

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125476Orig1s000

PHARMACOLOGY REVIEW(S)

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

ADDENDUM TO PHARMACOLOGY/TOXICOLOGY REVIEW

Application number: BLA 125476

Supporting document/s: 0001

Applicant's letter date: June 20, 2013

CDER stamp date: June 20, 2013

Product: Vedolizumab (Entyvio[®],MLN0002)

Indication: Vedolizumab is indicated for the treatment of patients with moderately to severely active ulcerative colitis (UC) or Crohn's disease (CD)

Applicant: Takeda Pharmaceuticals U.S.A., Inc.

Review Division: Division of Gastroenterology and Inborn Errors Products (DGIEP)

Reviewer: Tamal K. Chakraborti, Ph.D.

Supervisor/Team Leader: Sushanta K. Chakder, Ph.D.

Division Director: Donna Griebel, MD

Project Manager: Kevin Bugin, MS, RAC

This addendum is in reference to the required juvenile animal toxicology study as a post marketing requirement (PMR) to support the pediatric clinical study in patients aged 5 to 17 years. (b) (4)



The age of the monkeys used in 13-week and 26-week toxicology studies do not support the proposed pediatric age group of 5 to 17 years. In the PPND study in monkeys, adequate exposure and target saturation were not achieved in infants on postpartum (pp) period beyond 28 days. Vedolizumab was excreted at low levels into the breast milk of monkeys in this study. In addition, vedolizumab was detected only in one infant at 100 mg/kg on Day 120 pp, suggesting inadequate drug exposure to the infants during the entire observation period. Overall, the existing nonclinical studies were not considered adequate to support pediatric clinical studies for the pediatric age group of 5 to 17 years. Vedolizumab is a novel therapeutic agent with limited clinical experience, and a juvenile animal toxicology study in an appropriate species is required to support the proposed study in the pediatric age group of 5 to 17 years.

Recommendation: The Applicant should be asked to conduct a juvenile animal toxicology study of 3 months duration in an appropriate species before initiation of the pediatric trial in patients 5 to 17 years of age.

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/s/

TAMAL K CHAKRABORTI
04/11/2014

SUSHANTA K CHAKDER
04/11/2014

Comments on BLA 125476 Vedolizumab

From: A Jacobs, AD

Date: Nov 26, 2013

1. I concur that there are no pharm-tox approval issues
2. I concur with the pregnancy category B
3. I have conveyed other comments to the reviewer.

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/s/

ABIGAIL C JACOBS
11/26/2013

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

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Reviewer: Tamal K. Chakraborti, Ph.D.
Supervisor/Team Leader: Sushanta K. Chakder, Ph.D.
Division Director: Donna Griebel, MD
Project Manager: Kevin Bugin, MS, RAC

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1 Executive Summary

1.1 Introduction

MLN0002 (Vedolizumab/LDP-02/Entyvio[®]) is an IgG1 humanized monoclonal antibody (mAb) indicated for the treatment of adult ulcerative colitis (UC) and Crohn's Disease (CD). Ulcerative colitis and CD are inflammatory bowel disease (IBD) characterized by chronic inflammation at various sites in the gastrointestinal (GI) tract. Alpha 4 beta 7 integrin is a key mediator of gastrointestinal inflammation and immunity and is expressed on the surface of a discrete subset of memory T lymphocytes. The $\alpha 4\beta 7$ integrin mediates leukocyte trafficking into the GI mucosa and gut associated lymphoid tissue (GALT) through adhesion interaction with its natural ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1). MAdCAM-1 is preferentially expressed on endothelial venules at sites of lymphocyte extravasation in the GI mucosa and GALT (Briskin M et al. 1997, Am J Pathol, 151:97-110; Salmi M et al. 2005, Immunol Rev, 206:100-13; Erle DJ et al. 1994, J Immunol, 153:517-28; Feagan BG et al. 2005, New Eng J Med, 352:2499-507; von Andrian UH and B Engelhardt, 2003, New Eng J Med, 348:68-72; Arihiro S et al. 2002, Pathol Internatl, 52:367-374).

MLN0002 is a selective integrin antagonist that binds to $\alpha 4\beta 7$ integrin. MLN0002 does not bind to $\alpha 4\beta 1$ or $\alpha E\beta 7$ integrin. MLN0002 inhibits the leukocyte trafficking into the area of intestinal inflammation by selectively antagonizing binding of $\alpha 4\beta 7$ to its ligand, MAdCAM-1. MLN0002 does not antagonize adhesion interactions of $\alpha 4\beta 1$ to its ligand, vascular cell adhesion molecule-1 (VCAM-1). This selective antagonism of $\alpha 4\beta 7$ by MLN0002 restricts inhibition of $\alpha 4\beta 7$ /MAdCAM-1 and $\alpha 4\beta 7$ /fibronectin pathways resulting in inhibition of migration of leukocytes into GI mucosa, and is therefore expected to reduce inflammation in the GI tract.

1.2 Brief Discussion of Nonclinical Findings

MLN0002 has been shown to be a selective $\alpha 4\beta 7$ integrin antagonist and it does not bind to $\alpha 4\beta 1$ or $\alpha E\beta 7$ integrins. MLN0002 inhibited cellular adhesion interactions between $\alpha 4\beta 7$ and MAdCAM-1 and fibronectin, an extracellular matrix glycoprotein. However, MLN0002 did not inhibit $\alpha 4\beta 7$ -VCAM-1, $\alpha 4\beta 1$ -VCAM-1, or $\alpha 4\beta 1$ -fibronectin-mediated adhesive interactions. In *in vitro* pharmacology studies using human peripheral blood mononuclear cells (PBMCs), MLN0002 did not mediate antibody-dependent cell-mediated cytotoxicity (ADCC) and did not mediate complement dependent cytotoxicity (CDC), a mechanism of cytotoxic action of many monoclonal antibodies. MLN0002 did not induce T lymphocyte activation or cytokine release in whole human blood. In an *in vivo* animal efficacy study with ACT-1 (murine homologue of MLN0002) in cotton-tap Tamarin monkeys (*Sanguinus Oedipus*) with naturally occurring chronic colitis, ACT-1 showed efficacy (resolution of diarrhea, reduced inflammatory activity i.e., reduced leukocyte infiltration of the gut lamina propria and

decreased mucosal density of $\alpha 4\beta 7$ lymphocytes) when administered at 2 mg/kg for 8 days.

Immune surveillance of the central nervous system (CNS) was tested in experimental autoimmune encephalomyelitis (EAE) model in Rhesus monkeys, an animal model of multiple sclerosis (MS), to examine the potential of MLN0002 to cause progressive multifocal leukoencephalopathy (PML). An animal model of PML does not exist. MLN0002 did not appear to inhibit immune surveillance of the CNS in this EAE model. In contrast, natalizumab blocked immune surveillance of the CNS in this animal model. However, since EAE is not an animal model of PML; the results of this study do not directly demonstrate that MLN0002 has no potential to cause PML.

Tissue cross-reactivity (TCR) studies were conducted using a panel of monkey and human tissues. Binding was restricted to leukocytes in lymphoid tissues, within the lumens of blood vessels, or as low-grade inflammatory infiltrates in various non-lymphoid tissues (liver, kidney, prostate, thyroid, etc.). Vedolizumab staining pattern observed in Cynomolgus monkey tissues appeared to be similar to that observed in the human tissues and results were stated to be consistent with the pattern of expression of $\alpha 4\beta 7$ integrin. No unanticipated tissue cross-reactivity or off-target staining was observed in TCR studies.

Toxicology studies were conducted with MLN0002 in two species (rabbit and Cynomolgus monkeys) by intravenous (IV) route to support the chronic use of MLN0002. A 3-month IV toxicology study was conducted with MLN0002 in New Zealand white rabbits at 30 and 100 mg/kg and 3- and 6-month IV toxicology studies were conducted with MLN0002 in Cynomolgus monkeys at 10, 30 and 100 mg/kg administered once every two weeks. In rabbits, histopathological changes were seen in the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) and ileum (hyperplasia of submucosal lymphoid nodules) of treated and control animals. However, incidence and severity were not dose related and these changes were also seen in control animals and the relation to the treatment is uncertain. In monkeys, histopathological changes were seen in the gastrointestinal tract (minimal to mild lymphoid depletion in the Peyer's patches of the gastrointestinal tract in male animals at 10, 30, and 100 mg/kg/day and increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg). Minimal to mild lymphoid depletion in Peyer's patches of males and an analogous decrease in leukocytes expressing the $\alpha 4\beta 7$ integrin in crypt epithelium appeared to be due to the pharmacologic effect of MLN0002 (decreased trafficking of peripheral lymphocytes to the gut). The relation to the treatment is not clear in the absence of a dose response. MLN0002 treated monkeys had increased severity of regeneration of superficial mucosal epithelium in response to lymphoplasmacytic gastritis. Lymphoplasmacytic gastritis (lymphoplasmacytic infiltrates into the lamina propria of the stomach) is a common incidental finding in Cynomolgus monkeys and epithelial regeneration is an expected physiologic response to lymphoplasmacytic gastritis. The incidence of both lymphoplasmacytic gastritis and epithelial regeneration

was comparable in both MLN0002 treated and control monkeys in the 26-week study. However, the severity of the epithelial response to the inflammation was slightly increased in MLN0002 treated monkeys when compared to control monkeys. The toxicological significance of this increase in the regenerative response of the epithelium is not clear. *Balantidium coli* (parasites) were observed in the cecum and colon of both control and MNL0002 treated monkeys. *Balantidium coli* are common commensal intestinal parasite of macaques and are generally non-pathogenic. The presence of *Balantidium coli* observed in the cecum and colon of monkeys did not appear to be treatment related due to lack of a dose response, presence of this parasite in both control and treatment group animals and reported background incidences.

Conventional carcinogenicity studies (i.e., rodent bioassays) have not been conducted with MLN0002 to assess its carcinogenic potential as it lacks pharmacological activity in mice and rats. However, carcinogenic potential of Act-1 (murine homologue of MLN0002) was assessed in an *in vitro* study using human tumor cells that expressed $\alpha 4\beta 7$ integrin. In this study, Act-1 did not stimulate the growth or cellular proliferation of RPMI 8866 human B-cell lymphoma cell that express the $\alpha 4\beta 7$ integrin. MLN0002 also did not affect other factors that could affect oncogenesis, such as cytokine production, activation, cell proliferation of primary human leukocytes expressing the $\alpha 4\beta 7$ integrin. Lymphoid hyperplasia was observed in the 13- and 26-week toxicology studies in Cynomolgus monkeys and in a 3-month toxicology study in rabbits. These findings appear to be most likely resulted from the immunogenicity (i.e., antigenic stimulation) associated with infusing nonhuman species with a humanized monoclonal antibody. In addition, there was no evidence of systemic immunosuppression in toxicology studies. Overall, MLN0002 does not appear to have significant carcinogenic potential.

MLN0002 did not have adverse effects on embryofetal development in rabbits when administered on gestation day 7 (GD 7) at single IV doses up to 100 mg/kg (about 6.5 times the recommended human dose based on body surface area). A pre and postnatal development study in monkeys showed no evidence of any adverse effect on pre and postnatal development at IV doses up to 100 mg/kg (about 6.5 times the recommended human dose based on the body surface area).

Overall, the results of pharmacology studies appear to support the mechanism of action and the proposed indication of MLN0002. In toxicology studies in monkeys, histopathological findings appeared to be due to the pharmacologic effect of MLN0002 (decreased trafficking of peripheral lymphocytes to the gut). The presence of *Balantidium coli* observed in the cecum and colon of monkeys did not appear to be treatment related. There was no apparent off-target toxicity in rabbits and monkeys following repeated administration. In addition, in tissue cross-reactivity studies with MLN0002, no unanticipated tissue cross-reactivity or off-target staining was observed and results were consistent with the known pattern of $\alpha 4\beta 7$ integrin expression. The potential of MLN0002 to cause PML was examined in an EAE model in the Rhesus monkey; an animal model of MS. MLN0002 did not appear to inhibit immune surveillance of the CNS unlike natalizumab, which blocked immune surveillance of the CNS in this animal model. However, since EAE is not an animal model of PML; the

results of this study do not directly demonstrate that MLN0002 has no potential to cause PML.

1.3 Recommendations

1.3.1 Approvability

From a nonclinical standpoint, this BLA is recommended for approval.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

The draft labeling of Vedolizumab[®] conforms to the content and format of labeling for human prescription drug and biological products under 21CFR201.57. However, the following changes are recommended.

8.1 Pregnancy

Applicant's Version:

8.1 Pregnancy

(b) (4)

Evaluation: The pregnancy category B is acceptable. However, the text should be modified as recommended below.

Recommended Version:

“8.1 Pregnancy

Pregnancy Category B

Risk Summary

There are no studies with Vedolizumab in pregnant women. No fetal harm was observed in animal reproduction studies with intravenous administration of vedolizumab to rabbits and monkeys at dose levels (b) (4) times the recommended human dose. Because animal reproduction studies are not always predictive of human response, this drug should be used during pregnancy only if (b) (4)

Animal Data

A reproduction study has been performed in pregnant rabbits at single intravenous doses up to 100 mg/kg administered on gestation day 7 (about (b) (4) times the recommended human dose (b) (4)) and has revealed no evidence of impaired fertility or harm to the fetus due to vedolizumab. A pre and postnatal development study in monkeys showed no evidence of any adverse effect on pre and postnatal development at intravenous doses up to 100 mg/kg (about (b) (4) times the recommended human dose (b) (4)).

8.3. Nursing Mothers

Applicant's Version:

8.3 Nursing Mothers

(b) (4)

Evaluation: The text should be modified as proposed below.

Recommended Version:

“8.3 Nursing Mothers

It is unknown whether vedolizumab is present in human milk. Vedolizumab is detected in the milk of lactating monkeys. Exercise caution when administering vedolizumab to a nursing woman.”

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Applicant's Version:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

(b) (4)

Evaluation: The text should be modified as proposed below.

Recommended Version:

“13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals have not been performed to evaluate the carcinogenic potential of vedolizumab. Studies to evaluate the possible impairment of fertility or mutagenic potential of vedolizumab have not been performed.”

13.2 Animal Toxicology and/or Pharmacology

Applicant’s Version:

(b) (4)

Evaluation: This section is not necessary and should be deleted.

Recommended Version: None

2 Drug Information

2.1 Drug

CAS Registry Number: 943609-66-3

INN/USAN: Vedolizumab

Code Names: MLN0002, MLN02, LDP02

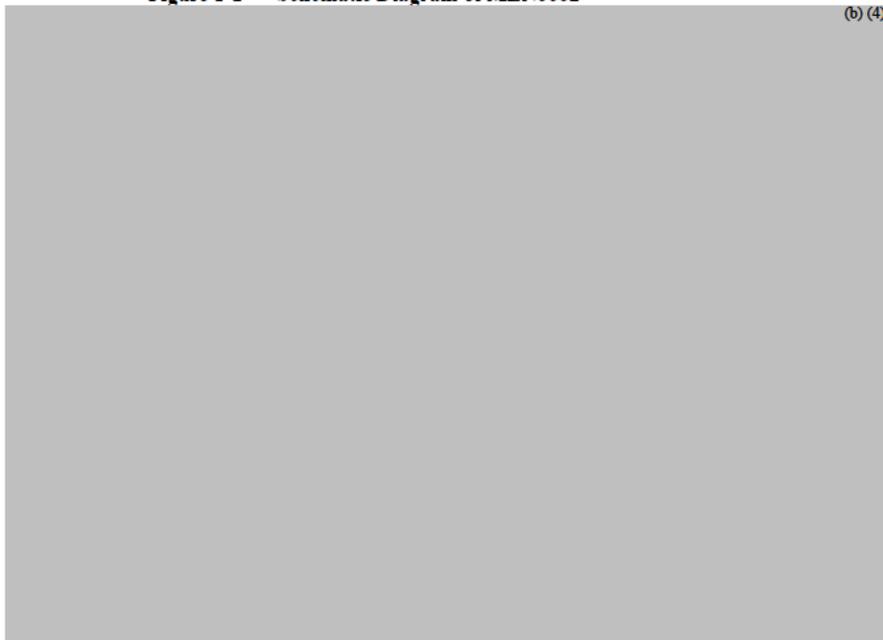
Chemical Name: Immunoglobulin G1, anti-(human integrin LPAM-1 (lymphocyte Peyer's patch adhesion molecule 1)) (human-Mus musculus heavy chain), disulfide with human-mus musculus κ -chain, dimer.

Molecular Weight: 146,837 Da

Structure or Biochemical Description: Vedolizumab is a recombinant humanized IgG1 monoclonal antibody to the human $\alpha 4 \beta 7$ integrin. It is composed of two (b) (4) light chains of the kappa (b) (4) and two (b) (4) heavy chains (b) (4). The subunits are linked together by four (b) (4) interchain disulfide bonds.

(b) (4) forming a Y-shaped structure that is typical of IgG1 immunoglobulins, as shown in Figure 1-1 (from page 2 of Section 2.3.S. of the submission).

Figure 1-1 Schematic Diagram of MLN0002



The predicted amino acid sequence of MLN0002 is shown below (from page 3 of Section 2.3.S. of the submission).

Figure 1-2 Complete Protein Sequence for MLN0002 Light and Heavy Chains



Pharmacologic Class: Integrin receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

- IND 09125 (MLN0002, Millennium Pharmaceuticals, Inc.)

2.3 Drug Formulation

The drug product is a sterile, lyophilized formulation supplied in a single use vial where each vial contains 300 mg of MLN0002. Sterile Water for Injection is to be used as a diluent for reconstitution. Reconstituted MLN0002 drug product contains (b) (4) of MLN0002, (b) (4) histidine (b) (4) (b) (4), (b) (4) L-arginine hydrochloride, (b) (4) sucrose, and (b) (4) polysorbate 80. The quantitative composition of the reconstituted drug product is presented in table (from page 1 of Section 2.3.P.1 of the submission).

Table 1-1 Quantitative Composition of MLN0002 Drug Product per Unit Dose (reconstituted)^a

Ingredients	Quantity per vial	Quality Standard	Function
MLN0002 antibody	(b) (4)	In-house	(b) (4)
L-histidine		USP, Ph. Eur.	
L-histidine monohydrochloride		Ph. Eur., F.C.C.	
L-arginine hydrochloride		USP, Ph. Eur., JP	
Sucrose		NF, Ph. Eur., JP	
Polysorbate 80		NF, Ph. Eur., JP	
Sterile Water for Injection		USP, Ph. Eur.	
(b) (4)	NA	NF, Ph. Eur.	

^a Includes a (b) (4) overfill (see [Module 3.2.P.2.2.1](#))

2.4 Comments on Novel Excipients

The excipients used in the MLN0002 drug product are listed in following table (from page 1 of Section 2.3.P.4). All excipients were compendial and no novel, human, or animal derived excipients or excipients prohibited for use in drug products were used in the manufacture of MLN0002 drug product.

Table 1-1 Specifications for Drug Product Excipients

Excipient	Compendial Reference	Function
L-histidine	USP, Ph. Eur.	(b) (4)
L-histidine monohydrochloride	Ph. Eur., F.C.C.	
L-arginine hydrochloride	USP, Ph. Eur., JP	
Sucrose	NF, Ph. Eur., JP	
Polysorbate 80	NF, Ph. Eur., JP	
(b) (4)	NF, Ph. Eur.	

2.5 Comments on Impurities/Degradants of Concern

No novel process or product related impurities were found in the drug product as compared to MLN0002 drug substance. The following table (from page 1 of Section 3.2.P.5.1) shows the specifications of the drug product.

Table 1-1 Tests and Acceptance Criteria for MLN0002 Drug Product

Attribute	Test	Acceptance Criteria
Identity	Charge Profile by Isoelectric Focusing	Conforms to Reference Standard
	Charge Profile by Cation-Exchange Chromatography	Conforms to Reference Standard
Purity/Impurity	SDS-PAGE (Reduced)	<ul style="list-style-type: none"> Conforms to Reference Standard Heavy + Light chain (b) (4)
	SDS-PAGE (Non-reduced)	Conforms to Reference Standard
	Charge Profile by Cation-Exchange Chromatography	Major (b) (4)
	% Monomer, % Aggregates by Size-Exclusion Chromatography	<ul style="list-style-type: none"> Monomer (b) (4) Aggregates (b) (4)
Potency	HuT78 Binding Assay	(b) (4) of Reference Standard
	Assay by UV	(b) (4) of label claim
Safety	Bacterial Endotoxin	(b) (4) MLN0002
	Sterility	No growth
General	pH	6.3 (b) (4)
	Appearance - Cake	White to off-white cake or powder
	(b) (4) - Cake	(b) (4)
	Reconstitution Time -Cake	≤ 30 minutes
	Clarity and Color of Solution	Clear or opalescent, colorless to brownish-yellow solution, essentially free of foreign matter
	Content Uniformity	Meets USP/EP AV (b) (4)
	Particulate Matter	Meet USP/EP

2.6 Proposed Clinical Population and Dosing Regimen

MLN0002 is indicated for the treatment of UC and CD in adult patients. The proposed indications are as follows:

Adult Ulcerative Colitis:

Vedolizumab is indicated for reducing signs and symptoms, inducing and maintaining clinical response and remission, and mucosal healing, and achieving corticosteroid-free remission in adult patients with moderately to severely active ulcerative colitis who have

had an inadequate response with, lost response to, or were intolerant to either conventional therapy or a tumor necrosis factor-alpha (TNF α) antagonist.

Adult Crohn's disease:

Vedolizumab is indicated for reducing signs and symptoms, inducing and maintaining clinical response and remission, and achieving corticosteroid-free remission in adult patients with moderately to severely active Crohn's disease who have had an inadequate response with, lost response to, or were intolerant to either conventional therapy or a tumor necrosis factor-alpha (TNF α) antagonist.

The proposed recommended dose regimen is 300 mg administered by IV infusion at zero, two and six weeks and every eight weeks thereafter.

2.7 Regulatory Background

The clinical development program of MLN0002 for UC and CD was initiated under IND 9125 and has received fast track designation on February 22, 2013. The FDA has accepted a rolling submission for this BLA. The first portion of the BLA rolling submission containing chemistry, manufacturing, and controls (CMC) information was submitted on March 27, 2013. The second portion of the BLA rolling submission containing nonclinical information was submitted on April 8, 2013. The third and final portion of the rolling BLA containing all information related to clinical efficacy and safety was submitted on June 20, 2013. The following are the major milestones:

1. Gastrointestinal Drugs Advisory Committee (GIDAC) meeting (IND 9125): July 20, 2011
1. Type C End of Phase 3 meeting on July 24 and 25, 2012
2. Type B pre BLA meeting on November 6, 2012

3 Studies Submitted

The following table in Section 3.1 shows the list of studies submitted in this application.

3.1 Studies Reviewed

The following table shows the list of studies reviewed.

