocking antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

The Applicant currently holds the relevant INDs listed below.

IND	Indication
(b) (4) (withdrawn)	(b) (4)
111271	Oncology
(b) (4)	(b) (4)
120827	Urothelial bladder carcinoma

2.3 Drug Formulation

Atezolizumab is provided as a sterile solution for intravenous infusion and formulated at a concentration of 60 mg/mL in single-use vials.

Table 1: Drug product composition of atezolizumab

Ingredient	Nominal Amount per Vial	Target Concentration	Function	Standard Specifications
Atezolizumab	1200 mg	60 mg/mL	Active ingredient	Section S.4.1 *Specification*
L-Histidine	62.0 mg	20 mM	(b) (4)	USP/Ph. Eur./JP
Glacial Acetic Acid	16.5 mg[a]	(b) (4)		USP/Ph. Eur./JP
Sucrose	821.6 mg	120 mM		NF/Ph. Eur./JP
Polysorbate 20	8.0 mg	0.04% (w/v)		NF/Ph. Eur./JPE
Water for Injection	QS to 20.0 mL	NA		USP/Ph. Eur./JP
(b) (4)				

Abbreviations: JPE = Japanese Pharmaceutical Excipients; NA = not applicable; NF = National Formulary; QS = quantity sufficient.

(b) (4)

[Excerpted from Applicant's submission]

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is patients with locally advanced or metastatic urothelial carcinoma who have disease progression during or following platinum-containing chemotherapy for metastatic disease or have disease progression within 12 months of treatment with a platinum-containing neoadjuvant or adjuvant chemotherapy

regimen. The recommended dose is 1200 mg administered as an intravenous infusion every 3 weeks.

2.7 Regulatory Background

Atezolizumab is a first-in-class, new molecular entity and has not been previously approved or marketed in the United States or any other country. Breakthrough Therapy designation was granted on May 22, 2014 for the treatment of patients with locally advanced or metastatic, PD-L1 positive, urothelial bladder cancer following progression on or intolerance to a platinum-containing chemotherapy regimen. Pre-BLA meetings between the Applicant and FDA were held on May 11, 2015 and September 24, 2015.

In the pre-BLA meeting on May 11, 2015, the Applicant indicated that studies were ongoing (b) (4) The Division acknowledged that these studies would not be available for review of this BLA submission. During the BLA review, the Applicant provided a status update (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

Toxicology

Study number	Study title	eCTD location
08-0806	A 15 day pilot toxicity study of anti-PDL1 (MPDL3280A) administered by intravenous injection once a week for a total of 3 doses to female C57BL/6 and CD-1 mice with 4 weeks recovery	4.2.3.2.
08-1148	An eight-week toxicity, toxicokinetic, and safety pharmacology study of MPDL3480A administered by intravenous injection or subcutaneous injection to Cynomolgus monkeys, with a 12-week recovery period	4.2.3.2.
13-3278	A 26-week toxicity and toxicokinetic study with MPDL3280A, administered by intravenous injection to Cynomolgus monkey with a 13-week recovery phase	4.2.3.2.
6281-961	Hemolytic potential testing with MPDL3280A in Cynomolgus monkey and human blood	4.2.3.7.
08-1174	Tissue cross-reactivity of MPDL3280A with human and Cynomolgus monkey tissues *ex vivo*	4.2.3.7.
08-1827	In vitro cytokine release study with anti-PD-L1 antibody in human PBMCs	4.2.3.7.

Pharmacology

Study number	Study title	eCTD location
09-0426	In vitro binding and biological activity of MPDL3280A (rhuMAb PD-L1)	4.2.1.1.
15-0984	In vitro binding affinity of MPDL3280A	4.2.1.1.
08-1033	Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic MC38.OVA colorectal cancer model in C57BL/6 mice	4.2.1.1.
08-1734	Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic CT26 colorectal cancer model in Balb/c mice	4.2.1.1.
09-2165	Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic Cloudman S91 melanoma model in DBA/2 mice	4.2.1.1
10-1883	Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic MC38 colorectal model in C57BL/6 mice	4.2.1.1.
08-0559A, 08-559B, 08-1160	Therapeutic efficacy and dose titration of anti-PD-L1 mAb in the lymphocytic choriomeningitis virus infection model	4.2.1.1.
08-1309	Evaluation of the combined effects of adenovirus expressed interferon-alpha (IFN-a) and anti-PD-L1 mAb in mice infected with lymphocytic choriomeningitis virus (LCMV)	4.2.1.1.
09-2500, 09-2500B, 09-2501, 09-2501A	Evaluation of the host response to Armstrong and CL-13 lymphocytic choriomeningitis virus (LCMV) infection in mice following administration of a single dose of anti-PD-L1 antibody at different times during infection	4.2.1.1.
10-1394	Studies to address mechanism of anti-PD-L1 enhanced pathology in lymphocytic choriomeningitis virus (LCMV) infection: comparison between clone-13 and Armstrong strains	4.2.1.1.

Pharmacokinetics

Study number	Study title	eCTD location
08-0598	A single dose pharmacokinetic study of MPDL3280A administered by intravenous injection to Cynomolgus monkeys	4.2.2.7.

3.2 Studies Not Reviewed

Pharmacology

15-0984	In vitro binding affinity of MPDL3280A	4.2.1.1.

Pharmacokinetics

08-1946	Evaluation of pharmacokinetics and pharmacodynamics following single-dose intravenous administration of an anti-PD-L1 reverse chimera antibody	4.2.2.7.
4.MPDL.1.AVR	4.MPDL.1 (MPDL3280A) Cynomolgus monkey serum antigen ELISA	4.2.2.1.
4.MPDL.2.AVR	4.MPDL.2 (MPDL3280A) Cynomolgus monkey serum ATA DIG ELISA	4.2.2.1.
ICD 590	Validation of an ELISA method for the quantitation of MPDL3280A (Anti-PD-L1) in Cynomolgus monkey serum	4.2.2.1.
ICDIM 207	Validation of an immunoassay method for the detection of antibodies to MPDL3280A (Anti-PD-L1) in Cynomolgus monkey serum	4.2.2.1.

3.3 Previous Reviews Referenced

IND (b) (4) nonclinical and microbiology review

4 Pharmacology

4.1 Primary Pharmacology

Study title: In vitro binding and biological activity of MPDL3280A (rhuMAb PD-L1)

Study No.:	09-0426
Study report date:	June 30, 2009, March 24, 2011 (amendment)
Study report location:	eCTD 4.2.1.1.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

The aim of this study was to assess the binding and inhibitory activity of MPDL3280A and chimeric antibodies, PRO304397 and PRO314483. Both chimeric products are mouse IgG2a antibodies containing the binding regions of MPDL3280A. (b) (4) Binding and inhibitory activity were evaluated in the following assays:

1. Equilibrium binding assay – Binding of [^{125}I]-labeled MPDL3280A or PRO304397 and increasing concentrations of cold antibody to HEK293 cells exogenously expressing human and murine PD-L1 (Table 2)
2. Flow cytometry analysis – Binding of anti-PD-L1 antibodies to activated human or Cynomolgus monkey T cells (Table 2)
3. Flow cytometry analysis – Binding of MPDL3280A and chimeric antibodies to HEK293 cells stably expressing human or murine PD-L1 (Table 2)

4. Competitive ELISA assay – Blocking of recombinant PD-L1 binding to recombinant PD-1 or B7-1 by MPDL3280A or chimeric antibodies (Table 3)
5. ELISA binding assay – Binding of MPDL3280A to human Fcγ receptors

Results and conclusions

- In vitro, MPDL3280A bound to human, Cynomolgus monkey, and murine PD-L1 with subnanomolar affinity, indicating that all three species are pharmacologically relevant (Table 2).
- Chimeric antibodies bound to PD-L1 with subnanomolar affinity, which was comparable to MPDL3280A (Table 2).
- MPDL3280A blocked PD-L1 binding to its receptors, PD-1 and B7-1, with picomolar inhibitory activity (Table 3).
- MPDL3280A showed minimal binding to Fcγ receptors. EC_{50} values were > 100 µg/mL.

Table 2: Binding of MPDL3280A and chimeric antibodies to human, Cynomolgus monkey, and murine PD-L1

	EQUILIBRIUM BINDING ASSAY (K_D), N = 2		**ELISA BINDING ASSAY (EC_{50})**		
	human PD-L1	murine PD-L1	activated human T cells	activated monkey T cells	HEK293 cells expressing murine PD-L1
MPDL3280A	0.433, 0.4 nM	0.134, 0.12 nM	0.395 nM	0.704 nM	0.519 nM
PRO304397	0.374, 0.336 nM	0.147, 0.188 nM	-	-	0.412 nM
PRO314483	-	-	-	-	0.433 nM

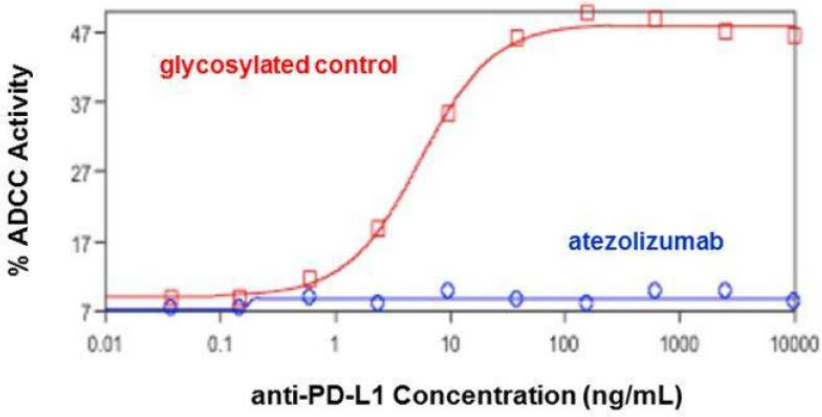
Table 3: Inhibitory activity of MPDL3280A and murine chimeric antibodies

	BLOCKADE OF RECEPTOR/LIGAND BINDING (IC_{50})			
	Human		**Mouse**	
	B7-1/PD-L1	**PD-1/PD-L1**	**B7-1/PD-L1**	**PD-1/PD-L1**
MPDL3280A	48.4 pM	82.8 pM	75.6 pM	104 pM
PRO304397	47.5 pM	77.5 pM	79.4 pM	113 pM
PRO314483	41.0 pM	78.9 pM	96.6 pM	125 pM

Antibody-dependent cell-mediated cytotoxicity (eCTD Module 3; 3.2.S.3)

As part of the quality assessment, the Applicant evaluated the ADCC activity of atezolizumab using an in vitro, NK cell-based assay. Atezolizumab showed no ADCC activity at concentrations up to 10 µg/mL whereas a glycosylated control antibody (N298) demonstrated concentration-dependent cytotoxicity.