STUDY TITLE	REPORT NO.	PAGE
PHARMACOLOGY		17
PHARMACOKINETICS		57
A Multiple Dose Pharmacokinetic/Pharmacodynamic Study of MLN0002 Administered Intravenously in Cynomolgus Monkeys	KLA00184-05-901	57
Pharmacokinetics of MLN0002 following Intra venous and Subcutaneous administration to Cynomolgus Monkeys	LDP-02-14	66
A Single Dose Pharmacokinetic, Pharmacodynamic, and Immunogenicity Study of MLN0002 Administered Intravenously to Cynomolgus Monkeys	RPT-00892	67
TOXICOLOGY		69
Acute		69

Monkey		69
An Acute Intravenous Infusion Toxicology Study of MLN0002 Manufactured Using Process B and Process C in Cynomolgus Monkeys	KLA00348	69
Subacute/Subchronic/Chronic		71
Monkey		71
14-Day Repeated dose Toxicity Study of MLN0002 Administered via Intravenous Infusion to Cynomolgus Monkeys	LDP-02-15	71
13-Week Intravenous Injection Toxicity Study of MLN0002 in Cynomolgus Monkeys with a 12-Week Recovery Period	502045	80
3-Month Pharmacokinetic and Safety Study of MLN0002 in Cynomolgus Monkeys	KLAW-111-01-607	91
26-Week Toxicity Study of MLN0002 Administered by Intravenous Infusion to Cynomolgus Monkeys, with a 12-Week Recovery Period	KLA00290	101
Rabbit		
3-Month Intravenous Infusion Toxicity and Toxicokinetics Study of MLN0002 with a 4 Week Recovery Period in Rabbits	(b) (4)-416055	110
REPRODUCTIVE TOXICITY		124
Rabbit		124
Intravenous Infusion Dose Range-Finding Study of the Effects of MLN0002 on Embryo/Fetal Development in Rabbits	(b) (4)-416036	124
Intravenous Infusion Study of the Effects of MLN0002 on Embryo/Fetal Development in Rabbits	(b) (4)-416044	125
Monkey		131
Intravenous Infusion Study for Effects on Pre- and Postnatal Development in Cynomolgus Monkeys	2091-003	131
LOCAL TOLERANCE		135
Single-Dose Local Toxicity Study of MLN0002 Administered Either Subcutaneously or Intramuscularly to Male New Zealand White Rabbits	(b) (4)-416096	135
SPECIAL TOXICOLOGY		137
3-Week Comparative Immunotoxicity Study of Natalizumab (Tysabri®) and Vedolizumab (MLN0002) Administered by Intravenous Infusion to Cynomolgus Monkeys	20002458	137
Single Dose TDAR Study in Cynomolgus Monkeys with Natalizumab (Tysabri®)"	KLA00441	138
Delayed-Type Hypersensitivity Responses, Anti-Tetanus Antibody Titers and Laboratory Assay Results in Act-1-Treated Rhesus Monkeys	RPT-02275	141
OTHER		142
Cross Reactivity Study of MLN0002 with Normal Human Tissues	IM1493	142
Cross-Reactivity Study of MLN0002 with Normal Cynomolgus Monkey Tissues	IM1741	143
Study of Binding Specificity of ACT-1 in Malignant Human Tumors	IM1806	144
Preliminary Studies of Cross Reactivity of a Humanized Monoclonal Antibody, MLN0002	IM353	145
Cross Reactivity of Mouse Monoclonal Antibody ACT-1 with Normal Human Tissues	IM433	146
Cross Reactivity of Humanized Monoclonal IgG1 LDP-02/3A9-8E3.C1 Antibody with Normal Human Tissues	IM434	146
Effect of ACT-1 Antibody on the Growth of the Alpha 4 Beta 7-Expressing RPMI 8866 Human B-Cell Lymphoma Cell Line	RPT-01335	147

3.2 Studies Not Reviewed

The following analytical methods and validation and PK modeling reports were not reviewed.

- Validation of a Flow Cytometry Method for the Beckman Coulter FC500 Flow Cytometer using CD4/CD45RA/CD8/CD3 in Cynomolgus Monkey Whole Blood (20002484)
- Monkey Serum Validation Study: Determination of MLN0002 in Monkey Serum by ELISA (Amendment #1, 96-0606)
- Monkey Serum Validation Study: Detection of Primate Anti-Human Antibodies to MLN0002 In Cynomolgus Monkey Serum by ELISA (96-0607)
- Rabbit Serum Validation Study: Determination of MLN0002 in Rabbit Serum by ELISA (96-0611)
- Rabbit Serum Validation Study: Detection of Rabbit Anti-Human (MLN0002) Antibodies in Rabbit Serum by ELISA (96-0612)
- Determination of MLN0002 in Monkey Breast Milk by ELISA (96-0723)
- Development of a Test Method for the Quantitation of MLN02 in Cynomolgus Monkey Serum using Anti-1D9 Idiotypic Antibody-coated Plates (RPT-00848)
- Development of a Test Method for the Qualitative Determination of Primate Anti-Human (MLN02) Antibodies (PAHA) in Cynomolgus Monkey Serum Using an Enzyme-Linked Immunosorbent Assay (ELISA, RPT-00849)
- Development and Qualification of a Test Method for the Quantitation of Natalizumab in Rhesus and Cynomolgus Macaque Serum by ELISA Assay (RPT-01448)
- Development and Qualification of an Enzyme-Linked Immunosorbent Assay for the Detection of Anti-Human Antibodies to Natalizumab in Rhesus and Cynomolgus Macaque Serum (RPT-01536)
- Development and Qualification of an Enzyme-Linked Immunosorbent Assay for the Quantitation of MLN0002 in Rhesus Macaque Serum (RPT-01682)
- Development and Qualification of an Enzyme-Linked Immunosorbent Assay for the Detection of Primate Antihuman Antibodies to MLN0002 in Rhesus Macaque Serum (RPT-01683)
- Validation of a Flow Cytometric Assay for Monitoring Bound Humanized IgG1 Monoclonal Antibody (MLN0002) on CD4⁺/CD45RA⁻ and CD4⁺/CD45RA⁺ Lymphocytes in the Peripheral Blood of Cynomolgus Monkeys (RPT-FCV-442-01)
- Validation of a Flow Cytometric Assay to Measure MLN0002 Free Sites on CD4⁺/CD45RA⁻ and CD4⁺/CD45RA⁺ T-cells and CD20⁺ B-cells from Whole Blood of Cynomolgus Monkeys (RPT-FCV443-01)
- Validation of a Flow Cytometric Assay for Monitoring Saturation of a Humanized IgG1 Monoclonal Antibody [MLN0002] (Bound drug) on CD4⁺ Lymphocytes in the Peripheral Blood of New Zealand White Rabbits (RPT-FCV470-01)
- Validation of an Assay to Measure a4b7 Free Sites on Leukocytes from Whole Blood of New Zealand White Rabbits by Flow Cytometry in the Presence of MLN0002 (RPT-FCV471-01)

- Pharmacokinetic modeling of LDP-02 (ML-LEU-3K88-97-267)

3.3 Previous Reviews Referenced

The following reviews are incorporated below from the pharmacology review of IND 09125 dated April 11, 2008.

4 Pharmacology

4.1 Primary Pharmacology

The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 065 dated April 11, 2008.

PHARMACOLOGY

Comparison of the Relative Potencies of Multiple Lots of MLN0002 and Act-1 in Competition Binding Assays in Human Whole Blood Lymphocytes (CMP-06-0004)

The objective of this study was to establish comparability between Chinese hamster ovary (CHO)-derived MLN0002 and mouse myeloma cell line (NSO)-derived MLN0002 (formerly called MLN02), which was used in previous studies. In this study, comparability was demonstrated by determining the relative potencies of multiple lots of MLN0002 and mouse anti-human α 4 β 7 as competitors with labeled MLN0002 or Act-1 for binding to the α 4 β 7 integrin on peripheral blood lymphocytes in human whole blood. In this study, human whole blood was stained with labeled MLN0002 or Act-1 with or without increasing concentrations of competitor antibodies, and expression of α 4 β 7 integrin on B and CD4 memory T lymphocytes was examined by flow cytometry.

Mean IC₅₀ values for the inhibition of either MLN0002-Alexa-647 or Act-1-biotin binding to B and memory CD4 T lymphocytes by MLN0002 were found to be within a range of 0.045 to 0.060 μ g/mL (0.3 to 0.4 nM) for all antibodies. The range of Act-1 mean IC₅₀ values was 0.059 to 0.078 μ g/mL (0.39 to 0.52 nM).

Assessment of the Potential Binding of MLN0002 and ACT-1 to the Alpha 4 Beta 7 Integrin on Peripheral Blood Mononuclear Cells of the Mouse, Rat, Guinea Pig, Rabbit, and Cynomolgus Monkey (RPT-00709)

The objective of this study was to determine the species cross-reactivity of MLN0002. In this study, human, mouse, rat, guinea pig, rabbit, and cynomolgus monkey whole blood were stained with MLN0002 and ACT-1 (the murine antibody from which MLN0002 was derived) and expression of α 4 β 7 integrin on lymphocytes was examined by flow cytometry.

Staining with MLN0002 and ACT-1 identified α 4 β 7 integrin bearing lymphocytes in the rabbit, cynomolgus monkey, and human blood samples, but failed to specifically stain α 4 β 7 integrin bearing lymphocytes in mouse, rat, or guinea pig blood samples.

Determination of the Binding Affinity of CHO-Derived MLN0002 to the Alpha4 Beta7 Integrin on Peripheral Blood Mononuclear Cells of Human and Cynomolgus Monkey (RPT-01052)

The objective of this study was to determine and compare the binding affinity of CHO-derived MLN0002 for the α 4 β 7 integrin on peripheral blood lymphocytes of Cynomolgus monkey and human whole blood. In this study, human and cynomolgus monkey whole blood was stained with labeled MLN0002 and competition (with MLN0002) binding experiments were conducted at saturating conditions. Expression of α 4 β 7 integrin on B or memory CD4 lymphocytes was examined by flow cytometry. The effective concentration 50% (EC₅₀) (concentration of labeled antibody which yields 50% of maximal staining) or the inhibition concentration 50% (IC₅₀)

(concentration of test antibody that inhibits binding by 50 percent) for monkey and human was determined from this binding experiment.

Mean EC50 values for binding of MLN0002-Alexa-647 to either B or memory CD4 lymphocytes were 0.055 µg/mL (0.37 nM) and 0.060 µg/mL (0.4 nM) for monkey and human B lymphocytes, respectively, and 0.058 µg/mL (0.39 nM) and 0.049 µg/mL (0.33 nM) for monkey and human memory CD4 lymphocytes, respectively. Mean IC50 values for inhibition of MLN0002-Alexa-647 binding to either B or memory CD4 T lymphocytes by MLN0002 were 0.059 µg/mL (0.39 nM) and 0.060 µg/mL (0.4 nM) for monkey and human B cells, respectively, and 0.057 µg/mL (0.37 nM) and 0.055 µg/mL (0.38 nM) for monkey and human memory CD4 T cells, respectively.

The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 075 dated July 14, 2008.

Selectivity of Binding of MLN0002 for $\alpha 4\beta 7$ versus $\alpha 4\beta 1$ and $\alpha E\beta 7$ (Report No. 01097)

The objective of this study was to demonstrate specificity of Chinese hamster ovary (CHO)- cell derived MLN0002 for binding to the human $\alpha 4\beta 7$ integrin vs. the human $\alpha 4\beta 1$ or $\alpha E\beta 7$ integrins utilizing cell lines selectively expressing only one of these integrins. Using antibodies specific for the integrin subunits $\alpha 4$, αE , $\beta 1$ and $\beta 7$ it was demonstrated that: 1) RPMI 8866 cells (a human B cell lymphoma-derived cell line) expressed $\alpha 4$ and $\beta 7$ but not $\beta 1$ or αE ; 2) RAOS cells (a human B cell lymphoma-derived cell line) expressed $\alpha 4$ and $\beta 1$ but not $\beta 7$ or αE and 3) $\alpha 4\beta 7$ -L1.2-(murine pre-B cell lymphoma) cell transfectants expressed $\alpha 4$ and $\beta 7$ but not $\alpha 4$ or $\beta 1$. CHO-cell-derived MLN0002 bound to RPMI8866 cells but not to RAOS or $\alpha 4\beta 7$ -L1.2 cell transfectants. Based on these, CHO-cell derived MLN0002 appeared to bind to $\alpha 4\beta 7$ but not $\alpha 4\beta 1$ or $\alpha E\beta 7$.

Selectivity of MLN0002 for Inhibition of $\alpha 4\beta 7$ -Mediated Cell Adhesion Interactions (Report No. 01098)

The objective of this study was to examine the specificity, selectivity, and potency with which MLN0002 blocked the adhesive interactions mediated by the binding of the human integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$ to the human cell adhesion molecules mucosal addressin cell adhesion molecule-1 (MAdCAM-1), vascular cell adhesion molecule-1 (VCAM-1), or fibronectin. In this study, cell lines only expressing either $\alpha 4\beta 1$ (RAOS) or $\alpha 4\beta 7$ (RPMI8866) were evaluated for their ability to adhere to plate-bound human MAdCAM-1, VCAM-1, or fibronectin under low affinity [no manganese (Mn^{+2})] or high affinity (1 mM Mn^{+2}) integrin activation conditions. Chinese hamster ovary (CHO)-derived MLN0002 and anti- $\alpha 4$ antibodies were then evaluated for their ability to block the adhesive interactions. The inhibitory potencies of MLN0002 and anti- $\alpha 4\beta 4$ antibodies were calculated from curves of the antibody concentration versus the extent of inhibition.

MLN0002 inhibited RPMI8866 cell binding, and hence $\alpha 4\beta 7$ binding, to MAdCAM-1 and fibronectin, but did not inhibit binding of $\alpha 4\beta 7$ to VCAM-1 or RAOS cell binding, and hence $\alpha 4\beta 1$ binding, to VCAM-1 or fibronectin. This selectivity was maintained up to the highest concentration tested (400 $\mu g/mL$), a concentration approaching the maximum concentration expected to be reached in human subjects. In contrast, anti- $\alpha 4$ antibody inhibited all interactions. The IC_{50} of $\alpha 4\beta 7$ binding to MAdCAM-1 was 0.023 $\mu g/mL$ (0.152 nM) and 0.058 $\mu g/mL$ (0.389 nM) in the absence and presence, respectively, of 1 mM Mn^{+2} . The IC_{50} for anti- $\alpha 4$ inhibition of $\alpha 4\beta 7$ binding to MAdCAM-1 was 0.021 $\mu g/mL$ (0.143 nM) and 0.069 $\mu g/mL$ (0.460 nM) in the absence and presence, respectively, of 1 mM Mn^{+2} . The IC_{50} for inhibition of $\alpha 4\beta 7$ binding to fibronectin was 0.020 $\mu g/mL$ (0.136 nM) and 0.023 $\mu g/mL$ (0.153 nM) for MLN0002 and anti- $\alpha 4$, respectively. The IC_{50} for anti- $\alpha 4$ inhibition of $\alpha 4\beta 7$ binding to VCAM-1 was 0.034 $\mu g/mL$ (0.227 nM) and 0.128 $\mu g/mL$ (0.853 nM) in the absence and presence of 1 mM Mn^{+2} , respectively. The IC_{50} for anti- $\alpha 4$ inhibition of $\alpha 4\beta 1$ binding to

VCAM-1 was 0.038 $\mu\text{g/mL}$ (0.256 nM) and 0.473 $\mu\text{g/mL}$ (3.153 nM) in the absence or presence of 1 mM Mn^{+2} , respectively. The sponsor stated that accurate IC50 values for anti- $\alpha 4$ inhibition of $\alpha 4\beta 1$ binding to fibronectin could not be calculated because of experimental variability.

In summary, MLN0002 was found to be an inhibitor of cellular adhesion mediated by interactions between $\alpha 4\beta 7$ and MAdCAM-1 or fibronectin. MLN0002 did not inhibit $\alpha 4\beta 7$ -VCAM-1, $\alpha 4\beta 1$ -VCAM-1, or $\alpha 4\beta 1$ -fibronectin-mediated adhesive interactions.

Selectivity of Binding of MLN0002 for $\alpha 4\beta 7$ versus $\alpha 4\beta 1$ in Human Whole Blood (Report No. 01099)

This study was conducted to examine the human whole blood leukocyte expression patterns of the integrins $\alpha 4\beta 1$ and $\alpha 4\beta 7$. Specifically, it examined the co-expression of both integrins in leukocyte subtypes and provided a quantitative assessment of the expression of both integrins across all leukocyte subsets in side-by-side comparisons. It also demonstrated the specificity of CHO-derived MLN0002 for $\alpha 4\beta 7$ in human whole blood. In this study, blood from 5 normal human volunteers was stained with saturating concentrations of various combinations of antibodies against $\alpha 4$, $\beta 1$, leukocyte-specific markers, and MLN0002 or Act-1 (the murine antibody from which MLN0002 was derived). Expression was examined by flow cytometry.

The specificity of CHO-derived MLN0002 for $\alpha 4\beta 7$ versus $\alpha 4\beta 1$ in human whole blood was demonstrated by the following results: 1) the human whole blood patterns of staining of Act-1 and CHO-derived MLN0002 were identical; 2) both of these antibodies completely cross-competed with each other; and 3) there was a significant degree of overlap in the expression of $\alpha 4\beta 1$ and $\alpha 4\beta 7$. Subsets of leukocytes with high expression of $\alpha 4\beta 1$ and low or no expression of $\alpha 4\beta 7$ (as determined by MLN0002 binding) suggested that MLN0002 does not bind to $\alpha 4\beta 1$ even at concentrations (400 $\mu\text{g/mL}$) approaching the maximum concentrations projected to be reached in the clinic.

The expression and co-expression of $\alpha 4\beta 1$ and $\alpha 4\beta 7$ was studied in human whole blood leukocytes, including monocytes, neutrophils, eosinophils, Band T ($\text{CD}4^+$, $\text{CD}8^+$, naive, and memory) lymphocytes, basophils, and natural killer (NK) cells. While all monocytes expressed $\alpha 4\beta 1$ at high levels, MLN0002 bound to only 15% of these cells, and expression of $\alpha 4\beta 7$ in these positive monocytes was low. Eosinophils expressed high levels of both integrins, while expression of both integrins was absent in neutrophils. All lymphocyte-gate cells (approximately 93% overall) expressed $\alpha 4\beta 1$ at various levels, while MLN0002 bound to approximately 60% of these cells, with various levels of $\alpha 4\beta 7$ expression in the specific lymphocyte subpopulations. Within this gate, expression of both integrins was examined in Band T ($\text{CD}4^+$, $\text{CD}8^+$, naive, and memory) lymphocytes, basophils and NK cells. Expression of $\alpha 4\beta 1$ was found in approximately 80% of memory $\text{CD}4^+$ T lymphocytes and in practically all of the rest of the lymphocyte gate subpopulations indicated above.

Expression of $\alpha 4\beta 7$ was homogeneously positive and intermediate to low in intensity in the vast majority of naive, $\text{CD}4^+$ and $\text{CD}8^+$ T lymphocytes, $\text{CD}19^+$ B lymphocytes, basophils, and $\text{CD}56^+$ NK cells. Approximately 35% of memory $\text{CD}4^+$ and $\text{CD}8^+$ T lymphocytes expressed $\alpha 4\beta 7$. In

these memory lymphocytes, the $\alpha 4\beta 1$ subsets comprised approximately 25% and 9%, respectively, of the CD^{4+} and CD^{8+} memory T lymphocytes.

Further examination of expression in all possible $\alpha 4\beta 1$ expression subsets in the CD4 and CD8 populations indicated that $\alpha 4\beta 1$ expressing cells segregated within the $\alpha 4^{hi}\beta 1^{lo}$ subset. The $\alpha 4\beta 7^{hi}$ and $\alpha 4\beta 1^{hi}$ subsets were mutually exclusive. The $\alpha 4\beta 1^{hi}$ subset of memory cells, which included approximately 70% of all $\alpha 4^{+}$ memory CD^{4+} T lymphocytes, was mostly negative for MLN0002 binding. Conversely, the $\alpha 4\beta 1^{lo}$ subset of memory CD^{4+} T lymphocytes, which included approximately 30% of all $\alpha 4^{+}$ memory CD^{4+} T lymphocytes, was bound by MLN0002 and included the vast majority of $\alpha 4\beta 7^{hi}$ CD^{4+} T lymphocytes. A similar correlation was found with memory CD^{8+} T lymphocytes. The $\alpha 4\beta 7^{hi}$ cells were enriched in the subset of memory CD^{8+} T lymphocytes with the $\alpha 4\beta 1^{lo}$ phenotype, which represented approximately 25% of all memory CD^{8+} lymphocytes. Because $\alpha 4\beta 1$ is so widely expressed, $\alpha 4\beta 7$ expressing cells also co-expressed $\alpha 4\beta 1$. Lack of cross-reactivity of MLN0002 to $\alpha 4\beta 1$ was demonstrated on those subsets of leukocytes that expressed high level of $\alpha 4\beta 1$ but not $\alpha 4\beta 7$, such as monocytes and memory T lymphocytes of the $\alpha 4\beta 1^{hi}$ phenotype. While 80% of memory CD^{4+} T lymphocytes and all memory CD^{8+} T lymphocyte expressed $\alpha 4\beta 1$, only 35% of either subset expressed $\alpha 4\beta 7$.

Overall, these experiments showed that the whole blood pattern of expression of $\alpha 4\beta 7$ is more restricted than that of $\alpha 4\beta 1$. MLN0002 appears to bind selectively to the $\alpha 4\beta 7$ integrin, and not bind to the $\alpha 4\beta 1$ integrin. These results indicated that a dual $\alpha 4$ antagonist could potentially inhibit $\alpha 4\beta 1$ or $\alpha 4\beta 7$ -mediated migration in 80% of memory CD^{4+} T lymphocytes and all memory CD^{8+} T lymphocytes, while MLN0002 would probably inhibit $\alpha 4\beta 7$ -mediated migration in 35% of memory CD^{4+} and CD^{8+} T lymphocytes.

Comparison of the Binding Affinity of CHO-Derived MLN0002 to the $\alpha 4\beta 7$ Integrin on Human and Rabbit Peripheral Blood Lymphocytes (Report No. 01101)

The objective of this study was to determine and compare the binding affinity of CHO-derived MLN0002 for the $\alpha 4\beta 7$ integrin on peripheral blood lymphocytes of rabbit and human whole blood. In this study, human or rabbit whole blood was stained with labeled MLN0002 in saturation or competition (with MLN0002) binding experiments and expression of $\alpha 4\beta 7$ on B or CD^{4+} T lymphocytes was examined by flow cytometry. The effective concentration 50% (EC50) (concentration of labeled antibody which yields 50% of maximal staining) or the inhibition concentration 50% (IC50) (concentration of test antibody that inhibits by 50 percent binding of the labeled antibody used at its EC50) values for rabbit and human were determined from saturation or competition binding curves.

Mean EC50 values for binding of MLN0002 to either B or CD^{4+} T lymphocytes were 0.031 $\mu\text{g}/\text{mL}$ (0.21 nM) and 0.062 $\mu\text{g}/\text{mL}$ (0.41 nM) for rabbit and human B lymphocytes, respectively, and 0.019 $\mu\text{g}/\text{mL}$ (0.13 nM) and 0.047 $\mu\text{g}/\text{mL}$ (0.31 nM) for rabbit and human CD^{4+} T lymphocytes, respectively. Mean IC50 values for inhibition of MLN0002 binding to either B or CD^{4+} T lymphocytes by MLN0002 were 0.039 $\mu\text{g}/\text{mL}$ (0.26 nM) and 0.051 $\mu\text{g}/\text{mL}$ (0.34 nM) for rabbit and human B cells, respectively, and 0.063 $\mu\text{g}/\text{mL}$ (0.42 nM) and 0.070 $\mu\text{g}/\text{mL}$ (0.47 nM) for rabbit and human CD^{4+} T lymphocytes, respectively. Overall, all average

EC50 and IC50 values for binding to either human or rabbit B or CD⁴⁺ T lymphocytes were in the range of 0.13 to 0.47 nM.

The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 084, 209 and 317 dated April 29, 2011.

Effect of MLN0002 on Antibody-Dependent Cell-Mediated Cytotoxicity (ADCC) of Alpha 4 Beta 7-Expressing RPMI 8866 Cells (RPT-01138)

Methods: Antibody-dependent cell-mediated cytotoxicity (ADCC) has been proposed to be an important mechanism of *in vivo* cytotoxic action for many monoclonal antibodies (mAbs). The objective of this study was to demonstrate whether MLN0002 is capable of inducing ADCC *in vitro* in Roswell Park Memorial Institute (RPMI) 8866 cells (a human B cell lymphoma-derived cell line), which express $\alpha 4\beta 7$.

ADCC is the killing of antibody-coated target cells by effector cells that express Fc receptors that recognize the Fc portion of the coating antibody. Cytotoxicity of MLN0002-coated cells would be expected to result in a significant decrease in lymphocyte counts. MLN0002 was designed to abrogate binding to Fc receptors and consequently to abrogate effector cytotoxic function so that it does not cause any ADCC. Lack of cytotoxicity is consistent with the mutations introduced in MLN0002 to abrogate binding to Fc receptors in order to eliminate ADCC. The predominant effector cell that mediates ADCC is the natural killer (NK) cell; however, monocytes, macrophages, eosinophils, and neutrophils can also mediate this function. NK cells express the Fc γ Receptor III (Fc γ RIII), which binds to the Fc domain of IgG antibodies bound to target cells and mediates cell lysis. The cytotoxicity mediated by NK cells is a major mechanism of action proposed for the *in vivo* elimination of tumor cells by therapeutic mAbs such as Rituximab (Rituxan[®]). Rituximab (a chimeric anti-CD20 mAb IgG1 isotype) mediates ADCC against normal and malignant B cells expressing the cell-surface molecule CD20.

The current study was performed to examine the mode of action of MLN0002, and to confirm abrogation of effector cytotoxic function. The ability of human peripheral blood mononuclear cells or PBMCs (which include NK cells) to lyse α 4 β 7-expressing RPMI 8866 cells previously treated with MLN0002, Act-1, or Rituxan was examined. In this study, cytotoxicity was measured using a cytotoxicity assay kit. The assay measured lactate dehydrogenase (LDH) released from RPMI 8866 cells incubated with isolated PBMCs and an antibody (MLN0002, Act-1, or Rituxan as a positive control) at various concentrations.

Results: Figure 2 (from page 20 of the study report) shows the average percent cytotoxicity for all three experiments. Mean baseline cytotoxicity ranged from 20.9% to 27.6% (n = 3). Rituxan increased cytotoxicity over the baseline in a concentration-dependent manner, confirming that ADCC occurred under the experimental conditions. Baseline cytotoxicity or cytotoxicity in the presence of Rituxan required the presence of both target and effector cells, as shown by the very low levels of cytotoxicity observed when either target or effector cells alone were incubated with 10 μ g/mL of Rituxan. In contrast, no cytotoxicity was induced (no increase over the baseline) by either Act-1 or MLN0002 at 10 μ g/mL (Figure 2), which is approximately 100- to 200-fold higher than the concentration needed for saturation binding of MLN0002 to RPMI 8866 cells. These results demonstrated that MLN0002 does not mediate ADCC *in vitro*.

Figure 2 Effect of MLN0002, Act-1, and Rituxan® on Cytotoxicity of RPMI 8866 Cells in the Presence of Peripheral Blood Mononuclear Cells

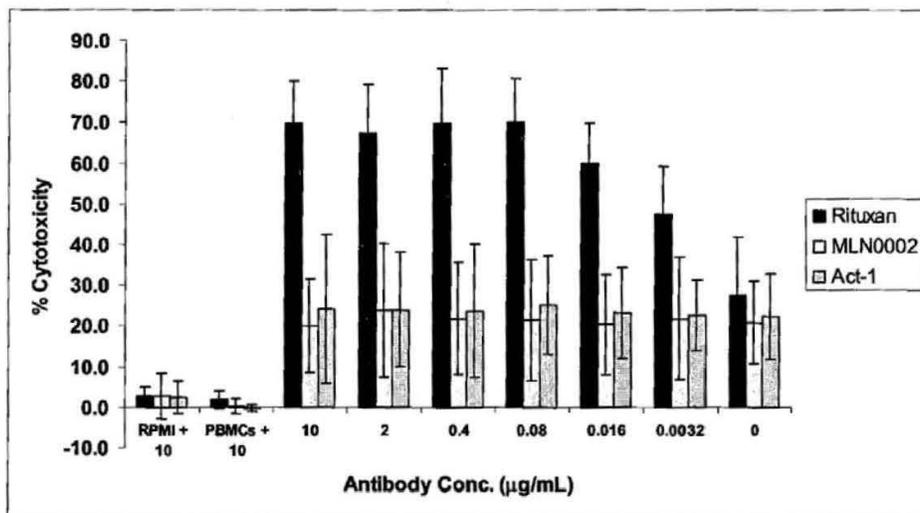


Figure 2: Data are from 3 separate experiments; bars represent means ± SDs.

Values (Means of 3 Separate Experiments With Standard Deviation) Used to Construct Figure 2

Antibody Conc. (µg/mL)	% Cytotoxicity					
	Rituxan®		MLN0002		Act-1	
	Mean	SD	Mean	SD	Mean	SD
RPMI + 10 ^a	2.6	2.4	2.6	5.5	2.3	4.1
PBMC + 10 ^b	1.8	2.1	0.4	1.9	-0.3	0.8
10	69.9	10.1	20.0	11.5	24.2	18.3
2	67.4	11.7	23.8	16.5	23.9	14.1
0.4	69.8	13.4	21.8	13.7	23.6	16.3
0.08	70.1	10.7	21.3	14.9	25.1	12.0
0.016	60.0	9.9	20.4	12.3	23.3	11.3
0.0032	47.4	11.9	21.8	14.9	22.6	8.7
0	27.6	14.4	20.9	10.2	22.2	10.5

SD = standard deviation.

Note: Means and standard deviations (SD) are from 3 independent experiments. The means (of triplicate determinations) of each independent experiment used to calculate the means and SD used in this figure are in the tables below.

a RPMI + 10 refers to samples of target (RPMI 8866) cells alone (no PBMCs) and 10 µg/mL of antibody. The values for this combination represent the means of 2 experiments from 11 Sep 07 and 18 Sep 07; this combination was not tested on 23 Aug 07.

b PBMC + 10 refers to samples of effector cells (PBMCs) alone (no RPMI 8866 cells) and 10 µg/mL of antibody.

Effect of MLN0002 on Complement-Dependent Cytotoxicity (CDC) of Human Peripheral Blood Mononuclear Cells (PBMCs) in Vitro (RPT-01139)

Complement-dependent cytotoxicity (CDC) has been proposed as a mechanism of *in vivo* cytotoxic action for many monoclonal antibodies (mAbs). The objective of this study was to demonstrate whether MLN0002 is capable of inducing CDC in human peripheral blood mononuclear cells (PBMCs) *in vitro*. In this study, lactate dehydrogenase (LDH) release from PBMCs incubated with rabbit complement in the presence of MLN0002, OKT3 (positive control), or human IgG1/K (MLN1202, the isotype control for MLN0002) were measured at various concentrations using a cytotoxicity assay kit.

OKT3, a murine mAb against human CD3 known to mediate CDC of lymphocytes *in vitro*, induced CDC in PBMC preparations in a dose-related manner. Cytotoxicity was observed at 0.08 µg/mL (17.2%, n = 3), with maximal release occurring at 10 µg/mL (28.2%, n = 3). In contrast, no cytotoxicity was observed in the presence of MLN0002 or IgG1 isotype control at a concentration of 10 µg/mL. The results are shown in the following Figure-2 (from page 17 of the study report). This concentration (10 µg/mL) is approximately 20-fold greater than the concentration needed for the saturation binding of MLN0002 to human whole blood cells, as assessed by competition binding analysis. These results suggested that MLN0002 does not mediate CDC *in vitro* in PBMCs.

Figure 2 Effect of MLN0002, OKT3IgG2a, and Human IgG1/k on Cytotoxicity of Isolated Peripheral Blood Mononuclear Cells (PBMCs) Treated With Rabbit Complement

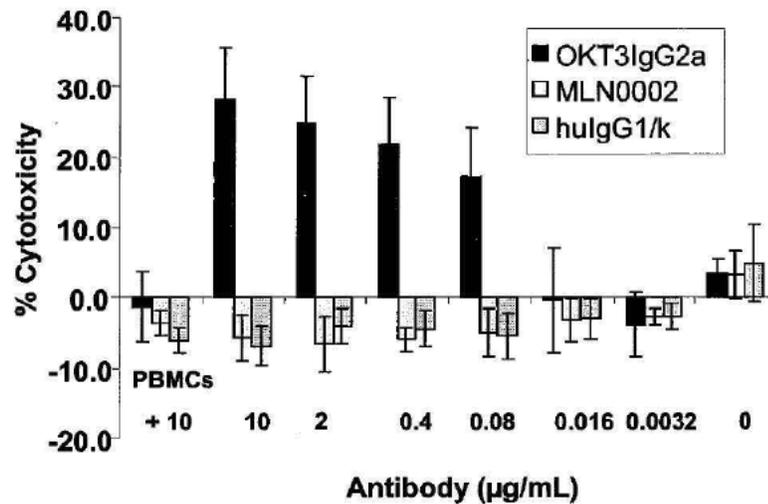


Figure 2: Data are from 3 separate experiments; bars represent means \pm SDs.

Values (Means of 3 Separate Experiments With Standard Deviation) Used to Construct Figure 2

Antibody Conc. (µg/mL)	% Cytotoxicity					
	OKT3IgG2a		MLN0002		huIgG1/k	
	Mean	SD	Mean	SD	Mean	SD
PBMC + 10 ^a	-1.3	4.9	-3.4	1.8	-6.0	1.9
10	28.2	7.5	-5.6	3.2	-6.8	2.9
2	24.8	6.8	-6.5	3.9	-4.0	2.5
0.4	21.7	6.8	-5.9	1.7	-4.4	2.6
0.08	17.2	7.0	-4.8	3.4	-5.4	3.2
0.016	-0.4	7.5	-3.1	3.1	-3.0	2.8
0.0032	-3.8	4.5	-2.7	1.1	-2.6	1.9
0	3.4	2.2	3.3	3.4	4.9	5.5

SD = standard deviation.

a PBMC + 10 refers to samples that had PBMCs and 10 µg/mL of the indicated antibody, but no complement. All other samples had PBMC, complement, and the indicated antibody concentration.

Evaluation of the Effect of MLN0002 on Cytokine Release and T Lymphocyte Activation Upon Incubation in Diluted Human Whole Blood (RPT-01140)

Previous studies have shown that MLN0002 blocks the binding of MADCAM-1 and fibronectin, but not VCAM-1, to $\alpha_4\beta_7$. MLN0002 lacks the cytotoxic effector functions antibody-dependent cell-mediated cytotoxicity (ADCC) and CDC. In addition, MLN0002 did not show any potential to induce lymphocyte or monocyte activation. The objective of the current study was to demonstrate whether MLN0002 has the potential to mediate cytokine release or T lymphocyte activation. This was evaluated by incubating 400 $\mu\text{g/mL}$ of antibody in diluted whole human blood samples at 37°C for up to 24 hours and evaluating activation by measuring cytokine release into the processed plasma and the expression of T lymphocyte activation cell surface markers.

MLN0002 did not increase the concentrations of the following cytokines: IF γ , TNF α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-12 (P70), IL-12 (P40), IL-17, and IL-23 as shown in Table 1 and 2 (from pages 15 and 16 of the study report). In addition, no effect was observed on the expression of the T lymphocyte activation markers CD25 and CD69 as shown in Table 4 and Table 5 (from pages 24 and 25 of the study report). In contrast, the positive control, lipopolysaccharide (LPS) and phytohemagglutinin (PHA) elicited cytokine release and increased expression of CD25 and CD69 on T lymphocytes.

Overall, the results of this study indicated that MLN0002 does not cause cytokine release and lymphocyte activation in human blood.

Table 1 Plasma Cytokine Concentrations After the Incubation at 37°C of Diluted Human Whole Blood With Buffer (Negative Control), LPS and PHA (Positive Control), or MLN0002

Incubation Time	Sample	Plasma Cytokine Concentration (ng/mL)								
		IFN γ	IL-1 β	IL-2	IL-4	IL-6	IL-8	IL-12 (p70)	IL-17	TNF α
Donor No. 1										
0 hr	Negative control ^a	0.4	0.2	0.4	0.8	0.2	0.4	0.6	0.8	2.4
5.5 hr	Negative control	0.4	0.2	0.4	0.8	4.8	7.6	0.6	0.8	2.4
5.5 hr	LPS (5 $\mu\text{g/mL}$) + PHA (5 $\mu\text{g/mL}$) ^b	53.6	80.0	24.6	9.2	30,500.0	8625.0	1.2	27.8	394.2
5.5 hr	MLN0002 (400 $\mu\text{g/mL}$)	0.4	0.2	0.4	0.8	10.2	26.8	0.6	0.8	2.4
25 hr	Negative control	0.4	0.2	6.8	0.4	26.2	202.2	0.6	0.8	19.0
25 hr	LPS (5 $\mu\text{g/mL}$) + PHA (5 $\mu\text{g/mL}$)	5985.0	2685.0	28.8	15.0	52,900.0	324.6	2.2	33.0	746.8
25 hr	MLN0002 (400 $\mu\text{g/mL}$)	0.4	0.2	4.6	0.2	31.8	182.6	0.6	0.8	15.6
Donor No. 2										
0 hr	Negative control	0.4	0.2	0.4	0.8	0.2	0.4	0.6	0.8	2.4
5.5 hr	Negative control	0.4	0.2	0.4	0.8	0.2	0.4	0.6	0.8	2.4
5.5 hr	LPS (5 $\mu\text{g/mL}$) + PHA (5 $\mu\text{g/mL}$)	51.6	145.2	18.6	11.6	37,400.0	2920.0	1.2	31.2	456.0
5.5 hr	MLN0002 (400 $\mu\text{g/mL}$)	0.4	0.2	0.4	0.8	0.2	2.8	0.6	0.8	2.4
25 hr	Negative control	0.4	0.2	0.4	0.8	1.2	5.2	0.6	0.8	2.4
25 hr	LPS (5 $\mu\text{g/mL}$) + PHA (5 $\mu\text{g/mL}$)	7785.0	4875.0	98.0	18.8	61,600.0	387.8	2.4	45.6	922.8
25 hr	MLN0002 (400 $\mu\text{g/mL}$)	0.4	0.2	0.4	0.8	3.4	15.8	0.6	0.8	2.4

Table 1 Plasma Cytokine Concentrations After the Incubation at 37°C of Diluted Human Whole Blood With Buffer (Negative Control), LPS and PHA (Positive Control), or MLN0002

Incubation Time	Sample	Plasma Cytokine Concentration (ng/mL)								
		IFN γ	IL-1 β	IL-2	IL-4	IL-6	IL-8	IL-12 (p70)	IL-17	TNF α
Donor No. 3										
0 hr	Negative control	5.4	0.2	2.6	1.2	3.6	0.4	0.4	5.6	4.0
5.5 hr	Negative control	7.8	0.2	5.0	1.6	4.4	5.4	1.2	11.6	15.0
5.5 hr	LPS (5 μ g/mL) + PHA (5 μ g/mL)	26.4	128.4	28.0	11.4	17,200.0	7300.0	3.4	60.0	893.8
5.5 hr	MLN0002 (400 μ g/mL)	10.8	0.2	5.6	3.2	6.2	4.0	2.2	22.2	21.8
25 hr	Negative control	9.8	0.2	6.4	1.4	9.4	17.8	2.0	24.8	33.0
25 hr	LPS (5 μ g/mL)+PHA (5 μ g/mL)	2315.0	2650.0	30.0	18.6	41,500.0	391.4	7.4	72.6	2077.6
25 hr	MLN0002 (400 μ g/mL)	13.4	0.2	7.8	1.6	8.4	24.2	3.0	33.6	58.6
Donor No. 4										
0 hr	Negative control	0.2	N/A ^c	1.3	0.9	1.2	0.4	N/A	0.8	2.4
5.5 hr	Negative control	0.2	N/A	1.3	0.8	18.3	107.9	N/A	14.4	2.4
5.5 hr	LPS (5 μ g/mL) + PHA (5 μ g/mL)	23.3	N/A	5.1	4.7	16,966.0	4176.7	N/A	105.7	892.9
5.5 hr	MLN0002 (400 μ g/mL)	0.2	N/A	1.1	0.9	19.8	80.4	N/A	5.7	2.4
25 hr	Negative control	0.6	N/A	2.1	1.5	409.3	1463.3	N/A	80.1	4.8
25 hr	LPS (5 μ g/mL) + PHA (5 μ g/mL)	1295.0	N/A	41.9	14.9	41,033.0	8063.3	N/A	232.3	1885.8
25 hr	MLN0002 (400 μ g/mL)	0.2	N/A	1.5	0.9	264.7	281.4	N/A	89.8	12.2

N/A = not applicable.

Note: Data for Donors 1 through 3 are from a single sample for each donor (Experiment 1), while the data for Donor 4 are the means of 3 experimental samples (Experiment 2, see Table 3 for the data for the individual data for each replicate).

- a Negative control = assay buffer only.
- b LPS (5 μ g/mL) + PHA (5 μ g/mL) was the positive control.
- c Not determined for Donor No. 4.

Table 4 Upregulation of CD69 After Incubation of Diluted Whole Blood With MLN0002 or LPS and PHA (Positive Control)

	Control ^a				LPS + PHA 5 μ g/mL ^b				MLN0002 400 μ g/mL ^a			
	5.5 hr		24 hr		5.5 hr		24 hr		5.5 hr		24 hr	
	% CD69 ⁺	GMFI	% CD69 ⁺	GMFI	% CD69 ⁺	GMFI	% CD69 ⁺	GMFI	% CD69 ⁺	GMFI	% CD69 ⁺	GMFI
Donor No. 1	0.9%	2.8	0.3%	2.8	72.5%	39.0	88.5%	146.0	0.4%	2.7	0.7%	2.7
Donor No. 2	1.6%	3.3	1.8%	2.9	58.0%	41.0	73.8%	102.0	0.9%	3.2	0.9%	3.0
Donor No. 3	1.2%	2.9	0.0%	2.7	56.4%	33.0	92.6%	171.0	0.6%	2.7	0.3%	2.7
Mean	1.2%	3.0	0.7%	2.8	62.3%	38.0	85.0%	140.0	0.6%	2.8	0.6%	2.8
SD	0.4%	0.2	1.0%	0.1	8.9%	4.3	9.9%	34.8	0.3%	0.3	0.3%	0.2

GMFI = geometric mean fluorescence intensity

- a GMFI of entire population.
- b GMFI of CD69-positive population.

Table 5 Upregulation of CD25 After Incubation of Diluted Whole Blood With MLN0002 or LPS and PHA (Positive Control)

	Control ^a				LPS + PHA 5 µg/mL ^b				MLN0002 400µg/mL ^a			
	5.5 hr		25 hr		5.5 hr		25 hr		5.5 hr		25 hr	
	% CD25 ⁺	GMFI	% CD25 ⁺	GMFI	% CD25 ⁺	GMFI	% CD25 ⁺	GMFI	% CD25 ⁺	GMFI	% CD25 ⁺	GMFI
Donor No. 1	0.6%	2.2	0.3%	2.2	2.9%	23.5	49.8%	68.9	0.6%	2.1	0.1%	2.1
Donor No. 2	0.9%	2.3	0.9%	2.2	2.9%	28.3	38.7%	74.1	1.3%	2.3	0.6%	2.3
Donor No. 3	0.5%	2.3	1.5%	2.2	2.5%	50.1	56.8%	75.2	0.8%	2.2	0.8%	2.3
Mean	0.7%	2.2	0.9%	2.2	2.8%	34.0	48.4%	73.0	0.9%	2.2	0.5%	2.2
SD	0.2%	0.0	0.6%	0.0	0.2%	14.2	9.1%	3.4	0.4%	0.1	0.4%	0.1

a GMFI of entire population.

b GMFI of CD25-positive population.

Evaluation of Humanized Monoclonal Antibodies Against Alpha 4 Integrins in the rhMOG-Induced Experimental Autoimmune Encephalomyelitis Model in Rhesus Monkeys (*Macaca mulata*) (RPT-01673)

Methods: This is an exploratory, non-GLP study. Immune surveillance of the CNS is a homeostatic activity that protects a host from infectious disease, such as reactivation of latent neurotropic viruses, including JC virus. A decrease in immune surveillance of the CNS by T lymphocytes is postulated to contribute to the development of PML. A nonclinical model for PML is not available; however, experimental autoimmune encephalitis (EAE) is a leukoencephalopathy that also results from immune surveillance of the CNS. Animals in this model are immunized subcutaneously (SC) with an exogenous auto antigen [e.g., myelin oligodendrocyte protein (MOG) to induce an immune response]. In this EAE model, adaptive immune system is sensitized to the MOG autoantigen in the skin and the resulting memory T lymphocytes continue surveying additional organs for the presence of MOG. MOG antigens are expressed endogenously by oligodendrocytes of the CNS and, recognition by sensitized anti-MOG helper T lymphocytes initiates an autoimmune response which culminates in EAE. Normal immune surveillance of the CNS induces EAE in this model; thus, concurrent induction of EAE in the vehicle (negative control) and test article groups indicates that CNS immune surveillance has not been impaired by the test article. Conversely, a delay in EAE induction as compared to vehicle control indicates that the test article impairs CNS immune surveillance and is at risk of causing PML.