Figure 1: ADCC activity of atezolizumab



[Excerpted from Applicant's submission]

Study title: Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic MC38.OVA colorectal model in C57BL/6 mice

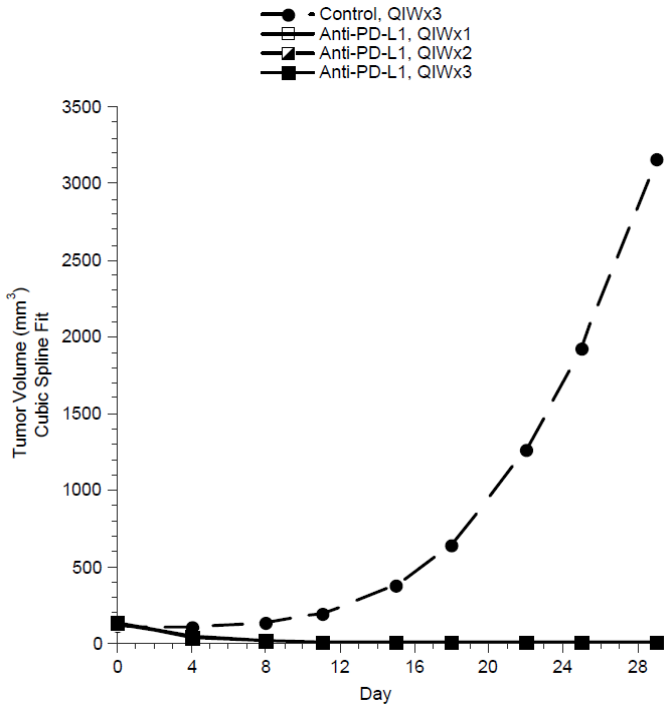
Study No.:	08-1033 E
Study report date:	February 11, 2011
Study report location:	eCTD 4.2.1.1.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

The aim of this study was to assess the anti-tumor activity of murine chimeric antibody, PRO314483, in a syngeneic immunocompetent mouse model of MC38.OVA colorectal cancer. As shown in Table 2 and Table 3, binding of PRO314483 to PD-L1 was comparable to MPDL3280A. Cells were mixed 50:50 with Matrigel and implanted subcutaneously (SC) into the right flank of female C57BL/6 mice (10/group). Once tumors reached a mean volume of ~150 mm^3, mice were treated with 10 mg/kg PRO314483 administered as an intraperitoneal (IP) injection three times weekly for one, two, or three cycles. Control anti-gp120 antibody was administered as an IP injection three times weekly for three cycles. Study endpoints were changes in tumor volume and body weight. If tumor volume decreased below the limit of detection (8 mm^3), it was considered a complete response. If tumor volume decreases by ≥ 50% of its initial volume, it was considered a partial response.

Results and conclusions

- All mice receiving 10 mg/kg anti-PD-L1 had complete responses, which were sustained through the end of study on Day 55.
- A dose of 10 mg/kg anti-PD-L1 antibody had no effect on body weights.

Figure 2: Anti-tumor activity of anti-PD-L1 antibody in a syngeneic mouse model of MC38.OVA colorectal cancer



Note: QIW=three times per week. Fitted tumor volumes after QIW intraperitoneal administration of either control (anti-gp120 mAb, 10 mg/kg) or anti-PD-L1 mAb (10 mg/kg).

[Excerpted from Applicant's submission]

Table 4: Anti-tumor activity of MPDL3280A in a syngeneic mouse model of MC38.OVA colorectal cancer

Percent Tumor Growth Inhibition versus Control at Day 29

Group	Treatment	Dose (mg/kg)	Schedule	N	Tumor Volume [a] (mm^3)	%TGI	PR	CR
1	Control [b]	10	QIW×3	10	>3000	0	1	0
2	Anti-PD-L1	10	QIW×1	10	0	118	0	10
3	Anti-PD-L1	10	QIW×2	10	0	116	0	10
4	Anti-PD-L1	10	QIW×3	10	0	119	0	10

%TGI=percent tumor growth inhibition at Day 29, the last day the control group was on study; CR=complete response; PR=partial response; QIW=three times per week.

[a] Tumor volumes are determined from the LME fitted values on Day 29, the last day the control mice were on study.

[b] Anti-gp120 mAb.

Time To Progression versus Control

Group	Treatment	Dose (mg/kg)	Schedule	N	Last Day on Study	TTP5×
1	Control [a]	10	QIW×3	10	29	18
2	Anti-PD-L1	10	QIW×1	10	55	NA
3	Anti-PD-L1	10	QIW×2	10	55	NA
4	Anti-PD-L1	10	QIW×3	10	55	NA

NA=never achieved; QIW=three times per week; TTP5×=time (in days) for animal to die or have tumor progress to 5-fold above starting volume.

[a] Anti-gp120 mAb.

[Excerpted from Applicant's submission]

Study title: Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic CT26 colorectal cancer model in Balb/c mice

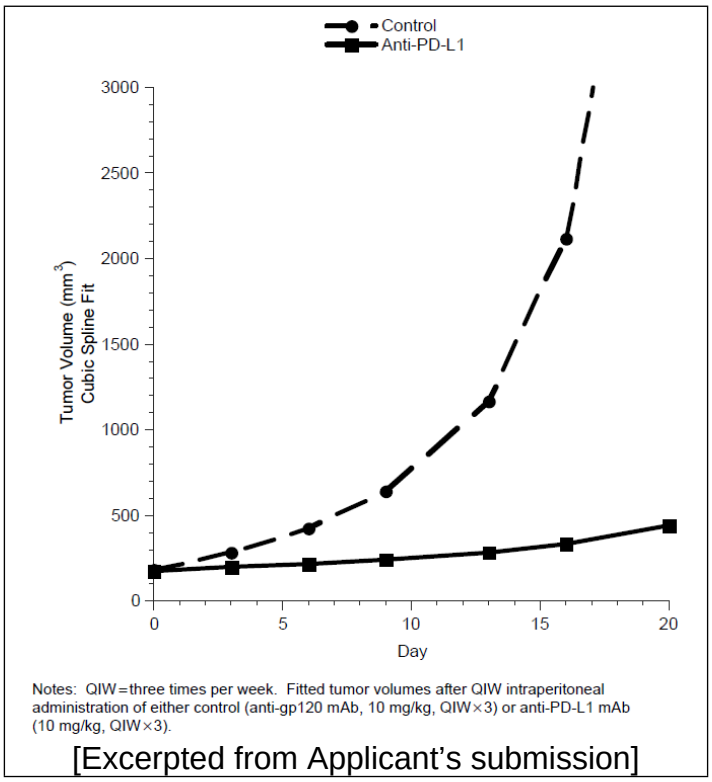
Study No.:	08-1734 D
Study report date:	February 11, 2011
Study report location:	eCTD 4.2.1.1.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

The objective of this study was to evaluate the anti-tumor activity of PRO314483 in a syngeneic CT26 colorectal cancer model. CT26 colorectal cells were injected SC into the right flank of female Balb/c mice (10/group). Once tumors reached a volume of ~200 mm^3, mice were injected IP with 10 mg/kg PRO314483 or control anti-gp120 antibody three times weekly for three cycles.

Results and conclusions

- Anti-PD-L1 antibody reduced tumor growth by > 90% and prolonged the time to progression by approximately two weeks compared to controls. There was a partial response and a complete response in the study.
- Body weights in the anti-PD-L1 treatment groups were comparable to controls.

Figure 3: Anti-tumor activity of anti-PD-L1 antibody in a syngeneic mouse model of CT26 colorectal cancer



Notes: QIW=three times per week. Fitted tumor volumes after QIW intraperitoneal administration of either control (anti-gp120 mAb, 10 mg/kg, QIW×3) or anti-PD-L1 mAb (10 mg/kg, QIW×3).

[Excerpted from Applicant's submission]

Table 5: Anti-tumor activity of anti-PD-L1 antibody in a syngeneic mouse model of CT26 colorectal cancer

Percent Tumor Growth Inhibition versus Control at Day 20

Group	Treatment	Dose (mg/kg)	Schedule	N	Tumor Volume [a] (mm^3)	%TGI	PR	CR
1	Control [b]	10	QIW×3	10	>3000	0	0	0
2	Anti-PD-L1	10	QIW×3	10	443	92	1	1

%TGI=percent tumor growth inhibition at Day 20, the last day the control group was on study; CR=complete response; PR=partial response; QIW=three times per week.

[a] Tumor volumes are determined from the LME fitted values on Day 20, the last day the control group mice were on study.

[b] Anti-gp120 mAb.

Time to Progression versus Control

Group	Treatment	Dose (mg/kg)	Schedule	N	Last Day on Study	TTP5×
1	Control [a]	10	QIW×3	10	20	11.5
2	Anti-PD-L1	10	QIW×3	10	56	27.5

QIW=three times per week; TTP5×=time (in days) for animal to die and/or have tumor progress to 5-fold above starting volume.

[a] Anti-gp120 mAb.

[Excerpted from Applicant's submission]

Study title: Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic Cloudman S91 melanoma model in DBA/2 mice

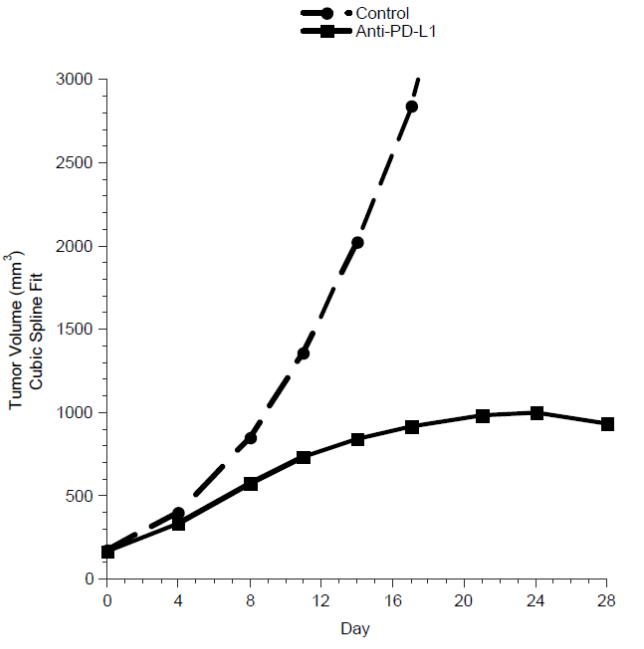
Study No.:	09-2165 I
Study report date:	February 11, 2011
Study report location:	eCTD 4.2.1.1.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

The aim of this study was to evaluate the anti-tumor activity of PRO314483 in a syngeneic mouse model of Cloudman S91 melanoma. Melanoma cells were injected subcutaneously in to the right flank of female DBA/2 mice (10/group). Tumors reached a volume of 200 mm^3 before treatment commenced. Mice were injected IP with 10 mg/kg anti-PD-L1 or control anti-gp120 antibody three times weekly for three cycles.

Results and conclusions

- Anti-PD-L1 reduced tumor growth by 78% and prolonged time to progression by 6 days compared to controls. There were two partial responses in the study.
- Body weights in the anti-PD-L1 treatment groups were comparable to controls.

Figure 4: Anti-tumor activity of anti-PD-L1 antibody in a syngeneic mouse model of melanoma



Note: QIW=three times per week. Fitted tumor volumes after QIW intraperitoneal administration of either control (anti-gp120 mAb, 10 mg/kg, QIW×3) or anti-PD-L1 mAb (10 mg/kg, QIW×3).

[Excerpted from Applicant's submission]

Table 6: Anti-tumor activity of anti-PD-L1 antibody in a syngeneic mouse model of melanoma

Percent Tumor Growth Inhibition versus Control at Day 28

Group	Treatment	Dose (mg/kg)	Schedule	N	Tumor Volume [a] (mm^3)	%TGI	PR	CR
1	Control [b]	10	QIW×3	10	>3000	0	0	0
2	Anti-PD-L1	10	QIW×3	10	1082	78	2	0

CR=complete response; PR=partial response; QIW=three times per week; %TGI=percent tumor growth inhibition at Day 28, the last day the control group was on study.

[a] Tumor volumes are determined from the LME fitted values on Day 28, the last day the control group mice were on study.

[b] Anti-gp120 mAb.

Time to Progression versus Control

Group	Treatment	Dose (mg/kg)	Schedule	N	Last Day on Study	TTP5×
1	Control [a]	10	QIW×3	10	28	8
2	Anti-PD-L1	10	QIW×3	10	53	14

QIW=three times per week; TTP5×=time (in days) for animal to die and/or have tumor progress to 5-fold above starting volume.

[a] Anti-gp120 mAb.