In this study, the sponsor examined potential effects of MLN0002 on immune surveillance of the CNS in a Rhesus experimental autoimmune encephalitis (EAE) model *in vivo*. Rhesus monkeys were chosen because this is the only established EAE model in which both MLN0002 and Natalizumab (positive control) are pharmacologically active. Consistent with its mechanism of action, Natalizumab delayed the onset of EAE in Guinea pigs and humanized mice. However, both of these models were considered inadequate to examine the effects of MLN0002 because MLN0002 does not bind to the $\alpha 4\beta 7$ integrin expressed by Guinea pigs. In addition, expression of the $\alpha 4\beta 7$ integrin was lost upon transplantation of human leukocytes into severe combined immunodeficient (SCID) mice. The current study has two phases and has the following

objectives. Phase 1 part of the study was designed to validate the Rhesus monkey model using Natalizumab that has shown efficacy in an EAE model in Guinea pigs and in humanized mice. The objective of the Phase 2 part of the study was to determine whether MLN0002 affected CNS immune surveillance. The following table (from page 8 of the report) shows the study design.

Table 1 Study Design

Group	Study Procedures	n	Sex
Phase 1			
Natalizumab (30 mg/kg)	Sedation, weighing blood withdrawal, intravenous and subcutaneous injections	7	M
Vehicle	Sedation, weighing blood withdrawal, intravenous and subcutaneous injections	4	M
Phase 2			
Vehicle	Sedation, weighing blood withdrawal, intravenous and subcutaneous injections	4	M (3) F (1)
MLN0002 (30 mg/kg)	Sedation, weighing blood withdrawal, intravenous and subcutaneous injections	7	M (5) F (2)

M = male(s).

Animals were treated once weekly with MLN0002 and Natalizumab at 30 mg/kg, IV (bolus) as above. Immunization was done by SC injection with rhMOG (recombinant human myelin/oligodendrocyte glycoprotein) to induce EAE. The primary assessment of immune surveillance of the CNS was the degree of inflammation in the CNS, as measured by the following:

- Clinical scores of EAE
- Levels of leukocytes in the cerebrospinal fluid (CSF)
- Magnetic resonance imaging (MRI) of brain hemispheres
- Histopathology of brain tissue
- Leukocytosis of the vasculature

Results: Fifty percent (4 of 8) of vehicle-treated animals and 57% (4 of 7) of MLN0002-treated animals developed symptoms of EAE in their respective groups, whereas 14% (1 of 7) of Natalizumab-treated animals developed symptoms of EAE within their groups. Vehicle- and MLN0002-treated animals developed EAE, whereas clinical symptoms of EAE were delayed or prevented in the Natalizumab group, an effect that was also observed in Guinea pigs (Figure 1, from page 10 of the report).

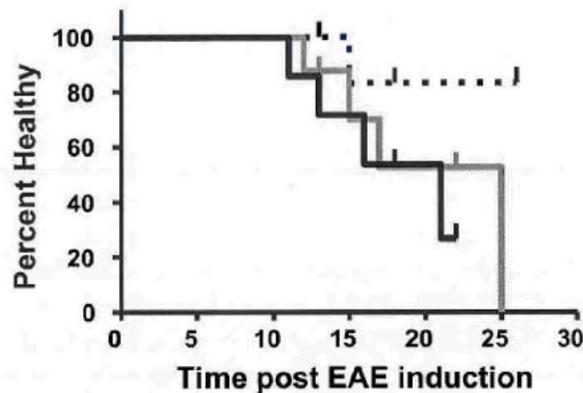
Figure 1 MLN0002 Did Not Delay Onset of Clinical Symptoms of EAE

Figure 1 Animals received an initial intravenous (IV) bolus of vehicle (grey bars), natalizumab at 30 mg/kg (checked bars), or MLN0002 at 30 mg/kg (solid black bars) before subcutaneous (SC) immunization with recombinant human myelin/oligodendrocyte glycoprotein (rhMOG) to induce experimental autoimmune encephalomyelitis (EAE) and were dosed with vehicle or test article once weekly (QW) thereafter.

Infiltration of the CNS as Measured by Leukocyte Count in the CSF: The level of leukocytes in the CSF was assessed as an assessment of immune surveillance of the CNS. CSF was collected via the cisterna magna before exposure to test articles, after 1 week and 2 weeks of exposure, and at necropsy. A fraction of the CSF sample was utilized for WBC count and the remainder was analyzed for levels of specific subsets of leukocytes by flow cytometry. Normal immune surveillance of the CNS was demonstrated by increases in the level of CSF leukocytes in this model. Conversely, impaired CNS immune surveillance was indicated by no increases in the level of CSF leukocytes in this model.

An increase in the level of WBCs in the CSF was observed in animals that developed EAE when compared to predose. The mean values of WBCs in the CSF of the vehicle control and MLN0002 groups were higher at necropsy than at predose sampling (Figure 2, from page 11 of the report). This CSF infiltrate consisted primarily of total T lymphocytes, helper and cytotoxic T lymphocyte subsets and monocytes, and total B lymphocytes. A decrease in the level of natural killer (NK) cells was also observed. In contrast, the mean level of WBCs in the CSF of the Natalizumab group was unchanged from predose (Figure 2 from page 11 of the report).

Figure 2 MLN0002 Did Not Delay Infiltration of Cerebral Spinal Fluid by Leukocytes

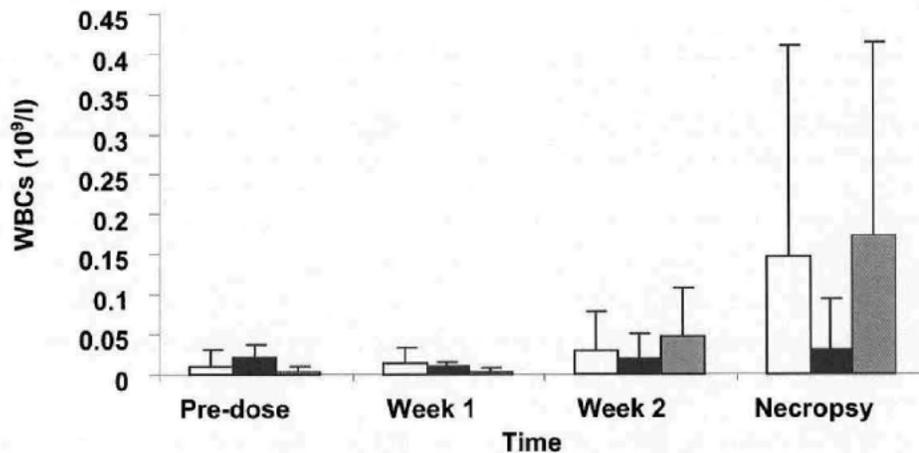


Figure 2 Cerebrospinal fluid (CSF) was collected via the cisterna magna from deeply sedated or euthanized monkeys at the indicated time points post-immunization. Automated white blood cell (WBC) counts were obtained with a hematology analyzer. Data are the mean values and standard deviations (SD) for vehicle (n = 8), natalizumab (n = 7), and MLN0002 (n = 7).

Legends: Open bar: control; solid bar: Natalizumab; and shaded bar: MNL0002

The sponsor concluded that antagonism of the $\alpha 4\beta 1$ integrin is associated with impairment of leukocyte infiltration into the CSF, since increases in levels of CSF leukocytes were observed for the vehicle control and $\alpha 4\beta 7$ integrin antagonist (MLN0002) groups, but not with the dual $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrin antagonist (Natalizumab) group. It is to be mentioned here that in a chronic toxicology study in monkeys, MLN0002 did not decrease CSF parameters (e.g., WBC and RBC counts, and total protein concentrations) or T lymphocyte populations. The sponsor stated that these data were consistent with clinical data showing that Natalizumab decreased the levels of WBCs, helper and cytotoxic T lymphocytes, and B lymphocytes in the CSF of patients with MS or who developed PML.

Infiltration of the CNS as Measured by Brain Magnetic Resonance Imaging (MRI)

The clinical symptoms of Rhesus EAE resulted from inflammation and demyelinating lesions in cerebral white matter, which were initiated by autoreactive helper T lymphocytes. These pathologic changes were quantified using MRI of brain hemispheres of Rhesus monkeys. The presence of white matter lesions was qualitatively graded between 0 (no lesions in white matter structures) and 10 (total white matter is affected by the lesion). Higher scores thus indicated more lesions, more severe EAE, and therefore, more immune surveillance of the CNS.

The mean values for lesion loads in brain hemispheres from the vehicle or MLN0002 groups were similar (Figure 3 from page 13 of the report). These values were higher than the mean value observed for the Natalizumab group (Figure 3), indicating that less immune surveillance of the CNS occurred in the Natalizumab group. Based on these, the sponsor concluded that antagonism of the $\alpha 4\beta 1$ integrin impairs the development of white matter lesions, since Natalizumab, the dual $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrin antagonist, delayed the development of EAE compared to MLN0002, a selective $\alpha 4\beta 7$ integrin antagonist.

Figure 3 MLN0002 Did Not Inhibit Formation of Brain Lesions

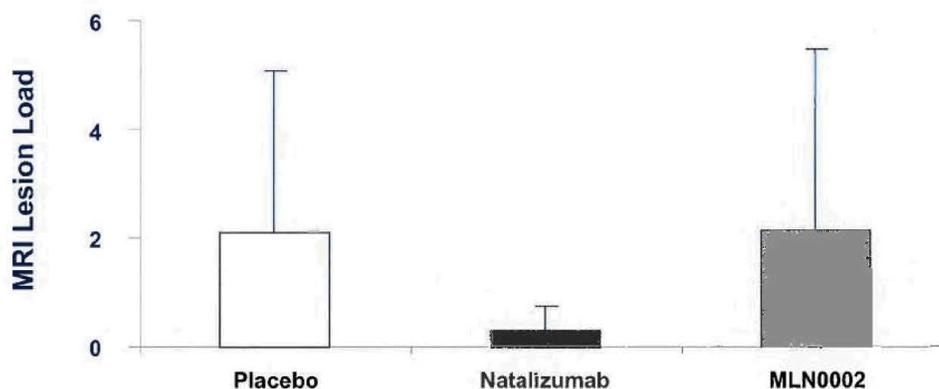


Figure 3 Postmortem magnetic resonance imaging (MRI) was performed on a brain hemisphere from each animal. The presence of white matter lesions was qualitatively graded between 0 (no lesions in white matter structures) and 10 (total white matter is affected by the lesion) by a neurologist who was blinded to the treatment each animal had received. Data are the mean values and standard deviations (SD) for the vehicle (n = 8), natalizumab (n = 7), and MLN0002 (n = 7) groups.

Infiltration of the CNS as Measured by Brain Tissue Histopathology

The extent of inflammation and demyelination in cerebral tissue sections was qualitatively determined. Representative photomicrographs are shown in Figure 4 (from page 14 of the report). The degree of demyelination in the white matter were comparable between the vehicle control animals (Figure 4A and D) and MLN0002-treated animals (Figure 4C and Figure 4F), but not in Natalizumab animals (Figure 4B and Figure 4D). Similarly, degree of inflammation in the white matter was comparable between the vehicle control animals (Figure 4G and Figure 4J) and MLN0002 animals (Figure 4I and Figure 4L), but not in Natalizumab animals (Figure 4H and Figure 4K). The individual mean inflammation and demyelination scores for the Natalizumab-treated animals were lower than those for the vehicle group (Figure 5 from page 15 of the report) and MLN0002 group. The composite group means for inflammation and demyelination score was significantly lower in the Natalizumab group than in the vehicle group (0.1 versus 1.4) and the MLN0002 group. The sponsor stated that there was a

good correlation between MRI analysis and histopathology scores. The individual mean inflammation and demyelination scores for the MLN0002 group were comparable to those for the vehicle group (Figure 5).

Figure 4 Representative Photomicrographs Illustrating That MLN0002 (Vedolizumab) Did Not Prevent Cerebral Demyelination or Inflammation

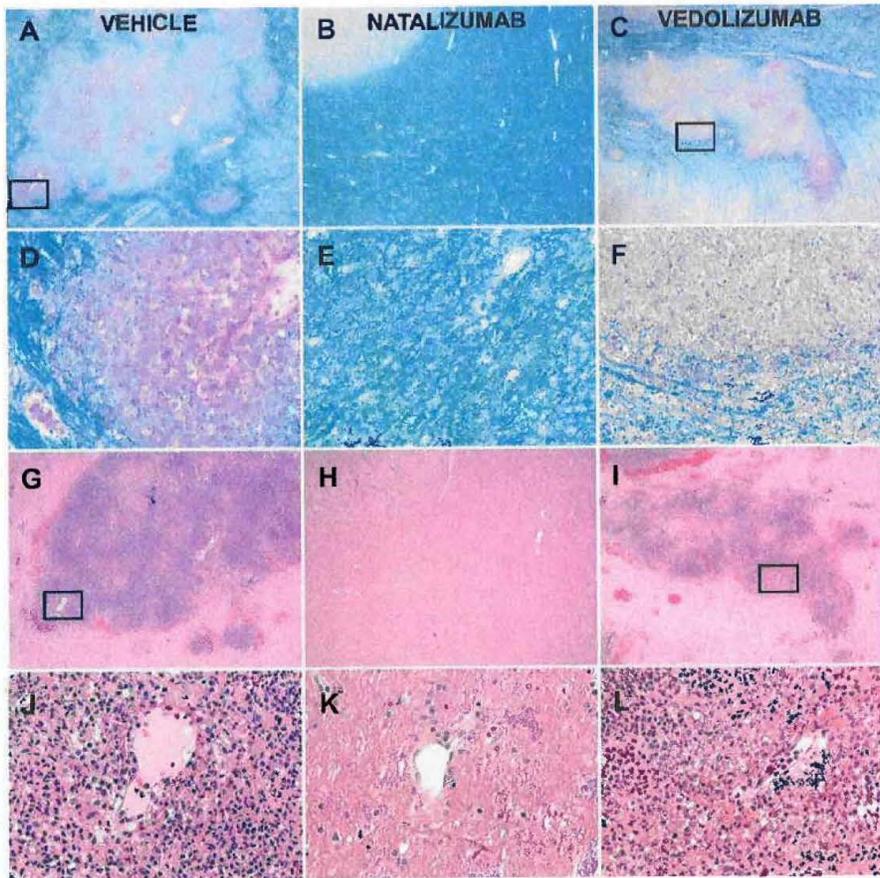


Figure 4 Three blocks of tissue were excised from each hemisphere analyzed by magnetic resonance imaging (MRI), from approximately the same region for all animals. Paraffin sections were stained with Kluver Barrera (A-F) or hematoxylin and eosin (G-L) histochemical stains. Photomicrographs A-C and G-I are at 25 \times and D-F and J-L are at 250 \times magnification. Boxes depicted in A, C, G, and I highlight the regions of tissue from which the higher magnification images D, F, J, and L were derived. Representative images are shown.

Figure 5 Pathology Assessment Illustrating That MLN0002 Did Not Prevent Cerebral Inflammation or Demyelination

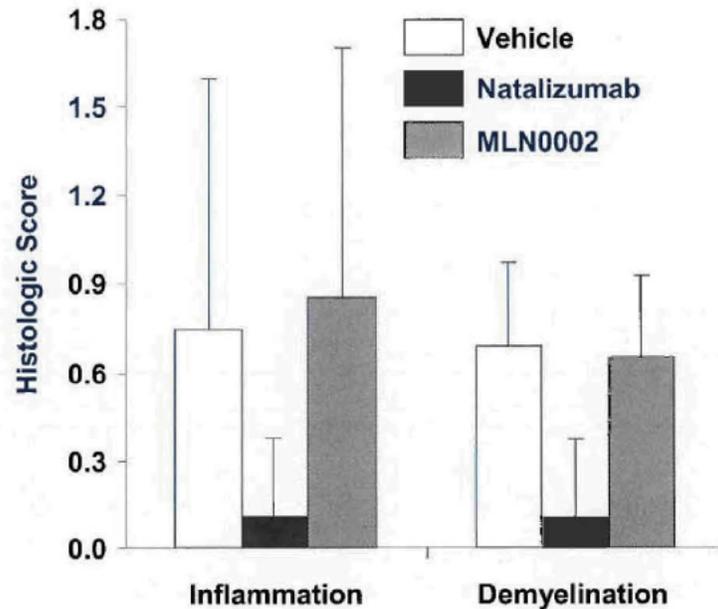


Figure 5 Three blocks of tissue were excised from each hemisphere analyzed by magnetic resonance imaging (MRI), from approximately the same region for all animals. Paraffin sections were stained with hematoxylin and eosin, Kluver Barrera, and Bielschowsky's silver stains. For calculation of group means and statistical analyses of histopathology, the ordered qualitative outcomes were converted to quantitative discrete outcomes (ie, +/- = 0.5; +/- to + = 0.75; + = 1; ++ = 2). Data are the mean values and standard deviations (SDs) for the vehicle (n = 8), natalizumab (n = 7), and MLN0002 (n = 7) groups.

Leukocytosis in the Vasculature

Vascular leukocytosis is generally considered to result from impaired migration of leukocytes out of the vessels and into peripheral tissue of organs, such as the CNS. Levels of total leukocytes and various leukocyte subsets in the vasculature were monitored during the study as an inverse indicator of immune surveillance of peripheral tissue. Natalizumab-treated animals exhibited a significant vascular leukocytosis (Figure 6A from page 17 of the report) and lymphocytosis (Figure 6B from page 17 of the report) when compared to the vehicle group. Leukocytosis consisted of significant elevations in monocytes, lymphocytes, basophils, and eosinophils, but not neutrophils (Figure 6C from page 17 of the report). Lymphocytosis consisted of significant elevations in total T lymphocytes, total and memory helper T lymphocytes, total and memory cytotoxic T lymphocytes, and total B lymphocytes, but not NK cells (Figure 6D from page 17 of the report). In contrast, no differences in leukocyte count, erythrocyte

count, reticulocyte count, platelet count, and differential counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were observed between the MLN0002-treated animals and the concurrent vehicle-treated animals (Figure 6 from page 17 of the report). Moreover, no differences between the MLN0002 and the vehicle group were observed in lymphocyte subpopulations, most notably total B lymphocytes, total T lymphocytes, helper T lymphocytes, cytotoxic T lymphocytes, memory helper T lymphocytes, memory cytotoxic T lymphocytes, and NK cells (Figure 6).

Figure 6 MLN0002 Affects a Smaller Population of Leukocytes Than Does Natalizumab

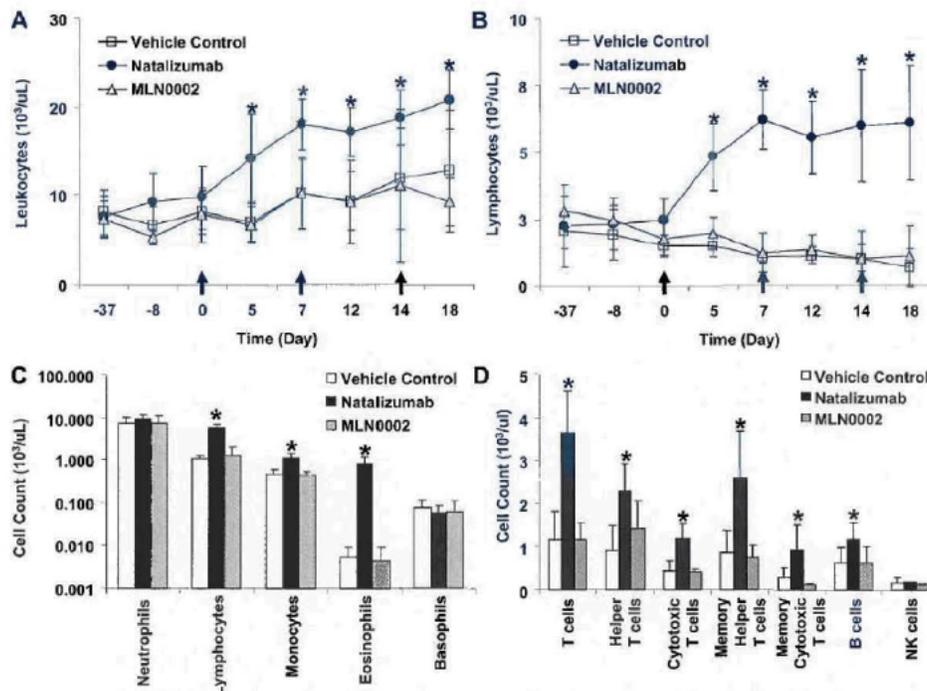


Figure 6 Peripheral blood was drawn from animals before (Days -3, -7, -8, and 0) and after (Days 5, 7, 12, 14, and 18) exposure to test article (arrows). Natalizumab, but not MLN0002, increased the absolute count of leukocytes (A) and lymphocytes (B) after exposure (Days 5, 7, 12, 14, and 18) as compared to pre-exposure, baseline levels (Days -3, -7, -8, and 0). (C) Natalizumab, but not MLN0002, elevated the levels of monocytes, lymphocytes, and eosinophils in peripheral blood after 12 days of exposure. (D) Natalizumab, but not MLN0002, elevated the calculated levels of total T lymphocytes ($\text{CD}3^+$), helper T lymphocytes ($\text{CD}3^+\text{CD}4^+$), cytotoxic T lymphocytes ($\text{CD}3^+\text{CD}8^+$), memory helper T lymphocytes ($\text{CD}3^+\text{CD}4^+\text{CD}45\text{RA}^+$), memory cytotoxic T lymphocytes ($\text{CD}3^+\text{CD}8^+\text{CD}45\text{RA}^+$), and B lymphocytes ($\text{CD}20^+$), but not natural killer (NK) cells ($\text{CD}3^-\text{CD}16^+$) after 12 days of exposure. Cell count = absolute lymphocyte count \times percent of lymphoid gate. All histograms display the mean and standard deviation for each group ($n = 7$ or 8). * denotes a significant difference ($p < 0.05$) relative to vehicle control on that day.

Overall, the results indicated that Natalizumab induced vascular leukocytosis in contrast to MLN0002, which did not induce leukocytosis. This Natalizumab-induced leukocytosis was considered to result from inhibition of migration of leukocytes from the vasculature, which, in turn, impairs immune surveillance of peripheral tissue.