[Excerpted from Applicant's submission]

Study title: Evaluation of the anti-tumor efficacy of anti-PD-L1 monoclonal antibody in the syngeneic MC38 colorectal model in C57BL/6 mice

Study No.:	10-1883
Study report date:	February 11, 2011
Study report location:	eCTD 4.2.1.1.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

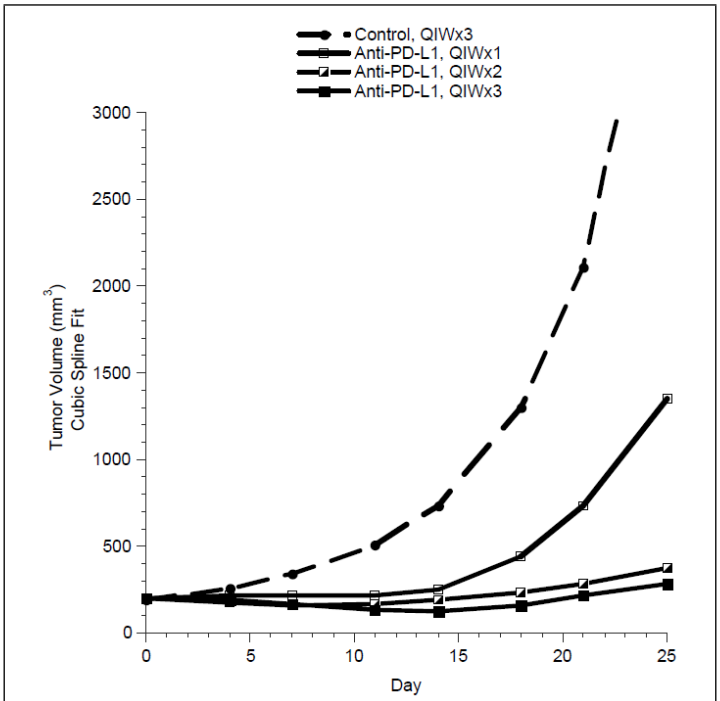
The aim of this study was to examine the anti-tumor activity of PRO314483 in a syngeneic mouse model of MC38 colorectal cancer. Colorectal cells were mixed 50:50 with Matrigel and injected SC into the right flank of female C57BL/6 mice (10/group). Tumors reached a mean volume of ~200 mm^3 before treatment commenced. Mice were administered doses of 10 mg/kg PRO314483 by IP injection three times weekly for one, two, or three cycles. Control anti-gp120 antibody was administered according to the same dosing schedule for three cycles.

Results and conclusions

- Anti-PD-L1 reduced tumor growth by 76, 98, and 103% following treatment of one, two, and three cycles, respectively. Time to progression was also prolonged with each increase in treatment length.

- Three complete and partial responses were reported after two and three treatment cycles.
- There were no treatment-related effects on body weight.

Figure 5: Anti-tumor activity of anti-PD-L1 in a syngeneic mouse model of MC38 colorectal cancer



Note: QIW=three times per week. Fitted tumor volumes after QIW intraperitoneal administration of either control (anti-gp120 mAb, 10 mg/kg) or anti-PD-L1 mAb (10 mg/kg).

[Excerpted from Applicant's submission]

Table 7: Anti-tumor activity of anti-PD-L1 in a syngeneic mouse model of MC38 colorectal cancer

Percent Tumor Growth Inhibition versus Control at Day 25

Group	Treatment	Dose (mg/kg)	Schedule	N	Tumor Volume [a] (mm^3)	%TGI	PR	CR
1	Control [b]	10	QIW×3	10	>3000	0	0	0
2	Anti-PD-L1	10	QIW×1	10	1349	76	1	0
3	Anti-PD-L1	10	QIW×2	10	372	98	3	3
4	Anti-PD-L1	10	QIW×3	10	282	103	3	3

CR=complete response; PR=partial response; QIW=three times per week; %TGI=percent tumor growth inhibition at Day 25, the last day the control group was on study.

[a] Tumor volumes are determined from the LME fitted values on Day 25, the last day the control group mice were on study.

[b] Anti-gp120 mAb.

Time to Progression versus Control

Group	Treatment	Dose (mg/kg)	Schedule	N	Last Day on Study	TTP5×
1	Control [a]	10	QIW×3	10	25	16.5
2	Anti-PD-L1	10	QIW×1	10	35	23.5
3	Anti-PD-L1	10	QIW×2	10	63	37
4	Anti-PD-L1	10	QIW×3	10	67	50

QIW=three times per week;TTP5×=time (in days) for animal to die and/or have tumor progress to 5-fold above starting volume.

[a] Anti-gp120 mAb.

[Excerpted from the Applicant's submission]

Study title: In vitro cytokine release study with anti-PD-L1 antibody in human PBMCs

Study No.:	081827
Study report date:	June 30, 2009, March 23, 2011 (amendment)
Study report location:	eCTD 4.2.3.7.
Conducting laboratory:	Genentech, Inc. South San Francisco, CA
GLP:	No

The aim of this study was to test whether MPDL3280A induces cytokine release from unstimulated human PBMCs. Briefly, human PBMCs isolated from three donors were incubated with soluble or immobilized MPDL3280A on tissue culture plates at concentrations ranging from 0.25 to 250 µg/mL. Anti-CD3 (1 µg/mL) and LPS (50 ng/mL) were positive controls. Anti-hFGFR3 (250 µg/mL) and medium only were negative controls. After incubating cells for 24 or 48 h at 37ºC, supernatants were analyzed for cytokine secretion using a Luminex multiplex assay. The cytokine profiling kit tested for GM-CSF, TNFα, IFNγ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, and IL-12.

Results and conclusions

- Soluble and immobilized MPDL3280A did not induced cytokine release from unstimulated human PBMCs at concentrations up to 250 µg/mL.
- Anti-CD3 induced secretion of GM-CSF, IFNγ, IL-10, IL-1β, IL-2, and TNFα.
- LPS stimulated secretion of IL-10, IL-1β, IL-6, and TNFα.
- High concentrations of IL-8 were detected in all wells containing PBMCs, including negative controls.

Chronic LCMV infection studies conducted with anti-PD-L1 antibodies
The Applicant conducted a series of in vivo pharmacology studies to evaluate PD-L1 blockade in a mouse model of chronic LCMV CL-13 infection. The data were previously reviewed under IND (b) (4) Key results and conclusions are summarized below.

Study number	Study Title
08-559A, 08-0559B, 08-1160	Therapeutic efficacy and dose titration of anti-PD-L1 mAb in the lymphocytic choriomeningitis virus infection model
09-2500, 09-2500 B, 09-2501, 09-2501 A	Evaluation of the host response to Armstrong and CL-13 lymphocytic choriomeningitis virus (LCMV) infection in mice following administration of a single dose of anti-PD-L1 antibody at different times during the infection
10-1394	Studies to address mechanism of anti-PD-L1 enhanced pathology in lymphocytic choriomeningitis virus (LCMV) infection: comparisons between Clone-13 and Armstrong strains
08-1309 A	Evaluation of the combined effects of adenovirus expressed interferon-alpha (IFN-a) and anti-PD-L1 mAb in mice infected with lymphocytic choriomeningitis virus (LCMV)

Results and conclusions
The Applicant tested two human/murine chimeric antibodies of atezolizumab (PR0314483 and PRO304497; Table 2 and Table 3) and an independently derived anti-PD-L1 antibody in mice infected with LCMV CL-13. The LCMV CL-13 strain produces high, sustained viral titers in multiple tissues and has an enhanced replication capacity. The infection peaks on Day 7 and persists for several weeks due to an impaired T-cell response. A single dose of 10 mg/kg anti-PD-L1 on Day 2, before the peak of infection, resulted in a mortality rate of ~40% by Day 7. Treatment at the peak of infection on Day 7 resulted in 60 to 100% mortality by Day 10. In contrast, anti-PD-L1 treatment starting on Day 14 or 17 was not lethal, and there was no reported toxicity. PD-L1 blockade markedly reduced viral titers and increased splenic $CD8^+$ T-cell function compared to controls. Collectively, these studies demonstrated that PD-L1 blockade causes immunopathology and death when administered during the acute phase of LCMV CL-13 infection and T-cell response.

The Applicant conducted additional mechanistic studies to better understand anti-PD-L1-mediated immunopathology and whether the effects were specific to LCMV CL-13 infection. Anti-PD-L1 treatment increased levels of T cell-derived cytokines, TNF-α and IFNγ. Depletion of $CD8^+$ T cells prevented anti-PD-L1-induced immunopathology and death. PD-L1 blockade was not lethal in three other mouse models of acute viral infection.

4.2 Secondary Pharmacology

No data or information submitted

4.3 Safety Pharmacology

Safety pharmacology studies were incorporated into repeat-dose toxicology studies conducted in Cynomolgus monkeys and reviewed in the General Toxicology section. The studies included evaluation of ECG, blood pressure, respiratory rate, and

neurological behavior (general sensorimotor aspects, cerebral reflexes, and spinal reflexes).

5 Pharmacokinetics/ADME/Toxicokinetics

Study title: A single dose pharmacokinetic study of MPDL3280A administered by intravenous injection to Cynomolgus monkeys

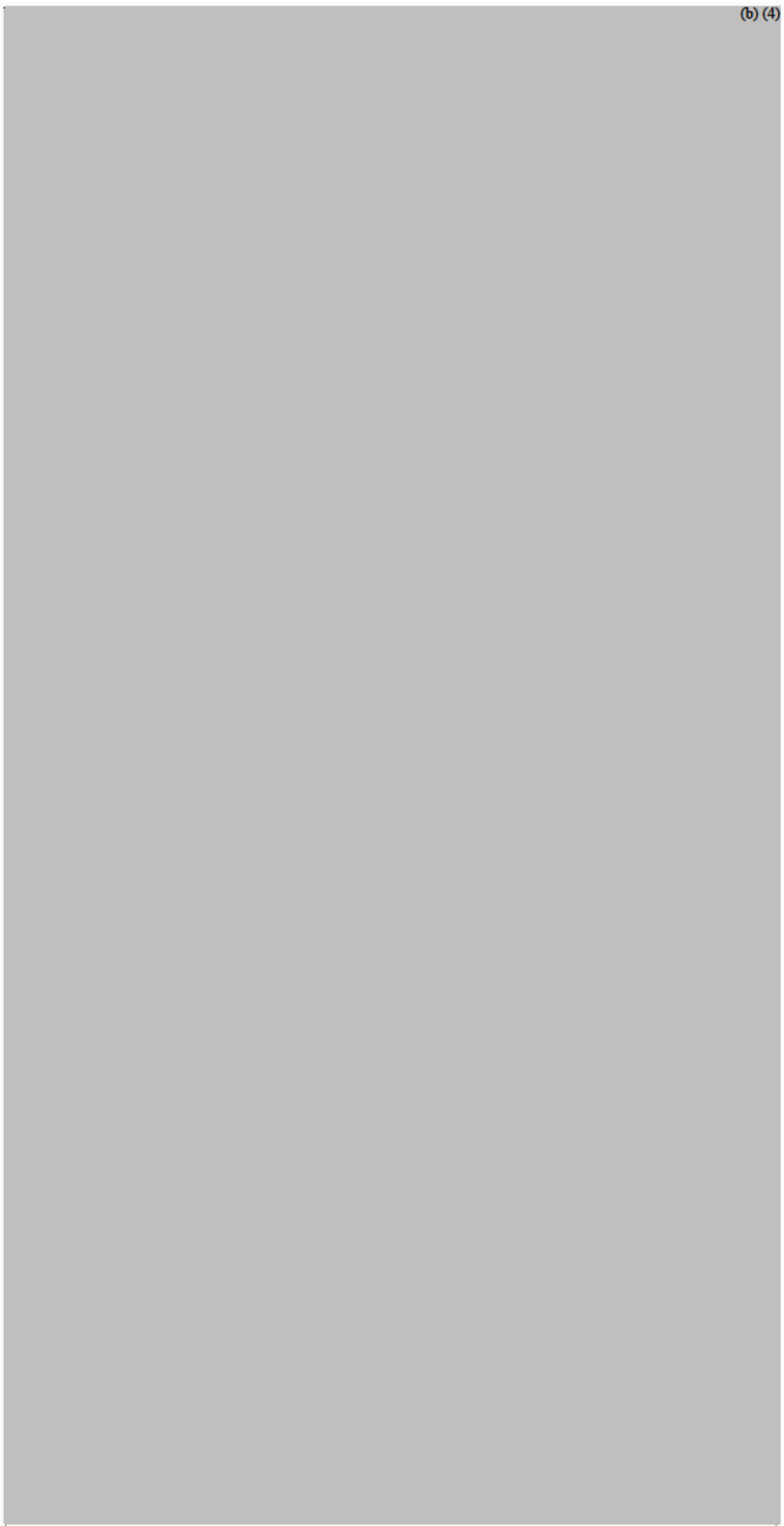
Study No.:	08-0598
Study initiation date:	May 9, 2008
Study report location:	eCTD 4.2.2.7
Conducting laboratory:	(b) (4)
GLP:	No

The aim of this study was to assess pharmacokinetic parameters following a single intravenous injection of MPDL3280A in Cynomolgus monkeys. The study also evaluated changes in clinical signs, food consumption, body weight, and the presence of anti-MPDL3280A antibodies. Monkeys (2/Sex/Group) were administered a dose of 0.5, 5, or 20 mg/kg, and blood samples were collected on Day 1 (0.5 and 8 h post-dose), 2, 4, 6, 8, 15, 22, 29, 36, 43, 50, and 57.

Summary of results and conclusions

There were no test article-related clinical signs or effects on body weight and food consumption. All animals were positive for anti-therapeutic antibodies (ATAs) on Day 15 and remained positive through the end of study with the exception of one male (# 3002) in the 20 mg/kg dose group. Male 3002 tested negative after Day 15, which may reflect assay interference from circulating MPDL3280A rather than a lack of ATAs. Following a single dose, C_{max} and AUC increased with increasing dose and were slightly greater than dose proportional across the dose range tested. As shown in Figure 6, MPDL3280A was rapidly cleared after Day 8 in 9 out of 12 animals, consistent with the presence of ATAs during the study. Starting on Day 14, serum concentrations were below the limit of quantitation for all animals in the low dose group, 3 out of 4 animals in the mid dose group, and 2 out of 4 animals in the high dose group. Data from 3 animals with potentially unaffected pharmacokinetics were fitted to a two-compartment and a non-compartmental model and used to predict pharmacokinetic parameters for all dose levels (Figure 7). In the two-compartment model, clearance of MPDL3280A ranged from 4.63 to 8.92 mL/day/kg, and mean volume at steady state was 70.9 mL/kg. The mean terminal half-life was 8.56 days. In the non-compartmental analysis, mean clearance and volume at steady state were 3.77 mL/day/kg and 53.2 mL/kg, respectively. Mean terminal half-life was 7.4 days.

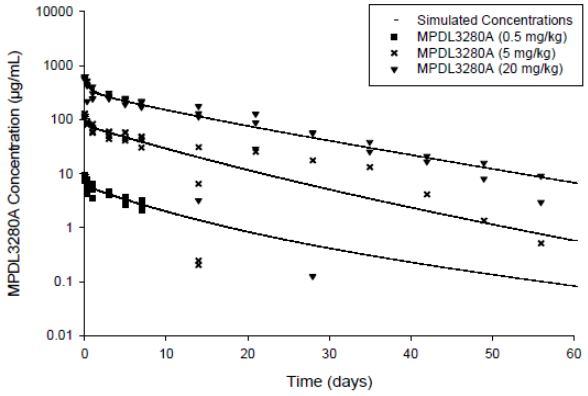
Figure 6: Individual serum concentration-time profiles following a single IV dose of MPDL3280A in Cynomolgus monkeys



[Excerpted from Applicant's submission]

Figure 7: Observed and predicted serum concentration-time profiles

Observed and Predicted Serum Concentration–Time Profiles following a Single IV Administration of MPDL3280A to Cynomolgus Monkeys



Notes: n=4 animals per group. The limit of quantitation (LOQ) in the MPDL3280A concentration ELISA was<0.07 µg/mL. LOQ values were interpreted as missing for summary statistics. Lines represent the predicted concentration, based upon simulations using parameters obtained from the 3 animals with complete serum concentration–time profiles (two-compartment analysis).

[Excerpted from Applicant's submission]

Dose Level (mg/kg)	$T_{1/2, beta}$ (day)	C_{max} (µg/mL)	AUC_{inf} (day·µg/mL)
All animals[a]			
0.5	8.3	8.6	62
5	7.3	123	830
20	10.1	610	4680
Animals with unaffected serum concentration-time curves (combined)[b]			
5/20	11.5	Not calculated	4080

[a] Non-compartmental analyses
[b] Two compartmental analyses, included animals 2001, 3002, and 3501

6 General Toxicology

6.1 Single-Dose Toxicity

No data or information submitted

6.2 Repeat-Dose Toxicity

Study title: A 15 day pilot toxicity study of anti-PDL1 (MPDL3280A) administered by intravenous injection once a week for a total of 3 doses to female C57BL/6 and CD-1 mice with 4 weeks of recovery

Study no.:	08-0806
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	Genentech Inc. 1 DNA Way South San Francisco, CA
Date of study initiation:	June 3, 2008
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	MPDL3280A (atezolizumab), lot # 59746-16, not specified

The Applicant conducted a non-GLP, 15-day toxicology study of MPDL3280A administered to female C57BL/6 and CD-1 mice. The data were previously reviewed under IND (b) (4). The result and conclusions are summarized below.

Methods

Doses:	0, 10, 50 mg/kg (Group 1, 2, 3; C57BL/6 mice) 0, 50 mg/kg (Group 4, 5; CD-1 mice)
Frequency of dosing:	Day 1, 8, 15
Route of administration:	Slow bolus IV injection via tail vein
Dose volume:	5 mL/kg
Formulation/Vehicle:	(b) (4) mM histidine acetate, (b) (4) mM sucrose, (b) (4) % polysorbate 20, pH (b) (4)
Species/Strain:	C57BL/6 and CD-1 mice
Number/Sex/Group:	4/Group (main study) 4/Group (recovery)
Age:	6 weeks old
Weight:	18 to 27 g at Day 1
Satellite groups:	9/Group (TK analysis) 15/Group (Immune subgroup)
Unique study design:	None
Deviation from study protocol:	Not specified

Observations and Schedule

Mortality	Not specified
Clinical signs	Day 1-4, 7-11, 14-17, 21, 23, 25, 28, 30, 31, 35, 37, 39, and 43
Body weights	Day 1-4, 7-11, 14-17, 21, 23, 25, 28, 30, 31, 35, 37, 39, and 43
Food consumption	Not evaluated
Hematology	Day 3, 8, 17, and 43
Clinical chemistry	Day 3, 8, 17, and 43
Urinalysis	Not evaluated
Immunophenotyping	Day 2, 8, 16, 22, and 43

Cytokine analysis	Day 2, 8, 16, 22, and 43
Immunogenicity	Day 18 and 43
Toxicokinetics	Day 1 (30 min post-dose), 4, 8 (pre-dose), 15 (30 min post-dose), 18, 22, and 43

Summary of results and conclusions:
Female C57BL/6 mice were administered IV doses of 0, 10, or 50 mg/kg MPDL3280A on Day 1, 8, and 15 followed by a 4-week recovery period. CD-1 mice received doses of 0 or 50 mg/kg MPDL3280A according to the same dosing schedule. All mice survived to scheduled necropsy. The major toxicological finding was minimal sciatic neuropathy (vacuolation and lymphocyte infiltration). This observation was reported in 2 out of 4 C57BL/6 mice receiving 10 and 50 mg/kg MPDL3280A. At end of recovery, sciatic neuropathy was present in one C57BL/6 mouse receiving 10 mg/kg MPDL3280A and 3 out of 4 mice in the 50 mg/kg dose group. No adverse findings were reported for CD-1 mice. All treated animals were positive for ATAs on Day 18 or 43, which significantly affected toxicokinetic parameters after the third dose. Drug exposure was generally maintained during the first 14 days of the dosing period but was reduced by Day 17. Mean C_{max} on Day 17 was reduced > 90% compared to Day 1 in C57BL/6 mice receiving 10 and 50 mg/kg MPDL3280A and > 75% in CD-1 mice.

Study title: An eight-week toxicity, toxicokinetic, and safety pharmacology study of MPDL3280A administered by intravenous or subcutaneous injection to Cynomolgus monkeys, with a 12-week recovery period

Study no.:	08-1148
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 7, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MPDL3280A (atezolizumab), lot # 729339, purity 99% monomer

The Applicant conducted an 8-week repeat-dose toxicology study of MPDL3280A administered to Cynomolgus monkeys. The data were previously reviewed under IND (b) (4). The results and conclusions are summarized below.

Methods

Doses:	See table below
Frequency of dosing:	Weekly
Route of administration:	Intravenous or subcutaneous injection
Dose volume:	See table below
Formulation/Vehicle:	(b) (4) mM histidine acetate, (b) (4) mM sucrose, (b) (4) % polysorbate 20, pH (b) (4)
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/Sex/Group (main study) 2/Sex/Group (recovery)
Age:	Males – 2.9 to 5.5 years Females – 2.8 to 7.3 years
Weight:	Males – 2.6 to 5.2 kg Females 2.3 to 3.9 kg
Satellite groups:	Groups 7 and 8 were implanted with telemetry units
Unique study design:	None
Deviation from study protocol:	Deviations were not considered to affect the integrity or the interpretation of the results.

Group No.	Dose Route	Number of Males/Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	No. Necropsied:	
					Terminal Day 60	Recovery Day 141
1	IV/SC[a]	5/5	0 (control)	0.67 (1.34 total)	3/3	2/2
2	IV	5/5	5 (low)	0.67	3/3	2/2
3	IV	5/5	15 (mid)	0.67	3/3	2/2
4	IV	5/5	50 (high)	0.67	3/3	2/2
5	SC	5/5	15 (mid)	0.67	3/3	2/2
6	SC	5/5	50 (high)	0.67	3/3	2/2
7[b]	IV	3/3	0 (control)	0.67	-	-
8[b]	IV	3/3	50 (high)	0.67	-	-

[a] Animals in Group 1 received IV and SC dose of the vehicle (i.e., 0.67 mL/kg at each site).
[b] Animals in Groups 7 and 8 were implanted with telemetry units prior to Day 1. Implanted animals were released to the Testing Facility telemetry colony at the end of the recovery period (Day 141).
IV = intravenous, SC = subcutaneous

[Excerpted from Applicant's submission]

Observations and Schedule

Mortality	Daily
Clinical signs	Daily On dosing days, observations at 30 min and 4 hours post-dose
Body weights	Pre-test, Weekly
Food consumption	Daily, qualitative
Ophthalmology	Pre-test, Day 59, and during Week 20
Physical evaluation	Pre-test, Day 2, Day 58, and during Week 20
Neurological evaluation	Pre-test, Day 58, and during Week 20
Cardiovascular and body temperature evaluation	Pre-test, Day 1, 29, 57, and 133
ECG	Pre-test, Day 1, 29, 57, and 133
Respiratory rate	Pre-test, Day 2, Day 58, and during Week 20

Hematology	See table below
Coagulation and clinical chemistry	See table below
Urinalysis	See table below
Immunophenotyping	See table below
Cytokine analysis	See table below
Immunogenicity	See table below
Toxicokinetics	See table below