Pharmacodynamic Analysis of Target Occupancy

Target occupancy in monocyte, naïve and memory helper T lymphocyte, and naïve and memory cytotoxic T lymphocyte populations in the peripheral blood was analyzed using a competitive binding assay between MLN0002 or Natalizumab and a diagnostic anti- $\alpha 4\beta 7$ integrin antibody. $\alpha 4\beta 7$ integrin was saturated for the duration of the experiment in each animal exposed to MLN0002. Approximately 20% to 45% of the memory helper T lymphocyte populations in the peripheral blood of MLN0002-treated animals were bound by the diagnostic anti- $\alpha 4\beta 7$ integrin antibody prior to exposure to MLN0002 (Figure 7 from page 19 of the report, Days -7 and 0). At trough MLN0002 exposures, anti- $\alpha 4\beta 7$ integrin antibody bound to less than 3% of these populations (Figure 7, Days 7 to 22). Results indicated that $\alpha 4\beta 7$ integrin was continuously saturated by MLN0002 for the duration of the investigation in each animal. Based on these, the sponsor concluded that MLN0002 did not affect the immune surveillance of the CNS in the presence of full target saturation. Therefore, the absence of effect by MLN0002 on immune surveillance of the CNS was not due to the failure to saturate the target.

Figure 7 The Alpha 4 Beta 7 Integrins Expressed by Memory Helper T Lymphocytes in Peripheral Blood Were Saturated by MLN0002 for the Duration of the Investigation in Each Animal Exposed to MLN0002

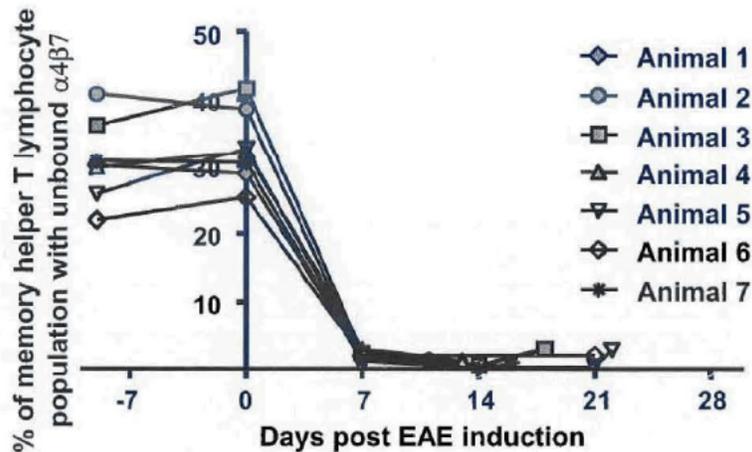


Figure 7 Peripheral blood was drawn from animals on Days -10, 0, 7, 14, and 21 before an intravenous (IV) bolus of vehicle (open symbols) or MLN0002 at 30 mg/kg (closed symbols) on Days 0, 7, and 14. Saturation of the $\alpha_4\beta_7$ integrin expressed by the memory helper T lymphocyte population ($CD3^+CD4^+CD45RA^-$ lymphocytes) was then measured ex vivo by flow cytometry. Data represent the percentage of the population expressing unsaturated $\alpha_4\beta_7$ integrin and are values for samples from individual animals at that time point.

The Inhibitory Effect of Natalizumab on EAE May be Caused by Impaired Migration of Leukocytes Into the CNS

In order to gain mechanistic insight into any potential effects on EAE, frequency of mononuclear leukocytes that recognized MOG (myelin oligo dendrocyte) in each animal was assessed to examine whether MLN0002 and Natalizumab affected the induction of these autoreactive cells. The presence of recombinant human MOG (rhMOG)-reactive leukocytes (i.e., pathogenic cells) was determined in *ex vivo* proliferation assays of peripheral blood mononuclear cells (PBMC) and splenocytes from animals receiving vehicle, Natalizumab, or MLN0002. Anti-MOG proliferative responses were observed in PBMCs and splenocytes from each animal in the investigation (data not presented). The results indicated that 21 of 22 animals contained a quantity of autoreactive cells that was sufficient to induce EAE, thus demonstrating that the inhibition of EAE by Natalizumab did not result from failure to induce autoreactive cells. Based on these, the sponsor concluded that the inhibitory effect of Natalizumab on the development of EAE in Rhesus monkeys may have resulted from impaired migration of autoreactive leukocytes from the vasculature into the CNS (i.e., immune surveillance of the CNS) and the development of EAE.

Summary of the Immune Surveillance of the Central Nervous System

A decrease in immune surveillance of the CNS is postulated to contribute to the development of PML (Berger JR and Houff S. *Neurol Res.* 2006;28:299-305; Tan CS, et al. *Lancet Neurology.* 2010;9:425-37; Carson KR, et al. *Lancet Oncol.* 2009;10:816-24; Major EO. *Annul Rev Med.* 2010; 61:35-47). Natalizumab, a dual $\alpha_4\beta_1$ and $\alpha_4\beta_7$ antagonist, was reported to decrease immune surveillance of the CNS in rodents (Kent SJ, et al. *J Neuroimmunol.* 1995; 58:1-10; Yednock TA, et al., *Nature.* 1992; 356:63-6; Coisne C, et al. *J Immunol.* 2009; 182:5909-13), nonhuman primates, and patients with MS. In contrast, MLN0002, a specific $\alpha_4\beta_7$ antagonist, did not inhibit any component of immune surveillance of the CNS in this EAE model as shown in the following Table 2 (from page 20 of the report). Millennium thus deduced that immune surveillance of the CNS is mediated by the $\alpha_4\beta_1$ integrin and concluded that MLN0002 has less risk of causing PML than Natalizumab, as MLN0002 does not antagonize the $\alpha_4\beta_1$ integrin, and does not impair immune surveillance of the CNS.

Table 2 Effects of Natalizumab and MLN0002 on Immune Surveillance of the CNS in Experimental Autoimmune Encephalomyelitis

Assessment of Immune Surveillance of the CNS	Natalizumab ($\alpha_4\beta_1$ and $\alpha_4\beta_7$ Antagonist)	MLN0002 ($\alpha_4\beta_7$ Antagonist)	Responsible Integrin
Clinical symptoms of encephalomyelitis	Inhibition	No inhibition	$\alpha_4\beta_1$
Leukocytic infiltration of the cerebral spinal fluid	Inhibition	No inhibition	$\alpha_4\beta_1$
Cerebral inflammation and demyelination (MRI)	Inhibition	No inhibition	$\alpha_4\beta_1$
Cerebral inflammation and demyelination (histopathology)	Inhibition	No inhibition	$\alpha_4\beta_1$
Peripheral leukocytosis, lymphocytosis, and monocytosis	Induction	No induction	$\alpha_4\beta_1$

CNS = central nervous system; MRI = magnetic resonance imaging.

Addendum: In this study, Rhesus monkeys received an initial IV bolus of placebo (0.9% saline), natalizumab (30 mg/kg), or vedolizumab (30 mg/kg) before intracutaneous immunization with rhMOG followed by the antibodies once weekly thereafter (days 0, 7, 14 and 21) until sacrifice. Overall, vedolizumab did not appear to inhibit immune surveillance of the CNS in this EAE model. In contrast, natalizumab blocked immune surveillance of the CNS in this animal model. However, since EAE is not an animal model of PML; the results of this study do not directly demonstrate that vedolizumab has no potential to cause PML.

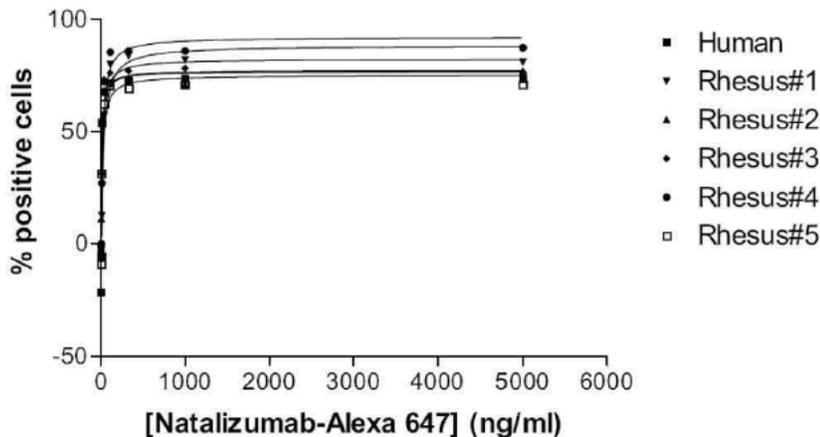
The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 088, 101 and 343 dated May 13, 2011.

A Comparison of the Binding Affinity of Natalizumab and Vedolizumab to Integrins on Peripheral Blood Mononuclear Cells of Rhesus Macaques, Cynomolgus Macaques, and Humans (RPT-01337)

The objective of this study was to determine and compare the binding affinity of Natalizumab and MLN0002 to their respective targets, the $\alpha 4$ integrins and the $\alpha 4\beta 7$ integrin, respectively, in Rhesus monkeys, Cynomolgus monkeys, and human whole blood. In addition, the inhibitory activity of Natalizumab against Natalizumab-A647 and Vedolizumab against vedolizumab-A647 was also determined in the above three species. In this study, Rhesus monkeys, Cynomolgus monkeys, and human whole blood samples were stained with Natalizumab-Alexa Fluor[®]-647 (A647) and MLN0002-A647 in saturation or competition binding experiments. Expression of $\alpha 4$ and $\alpha 4\beta 7$ on CD4+ memory T lymphocytes was examined by fluorescence-activated cell sorting (FACS). The concentration producing 50% efficacy (EC50) (i.e., the concentration of labeled antibody that yielded 50% of maximal staining) or the concentration producing 50% inhibition (IC50) (i.e., the concentration of test antibody that inhibited by 50% the binding of the labeled antibody used at its EC50) values for all samples were determined from saturation and competition binding curves.

Figure 1 (from page 11 of the report) and Figure 2 (from page 12 of the report) show saturation binding curves of Natalizumab-A647 binding to Rhesus, Cynomolgus monkeys, and human lymphocytes. Figure 3 (from page 13 of the report) shows saturation binding curves of MLN0002-A647 binding to Rhesus monkeys and human lymphocytes.

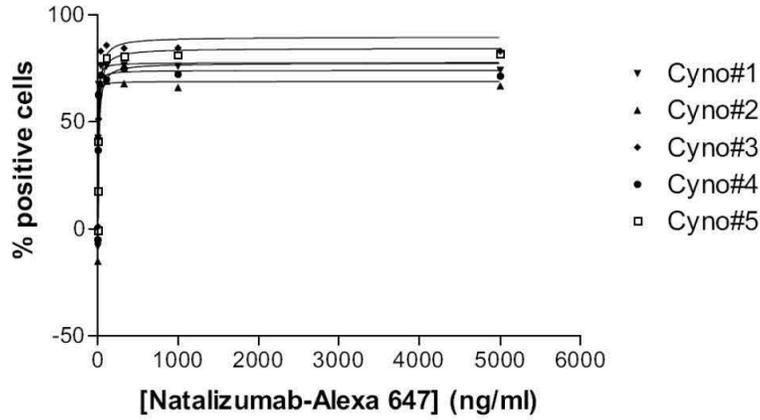
Figure 1 Saturation Binding of Natalizumab-A647 to Rhesus Macaque and Human Whole Blood CD4⁺ Memory T Lymphocytes



Natalizumab-A647 (ng/mL)	Rhesus 1 (%)	Rhesus 2 (%)	Rhesus 3 (%)	Rhesus 4 (%)	Rhesus 5 (%)	Human (%)
5000.0	81.2	77.0	76.5	87.5	70.9	73.7
1000.0	82.1	71.0	78.3	86.1	71.1	73.6
333.3	83.9	73.3	77.2	85.9	69.5	73.1
111.1	80.0	71.7	76.3	85.5	70.7	71.8
37.0	57.5	68.7	73.3	72.6	62.3	67.8
12.3	12.7	56.3	31.4	27.2	31.5	54.1
4.1	-3.0	11.3	-0.3	0.0	-5.7	-5.5
0	-4.0	-1.7	-3.4	-1.2	-9.0	-21.6
5000.0 plus 600.0- μ g/mL unlabeled natalizumab	0	0	0	0	0	0
B_{max}	88.5	76.7	82.5	92.3	75.3	77.5
EC₅₀ (ng/mL)	30.4	8.1	16.2	20.5	17.6	11.4

A647 = Alexa Fluor[®] 647; B_{max} = binding maximum; EC₅₀ = concentration producing 50% efficacy.

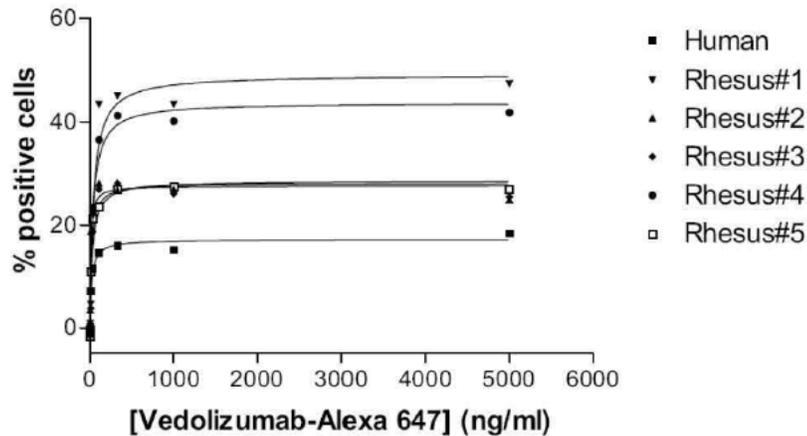
Figure 2 Saturation Binding of Natalizumab-A647 to Cynomolgus Macaque Whole Blood CD4⁺ Memory T Lymphocytes



Natalizumab-A647 (ng/mL)	Cynomolgus 1 (%)	Cynomolgus 2 (%)	Cynomolgus 3 (%)	Cynomolgus 4 (%)	Cynomolgus 5 (%)
5000.0	74.2	66.8	83.1	71.4	81.9
1000.0	76.0	66.0	84.6	72.3	81.2
333.3	76.6	67.8	84.4	74.8	80.4
111.1	76.1	69.0	85.8	69.8	79.8
37.0	76.3	67.9	83.1	71.8	70.2
12.3	67.5	64.5	51.4	62.4	40.8
4.1	42.5	43.4	1.0	36.8	17.6
0.0	-8.3	-15.2	-4.5	-5.3	-1.0
5000.0 plus 600.0- μ g/mL unlabeled natalizumab	0	0	0	0	0
B_{max}	77.7	68.9	89.7	74.1	84.4
EC₅₀ (ng/mL)	2.8	2.0	11.6	3.4	11.8

A647 = Alexa Fluor[®] 647; B_{max} = binding maximum; EC₅₀ = concentration producing 50% efficacy.

Figure 3 Saturation Binding of Vedolizumab-A647 to Rhesus Macaque and Human Whole Blood CD4⁺ Memory T Lymphocytes



Vedolizumab (ng/mL)	Rhesus 1 (%)	Rhesus 2 (%)	Rhesus 3 (%)	Rhesus 4 (%)	Rhesus 5 (%)	Human (%)
5000.0	47.4	24.9	25.4	41.8	26.9	18.4
1000.0	43.4	26.8	26.0	40.1	27.4	15.2
333.3	45.1	26.7	28.0	41.2	26.8	16.0
111.1	43.4	28.0	26.9	36.5	23.5	14.5
37.0	21.9	22.7	18.7	23.4	21.3	11.5
12.3	4.6	18.7	3.8	7.2	10.9	7.2
4.1	0.9	3.5	-1.2	0.3	-0.4	0.3
0.0	-0.5	-0.6	-1.3	-0.2	-1.6	-1.1
5000.0 plus 600.0- μ g/mL unlabeled vedolizumab	0	0	0	0	0	0
B_{max}	49.2	27.5	28.5	43.7	28.1	17.2
EC₅₀ (ng/mL)	42.9	9.3	29.4	36.5	20.1	21.3

A647 = Alexa Fluor[®] 647; B_{max} = binding maximum; EC₅₀ = concentration producing 50% efficacy.

Table 1 (from page 14 of the report) summarizes the EC₅₀ values calculated from these experiments for binding of Natalizumab-A647 to Rhesus, Cynomolgus monkeys, and human CD4⁺ memory T lymphocytes. Mean EC₅₀ values for binding of Natalizumab-A647 to CD4⁺ memory T lymphocytes from Rhesus (n = 5), Cynomolgus monkeys (n = 5), and humans (n = 1) were 18.6 ± 8.1, 6.3 ± 5.0, and 11.4 ng/mL, respectively. Mean EC₅₀ values for binding of MLN0002-A647 to Rhesus monkeys (n = 5) and human (n = 1) CD4⁺ memory T lymphocytes were 27.6 ± 13.3 and 21.3 ng/mL, respectively.

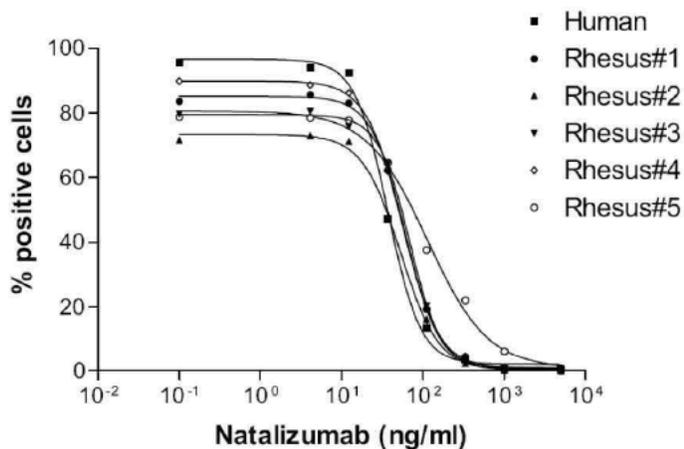
Table 1 Summary of EC₅₀ Values From the Saturation Binding Experiments Using Natalizumab-A647 and Vedolizumab-A647 in Rhesus Macaque, Cynomolgus Macaque, and Human Whole Blood CD4⁺ Memory T Lymphocytes

Animal ID	Saturation of Natalizumab-A647 EC ₅₀ (ng/mL)			Saturation of Vedolizumab-A647 EC ₅₀ (ng/mL)	
	Rhesus	Cynomolgus	Human	Rhesus	Human
1	30.4	2.8	11.4	42.9	21.3
2	8.1	2.0		9.3	
3	16.2	11.6		29.4	
4	20.5	3.4		36.5	
5	17.6	11.8		20.1	
Mean	18.6	6.3	N/A	27.6	N/A
SD	8.1	5.0	N/A	13.3	N/A

A647 = Alexa Fluor[®] 647; EC₅₀ = concentration producing 50% efficacy; ID = identification; N/A = not applicable; SD = standard deviation.

Figure 4 (from page 15 of the report) and Figure 5 (from page 16 of the report) show inhibition of Natalizumab-A647 binding (at saturating concentration) to CD4⁺ memory T lymphocytes in Rhesus monkeys (n = 5), Cynomolgus monkeys (n = 5), and human (n = 1) blood samples. Figure 6 (from page 17 of the report) and Figure 7 (from page 18 of the report) show the inhibition of MLN0002-A647 binding (at saturating concentration) to CD4⁺ memory T lymphocytes by MLN0002 in Rhesus and Cynomolgus monkeys, respectively.

Figure 4 Inhibition of Natalizumab-A647 (100 ng/mL) Binding to Rhesus Macaque and Human Whole Blood CD4⁺ Memory T Lymphocytes by Natalizumab

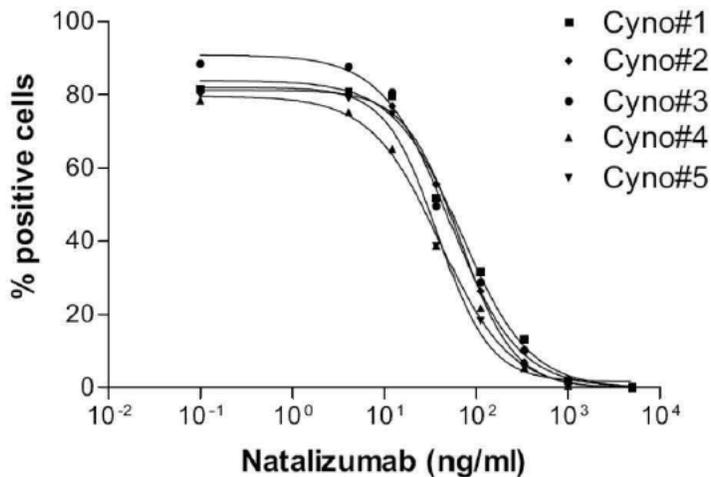


Natalizumab (ng/mL)	Rhesus 1 (%)	Rhesus 2 (%)	Rhesus 3 (%)	Rhesus 4 (%)	Rhesus 5 (%)	Human
5000.0	0.0	0.0	0.1	0.1	0.8	0.5
1000.0	0.2	0.4	0.8	1.2	6.2	1.0
333.3	4.0	2.3	4.2	4.6	22.0	3.4
111.1	19.3	16.2	20.3	19.4	37.6	13.6
37.0	62.4	47.2	64.3	62.0	64.6	47.4
12.3	83.1	71.2	75.8	86.2	77.8	92.4
4.1	85.7	73.0	80.5	88.6	78.5	94.1
0.0	83.6	71.5	79.7	89.9	78.8	95.6
Hill slope	-2.1	-1.8	-2.2	-1.9	-1.2	-2.1
IC₅₀ (ng/mL)	60.6	53.2	68.7	55.9	113.6	37.2

IC₅₀ = concentration producing 50% inhibition.

Figure 4 The saturating concentration of natalizumab-A647, 100 ng/mL, was used.

Figure 5 Inhibition of Natalizumab-A647 (100 ng/mL) Binding to Cynomolgus Macaque Whole Blood CD4⁺ Memory T Lymphocytes by Natalizumab

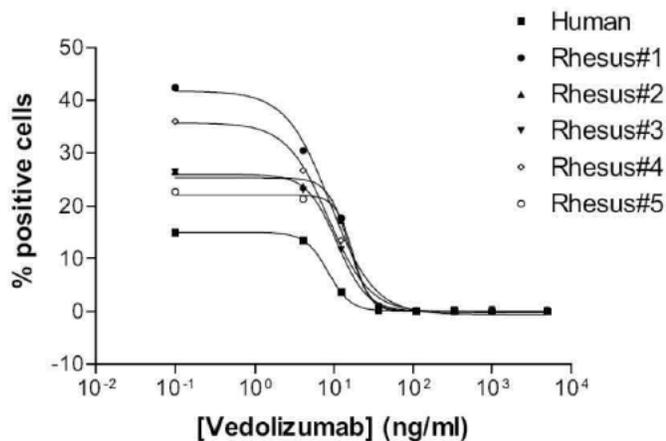


Natalizumab (ng/mL)	Cynomolgus 1 (%)	Cynomolgus 2 (%)	Cynomolgus 3 (%)	Cynomolgus 4 (%)	Cynomolgus 5 (%)
5000.0	0.0	0.1	0.0	0.1	0.1
1000.0	2.0	0.9	1.2	0.6	0.9
333.3	13.0	6.8	10.1	5.2	5.5
111.1	31.6	26.3	28.7	21.6	18.2
37.0	51.9	55.5	49.5	38.6	38.7
12.3	79.6	76.9	80.7	65.1	74.7
4.1	80.8	79.6	87.7	75.2	79.1
0.0	81.6	80.5	88.5	78.4	80.3
Hill slope	-1.2	-1.5	-1.2	-1.2	-1.5
IC₅₀ (ng/mL)	68.9	65.8	51.8	40.7	38.4

IC₅₀ = concentration producing 50% inhibition.