Table 8: Blood and urine collection times

Sample Collection Schedule			
Study Day	Group(s)	Time Point(s)[a] (relative to dosing)	Samples Collected[b]
Week -2	1-6	NA	Hem, Chem, Coag, Flow, NK, CK, TK, ATA, AutoAb
Week -2	7-8	NA	TK, ATA
Week -1	1-6	NA	Hem, Flow, NK, CK
1	2-4, 7-8	30 min	TK
1	1-6	90 min	CK
1	1, 5-6	8 hr	TK
3	1-6	48 hr	Hem, Chem, Flow, NK, CK, TK
8	1-6	Pre	Hem, Flow, NK, CK
15	1-6	Pre	ATA, TK
15	2-4	30 min	TK
15	1, 5-6	8 hr	TK
17	1-6	48 hr	Hem, Chem
22	7-8	Pre, 30 min	TK
29	1-6	Pre	Hem, Flow, CK, TK
29	2-4	30 min	TK
29	1-6	90 min	CK
29	1, 5-6	8 hr	TK
31	1-6	48 hr	Hem, Chem, Coag, Flow, CK
36	1-6	NA	Hem, Flow, CK
43	1-6	Pre	TK
43	2-4	30 min	TK
43	1, 5-6	8 hr	TK
45	1-6	48 hr	Hem, Chem
57	1-6	Pre	Hem, ATA, Flow, NK, CK, TK
57	7-8	Pre	TK, ATA
57	2-4, 7-8	30 min	TK
57	1-6	90 min	CK
57	1, 5-6	8 hr	TK
59	1-6	48 hr	Hem, Chem, Coag, Flow, NK, CK, TK, AutoAb
60	1-6	72 hr	TK

Sample Collection Schedule			
Study Day	Group(s)	Time Point(s)[a] (relative to dosing)	Samples Collected[b]
60	1-6 (necropsy)	NA	Urin
65	1-6	NA	Hem, Flow, NK, CK
72	1-6	NA	TK
86	1-6	NA	Hem, Chem, TK, ATA
93	1-6	NA	Hem, Flow, CK
100	1-6	NA	TK
114	1-6	NA	Hem, Chem, TK, ATA
128	1-6	NA	TK
140	1-6	NA	Hem, Chem, Coag, ATA, Flow, NK, CK, TK, AutoAb
140	7-8	NA	ATA
141	1-6	At necropsy	TK, Urin

[a] NA - not applicable, Pre - predose, min - minute, hr - hour
[b] ATA - anti-therapeutic antibody, Chem - serum chemistry, Coag - coagulation, Hem-hematology, TK - toxicokinetic, Urin - urinalysis, Flow - Immunophenotyping, CK-cytokine analysis samples, NK - Natural Killer Cell assay, AutoAb – Auto-antibody and double-stranded DNA analyses

[Excerpted from Applicant's submission]

Summary of results and conclusions

Cynomolgus monkeys received IV doses of 0, 5, 15, and 50 mg/kg MPDL3280A or SC doses of 0, 15, and 50 mg/kg weekly for 8 weeks followed by a 12-week recovery period. All animals survived to scheduled necropsy. The major toxicological finding was minimal to mild multifocal arteritis/periarteritis in multiple organs including the heart, aorta, kidney, liver, pancreas, epididymis, GI tract, female reproductive organs, and tongue. Arteritis/periarteritis was noted in animals administered ≥ 15 mg/kg MPDL3280A SC and 50 mg/kg IV. The Applicant characterized the findings as increased thickening of the tunica adventitia and intima of medium-sized arteries by spindle cells with large nuclei and associated mixed cell infiltrates. These microscopic findings were primarily observed within the interstitium of parenchymal organs and within the submucosa or muscularis of tubular organs (GI and female reproductive tract). No adverse findings were observed following a 12-week recovery period. ATAs were detected in 50 out of 56 monkeys receiving test article and 7 out of 16 animals in the vehicle control group. Although ATAs were present in the majority of treated animals, mean treatment exposure was maintained during the dosing period.

Study title: A 26-week toxicity and toxicokinetic study with MPDL3280A, administered by intravenous injection to Cynomolgus monkey with a 13-week recovery phase

Study no.:	13-3278
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	[redacted] (b) (4)
Date of study initiation:	February 21, 2014
GLP compliance:	Yes (OECD)
QA statement:	Yes
Drug, lot #, and % purity:	MPDL3280A (atezolizumab), lot # 598665, 99.2% purity

Key Study Findings

- Doses of ≥ 15 mg/kg caused minimal to moderate arteritis/periarteritis in multiple organs.
- MPDL3280A-treated females showed irregular menstruation during the study period and a lack of newly formed corpora lutea at scheduled necropsy.

Methods

Doses:	0, 5, 15, 50 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Weekly
Route of administration:	Intravenous injection
Dose volume:	5.0 mL/kg
Formulation/Vehicle:	20 mM histidine acetate, 120 mM sucrose, 0.04% polysorbate 20, pH 5.8
Species/Strain:	Cynomolgus monkey
Number/Sex/Group:	3/Sex/Group (main study) 2/Sex/Group (recovery)
Age:	Males – at least 5 years old Females – at least 4 years old
Weight:	Males – 4.5 to 7.4 kg Females – 2.6 to 4.3 kg
Satellite groups:	None
Unique study design:	The study included a testicular and semen evaluation and assessed effects on testosterone levels and menstruation.
Deviation from study protocol:	Deviations were not considered to affect the study design or the interpretation of the results.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Twice daily, detailed evaluations weekly
Body weights	Pre-test, weekly
Food consumption	Not performed
Ophthalmology	Pre-test, last week of dosing, last week of recovery
Physical and neurological evaluations	Pre-test, Week 27 of dosing period, end of recovery period
ECG	Pre-test, Week 11, 20, and 27 of dosing period, last week of recovery
Respiratory rate	Pre-test, Week 11, 20, and 27 of dosing period, last week of recovery
Hematology	Pre-test, Day 3, 60, 116, 183, and 185 Last week of recovery
Coagulation and clinical chemistry	Pre-test, Day 3, 60, 116, 183, and 185 Last week of recovery
Urinalysis	Pre-test, Week 27, last week of recovery
Menstrual cycle	Pre-test, Daily
Testicular and semen evaluation	Pre-test, Week 26 of dosing, end of recovery period
Testosterone analysis	Pre-test, Week 27 of dosing, end of recovery period
Immunophenotyping	Pre-test, Day 3, 60, 116, and 185 Recovery Day 35 and end of recovery period Cell types: Total T cells, CD4+ T cells, CD8+ T cells, B cells, NK cells
Cytokine analysis	Pre-test, Day 2, 58, 114, and 184 Recovery Day 35 and end of recovery period Cytokines: IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, GM-CSF, IL-1β
Immunogenicity	Day 15, 57, 113, 183, 225, and 267
Toxicokinetics	Day 1 (0, 0.25 and 24 h post-dose), Day 3, 15, 57, 113, 183, 184, 186, 190, 225, and 267

Mortality

All animals survived to scheduled necropsy.

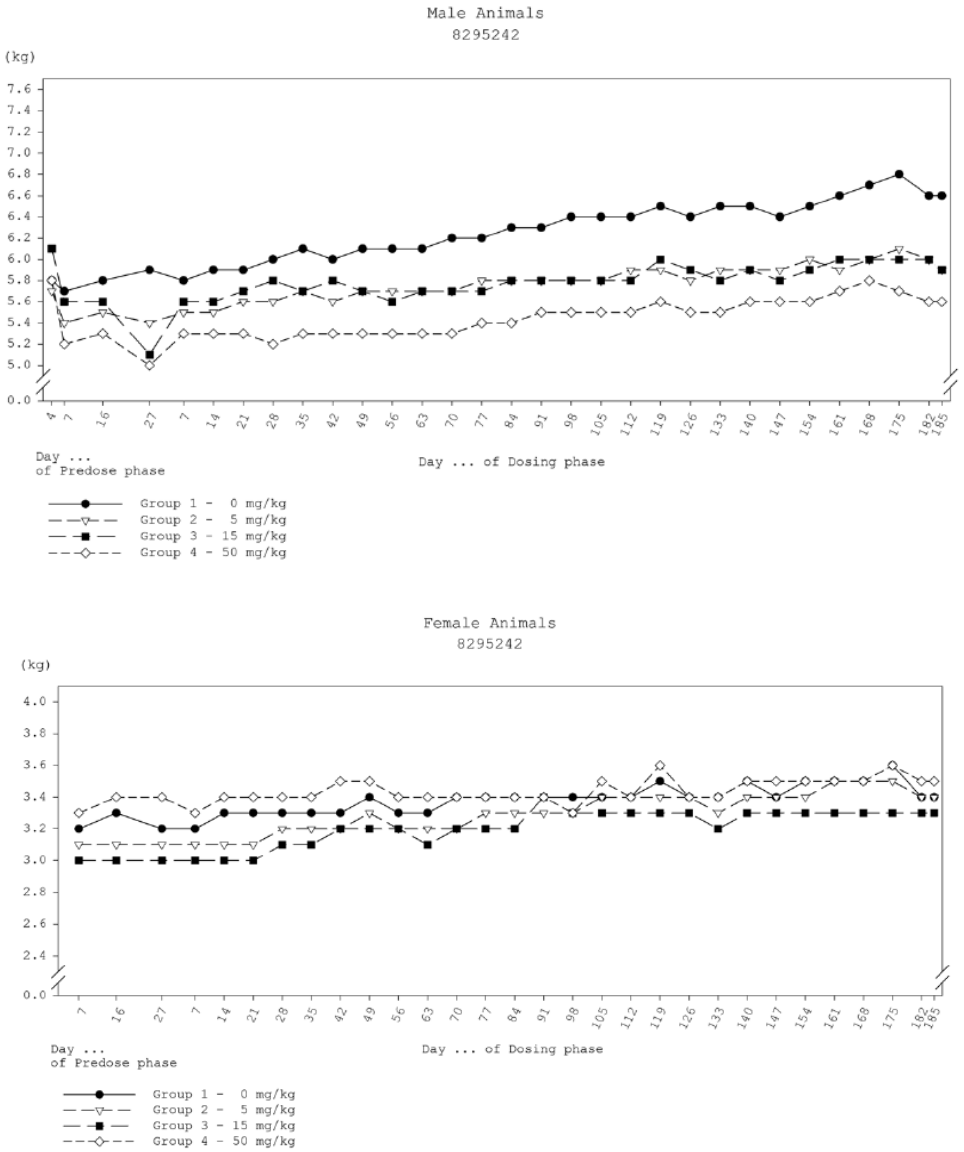
Clinical Signs

- One male in the 5 and 15 mg/kg dose groups experienced infusion-related reactions after dosing on Day 113 and 141, respectively. Clinical signs included severe hypoactivity, staggered movements, and increased heart rate (15 mg/kg male). Animals recovered after receiving glucose and sodium chloride.

Body Weights

- Body weight gain in males was slightly reduced at all dose levels compared to controls. There were no test article-related effects in female monkeys.

Figure 8: Body weights of monkeys administered weekly IV doses of MPDL3280A



[Excerpted from Applicant's submission]

Ophthalmoscopy

Unremarkable

Physical and Neurological Evaluation

Unremarkable

Respiratory Rate

Unremarkable

ECG

Unremarkable

Hematology

- Females administered 50 mg/kg MPDL3280A showed elevated leukocyte counts, correlating with microscopic findings of arteritis/periarteritis in multiple organs.

Table 9: Summary of hematology parameters (% change relative to controls)

		5 mg/kg/week		15 mg/kg/week		50 mg/kg/week	
Parameters	**Day**	**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Leukocytes	60	-	-	-	-	-	36.4
	116	-	-	-	-	-	54.5*
Neutrophils	3	-	-	-	↓31.6	-	↓37.6
	116	↓32.8	-	↓41.3	↓22.4	↓25.8	40.5
	183	-	-	-	↓37.5	-	↓35.8
Lymphocytes	3	-	-	-	-	-	25.8
	60	-	-	-	-	-	67.6
	116	-	-	-	-	-	57.8
	183	-	-	-	-	-	39.9
	185	-	-	-	-	-	57.6
Eosinophils	60	-	-	-	-	-	123.1
	116	-	-	-	-	-	326.7

Significant finding, *p < 0.05; (-) no test article-related change; (↓) decrease

Clinical Chemistry

- There was a ~2.5 to 3-fold increase in C reactive protein in female monkeys administered 50 mg/kg compared to controls, correlating with microscopic findings of multi-organ arteritis/periarteritis.

Urinalysis

Unremarkable

Gross Pathology

Table 10: Summary of macroscopic findings in Cynomolgus monkeys at scheduled necropsy

	Males (mg/kg/week)				Females (mg/kg/week)			
	Grp1 0	Grp2 5	Grp3 15	Grp4 50	Grp1 0	Grp2 5	Grp3 15	Grp4 50
No. of animals examined	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R
Cecum								
• Abnormal contents								1
Colon								
• Abnormal contents								1
General comment								
• Cachexia								1
Lymph node, mesenteric								
• Enlargement								1

Organ Weights

- Organ weight changes were observed in the ovary, thymus, and thyroid/parathyroid without microscopic correlates.

Table 11: Summary of organ weights in Cynomolgus monkeys at end of dosing necropsy (% change relative to control)

		5 mg/kg		15 mg/kg		50 mg/kg	
Organs		**Males**	**Females**	**Males**	**Females**	**Males**	**Females**
Ovary	A		-		↓35.9		↓35.9
	R/BW		-		-		↓41.7
Ovary (cysts excluded)	A		-		↓27.3		↓35.9
	R/BW		-		-		↓41.7
Thymus	A	-	-	-	26.5	-	44.7
	R/BW	-	-	-	41.9	-	31.0
Thyroid/ Parathyroid	A	-	↓43.8	-	↓35.8	-	↓28.5
	R/BW	-	↓35.2	-	↓26.1	-	↓32.7

There were no statistically significant findings.
(-) no test article-related change; (↓) decrease

Histopathology

Adequate Battery: yes

Peer Review: yes

Histological Findings

Table 12: Summary of histological findings in Cynomolgus monkeys at scheduled necropsy

	Males (mg/kg/week)				Females (mg/kg/week)			
	Grp1 0	Grp2 5	Grp3 15	Grp4 50	Grp1 0	Grp2 5	Grp3 15	Grp4 50
No. of animals examined	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R
Adrenal								
• Infiltrate, mononuclear cells Minimal……								1
Cecum								
• Balantidium coli Minimal……								1
Cervix								
• Arteritis/periarteritis Minimal……								1
Colon								
• Arteritis/periarteritis Slight……								1
• Balantidium coli Minimal……								1
• Increased lymphocytes Slight……								1

	Males (mg/kg/week)				Females (mg/kg/week)			
	Grp1 0	Grp2 5	Grp3 15	Grp4 50	Grp1 0	Grp2 5	Grp3 15	Grp4 50
No. of animals examined	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R
Duodenum								
• Arteritis/periarteritis								
Minimal……								1
Femur, marrow								
• Arteritis/periarteritis								
Minimal……								1
Gall bladder								
• Arteritis/periarteritis								
Minimal……								1
Heart								
• Arteritis/periarteritis								
Minimal……							1	
Slight……								1
Ileum								
• Arteritis/periarteritis								
Minimal……								1
• Increased lymphocytes								
Slight……								1
Jejunum								
• Arteritis/periarteritis								
Minimal……								1
Kidney								
• Arteritis/periarteritis								
Moderate……								1
• Fibrosis								
Minimal……								1
• Congestion								
Minimal……								1R
Larynx								
• Arteritis/periarteritis								
Minimal……								1
Lung								
• Infiltrate, mononuclear cells								
Minimal……				1R				
Lymph node, mesenteric								
• Arteritis/periarteritis								
Minimal……								1
Mammary gland								
• Vacuolation, epithelium glands								
Slight……								1R
Mandibular salivary gland								
• Arteritis/periarteritis								
Minimal……								1

	Males (mg/kg/week)				Females (mg/kg/week)			
	Grp1 0	Grp2 5	Grp3 15	Grp4 50	Grp1 0	Grp2 5	Grp3 15	Grp4 50
No. of animals examined	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R
Muscle, skeletal								
• Degeneration/necrosis Slight……				1R				
• Infiltration of inflammatory cells Slight……				1R				
Ovary								
• Corpus luteum, new Present……					2/1R	2	1R	1R
• Corpus luteum, old Present……					1	1/1R	3/1R	3/1R
Pancreas								
• Arteritis/periarteritis Minimal……								1
• Infiltrate, mononuclear cells Minimal……							1	
Parathyroid								
• Infiltrate, lymphocytes Minimal……			1	1				
Pituitary								
• Vacuolation Minimal……				1R			2R	
Rectum								
• Arteritis/periarteritis Minimal……								1
Skin/subcutis								
• Arteritis/periarteritis Minimal……								1
Sternum, marrow								
• Arteritis/periarteritis Minimal……								1
• Infiltrate, lymphocytes Minimal……				1				
Stomach								
• Arteritis/periarteritis Minimal……							1	1
Thyroid								
• Infiltrate, lymphocytes Minimal……				1R				
Urinary bladder								
• Arteritis/periarteritis Minimal……								1

	Males (mg/kg/week)				Females (mg/kg/week)			
	Grp1 0	Grp2 5	Grp3 15	Grp4 50	Grp1 0	Grp2 5	Grp3 15	Grp4 50
No. of animals examined	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R	3/2R
Uterus								
• Arteritis/periarteritis Minimal……								1
• Menstrual phase Present……						1R	1R	1
• Early follicular phase Present……					1/1R	1	1R	2R
• Mid follicular phase Present……						1R	2	2
• Late follicular phase Present……					1/1R			
• Mid luteal phase Present……							1	
• Late luteal phase Present……					1	2		
Vagina								
• Arteritis/periarteritis Minimal……							1	1
• Pigment Slight……								1R

Special Evaluation

Immunogenicity

- *Methods:* A bridging ELISA was used to detect anti-therapeutic antibodies (ATAs) throughout the dosing and recovery period. According to the Applicant, the relative sensitivity of the assay was 51.2 ng/mL. The assay was able to detect 400 ng/mL ATAs in the presence of up to 31.3 µg/mL MPDL3280A. Drug interference was observed at higher concentrations of MPDL3280A.
- *Results:* A total of 21 out of 30 monkeys were positive for ATAs. The results in Table 13 suggest that the number of ATA-positive monkeys decreased with increasing MPDL3280A dose; however, C_{max} values for MPDL3280A were greater than 31.3 µg/mL at doses of 15 and 50 mg/kg/dose on Days 1 and 182. This indicates that MPDL3280A likely interfered with the ELISA used to detect ATAs in these groups. The number of ATA-positive monkeys in the 15 and 50 mg/kg/dose groups in Table 13 may not be representative.

Table 13: Immunogenicity results from Cynomolgus monkeys receiving MPDL3280A weekly

	5 mg/kg/dose	15 mg/kg/dose	50 mg/kg/dose
Total no. of ATA-positive monkeys	10/10	8/10	3/10
Day -8			1/10
Day 15	10/10	8/10	
Day 57	10/10	5/10	2/10
Day 113	10/10	4/10	2/10

Day 183	10/10	4/10	1/10
Day 225	4/4	2/4	
Day 267	4/4	3/4	2/4

Cytokine analysis

- *Methods:* The Applicant used a multiplex immunoassay (Luminex) to measure serum levels of IL-2, IL-4, IL-6, IL-8, IL-10, TNF-α, IFN-γ, GM-CSF, and IL-1β. The lower and upper limit of quantification was 11.43 and 20850 pg/mL.
- *Results:* Unremarkable

Immunophenotyping

- *Methods:* Changes in peripheral lymphocyte subpopulations (CD4+ T cells, CD8+ T cells, B cells, and NK cells) were measured by flow cytometry analysis.
- *Results:* Females administered 50 mg/kg MPDL3280A demonstrated an increase in total T cells (54-56%), CD4+ T cells (40-45%), and B cells (67-82%) compared to controls starting on Day 60 until the end of dosing. No test article-related changes were noted in males compared to controls or pre-test values.

Menstrual cycle

- *Methods:* Menstruation was checked by daily vaginal swabs.
- *Results:* Females administered 50 mg/kg MPDL3280A experienced irregular menstruation during the dosing period, including an increase in mean menstrual cycle length compared to controls (Figure 9 and Figure 10). Findings correlated with a lack of newly formed corpora lutea at terminal necropsy.

    - Extended menstrual cycles were noted for Animal 18178F (67 days, Week 7-15), Animal 18196F (79 days, Week 4-14), and Animal 18227 (79 days, Week 11-20). Subsequent menstrual cycles were comparable to control animals and within the normal range for Cynomolgus monkeys.
    - Animal 18159F showed menstrual bleeding during the pre-dose phase followed by amenorrhea until Week 25 after end of dosing. Regular menstrual cycles were observed for the remainder of study.
    - Animal 18187F experienced menstrual bleeding prior to the pre-dose observation phase but not during the pre-dose, dosing, or recovery periods.
    - Animal 18232F (15 mg/kg group) experienced menstrual bleeding during the pre-dose period followed by amenorrhea through the end of study.
        - The Applicant did not consider this finding to be test article-related; however, it is difficult to exclude a causal relationship, given the irregular menstrual cycles and amenorrhea observed in the 50 mg/kg dose group.

Table 14: Individual menstrual cycle lengths in days

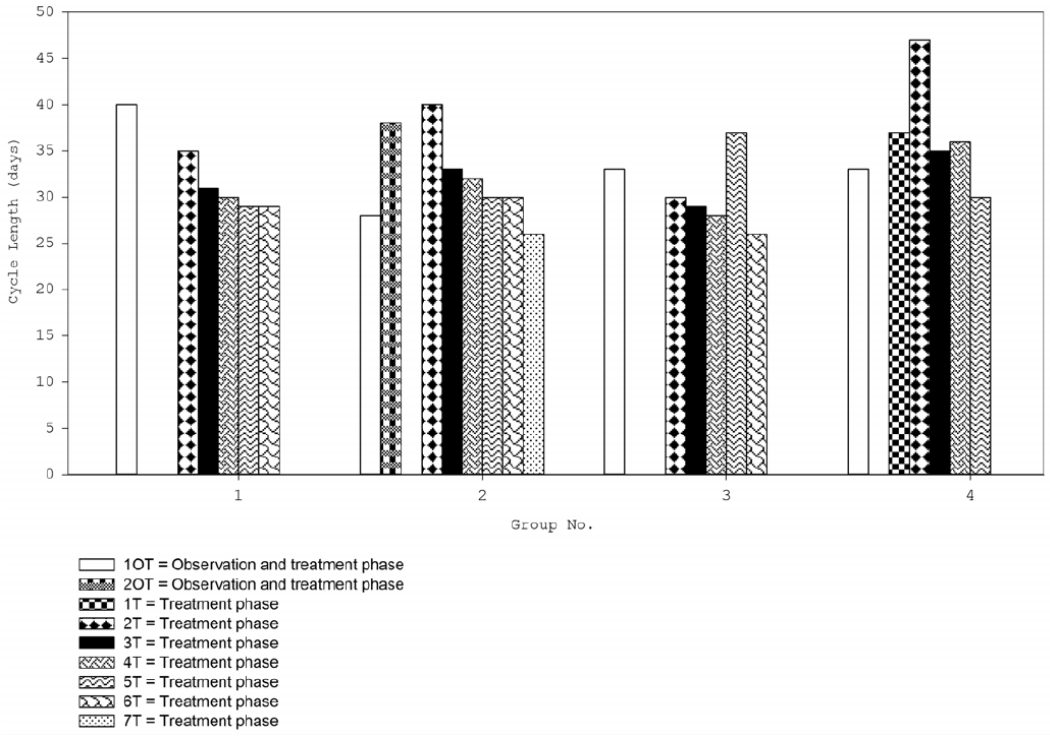
	Animal #	1O	1OT	2OT	1T	2T	3T	4T	5T	6T	7T
0 mg/kg	(b) (4)										