Figure 5 The saturating concentration of natalizumab-A647, 100 ng/mL, was used.

Figure 6 Inhibition of Vedolizumab-A647 (200 ng/mL) Binding to Rhesus Macaque and Human Whole Blood CD4⁺ Memory T Lymphocytes by Vedolizumab

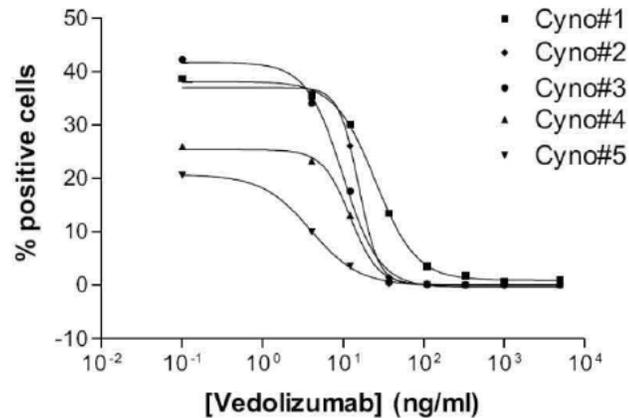


Vedolizumab (ng/mL)	Rhesus 1 (%)	Rhesus 2 (%)	Rhesus 3 (%)	Rhesus 4 (%)	Rhesus 5 (%)	Human (%)
5000.0	0.0	0.3	0.2	0.2	0.1	0.0
1000.0	0.1	0.2	0.4	0.1	0.0	0.0
333.3	0.1	0.0	0.3	0.2	0.0	0.1
111.1	0.1	0.0	0.1	0.2	0.1	0.1
37.0	0.3	1.0	0.6	0.9	1.0	0.2
12.3	17.8	17.2	11.7	13.5	17.6	3.6
4.1	30.5	23.7	23.0	26.7	21.4	13.5
0.0	42.4	26.5	26.4	36.0	22.7	15.0
Hill slope	-1.6	-3.3	-2.3	-1.7	-4.0	-3.0
IC₅₀ (ng/mL)	8.9	15.3	11.0	8.5	17.4	8.4

IC₅₀ = concentration producing 50% inhibition.

Figure 6 The saturating concentration of vedolizumab-A647, 200 ng/mL, was used.

Figure 7 Inhibition of Vedolizumab-A647 (200 ng/mL) Binding to Cynomolgus Macaque Whole Blood CD4⁺ Memory T Lymphocytes by Vedolizumab



Vedolizumab (ng/mL)	Cynomolgus 1 (%)	Cynomolgus 2 (%)	Cynomolgus 3 (%)	Cynomolgus 4 (%)	Cynomolgus 5 (%)
5000.0	1.1	0.3	0.1	0.2	0.1
1000.0	0.7	0.1	0.1	0.4	0.0
333.3	1.8	0.2	0.1	0.2	0.0
111.1	3.6	0.3	0.2	0.3	0.2
37.0	13.5	1.5	0.6	0.9	0.3
12.3	30.1	26.1	17.6	13.1	3.6
4.1	35.7	34.9	34.1	23.2	10.0
0.0	38.7	38.7	42.3	25.9	20.6
Hill slope	-1.7	-3.5	-1.9	-2.5	-1.4
IC₅₀ (ng/mL)	25.1	15.6	9.9	12.3	4.0

IC₅₀ = concentration producing 50% inhibition.

Figure 7 The saturating concentration of vedolizumab-A647, 200 ng/mL, was used.

Binding of MLN0002-A647 to Cynomolgus monkeys was determined previously (Report RPT-01052). Mean IC₅₀ values for inhibition of Natalizumab-A647 binding to CD4⁺ memory T lymphocytes by Natalizumab in Rhesus, Cynomolgus monkeys, and human samples were 70.4 ± 24.9, 53.1 ± 14.0, and 37.2 ng/mL, respectively. Mean IC₅₀ values for inhibition of MLN0002-A647 binding to CD4⁺ memory T lymphocytes by MLN0002 in Rhesus, Cynomolgus monkeys, and human samples were 12.2 ± 4.0, 13.4 ± 7.8, and 8.4 ng/mL, respectively. The results are shown in the following tables (from page 14 and 19 of the study report).

Table 2 Summary of IC₅₀ and Hill Slope Values From the Binding Competition Experiments

Inhibition of Natalizumab-A647 Binding to Whole Blood CD4⁺ Memory T Lymphocytes by Natalizumab						
Animal ID	Rhesus		Cynomolgus		Human	
	IC₅₀ (ng/mL)	Hill Slope	IC₅₀ (ng/mL)	Hill Slope	IC₅₀ (ng/mL)	Hill Slope
1	60.6	-2.1	68.9	-1.2	37.2	-2.1
2	53.2	-1.8	65.8	-1.5		
3	68.7	-2.2	51.8	-1.2		
4	55.9	-1.9	40.7	-1.2		
5	113.6	-1.2	38.4	-1.5		
Mean	70.4	-1.8	53.1	-1.3	N/A	N/A
SD	24.9	0.4	14.0	0.2	N/A	N/A

Inhibition of Vedolizumab-A647 Binding to Rhesus Macaque, Cynomolgus Macaque, and Human Whole Blood CD4⁺ Memory T Lymphocytes by Vedolizumab						
Animal ID	Rhesus		Cynomolgus		Human	
	IC₅₀ (ng/mL)	Hill Slope	IC₅₀ (ng/mL)	Hill Slope	IC₅₀ (ng/mL)	Hill Slope
1	8.9	-1.6	25.1	-1.7	8.4	-3.0
2	15.3	-3.3	15.6	-3.5		
3	11.0	-2.3	9.9	-1.9		
4	8.5	-1.7	12.3	-2.5		
5	17.4	-4.0	4.0	-1.4		
Mean	12.2	-2.6	13.4	-2.2	N/A	N/A
SD	4.0	1.0	7.8	0.8	N/A	N/A

IC₅₀ = concentration producing 50% inhibition; ID = identification; N/A = not applicable; SD = standard deviation.

Note The saturating concentrations of natalizumab-A647 and vedolizumab-A647, 100 and 200 ng/mL, respectively, were used.

Overall, Natalizumab appears to have higher affinity (lower EC₅₀ values) when compared to MLN0002 (higher EC₅₀ values) in all three species tested (only one sample for human). In the competition binding experiment, although MLN0002 had lower IC₅₀ value than Natalizumab, it is to be noted here that twice the amount of MLN0002 (labeled, 200 μ L) was added than Natalizumab (100 μ L/mL) and still binding of MLN0002 (18-47%) did not reach the level of Natalizumab binding (70-87%). This is also worth mentioning here that Natalizumab is binding to both α 4 β 1 and α 4 β 7 integrins whereas MLN0002 is only binding to α 4 β 7. The interpretation of this study results is somewhat difficult as the binding affinity of Natalizumab to α 4 β 7 integrin has not been established in this study. Overall, Natalizumab appears to have higher affinity for α 4 integrins than MLN0002.

Relative Binding Affinity of LDP-02 on T Cells from Humans and Cynomolgus Monkeys (RPT-00240)

The objective of this study was to determine the relative binding affinity of LDP-02 on T cells from humans and Cynomolgus monkeys (*Macaca fascicularis*). In this study, fluorescein isothiocyanate-(FITC) labeled LDP-02 was used to stain $\alpha 4\beta 7$ T cells in the whole blood from humans and monkeys for flow cytometric analysis. CD8 cells were assessed from the CD3⁺CD4⁻ population. The effective concentration of FITC-LDP-02 resulting in 50% saturation (EC₅₀) of $\alpha 4\beta 7$ on CD4⁺ and CD8⁺ T cells from humans and Cynomolgus monkeys ranged from 0.001 to 0.005 μ M, with a mean value of 0.002 μ M (0.0003 μ g/mL). The average EC₅₀ was 0.003 μ M for human T cells, 0.002 μ M for monkey T cells and 0.002 μ M for both species and all populations combined. The 100% saturation (EC₁₀₀) mean value was 0.2 μ M (0.03 μ g/mL). The following table (from page 10 of the report) shows the results.

Table 2 EC₅₀ of FITC-Labeled LDP-02 on Human and Cynomolgus Monkey T Cells

Species	CD4	EC ₅₀ (μ M)		Overall Mean (Human and Cynomolgus)
		CD8	Mean	
Human	0.005	0.0011	0.003	0.002 \pm 0.002
Cynomolgus	0.001	0.0019	0.002	

At EC₅₀ concentration (0.002 μ M) of FIT-LDP-02, the IC₅₀ values for competitive inhibition of homologous binding of LDP-02 to human and Cynomolgus CD4⁺ T cells were 0.85 and 0.88 nM, respectively, as shown in the table (Table 3, from page 14 of the report) below. At EC₅₀ concentration (0.002 μ M) of FIT-LDP-02, the IC₅₀ values for competitive inhibition of homologous binding of LDP-02 to human and monkey CD8⁺ T cells were 0.88 and 0.43 nM, respectively. At EC₅₀ concentration (0.002 μ M) of FIT-LDP-02, the IC₅₀ values obtained from the CD3⁺ population of both the monkey and human were 0.9 and 1.3 nM, respectively, as shown in table (Table 4, from page 14 of the report) below. At saturating (EC₁₀₀) concentration (0.2 μ M) of FIT-LDP-02, the mean IC₅₀ values for competitive binding inhibition on human and Cynomolgus monkey CD3⁺ T cells were 73.2 and 60.4 nM, respectively. Overall, the IC₅₀ values for monkey and human were comparable for CD4⁺ T cells. However, for CD8⁺ and CD3⁺ cells, the IC₅₀ values were lower for the monkey than human.

Table 3 Average IC₅₀ Values for Each Concentration of Labeled LDP-02

The % CD4 or CD8 represent IC₅₀ values obtained from the % of cells positive in the noted population and MFI represents IC₅₀ values from the MFI of the noted population.

A IC₅₀ Values Expressed as nM Units

FITC-LDP-02 at EC₅₀ (0.002 μM)				
	%CD4	MFI CD4	%CD8	MFI CD8
Human	0.85 ± 0.14	0.70 ± 0.06	0.88 ± 0.15	0.66 ± 0.12
Cynomolgus monkey	0.88 ± 0.21	0.70 ± 0.15	0.43 ± 0.08	2.35 ± 2.34
FITC-LDP-02 at EC₁₀₀ (0.2 μM)				
	%CD4	MFI CD4	%CD8	MFI CD8
Human	67.3 ± 16.0	49.3 ± 12.0	102 ± 24.5	43.0 ± 7.21
Cynomolgus monkey	50.3 ± 10.3	36.7 ± 7.37	48.3 ± 20.0	34.3 ± 4.16

B IC₅₀ Values Expressed as μg/mL Units

FITC-LDP-02 at EC₅₀ (0.002 μM)				
	%CD4	MFI CD4	%CD8	MFI CD8
Human	0.13 ± 0.02	0.11 ± 0.01	0.13 ± 0.02	0.1 ± 0.02
Cynomolgus monkey	0.13 ± 0.03	0.10 ± 0.02	0.06 ± 0.01	0.35 ± 0.35
FITC-LDP-02 at EC₁₀₀ (0.2 μM)				
	%CD4	MFI CD4	%CD8	MFI CD8
Human	10.10 ± 2.39	7.40 ± 1.79	15.35 ± 3.68	6.45 ± 1.08
Cynomolgus monkey	7.55 ± 1.54	5.50 ± 1.11	7.25 ± 3.00	5.15 ± 0.62

Table 4 Average IC₅₀ Values for Unlabeled LDP-02 Inhibition of FITC-LDP-02 Staining of CD3⁺ T Cells

FITC-LDP-02 at EC₅₀ (0.002 μM)	Average IC₅₀ (nM)	Average IC₅₀ (μg/mL)
Human	1.3 ± 0.5	0.2 ± 0.08
Cynomolgus monkey	0.9 ± 0.7	0.1 ± 0.1
FITC-LDP-02 at EC₁₀₀ (0.2 μM)	Average IC₅₀ (nM)	Average IC₅₀ (μg/mL)
Human	73.2 ± 18.6	11.3 ± 2.9
Cynomolgus monkey	60.4 ± 63.0	9.4 ± 9.7

MFI = mean fluorescence intensity

Binding of Vedolizumab (MLN0002) to Th17 and Treg Cells in Human Peripheral Blood in Vitro (RPT-01405)

The objective of this study was to determine whether vedolizumab binds to the Th17 and Treg subsets of lymphocytes in human peripheral blood. This study examined the binding of vedolizumab (MLN0002) to the memory T helper (Th) 17 (Th17) and regulatory T (Treg) subsets of lymphocytes in human peripheral blood. In this study, peripheral blood mononuclear cells (PBMCs) were isolated from blood samples from healthy human volunteers and then stained with fluorescently labeled vedolizumab (Alexa Fluor® 647-labeled MLN0002/MLN0002-A647, 0.75 mg/mL).

About 27% of the memory Th17 cell population in the peripheral blood was bound by vedolizumab, and similar data (~29%) were obtained for expression of the $\beta 7$ chain. Based on this, it was concluded that approximately 27% of the memory Th17 population in the peripheral blood expresses the $\alpha 4\beta 7$ integrin and that vedolizumab could affect the functional activity of this Th17 subset. About 9% of the memory Treg cell population in the peripheral blood was bound by vedolizumab and similar data (~13%) were obtained for expression of the $\beta 7$ chain. It was therefore concluded that approximately 9% of the memory Treg population in the peripheral blood expresses the $\alpha 4\beta 7$ integrin and that vedolizumab could affect the functional activity of this Treg subset. Overall, vedolizumab bound to 27% and 9% of Th17 and Treg cell populations, respectively, that expressed $\alpha 4\beta 7$ integrin in the peripheral blood from healthy human donors.

The Kinetics of Reconstitution of Alpha 4 Beta 7 Function in Human CD4-Positive Memory T Lymphocytes after Removal of MLN0002 *In Vitro* (RPT-01436)

This study was conducted to examine the persistence of the pharmacodynamic (PD) effect of vedolizumab. The primary objective of this study was to examine whether bound MLN0002 causes $\alpha 4\beta 7$ receptor internalization and, the mechanism and kinetics of the restoration of $\alpha 4\beta 7$ function after removal of MLN0002. In this study, MLN0002 was labeled with Alexa-Fluor 647 at 0.75 mg/mL concentration. Blood cells (purified CD4⁺ memory T lymphocytes, or PBMCs) were incubated for 24 hours at 4°C or 37°C with unlabeled or Alexa-647-labeled MLN0002 (MLN0002-Alexa-647). Mouse immunoglobulin (Ig) labeled with Alexa-647 was used as a control antibody. After 24-hour incubation, cells were washed and visualized with a flow cytometer.

The results indicated that upon binding of MLN0002 to $\alpha 4\beta 7$, the MLN0002/ $\alpha 4\beta 7$ complex was internalized. This internalization began within 4 hours and was complete by 24 hours. Upon removal of MLN0002, the $\alpha 4\beta 7$ complex returned to the surface of the cell within 24 hours [50% to 58% of cells positive for $\alpha 4\beta 7$, with almost complete reconstitution of $\alpha 4\beta 7$ expression by 4 days (90%)]. The reconstituted $\alpha 4\beta 7$ was functional in that it had the capacity to bind to MAdCAM-1, the ligand for $\alpha 4\beta 7$. These results suggested that the effect of bound MLN0002 is reversible and provided proof of concept that plasmapheresis (to remove vedolizumab from the blood) in the clinic may be an effective corrective action for potential adverse events caused by MLN0002.

Potential Effects of Vedolizumab upon Human Regulatory T Cell Function *in Vitro* (RPT-01954)

This study was conducted to examine the potential effects of three different $\alpha 4\beta 7$ antagonists (vedolizumab, natalizumab, and the anti- $\beta 7$ mAb FIB504) on the suppressive effects of the total regulatory T (Treg) as well as the gut-homing subset of Treg cell population in peripheral blood from healthy human volunteers.

There was no consistent effect of vedolizumab, natalizumab, and FIB504 on the proliferation of Treg cells when stimulated with either test article compared to vehicle control or an isotype control antibody. There was no consistent effect of vedolizumab, natalizumab, and FIB504 on the suppressive activity of $CD4^+CD25^+CD127^{low}$ Treg cells, as compared to vehicle control or an isotype control antibody. There was no consistent effect of vedolizumab on the suppressive activity of ($\alpha 4\beta 7^{high}$) $CD4^+CD25^+CD127^{low}$ Treg cells, as compared to vehicle control. Based on these, it was concluded that vedolizumab does not appear to affect the activity of human Treg cells.

Differentiation of the Mechanism of the Action of the Alpha 4 Beta 7 Integrin Antagonist Vedolizumab from the Alpha 4 beta 1 and Alpha 4 Beta 7 Integrin Antagonist Natalizumab and Relevance to the Incidence of PML (RPT-02274 Amended Report dated June 20, 2013)

This report contains an assessment of the mechanism of action (MOA) of $\alpha 4\beta 7$ integrin antagonist vedolizumab and the pleiotropic $\alpha 4\beta 1$ and $\alpha 4\beta 7$ integrin antagonist natalizumab. This report also highlighted pharmacologic differences between the MOAs of vedolizumab and natalizumab with respect to the relative risk of these compounds for the development of PML. This report contains a summary of nonclinical and clinical data from several studies (shown in the table below from page 8 of the report) conducted by the Applicant to illustrate the gut-selective anti-inflammatory MOA of vedolizumab.

Table 1 Nonclinical and Clinical Data Illustrating Various Components of the Gut-Selective Anti-Inflammatory Mechanism of Action of Vedolizumab

Mechanistic Component	Animal	Human	
	In Vitro and In Vivo	In Vitro	In Vivo
Binding specificity (ie, exclusivity of $\alpha_4\beta_7$ binding) ^(2, 3, 4, 5)	✓	✓	✓
Lack of agonism: cellular activation, cytokine production ^(6, 7, 8, 9, 10)		✓	✓
Selective antagonism (ie, MAdCAM-1, not VCAM-1) ⁽¹¹⁾		✓	
Lack of cytotoxicity (ie, CDC, ADCC) ^(12, 13)		✓	
Gastrointestinal anti-inflammatory effects ^(14, 15)	✓		✓
Gut-selective effect on mounting an adaptive immune response ^(16, 17, 18)	✓		✓
Lack of effect on systemic adaptive immune response to dermal and intramuscular challenge ^(16, 17, 18)	✓		✓
Absence of effect on bone marrow, CV, and CNS ^(5, 16, 19, 20)	✓		✓
Lack of effect on immune surveillance of the CNS ^(21, 22)	✓		✓
Reversibility of pharmacodynamic effects (ie, function) ^(16, 19, 23, 24, 25)	✓	✓	✓

ADCC = antibody-dependent cell-mediated cytotoxicity; CDC = complement-dependent cytotoxicity;

CNS = central nervous system; MAdCAM-1 = mucosal addressin cell adhesion molecule-1;

VCAM-1 = vascular cell adhesion molecule-1.

Note: The nonclinical test articles, clinical trial materials, and nomenclature evolved during the vedolizumab development program (eg, MLN0002, MLN02, LDP-02); accordingly, different terms were used in the cited study reports and articles. For the convenience of reviewers, “vedolizumab” is used as the consistent descriptor in this document regardless of the designation used in the original reference.

Overall, the report compares the pharmacology of vedolizumab to that of natalizumab, particularly with regard to the development of PML. Vedolizumab antagonizes $\alpha_4\beta_7$ integrin when compared to natalizumab, which antagonizes $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrins. The $\alpha_4\beta_1$ integrin is more widely expressed by leukocytes than the $\alpha_4\beta_7$ integrin and the $\alpha_4\beta_1$ integrin mediates pleiotropic activities that are not regulated by the $\alpha_4\beta_7$ integrin. As per the Applicant, this additional pharmacologic activity elicits effects outside of the GI tract that may contribute to the development of PML (shown in Table 2 from page 9 of the report).

Table 2 Physiologic Differences Between Vedolizumab and Natalizumab That are Relevant to the Development of PML

Pharmacologic Difference	Vedolizumab ($\alpha_4\beta_7$)	Natalizumab ($\alpha_4\beta_1$ and $\alpha_4\beta_7$)	Relevance to Events Leading to PML
1. Mobilization of cells from the bone marrow	No effect detected ⁽⁵⁾	Induces ^(30, 31, 32)	Emergence of neurotropic JCV
2. Leukocytic infiltration of the CNS	No effect detected ⁽²²⁾	Inhibits ⁽²²⁾	Neutralization of JCV in the oligodendroglia
3. Broad sequestration of leukocytes in the vasculature	No effect detected ^(17, 22)	Induces leukocytosis ^(17, 22)	Pleiotropic impairment of immune surveillance
4. Adaptive immune response to extra-gastrointestinal challenges	No effect detected ⁽²⁸⁾	Inhibits ⁽³³⁾	Pleiotropic impairment of immune surveillance

CNS = central nervous system; JCV = John Cunningham virus; PML = progressive multifocal leukoencephalopathy.

4.2 Secondary Pharmacology

N/A

4.3 Safety Pharmacology

The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 065 dated April 11, 2008.

Cardiovascular Safety Pharmacology with MLN0002 in Cynomolgus Monkeys (KLA00299/DSD-00729)

The objective of this study was to determine the potential effects of MLN0002 on the cardiovascular system following IV infusion to conscious, unrestrained, radiotelemetry-implanted Cynomolgus monkeys. In this study, MLN0002 and the vehicle control (isotonic 20 mM citrate, 125 mM sodium chloride, pH 6.0) were administered to male Cynomolgus conscious monkeys (n = 4/dose) at 10 and 100 mg/kg by 1 hour IV infusion on Day 1 (control) and Day 6 (MLN0002). Cardiovascular parameters (mean arterial pressure/MAP, heart rate, RR-, QT- and QTc- intervals) were recorded (via telemetry) prior to and following dose administration on Day 6. In addition, the animals were evaluated for changes in clinical signs (once daily) and food consumption (once daily), and health checks were conducted twice daily.