5 mg/kg	(b) (4)
15 mg/kg	
50 mg/kg	

O = Observation phase
OT = Observation and treatment phase
T = Treatment phase

(-) = No menstrual bleeding during phase
x = cycle incomplete due to necropsy

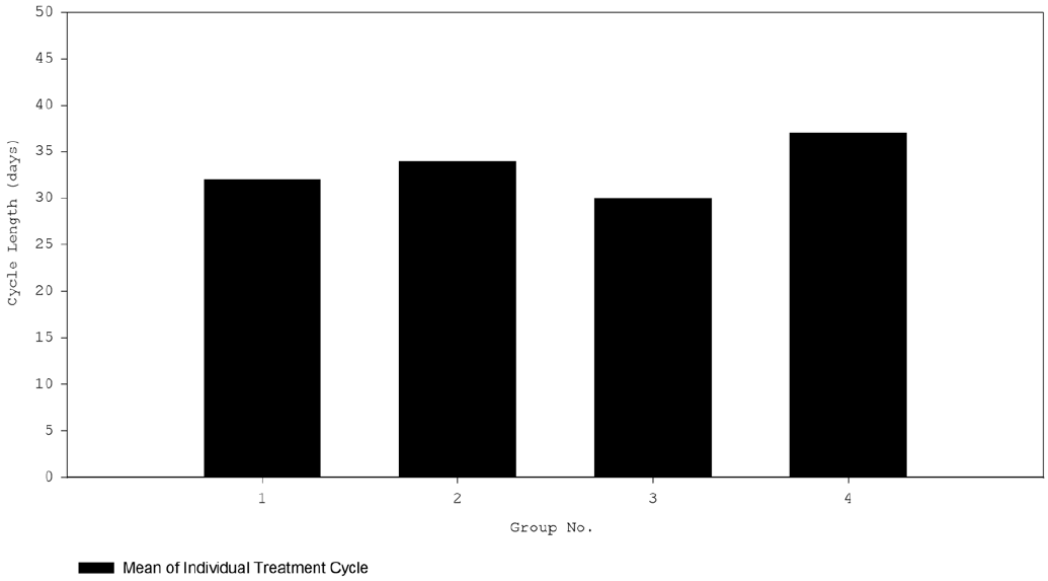
Figure 9: Group mean menstrual cycle length



[Excerpted from Applicant's submission]

Group 1 = 0 mg/kg, Group 2 = 5 mg/kg, Group 3 = 15 mg/kg, Group 4 = 50 mg/kg

Figure 10: Group mean of individual treatment cycle length



[Excerpted from Applicant's submission]

Group 1 = 0 mg/kg, Group 2 = 5 mg/kg, Group 3 = 15 mg/kg, Group 4 = 50 mg/kg

Testicular and semen evaluation

- *Methods:* Collected semen was analyzed for test article-related effects on sperm count, motility, and morphology. Males were also assessed for changes in testicular volume, homogeneity, and echogenicity by ultrasound.
- *Results:* Unremarkable

Testosterone analysis

- *Methods:* Serum testosterone levels were measured in males using a radioimmunoassay (RIA).
- *Results:* Unremarkable

Toxicokinetics

- Following a single dose, C_{max} and AUC increased with increasing dose and were generally dose proportional across the dose range tested.
- Following repeat dosing, systemic exposure in the 5 mg/kg dose group was greatly reduced due to formation of ATAs. C_{max} and AUC increased in monkeys receiving 15 and 50 mg/kg MPDL3280A and were dose proportional at these dose levels.
- Systemic exposure was greater on Day 182 compared to Day 1 in animals receiving 15 and 50 mg/kg antibody, indicating accumulation following repeat dosing.
- After the last dose, terminal half-life ranged from 11.8 to 23.5 days for recovery animals.

Table 15: Mean toxicokinetic parameters in monkeys administered intravenous MPDL3280A

	Sex	Dose (mg/kg/week)	C_{max} (µg/mL)	C_{max}/D (µg/mL)/D	$AUC_{(0-t)}$ (day·µg/mL)	$AUC_{(0-t)}$/D (day·µg/mL)/D
Day 1	Male	5	139	27.8	263	52.6
		15	351	23.4	758	50.5
		50	1290	25.8	2880	57.6
	Female	5	107	21.4	224	44.8
		15	251	16.7	629	41.9
		50	1110	22.2	2690	53.8
Day 182	Male	5	7.3	1.5	NA	NA
		15	1220	81.3	4250	283
		50	4060	81.2	10100	202
	Female	5	116	23.2	378	75.6
		15	1350	90.0	2810	187
		50	3300	66.0	6740	135

Note: Limited data were available for the 5 mg/kg dose level due to anti-therapeutic antibody formation in all animals. Female means in the 5 mg/kg group were calculated from 2 of 3 animals. Following a single dose, AUC was estimated from Day 0 to 3. For TK analysis on Day 182, AUC was estimated from Day 182 to 185.
NA – not applicable

Dosing Solution Analysis

Sample formulations were analyzed on Day 1, Week 7, 13, 19, 25, and 27 of the dosing phase and were within the acceptance criteria of ± 10% of the intended concentration.

7 Genetic Toxicology

Not conducted

8 Carcinogenicity

Not conducted

9 Reproductive and Developmental Toxicology

The Applicant did not conduct reproductive and developmental toxicology studies with atezolizumab. The current nonclinical literature demonstrates that the PD-L1/PD-1 pathway plays a role in fetal/maternal tolerance, and inhibition of PD-L1 is associated with an increased risk of fetal rejection. The Division agreed with the Applicant's request to provide a non-product specific literature-based assessment of the reproductive risks associated with blockade of the PD-L1 pathway to inform appropriate labeling for atezolizumab.

During pregnancy, the maternal immune system must maintain the ability to defend against infection while also maintaining tolerance for a fetus expressing and shedding foreign, paternally-inherited antigens. Several mechanisms protect a developing fetus from the maternal immune system, including expression of non-classical MHC molecules on fetal trophoblast cells, clonal deletion and anergy of fetal antigen-specific

T cells, and the presence of immunomodulatory molecules, including PD-L1 (Guleria and Sayegh 2007, Petroff and Perchellet 2010).

PD-L1 is expressed in the human placenta throughout pregnancy. Expression is low during the first trimester and markedly increases during the second and third trimesters, corresponding to the onset of maternal blood flow (Holets et al. 2006, Petroff et al. 2003). PD-L1 expression is primarily located on fetal trophoblast populations in direct contact with maternal blood and tissue at the fetal/maternal interface. PD-1 receptor has been detected on Tregs and $CD4^+$ and $CD8^+$ T cells within the decidua (Petroff and Perchellet 2010). The spatial and temporal regulation of PD-L1 and PD-1 receptor at the fetal/maternal interface suggests an important role for these immunomodulatory proteins in maintaining fetal tolerance.

Nonclinical studies have demonstrated that loss of PD-L1 activity reduces embryo-fetal survival. In an allogenic pregnancy model (CBA x B6), administration of a PD-L1 blocking antibody to pregnant mice increased the rate of fetal resorption (86%) compared to isotype control (~18%), resulting in a corresponding decrease in litter size (Guleria et al. 2005). Similarly, allogenic pregnancies of PD-$L1^{-/-}$ mice resulted in a mean litter size of 2.7 whereas heterozygous or WT females produced mean litter sizes of 8.5 and 9, respectively. D'Addio et al. assessed the effects of PD-L1 blockade in Th1.1 B6 females mated with Bm12 males and associated changes in fetal antigen-specific T cells (D'Addio et al. 2011). Administration of a PD-L1 blocking antibody increased fetal rejection (34%) compared to isotype control (1.4%) and reduced mean litter size from 8.5 in the isotype control group to 5.8 in the anti-PD-L1 group. PD-L1 blockade reduced fetal antigen-specific Tregs and increased fetal antigen-specific effector T cells in the spleen and lymph nodes.

In another allogenic pregnancy model, Taglauer et al. assessed the role of PD-1 receptor on fetal antigen-specific T cells using a transgenic mouse model, in which T cells expressing the OT-I T-cell receptor recognize ovalbumin (OVA)-derived peptide (Taglauer et al. 2009). B6 WT females were mated with OVA-Tg males. On gestation Day 10.5, pregnant mice were injected with OT-I expressing splenocytes, which recognize parentally-inherited, OVA antigen. Three days post-transfer, proliferating PD-1^+ OT-I cells, including $CD8^+$ T cells, were detected in the uterus draining lymph nodes and spleen. Adoptive transfer of PD-$1^{-/-}$ OT-I splenocytes resulted in greater accumulation of OT-I $CD8^+$ T cells in the uterus draining lymph nodes. In vitro analysis of PD-$1^{-/-}$ T cells suggested that T-cell accumulation was due to reduced apoptosis rather than an increase in cell proliferation. Collectively, these nonclinical studies demonstrate that the PD-L1/PD-1 pathway controls the accumulation of fetal antigen-specific T cells during pregnancy, and inhibition of PD-L1 leads to a loss of fetal tolerance and an increased risk of immune-mediated abortion.

It is unclear if atezolizumab crosses the placental barrier at levels that would have adverse effects on developing offspring. The allogenic pregnancy models did not evaluate offspring for teratogenicity or adverse developmental effects. Data from knockout mice demonstrate that syngeneic PD-$1^{-/-}$ and PD-$L1^{-/-}$ fetuses develop

normally with no reported malformations. Depending on the genetic background, PD-1$^{-/-}$ and PD-L1$^{-/-}$ mice develop late onset autoimmune phenotypes, including lupus-like glomerulonephritis, arthritis, and cardiomyopathy (Keir et al. 2008, Okazaki and Honjo 2006, Okazaki and Honjo 2007). Loss or blockade of PD-L1 and its receptors exacerbates disease in mouse models of autoimmunity. In humans, SNPs located in the gene encoding PD-1 are associated with several autoimmune diseases, including type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus (SLE). Therefore, fetal exposure to atezolizumab may result in adverse effects on the developing immune system.

10 Special Toxicology Studies

Study title: Hemolytic potential testing with MPDL3280A in Cynomolgus monkey and human blood

Study no.:	08-1172
Study report location:	eCTD 4.2.3.7.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 4, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	MPDL3280A, lot # 729339, 99% monomer

The objective of this study was to determine the potential for MPDL3280A to cause hemolysis of human or Cynomolgus monkey blood. Briefly, blood samples were collected from fasted, naïve Cynomolgus monkeys and a fasted human volunteer. Blood and plasma were incubated with 125 mg/mL MPDL3280A or vehicle control for 40 minutes at 37°C. Samples were incubated with 1% saponin as a positive control. Supernatants were collected after centrifugation, and hemoglobin concentrations were measured by spectrophotometer.

Results and conclusions

- A concentration of 125 mg/mL MPDL3280A did not cause hemolysis of human or Cynomolgus monkey blood.

Study title: Tissue cross-reactivity of MPDL3280A with human and Cynomolgus monkey tissues ex vivo

Study no.:	08-1174
Study report location:	eCTD 4.2.3.7.
Conducting laboratory and location:	Genentech, Inc. South San Francisco, CA
Date of study initiation:	October 27, 2008
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Biotin-MPDL3280A, from antibody lot # 729339, not provided

The objective of this study was to assess the tissue cross-reactivity of MPDL3280A with a panel of human and Cynomolgus monkey tissues.

Methods

Species of tissues	Human and Cynomolgus monkey		
Positive and negative control samples	HEK293 cells expressing human PD-L1 Wild type HEK293 cells		
Number of tissue donors	3/species		
Tissues	Adrenal Bladder Blood Bone marrow Breast Cerebellum Cerebral cortex Colon Endothelium (aorta) Eye Fallopian tube GI tract (small intestine)	Heart Kidney (glomerulus) Kidney (tubule) Liver Lung Lymph node Ovary Pancreas Parathyroid Pituitary Placenta Prostate	Skin Spinal cord Spleen Striated muscle Testis Thymus Thyroid Thymus Tonsil Ureter Uterus (cervix) Uterus (endometrium)
Tissue fixation/embedding	Cryosections in OCT compound or equivalent were mounted on slides and fixed in acetone.		
Test and control article	Biotin-MPDL3280A and Biotin-human IgG antibody		
Test article concentrations	0.25 µg/mL (optimal), 1.25 µg/mL		
IHC methods	Steps were performed at room temperature. • Washed slides 3X with PBS, incubated in 1X Morphosave for 15 min • Incubated with glucose oxidase solution for 1 h • Blocked with avidin and biotin for 15 min followed by 2% gamma globulin blocking solution for 30 min • Incubated with primary antibody for 1 h • Incubated with streptavidin-HRP for 30 min followed by DAB substrate for detection for 5 min Note: Slides were washed with 1X TBST in between each step.		
Tissue counterstain	Mayer's hematoxylin		
Tissue validation	Anti-CD31 antibody (3 µg/mL) to detect endothelial cells		
Microscopy	Light microscopy		

Results and conclusions

- HEK293 cells expressing PD-L1 showed moderate intensity membrane staining in the presence of 0.25 and 1.25 µg/mL Biotin-MPDL3280A but not in the presence of Biotin-IgG isotype control. Biotin-MPDL3280A did not bind to wild type HEK293 cells.
- Positive CD31 staining was reported in all human and monkey tissues tested, indicating staining of endothelial cells, platelets, or megakaryocytes and validation of tissues in the immunohistochemistry assay.
- No specific tissue staining was detected with Biotin-IgG isotype control.
- Membrane staining was reported only in syncytiotrophoblasts of human placenta. Cytoplasmic staining was observed in human lymph node, thymus, and tonsils.
- Staining of Cynomolgus monkey tissues was reported only in the lymph node (1-3+ cytoplasmic staining of sinusoidal cells; rare to frequent).

Table 16: Summary of Biotin-MPDL3280A cross-reactivity with human tissues

	Donor 1		Donor 2		Donor 3	
	0.25 µg/mL	1.25 µg/mL	0.25 µg/mL	1.25 µg/mL	0.25 µg/mL	1.25 µg/mL
Lymph node						
- sinusoidal cell, cytoplasmic						
Very rare……	1+	1+	1+	1+	neg	neg
Placenta						
- syncytiotrophoblasts, apical cytoplasm and membrane						
Frequent……	3+	3+	3+	3+	3+	3+
- chorionic plate, cytoplasm						
Frequent……	3+	3+	3+	3+	3+	3+
Thymus						
- thymic cortex, cytoplasm						
Rare……			2+	2+		
Occasional……....					2-3+	2-3+
Frequent……	2+	3+				
- medulla, cytoplasm						
Rare……	2+	3+				
Occasional……			2+	2+	2-3+	2-3+
Tonsil						
- sinusoidal cells, cytoplasm						
Very rare……	neg	1-2+	1-2+	1-2+	1-2+	1-2+

Staining intensity: 1+ = minimal, 2+ = mild, 3+ = moderate, 4+ = marked, neg = negative
Frequency of stained cells of particular type: very rare <25%, rare 25-50%, occasional >50-75%, frequent 76-100%

11 Integrated Summary and Safety Evaluation

Pharmacology

Atezolizumab is a humanized IgG1 monoclonal antibody that binds to PD-L1 and blocks its interaction with PD-1 and B7-1 receptors. Atezolizumab contains a mutation in the Fc domain to prevent glycosylation, limit binding to Fcγ receptors, and prevent Fc-mediated depletion of PD-L1 expressing cells (e.g., ADCC). Atezolizumab retains binding to

neonatal Fc receptors in the presence of the mutation affecting Fcγ receptor binding. In pharmacology studies, atezolizumab bound to HEK293 cells exogenously expressing human or murine PD-L1 (EC_{50} = 0.4 and 0.1 nM) and to activated human and Cynomolgus monkey T cells (EC_{50} = 0.4 and 0.7 nM). In a competitive binding ELISA assay, atezolizumab blocked recombinant PD-L1 binding to human and murine PD-1 (IC_{50} = 82.8 and 104 pM) and B7-1 (IC_{50} = 48.4 and 75.6 pM) receptors with subnanomolar inhibitory activity. Atezolizumab showed minimal binding to human Fcγ receptors and did not stimulate cytokine release from human PBMCs at concentrations up to 250 µg/mL or ADCC activity at concentrations up to 10 µg/mL.

The Applicant conducted in vivo pharmacology studies to evaluate the effect of PD-L1 blockade in syngeneic tumor models and a mouse model of chronic viral infection. To minimize immunogenicity in mice, the Applicant generated two mouse IgG2a chimeric antibodies (PRO304397 and PRO314483), containing the binding region of atezolizumab. In vitro, the chimeric antibodies bound to PD-L1 with subnanomolar affinity, which was comparable to atezolizumab. In syngeneic tumor models of melanoma and colorectal cancer, a dose of 10 mg/kg anti-PD-L1 reduced tumor growth by ≥ 80% and prolonged time to progression. There were no treatment-related effects on body weight or reported clinical signs.

The Applicant evaluated the effect of PD-L1 blockade in a mouse model of chronic LCMV CL-13 infection using the murine/human chimeric antibodies and an independently derived anti-mouse PD-L1 antibody. LCMV CL-13 is highly virulent and produces an infection that persists for several weeks due to impaired T-cell response. The peak of viral infection and acute T-cell response occur at one week post-infection. A dose of 10 mg/kg anti-PD-L1 caused ~40% mortality when given prior to the peak of infection on Day 2 and 60 to 100% mortality when administered at the peak of infection on Day 7. In contrast, all mice receiving anti-PD-L1 starting on Day 14 or 17 survived to scheduled necropsy with no reported toxicity. Anti-PD-L1 enhanced virus-specific $CD8^+$ T-cell function and reduced viral titers. Similarly, PD-$L1^{-/-}$ mice succumb to LCMV infection during the acute phase, suggesting that the PD-L1 pathway is required to control the acute immune response and prevent immune-mediated damage in the chronic LCMV CL-13 model (Barber et al. 2006). These findings were not observed in other models of acute viral infection.

In a mouse model of bacterial infection, PD-$1^{-/-}$ mice infected with *M. tuberculosis* showed a significant decrease in survival (Lazar-Molnar et al. 2010). The lungs from these mice showed a dose-dependent increase in bacterial infection and focal areas of necrotizing inflammation. Pro-inflammatory cytokines were markedly increased systemically and in the lungs. In contrast, infected wild type mice survived to the end of study with less severe lung inflammation and bacterial loads. Collectively, animal models demonstrate that blocking the PD-L1/PD-1 pathway may increase the inflammatory response and enhance the severity of some infections.

Safety pharmacology studies were incorporated into repeat-dose toxicology studies conducted in Cynomolgus monkeys. There were no test article-related effects on blood

pressure, ECG, respiratory rate, or neurological parameters at doses up to 50 mg/kg/week administered for up to 26 weeks.

General Toxicology

The Applicant conducted repeat-dose toxicology studies in mice and Cynomolgus monkeys. In a pilot study, mice received doses up to 50 mg/kg/week IV atezolizumab for three doses. The major toxicological finding was minimal sciatic neuropathy (vacuolation and lymphocytic infiltration) at doses ≥ 10 mg/kg observed at the end of dosing and recovery period. All mice were positive for ATAs, which significantly reduced drug exposure by Day 17.

In GLP-compliant toxicology studies, Cynomolgus monkeys received atezolizumab at doses up to 50 mg/kg/week given SC for 8 weeks or administered IV for up to 26 weeks. The major toxicological finding was minimal to mild multifocal arteritis/ periarteritis in multiple organs including the heart, aorta, kidney, liver, pancreas, epididymis, GI tract, skin, tongue, and female reproductive organs. Arteritis/periarteritis was noted in animals administered ≥ 15 mg/kg SC and 50 mg/kg IV atezolizumab. The findings were reversible following a 3-month recovery period. In the 26-week study, female monkeys administered 50 mg/kg IV atezolizumab experienced irregular menstruation during the dosing period, including an increase in mean menstrual cycle length compared to controls and a corresponding lack of newly formed corpora lutea. Effects on menstruation occurred at an AUC that was approximately 6 times the estimated AUC in patients administered atezolizumab at the recommended dose of 1200 mg every 3 weeks. During the study, the majority of animals were positive for ATAs. At end of dosing (Day 182), mean exposure was markedly reduced in males receiving 5 mg/kg atezolizumab and was increased at doses ≥ 15 mg/kg for both genders compared to Day 1.

Reproductive and Developmental Toxicology

The Applicant did not conduct reproductive and developmental toxicology studies with atezolizumab. Atezolizumab was immunogenic in mice after three weekly doses, resulting in reduced exposures by Week 3. In addition, the scientific literature demonstrates that the PD-L1/PD-1 pathway plays a role in fetal/maternal tolerance and inhibiting this pathway increases the risk of embryo-fetal death in vivo. The Pharmacology/Toxicology team agreed with the Applicant that a non-product specific literature-based assessment was appropriate to characterize the potential risk of embryo-fetal toxicity.

PD-L1 is expressed in the human placenta throughout pregnancy and primarily located on fetal trophoblast cells in direct contact with maternal blood and tissue at the fetal/maternal interface (Holets et al. 2006, Petroff et al. 2003). PD-1 receptor has been detected on T-cell populations within the decidua (Petroff and Perchellet 2010). In allogenic pregnancy models, administration of anti-PD-L1 blocking antibodies to pregnant mice increased the rate of fetal resorption and caused a corresponding

decrease in surviving pups (D'Addio et al. 2011, Guleria et al. 2005). Similarly, allogenic pregnancies of PD-L1$^{-/-}$ mice resulted in reduced litter sizes compared to wild type and heterozygous controls (Guleria et al. 2005). Blocking the PD-L1/PD-1 pathway reduces fetal antigen-specific Tregs and increases accumulation of fetal-antigen specific T effector cells in maternal lymphoid organs (D'Addio et al. 2011, Taglauer et al. 2009). Collectively, nonclinical studies demonstrate that PD-L1 blockade leads to a loss of fetal tolerance and an increased risk of immune-mediated abortion.

The allogenic pregnancy models did not evaluate offspring for teratogenicity or adverse developmental effects. Data from knockout mice, however, demonstrate that syngeneic PD-L1$^{-/-}$ and PD-1$^{-/-}$ mice develop normally with no malformations. Depending on the genetic background, inhibition of the PD-L1/PD-1 pathway by genetic deletion or blockade results in autoimmune phenotypes and exacerbation of disease in mouse models of autoimmunity. In humans, SNPs located in the gene encoding for PD-1 are associated with autoimmune diseases, including SLE, type 1 diabetes, and rheumatoid arthritis. It is unknown if atezolizumab crosses the placental barrier at concentrations that would cause adverse effects in developing offspring. The potential risk of offspring developing immune-mediated disorders or alterations in the normal immune response cannot be dismissed based on the mechanism of action of atezolizumab.

Special Toxicology Studies

The Applicant evaluated atezolizumab for cross-reactivity to a panel of normal human and Cynomolgus monkey tissues. Atezolizumab bound to the cell membrane of syncytiotrophoblasts in human placenta and within the cytoplasm of human and monkey lymph nodes and human thymus and tonsils. In a hemolysis assay, atezolizumab had no effect on human or Cynomolgus monkey blood at a concentration of 125 mg/mL.

12 Appendix/Attachments

None

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