There were no significant MLN0002-related effects on clinical signs, ECG parameters, mean arterial blood pressure or heart rate.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The following reviews are incorporated below from the pharmacology review of IND 09125 SDN 065 dated November 14, 2007.

Intravenous Multiple Dose Pharmacokinetic/Pharmacodynamic Study of MLN0002 in Cynomolgus Monkeys (Protocol No. KLA00184, MLN Study No. DSD-00469)

Method: The objective of this study was to provide pharmacokinetic/pharmacodynamic (PK/PD) information in Cynomolgus monkeys (*Macaca fascicularis*) in order to compare MLN0002 generated from two different cell lines (NS0- and CHO-derived). This report presented the PK and PD (CD4+ and CD8+ binding) analysis and primate anti-human antibody (PAHA) response in Cynomolgus monkeys.

On Day 1, male cynomolgus monkeys (n = 4/group, adult, 2.5-5.2 kg) received an intravenous (IV) infusion (30 minutes) of MLN0002 at a dose of 10 mg/kg. On Days 4, 8, 11 and 15 the animals received a slow bolus (approximately 1 minute) IV injection of MLN0002 at 10 mg/kg. Group 1 (Animal Nos. 1001, 1002, 1003 and 1004) animals received MLN0002 obtained from the NS0 cell line and Group 2 (Animal Nos. 2001, 2002, 2003 and 2004) monkeys received MLN0002 obtained from the CHO cell line. Periodic blood samples were collected at scheduled intervals and serum or plasma was prepared from the blood samples. MLN0002 concentrations were quantified in the serum using an enzyme-linked immunosorbent assay (ELISA) method with a lower limit of quantitation (LOQ) of 0.150 µg/mL. The PAHA was determined using an ELISA method. The PD of MLN0002 was determined in whole blood samples. The percent of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ sites or bound MLN0002 was determined by flow cytometry. The following table (from page 13 of the report) shows the study design.

Table 2 Study Design

Group No.	No. of Males	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	Dose Regimen	Last Day of Study
1	4	MLN0002 NS0 cell line	10	4.73	2.11	Approximate 30 minute intravenous infusion in 30 mL on Day 1; slow bolus IV on Days 4, 8, 11 and 15	Day 22
2	4	MLN0002 CHO cell line	10	4.90	2.04		

Blood samples were collected as per the following schedule shown in the following table (from 14 of the report).

Table 3 Study Procedures

Study Day	Dose Administration	PK	PD	PAHA
1	X	Pre, 0.5, 1, 2, 4, 8, 10 h	Pre, 0.5, 1, 2, 4 h	Pre
2		24 h	24 h	
3		48 h	48 h	
4	X	Pre, 0.5 h	Pre, 0.5 h	Pre
8	X	Pre, 0.5 h	Pre, 0.5 h	Pre
11	X	Pre, 0.5 h	Pre, 0.5 h	Pre
15	X	Pre, 0.5, 1, 2, 4, 8, 10 h	Pre, 0.5, 1, 2, 4 h	Pre
16		24 h	24 h	
17		48 h	48 h	
18		72 h	72 h	
22		168 h	168 h	X

Collection times were calculated from the end of the infusion.

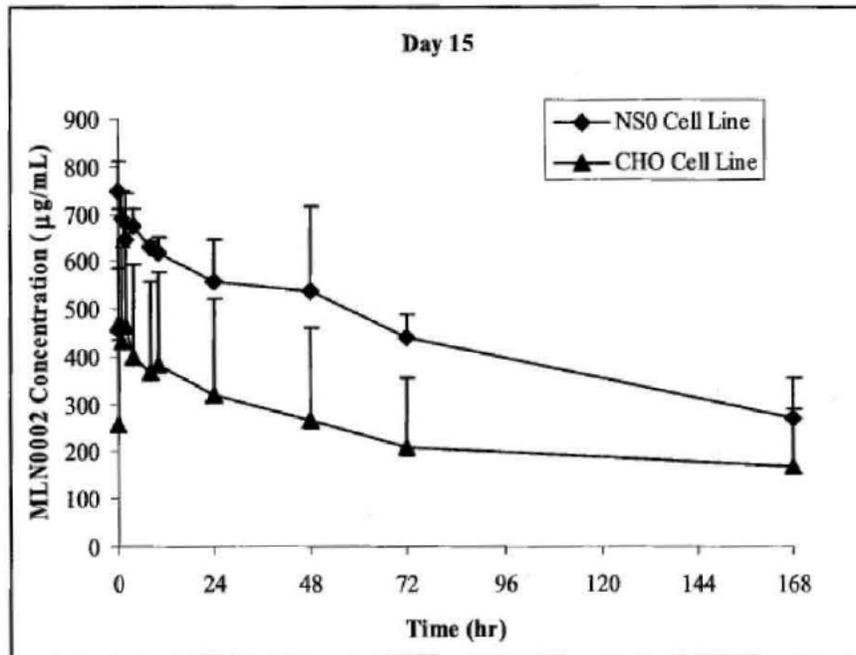
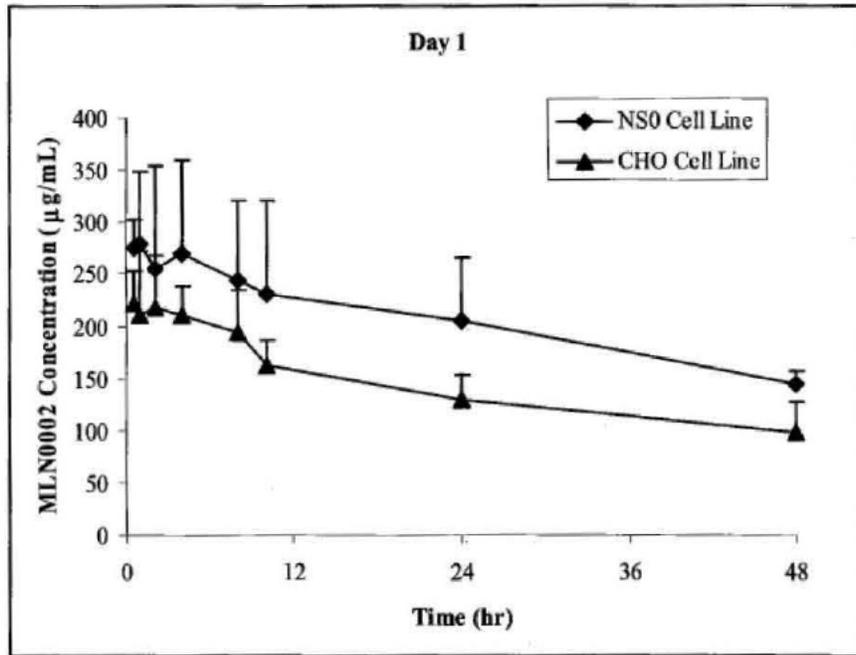
Results:

Pharmacokinetics:

MLN0002 was detected in all of the samples collected from animals dosed with MLN0002 from either cell line throughout the duration of study, except Animal No 2003 on Day 22. The T max on Day 1 occurred at 2.5 and 1.9 hr after initiation of infusion for NS0- and CHO-derived MLN0002, respectively. On Day 15 the mean Tmax was 12.4 and 0.9 hr postdose for NS0 and CHO derived material, respectively. The mean Cmax of MLN0002 was 300 and 250 µg/mL for NS0- and CHO-cell line derived MLN0002 respectively, on Day 1 and 749 and 497 µg/mL on Day 15, respectively. On Day 1, the area under the serum concentration of MLN0002 versus time curve (AUC_{0-48hr}) was 16,800 and 11,700 hr*µg/mL for NS0- and CHO-cell line derived MLN0002, respectively. On Day 15, AUC_{0-168hr} values were 73,800 and 39,900 hr*µg/mL for NS0 and CHO derived materials, respectively. The exposure to MLN0002 (as measured by either Cmax or AUC) was higher (Cmax: 1.2-1.5 times higher and AUC: 1.4-1.8 times higher) in Group 1 (NS0) than in Group 2 (CHO) after both the first (Day 1) and last dose (Day 15). As per the sponsor, the accumulation index (R₀: Day 15 AUC_{0-168hr}/Day 1 AUC_{0-48hr}) appeared to be similar (Group 1: 4.39; Group 2: 3.41) for both sources of material. It is to be noted here that this R₀ may not reflect the true accumulation index, since Day 15 AUC was determined using 0-168hr sampling and Day 1 AUC was determined using 0-48 hr sampling. Due to the limited duration of sample collection after the last dose, no estimate of the terminal elimination half life was made. It is to be mentioned here that in another IV study (KLAW-111) in Cynomolgus monkeys, the terminal elimination half-life was determined to be 22.5 days. Generally, trough serum concentrations tended to increase with each dose administration, with the exception of Animal No 2003. As per the sponsor, development of PAHA in this animal may have increased

the clearance of MLN0002. The following curves (from page 19 of the study report) show the mean serum concentration versus time curves.

Figure 1 Mean Serum Concentration versus Time Curves



The mean PK parameters are shown in the following table (from page 17 of the study report).

Table 5 Mean Pharmacokinetic Parameters of MLN0002

Day	Group	T_{max} (hr)		C_{max} ($\mu\text{g/mL}$)		AUC_{all} (hr* $\mu\text{g/mL}$)		$AUC_{0-\infty}$ (hr* $\mu\text{g/mL}$)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	1 (NS0)	2.5	1.7	300	70.3	16800	3000	39500	9530
	2 (CHO)	1.9	1.6	250	21.2	11700	2350	28000	9790
15	1 (NS0)	12.4	23.7	749	65.3	73800	6900	142000	47200
	2 (CHO)	0.9	0.7	497	238	39900	26100	93600	66500

Animals in Group 1 received MLN0002 derived from the NS0 cell line; animals in Group 2 received MLN0002 derived from the CHO cell line.

Means were calculated from PK parameters for individual animals

Individual Pharmacokinetic parameters are shown in the following table (from page 45 of the study report).

Millennium Pharmaceuticals, Inc.
MLN0002

RPT-00878

Day	Group	Animal No.	T_{max} (hr)	C_{max} ($\mu\text{g/mL}$)	AUC_{all} (hr* $\mu\text{g/mL}$)	$AUC_{0-\infty}$ (hr* $\mu\text{g/mL}$)
1	1 (NS0)	1001	1	251	15000	35400
		1002	4	401	21000	31700
		1003	1	295	16800	37700
		1004	4	253	14400	53300
	2 (CHO)	2001	0.5	258	10600	31200
		2002	2	248	13600	28400
		2003	4	221	8860	14600
		2004	1	271	13600	37900
15	1 (NS0)	1001	0.5	809	79000	210000
		1002	48	796	79000	91000
		1003	0.5	719	72000	140000
		1004	0.5	671	65000	130000
	2 (CHO)	2001	0.5	654	41000	97000
		2002	2	516	60000	110000
		2003	0.5	154	2700	2700
		2004	0.5	662	56000	160000

Animals in Group 1 received MLN0002 derived from the NS0 cell line; animals in Group 2 received MLN0002 derived from the CHO cell line.

Pharmacodynamics:

The pharmacodynamics of NS0- and CHO-derived MLN0002 appeared to be generally similar for both CD4+ and CD8+ cells. In Group 1 (NS0), prior to administration of the first dose, the percent of CD4+ or CD8+ cells staining positive for bound MLN0002 ranged from 0.33% to 1.02% (average 0.7675%) and 2.32% to 8.93% (average 4.835%), respectively. In Group 1, immediately (0.5 hr) after administration of the first dose the percent of CD4+ or CD8+ cells staining positive for bound MLN0002 ranged from 59.68% to 71.34% (average 66.25%) and 63.67% to 87.39% (average 78.88%), respectively. There was no apparent significant effect of source of material on the percent cells staining positive for bound MLN0002 except at 504 hr (Day 21, 7 days after the last dose) where average CD4+ and CD8+ staining for NSO bound drug was 85-87% compared to 63-68% for CHO derived material. These values increased to 75.46% - 85.76% and 80.45% - 91.95%, respectively at 72 hours post dose. In Animal No 2003, the percent of CD4+ or CD8+ cells staining positive for bound MLN0002 was 5.39% and 16.87% at 7 days post last dose, respectively. According to the sponsor, this reduced number may be attributed to high levels of neutralizing PAHA in this animal. At 7 days post last dose (504 hr), average percentage of CD4+ and CD8+ cells staining positive for bound MLN0002 was about 1.3 and 1.3 times higher, respectively, for NS0 derive material than CHO derived material. The following tables show the percent of CD4+ and CD8+ cells staining positive for bound MLN0002 and the averages.

Time (Hr)	Percent of CD4+ and CD8+ Cells Staining Positive for Bound MLN0002 (%)															
	GROUP 1 (NS0)								GROUP 2 (CHO)							
	1001*	1002	1003	1004	1001	1002	1003	1004	2001	2002	2003	2004	2001	2002	2003	2004
	CD4	CD4	CD4	CD4	CD8	CD8	CD8	CD8	CD4	CD4	CD4	CD4	CD8	CD8	CD8	CD8
0	0.73	0.33	0.99	1.02	4.31	2.32	8.93	3.78	0.35	0.98	0.74	0.30	7.67	7.79	8.14	4.15
0.5	63.88	71.34	70.13	59.68	86.73	77.76	87.39	63.67	55.09	61.41	80.49	70.03	79.55	88.73	82.80	74.30
72	75.46	82.32	85.76	77.91	91.95	85.05	90.20	80.45	82.33	70.53	85.62	84.63	91.74	85.93	89.08	84.55
168	82.86	83.95	79.33	78.22	93.22	87.85	89.57	85.66	82.51	74.72	88.25	82.05	93.61	86.53	88.50	80.92
336	76.62	73.88	84.70	69.05	91.51	77.61	89.77	71.97	78.41	70.47	83.38	72.63	92.87	82.66	79.78	72.26
504	84.44	83.89	88.92	82.79	93.33	86.68	88.73	78.69	85.12	77.77	5.39	83.07	91.29	82.40	16.87	80.71

*: Animal ID

Time (Hour)	Average Percent of CD4+ and CD8+ Cells Staining Positive for Bound MNLN0002 (%)			
	GROUP 1 (NS0)		GROUP 2 (CHO)	
	CD4+	CD8+	CD4+	CD8+
0	0.7675	4.835	0.5925	6.9375
0.5	66.25	78.88	66.75	81.345
72	80.3625	86.91	80.775	87.825
168	81.09	89.07	81.88	87.39
336	76.06	82.71	76.22	81.89
504	85.01	86.90	62.83	67.81

Prior to administration of the first dose of MLN0002, the percent of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ sites ranged from 39.17% to 66.41 % and 25.41 % to 70.81 % respectively. Immediately after administration of the first dose the mean percent of cells staining positive for free $\alpha 4\beta 7$ sites decreased to less than 1% for both cell types and sources of MLN0002. This low percent of cells staining positive for free $\alpha 4\beta 7$ sites was maintained through 3 days after administration of the last dose (Day 15). On study day 22 (7 days after administration of the last dose), the percent of cells staining positive for free $\alpha 4\beta 7$ sites increased, ranging from 0.64% to 4.78% and 0.04% to 1.47% for CD4+ and CD8+ cells, respectively. For Animal No 2003, the percent of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ sites was 12.85% and 5.80% respectively. The sponsor speculated that this was due to the presence of neutralizing PAHA in this animal. At 0.5 hour post dose, average percentage of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ was 2 and 5 times higher, respectively, for CHO derive material than NS0 derived material. At 504 hour (Day 21 or 7 days after the last dose), average percentage of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ was 4 and 10 times higher, respectively, for CHO derive material than NS0 derived material. The following tables show the percent of CD4+ and CD8+ cells staining positive for free $\alpha 4\beta 7$ sites and the averages.

Time (Hr)	Percent of CD4+ and CD8+ Cells Staining Positive for free $\alpha 4\beta 7$ (%)															
	GROUP 1 (NS0)								GROUP 2 (CHO)							
	1001 CD4	1002 CD4	1003 CD4	1004 CD4	1001 CD8	1002 CD8	1003 CD8	1004 CD8	2001 CD4	2002 CD4	2003 CD4	2004 CD4	2001 CD8	2002 CD8	2003 CD8	2004 CD8
0	47.23	39.17	58.51	49.11	47.79	54.22	55.74	25.41	41.59	53.56	66.41	44.31	70.81	55.72	52.42	35.28
0.5	0.40	0.13	0.22	0.31	0.05	0.00	0.08	0.00	0.37	0.94	0.44	0.40	0.12	0.00	0.18	0.00
72	0.24	0.09	0.19	0.16	0.00	0.00	0.00	0.00	0.15	0.21	0.32	0.08	0.02	0.00	0.13	0.00
168	1.42	0.77	0.75	0.93	0.00	0.00	0.00	0.02	1.76	1.39	0.96	1.17	0.03	0.00	0.00	0.03
336	0.03	0.00	0.00	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.04	0.09	0.00	0.00	0.00	0.00
504	1.45	1.48	0.64	1.95	0.14	0.27	0.04	0.31	4.78	2.28	12.85	2.82	1.47	0.29	5.80	0.36

Time (Hour)	Average Percent of CD4+ and CD8+ Cells Staining Positive for free $\alpha 4\beta 7$ (%)			
	GROUP 1 (NS0)		GROUP 2 (CHO)	
	CD4+	CD8+	CD4+	CD8+
0	48.5	45.79	51.46	53.55
0.5	0.265	0.0325	0.5375	0.15
72	0.17	0.00	0.19	0.075
168	0.9675	0.005	1.32	0.015
336	0.015	0.0075	0.0325	0.00
504	1.38	0.19	5.68	1.98

PAHA Response:

At Day 22, the average PAHA titer in Group1 (NS0) animals was 4500 and the average PAHA titer in Group 2 (CHO) was 16500, which was about four times higher than that of Group 1. One animal (Animal No 2003) treated with CHO-derived MLN0002 developed a high PAHA titer of 62,500. The sponsor speculated that the high PAHA response in this animal appeared to be neutralizing as it affected both serum concentrations of MLN0002 and PD parameters (CD4+

and CD8+ binding). Therefore, as per sponsor's speculation, about 25% animal apparently developed neutralizing antibody following treatment with CHO cell derived material. In contrast, there was no such indication for the formation of neutralizing antibody in any NS0-treated animals. This is to be mentioned here that the sponsor did not conduct any assay to demonstrate that the PAHA is neutralizing. The sponsor may be asked to conduct such studies to address this issue. The following tables (from page 53 and 54 of the study report) show the PAHA responses in Cynomolgus monkeys using MLN0002 from NS0 cells to capture PAHA and using MLN0002 from CHO cells to capture PAHA.

Using MLN0002 from CHO Cells to Capture PAHA				
Sampling Time	PAHA Response (Titer)			
	Animal No. 1001	Animal No. 1002	Animal No. 1003	Animal No. 1004
Day 1, Pre-dose	Negative	Negative	Negative	Negative
Day 4, Pre-dose	Negative	Negative	Negative	Negative
Day 8, Pre-dose	Negative	Negative	Negative	Negative
Day 11, Pre-dose	100	Negative	Negative	Negative
Day 15, Pre-dose	500 ^a	2500	300	300
Day 22	2500	12500	2500	500 ^a

Using MLN0002 from NS0 Cells to Capture PAHA				
Sampling Time	PAHA Response (Titer)			
	Animal No. 2001	Animal No. 2002	Animal No. 2003	Animal No. 2004
Day 1, Pre-dose	Negative	Negative	Negative	Negative
Day 4, Pre-dose	Negative	Negative	Negative	Negative
Day 8, Pre-dose	Negative	Negative	100	Negative
Day 11, Pre-dose	Negative	100	2500	100
Day 15, Pre-dose	2500	300	12500	300
Day 22	2500	500 ^a	62500	500 ^a

a: The sample tested positive at dilutions 100 and 1000 at screening

Using MLN0002 from NS0 Cells to Capture PAHA				
Sampling Time	PAHA Response (Titer)			
	Animal No. 1001	Animal No. 1002	Animal No. 1003	Animal No. 1004
Day 1, Pre-dose	Negative	Negative	Negative	Negative
Day 4, Pre-dose	Negative	Negative	Negative	Negative
Day 8, Pre-dose	Negative	Negative	Negative	Negative
Day 11, Pre-dose	300	Negative	Negative	Negative
Day 15, Pre-dose	2500	2500	300	300
Day 22	2500	12500	2500	500 ^a

Sampling Time	PAHA Response (titer)			
	Animal No. 2001	Animal No. 2002	Animal No. 2003	Animal No. 2004
Day 1, Pre-dose	Negative	Negative	Negative	Negative
Day 4, Pre-dose	Negative	Negative	Negative	Negative
Day 8, Pre-dose	Negative	Negative	100	Negative
Day 11, Pre-dose	100	100	2500	100
Day 15, Pre-dose	2500	300	12500	300
Day 22	2500	500 ^a	62500	900

a: The sample tested positive at dilutions 100 and 1000 at screening.

In summary, considerable differences in PK parameters (C_{max} and AUC) were observed between the two sources of material. Generally, the exposure (C_{max} and AUC) to MLN0002 was considerably lower after administration of CHO cell line derived MLN0002 when compared to NS0 cell line derived material. The PD response generally appeared to be similar for both sources of material. However, at 7 days after the last dose (504 hour), average percentage of CD4⁺ and CD8⁺ cells staining positive for bound MLN0002 was about 1.3 and 1.3 times higher, respectively, for NS0 derived material than CHO derived material. At 504 hour (Day 21 or 7 days after the last dose), average percentage of CD4⁺ and CD8⁺ cells staining positive for free $\alpha 4\beta 7$ was about 4- and 10 times higher, respectively, for CHO derive material than NS0 derived material. At Day 22, the average PAHA titer in Group1 (NS0) animals was 4500 and the average PAHA titer in Group 2 (CHO) was 16500, which was about four times higher than that of Group 1. As per sponsor's speculation, about 25% animal (1 of 4 monkeys) developed neutralizing antibody following treatment with CHO cell derived material. In contrast, there was no such indication for the formation of neutralizing antibody in any NS0-treated animals. Overall, MLN0002 derived from NS0 and CHO cell did not appear to be comparable based on the PK data and PAHA response in this study. This is to be mentioned here that the sponsor did not conduct any assay to demonstrate that the PAHA was neutralizing in Cynomolgus monkeys following administration of CHO cell derived MLN0002. The sponsor may be asked to conduct such studies to address this issue.

Pharmacokinetics of IDP-02 Following Intravenous and Subcutaneous Administration to Cynomolgus Monkeys (LDP-02-14)

Methods: This study was conducted using two groups of three female Cynomolgus monkeys per group. Group 1 animals received LDP-02 once at 2.5 mg/kg (0.75 mL/kg) *via* intravenous (IV) infusion for approximately one hour on Day 1. Group 2 monkeys received LDP-02 once at 2.5 mg/kg (0.75 mL/kg) *via* subcutaneous (SC) injection. Blood samples were collected on Day -1, two hours and one hour prior to treatment and at 1, 2, 4, 8, 20, 24, 48, 72, 96, 120, 144, 168, 192, 240, and 336 hours after treatment. In addition, saturation of $\alpha 4\beta 7$ on the surface of circulating lymphocytes was also studied using flow cytometry (report addendum).

Results:

Pharmacokinetics: Following IV administration (Group 1), serum LDP-02 C_{max} value was achieved at 1.41 hours and serum concentrations ranged from 40.04 to 67.27 $\mu\text{g/mL}$. The terminal elimination half-life ($t_{1/2}$) was estimated to be 102.6 hours. When LDP-02 was administered *via* SC injection (Group 2), t_{max} values ranged from 49.42 to 145.25 hours. Pharmacokinetic (PK) parameters could not be determined for SC administration as LDP-02 could not be measured at time points that exceeded 11 days in the majority of animals because of a probable immune response that interfered with the method of analysis. The following table (from page 32 of the report) shows the PK parameters following IV administration.

TABLE 1
LDP-02 SERUM PHARMACOKINETIC PARAMETERS
FEMALE CYNOMOLGUS MONKEYS

PARAMETER	Intravenous Administration				
	1101	1102	1103	Mean	SD
Animal Number	1101	1102	1103	Mean	SD
Dose (mg/kg)	2.5	2.5	2.5	2.5	0
Body Wt (kg)	4.1	2.6	2.9	3.2	0.8
C_{max} ($\mu\text{g/mL}$)	67.27	43.66	40.04	50.32	14.79
t_{max} (hr)	1.02	2.21	1.00	1.41	0.69
λ (hr^{-1})	0.0074	0.0063	0.0066	0.0068	0.0006
$t_{1/2}(\lambda)$ (hr)	93.7	109.7	104.3	102.6	8.1
AUC_t ($\mu\text{g}\cdot\text{hr}/\text{mL}$)	4759	3802	3073	3878	846
AUC_{∞} ($\mu\text{g}\cdot\text{hr}/\text{mL}$)	7240	5525	4838	5868	1237
% area extrap.	34.3	31.2	36.5	34.0	2.7
$AUMC_{\infty}$ ($\mu\text{g}\cdot\text{hr}^2/\text{mL}$)	984,135	915,434	770,583	890,051	109,015
MRT_t (hr)	135.4	165.1	158.8	153.1	15.6
Cl_{ms} ($\text{mL}/\text{hr}/\text{kg}$)	0.345	0.453	0.517	0.438	0.087
Vd_{ss} (mL/kg)	46.8	74.7	82.1	67.9	18.6

AUC_t : $t = 145.10$ hours for animal No. 1101
 $t = 193.01$ hours for animal No. 1102
 $t = 168.85$ hours for animal No. 1103

Flow Cytometry: Following treatment with LDP-02, there was a rapid decline in the percent of cells as detected by ACT-1 biotin. Within 1 hour postdose, the percentage of cells detected by ACT-1 biotin dropped below the detectable levels following IV dosing and to 9.5% following SC administration. Overall, single SC or IV administration of LDP-02 to Cynomolgus monkeys at 2.5 mg/kg blocked almost all available circulating $\alpha 4\beta 7$ integrins for up to 240 hours in most animals.

A Single Dose Pharmacokinetic/Pharmacodynamic Study of MLN002 Administered Intravenously to Cynomolgus Monkeys (RPT-00892, Study No. KLA00276)

The following review is incorporated below from the pharmacology review of IND 09125 SDN 065 dated April 11, 2008.

A Single Dose Pharmacokinetic/Pharmacodynamic Study of MLN002 Administered Intravenously to Cynomolgus Monkeys (KLA00276)

Methods: The objective of this study was to evaluate the pharmacokinetics and pharmacodynamics (PK/PD), including the duration of target receptor saturation of MLN002 after a single dose, of 10 or 100 mg/kg of MLN002.

In this study, two groups of three female Cynomolgus monkeys were administered either 10 mg/kg (Group 1) or 100 mg/kg (Group 2) MLN0002 by slow bolus intravenous (IV) infusion over approximately 30 minutes on Day 1. Clinical signs and body weights were recorded. Blood samples were collected at specified time points to perform assays to assess PK by ELISA, PD (binding to $\alpha 4\beta 7$ integrin) by flow cytometry, and immunogenicity by ELISA to measure primate anti-humanized antibody (MLN0002) antibodies (PAHA).

Results: The C_{max} of 214 and 2090 $\mu\text{g/mL}$ was observed at T_{max} at 0.5 hour postdose, and the AUC_{0-161hr} were 32,700 and 54,2000 $\mu\text{g}\cdot\text{hr/mL}$ at 10 and 100 mg/kg, respectively. The clearance of MLN0002 was 0.266 and 0.180 mL/h/kg at doses of 10 and 100 mg/g, respectively, and volume of distribution was 80.7 and 88.3 mL/kg, respectively, and the half life was 336 and 362 hours, respectively. The PK was approximately dose proportional between 10 and 100 mg/kg.

MLN0002 caused a rapid decrease in percentage of CD4+ (14%), CD20+ (0.9%), CD4+CD45RA+ (12%), CD4+CD45RA- (18%) cells staining positive for free $\alpha 4\beta 7$ integrin sites. The percentage of cells with free sites gradually declined, reaching 0.0-1.0% by Day 43. This PD response was maintained through day 85. MLN0002 resulted in rapid increase in percentage of CD4+ (50-57%), CD4+CD45RA+ (60-72%), CD4+ CD45RA- (30%) cells staining positive for bound MLN0002 after a single 0.5 hour IV infusion at 10 or 100 mg/kg, compared to the pre-study values of ~2%. The percent of cells staining positive for bound MLN0002 increased until 24-72 hours postdose, and then declined thereafter. The rate of decline was dose dependent. MLN0002 was immunogenic at both dose levels. The range of PAHA titers was 100-12500. There was no apparent dose dependency or relationship to time in PAHA response.

5.2 Toxicokinetics

Toxicokinetics is discussed in the review of individual toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

An Acute Intravenous Infusion Toxicology Study of MLN0002 Manufactured Using Process B and Process C in Cynomolgus Monkeys (KLA00348)

The following review is incorporated below from the pharmacology review of IND 09125 dated May 13, 2011.

An Acute Intravenous Infusion Toxicology Study of MLN0002 Manufactured Using Process B and Process C in Cynomolgus Monkeys (KLA00348)

The sponsor previously submitted draft reports of this study under SDN 88 dated September 19, 2008 and SDN 101 dated December 23, 2008. The final report of this study is submitted under SDN 341 dated March 16, 2001. The final report is reviewed below. The results and interpretations of the final report do not seem to differ from that of the draft reports submitted under SDN 88 and 101.

Report No.	Testing Laboratory	Species & Route	Date Started	Date Completed	Batch No.
KLA00348	(b) (4)	Cynomolgus Monkey, IV infusion	6/24/2008	10/29/2008	Process B : IA015SA01, pre- (b) (4) Process C : IC006LA, lyophilized powder

GLP Compliance: Statements of compliance with GLP regulations and the quality assurance unit (QAU) were included.

Methods: The objectives of this study were two-fold: a) to re-evaluate the potential acute toxicity of MLN0002 manufactured using Processes B and Process C and b) to characterize the toxicokinetics and provide limited characterization on the pharmacodynamic responses in Cynomolgus monkeys. In this study, Cynomolgus monkeys (n = 3/ sex /dose, 2.3 to 3.4 years of age for the males and 2.7 to 3.5 years of age for the females, and weighing 2.2 to 2.9 kg for the males and 2.2 to 2.8 kg for the females) were assigned to dose groups as shown in the table below (from page 8 of the report).

Group No.	Number of Males/Females	Dose Level (mg/kg)	MLN0002 Material
1	3/3	0	Control Article
2	3/3	10	Process B
3	3/3	30	Process B
4	3/3	10	Process C
5	3/3	30	Process C

All animals were treated via a 30 minute IV infusion on Day 1 at 0 (0.9% saline), 10, 30 mg/kg with Process B and Process C MLN0002. Animals were evaluated for changes in clinical signs, body weight, and clinical pathology indices. Blood samples for serum chemistry and hematology were collected from all animals at prestudy and on Day 8. Blood samples were collected for TK (Day 1 at predose and 0.5, 1, 4, 12, 24, 48, 72, 120, and 168 hours post dose), PD (Day 1 at predose and 0.5, 1, 4, 12, 24, 48, 72, and 168 hours postdose), and PAHA (Days -7 and 1 at predose and 72 and 168 hours post dose) analyses.

Results: There were no mortalities. There were no significant treatment-related effects on clinical signs, food consumption, body weight, serum chemistry, or hematology parameters following administration of either Process B or Process C MLN0002. Process B and Process C MLN0002 showed similar serum TK parameters (C_{max} , $AUC_{0-168hr}$) after a 10 and 30 mg/kg IV. Mean serum C_{max} values were 296 and 342 $\mu\text{g/mL}$ at 10 mg/kg and 794 and 828 $\mu\text{g/mL}$ at 30 mg/kg for Process B and Process C MLN0002, respectively. The mean serum $AUC_{0-168hr}$ values were 25,100 $\mu\text{g}\cdot\text{hr/mL}$ at 10 mg/kg for both Process B and Process C MLN0002, and values were 64,800 and 66,500 $\mu\text{g}\cdot\text{hr/mL}$ at 30 mg/kg for Process B and Process C MLN0002, respectively. Process B and Process C MLN0002 were not immunogenic and no pronounced primate anti-human antibody (PAHA) titers were observed in any animals. Mean TK parameters are shown in the following table (from page 34 of the report).

Table: Mean Serum Toxicokinetic Parameters of MLN0002 in Cynomolgus Monkeys (Genders Combined)

Dose (mg/kg)	Process	C_{max} ($\mu\text{g/mL}$)			$AUC_{0-168hr}$ ($\text{hr}\cdot\mu\text{g/mL}$)			C_{max}/Dose	$AUC_{0-168hr}/\text{Dose}$
		Mean ^a	SD	CV (%)	Mean	SD	CV (%)		
10	B	296	33.2	11.2	25,100	1620	6.45	29.6	2510
10	C	342	79.7	23.3	25,100	4430	17.6	34.2	2510
30	B	794	43.4	5.47	64,800	3420	5.28	26.5	2160
30	C	828	113	13.6	66,500	4520	6.80	27.6	2220

$AUC_{0-168hr}$ = area under the concentration-versus-time curve from 0 to 168 hours; C_{max} = maximum concentration; CV = coefficient of variation; SD = standard deviation;

^a n = 6

Pharmacodynamic results showed that $\alpha 4\beta 7$ sites on peripheral lymphocytes were saturated with MLN0002 shortly after dosing and this was maintained for the entire study period (7 days postdose). There were no marked differences in the extent of binding to the target ($\alpha 4\beta 7$ sites) between Process B and Process C MLN0002 and/or between the different cell types evaluated. Overall, the Process B and Process C MLN0002 appeared to be comparable in this study.

6.2 Repeat-Dose Toxicity

Study title: 14-Day Toxicity Study of LDP-02 Administered via Intravenous Infusion to Cynomolgus Monkeys

Study no.: 3-G10
Report No.: LDP-02-15
Study report location: EDR 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: October 29, 1996
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: LDP-02, 3A9/TAC Lot # 3, Purity data not provided

Key Study Findings:

- In a 14-day IV infusion study in Cynomolgus monkeys, animals (n =1/sex) were treated with LDP-02 at 2.5 mg/kg once a day for 14 days. In this study, three groups received identical treatment and were sacrificed at different time points. There were no control animals and only one dose was tested using one animal per sex.
- There was no mortality.
- Ova and parasites (*Balantidium coli*, *Oesophagostomum* sp, *Trichuris* sp, etc.) were observed in the stool samples of several male monkeys. Parasitic infections are common in monkeys and the relation to the treatment is unclear in the absence of control animals and a dose response.
- Gross necropsy findings included a nodule in the stomach in one Group 1 male and one Group 1 and Group 2 female had discoloration of the skin at the infusion site. One Group 3 male had lesion (adhesions) in the lungs and one Group 3 female had a multifocal discoloration on the heart (that was considered secondary to a cardiac puncture for blood collection at necropsy).
- Histopathological changes were seen in the testes (focal seminiferous tubular ectasia and rupture, accompanied by focal aggregates of immature spermatogonia within the adjacent epididymal tubules of the Group 1 male), pituitary (focal vacuolization within the hippocampus and pars intermedia in the Group 3 male), and lens (mild lenticular degeneration at the posterior equator of the lens in all six animals). One Group 2 female (No. 2101) showed linear hemorrhage and necrosis within the myocardium which was attributed to the cardiac puncture procedure performed on this animal for blood collection. Moreover, moderate lymphoid hyperplasia was observed within the spleen, salivary gland, stomach, duodenum, and vaginal stroma as well as within mandibular and mesenteric lymph nodes in several animals. The relation to the treatment is unclear.
- No conclusions could be made from this study, as this study used only one animal per sex, one dose and there was no control group. The results of this study are not interpretable.

Methods: The study consisted of three groups of one male and one female Cynomolgus monkey per group. LDP-02 was administered at a dose level of 2.5 mg/kg once a day for 14 consecutive days via IV infusion over a one hour period on Day 1, a 30 minute period on Day 2 and over 15 minute periods on Days 3-14. Group 1, 2, and 3 monkeys were euthanized on Days 14, 18, and 29, respectively.

Doses: 2.5 mg/kg
 Frequency of dosing: Once a day
 Route of administration: IV infusion
 Dose volume: 0.75 mL/kg
 Formulation/Vehicle: 0.9% Sodium chloride
 Species/Strain: Cynomolgus monkey
 Number/Sex/Group: 1/sex/dose
 Age: Not specified, young adult
 Weight: Not provided
 Satellite groups: None
 Unique study design: Study design is shown below
 Deviation from study protocol: Protocol deviations did not affect the quality or integrity of the study or the interpretation of the results in the report.

The following table (from page 15 of the report) shows the study design.

Group Number	Number of Animals		Treatment Administration				Necropsy
	Males	Females	Substance	Dose Level (mg/kg) ¹	Route	Dosing Regimen	
1	1	1	LDP-02	2.5	IV infusion	On Day 1: 1 hr infusion; On Day 2: 30 min infusion; On Days 3-14: 15 min infusion	Day 14
2	1	1					Day 18
3	1	1					Day 29

¹ The test article solution (received from the Sponsor) was diluted to 30 mL in 0.9% NaCl and infused over a 1 hour period on Day 1. On Day 2, the infusion was 30 minutes long and on Days 3 - 14, the infusion was 15 minutes. The infusions were administered once every 24 hours (\pm 2 hrs) on Days 1-14.

Observations and Results:

Mortality: Mortality was checked twice daily. There were no mortalities.

Clinical Signs: Clinical signs were observed once daily. Clinical signs included scab formation around nose and mouth, emesis, and soft feces. The relation to the treatment is unclear.

Body Weights: Body weights were recorded on Days -5, -1, 1 through 14, 18, and 29. The mean initial (Day -5) body weight of the male was 3.5 kg. The mean initial (Day -5) body weight of the female was 2.4 kg. No comments could be made regarding the effect of treatment in the absence of control.

Food Consumption: Food consumption was recorded daily. The mean initial (Day 4) food consumption of the male was 13.0 g/animal/day. The mean initial (Day 4) food consumption of the female was 6.0 g/animal/day. No comments could be made regarding the effect of treatment in the absence of control.

Ophthalmoscopy: Ophthalmoscopy was conducted weekly. No abnormalities were detected prior to the treatment or during the course of the study.

Electrocardiography (ECG): Electrocardiography was not performed. However, blood pressure, heart rate and respiration rate were recorded at specified times. Heart rate, body temperature and respiratory rates remained relatively constant throughout the study.

Hematology: Blood samples were collected on Days -5, 1, 3, 9, 14, 18, 24 and 29. In general, there was a decline in the red blood cell (RBC) count, hemoglobin and hematocrit during the first nine days of the study when compared to the pre-treatment values on Day -5. The decrease in RBC count ranged 5 to 16% for males and 12 to 19% for females. These were attributed to the repeated blood collections over the first nine days of the study and were not considered treatment-related.

Clinical Chemistry: Blood samples were collected on Days -5, 1, 3, 9, 14, 18, 24 and 29. There were no significant changes in serum chemistry parameters when compared to the pre-treatment values.

Urinalysis: Urine samples were collected on Day 1 and at necropsy. One female (No. 2101) had elevated white, red, epithelial cells in the sediment when compared to Day 1 value.

Stool: Rectal swabs for culture were collected on Days 1, 7, 10, 14, 17 and 29. Stool samples for ova and parasite determinations were collected on Days 1, 7, 10, 14, 18 and 29. Ova and parasites were observed in stool samples of several male monkeys. Group 1 monkey No. 1001 was positive for *Balantidium coli* on Day 1 and on Day 14 and positive for *Oesophagostomum* sp. on Day 14. Group 2 monkey No. 2001 was positive for *Trichuris* sp. on Day 7. In Group 3, monkey No. 3001 was positive for *Balantidium coli* on Days 1, 7 and 29. No ova and parasites were observed in the stool samples of female monkeys during the course of the study. Parasitic infections are

common in monkeys and the relation to the treatment is unclear in the absence of control animals and dose response.

Gross Pathology: Gross pathology was conducted at necropsy. Group 1 male No. 1001 was found to have a nodule in the stomach. Group 1 female No. 1101 and Group 2 female No. 2101 had discoloration of the skin at the site of infusion. Group 2 male No. 2001 was found to have parasites in the cecum. Group 3 male No. 3001 had a notable lesion (adhesions) in the lungs while Group 3 female No. 3101 had a multifocal discoloration on the heart that was secondary to a cardiac puncture at necropsy to obtain blood. The relation to the treatment is unclear in the absence of a control group and dose response (one dose was used) and one animal per sex was used in this study.

Organ Weights: The following (from page 24 of the report) organs were weighed from all animals. In males, the absolute weight of the liver and kidneys increased slightly across Groups 1, 2 and 3; however, only the relative weight (to body weight) of the kidneys was increased. In females, similar increases were noted for absolute liver and kidney weights. In female monkeys, however, only increases in the relative (to body weight) liver weights across Groups 1, 2 and 3 were observed. The Applicant did not state whether these increases were relative to the pretreatment values. The relation to the treatment is unclear in the absence of controls and limited number of animals.

Text Table 5
Tissues Examined at Necropsy

Adrenal glands *	Mammary gland
Aorta	Ovaries *
Bone marrow	Oviducts
Brain *	Pancreas
Cervix/Vagina	Peripheral nerve
Epididymides	Pituitary gland
Esophagus	Prostate gland
Eyes	Salivary glands
Femur	Seminal vesicles
Gallbladder	Skeletal muscle
Heart	Skin
Intestine, large	Spinal cord
cecum	Spleen
colon	Sternum
rectum	Stomach
Intestine, small	Testes *
duodenum	Thymus
ileum	Thyroid/Parathyroids *
jejunum	Tongue
Kidneys *	Tonsils
Larynx/Pharynx	Trachea
Liver *	Urinary bladder
Lungs	Uterus
Lymph nodes	
mandibular	Administration site
mesenteric	Gross lesions
	Tissue masses

* Tissues weighed

NOTE: Animal identification was saved.

Histopathology: The following (from page 25 of the report) tissues were collected for histopathology from all animals.

Text Table 6
Tissues Processed and Examined Histopathologically

Cardiovascular aorta heart	Integument skin mammary gland
Digestive large intestine (cecum, colon, rectum) liver/gallbladder pancreas/islets salivary gland small intestine (duodenum, jejunum, ileum) stomach esophagus	Hematopoietic/Lymphoid bone marrow (femur) lymph nodes (mandibular, mesenteric) spleen thymus
Respiratory trachea lungs	Neurologic brain (medulla/pons, cerebellum and cerebral cortex) spinal cord (cervical, mid-thoracic and lumbar) sciatic nerve
Endocrine adrenal glands (cortex and medulla) parathyroid glands pituitary gland thyroid gland	Musculoskeletal sternum skeletal muscle
Reproductive/Male epididymides testes prostate seminal vesicles	Urinary kidneys urinary bladder
Reproductive/Female ovaries/oviducts uterus vagina cervix	Other administration site tissue masses gross lesions eyes

Histological Findings: Histopathological changes were seen in the testes (focal seminiferous tubular ectasia and rupture, accompanied by focal aggregates of immature spermatogonia within the adjacent epididymal tubules of the Group 1 male), pituitary (focal vacuolization within the hippocampus and pars intermedia in the Group 3 male), and lens (mild lenticular degeneration at the posterior equator of the lens in all six animals). As per the Applicant, these findings in the testes, pituitary and lens were incidental and unrelated to the test article as there was no distinct difference in severity of these changes among treatment groups. One Group 2 female (No. 2101) showed linear hemorrhage and necrosis within the myocardium which was attributed to the cardiac puncture procedure performed on this animal for blood collection. Moreover, moderate lymphoid hyperplasia was observed within the spleen, salivary gland, stomach, duodenum, and vaginal stroma as well as within mandibular and mesenteric lymph nodes in several animals. It was not clear whether this lymphoid hyperplasia is related to test article administration in the absence of a control. The following table (from page 40 of the report) shows the incidence of microscopic changes.

Pathology Table 2
Incidence of Microscopic Lesions

Tissue/Lesion	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
Lungs						
-perivascular inflammation	1/1	1/1	1/1	1/1	1/1	1/1
-pleural fibroplasia	0/1	0/1	0/1	0/1	1/1	0/1
Heart						
-myofiber degeneration	0/1	0/1	0/1	1/1	0/1	0/1
-peracute hemorrhage	0/1	0/1	0/1	1/1	0/1	0/1
Trachea						
-nonsuppurative inflammation	1/1	1/1	1/1	1/1	1/1	1/1
Salivary Glands						
-nonsuppurative inflammation	1/1	0/1	0/1	0/1	1/1	1/1
-lymphoid hyperplasia	0/1	0/1	0/1	1/1	1/1	1/1
Mandibular lymph nodes						
-extramedullary hematopoiesis	0/1	0/1	0/1	1/1	0/1	0/1
-erythrophagocytosis	0/1	0/1	1/1	0/1	0/1	1/1
-paracortical expansion	0/1	0/1	1/1	0/1	0/1	1/1
-sinus histiocytosis	1/1	0/1	0/1	1/1	0/1	0/1
-follicular hyperplasia	1/1	1/1	1/1	1/1	1/1	0/1
-sinusoidal hemorrhage	0/1	0/1	1/1	0/1	0/1	0/1
Mesenteric Lymph nodes						
-sinus eosinophilia	0/1	0/1	1/1	0/1	0/1	1/1
-sinus histiocytosis	1/1	1/1	1/1	1/1	0/1	1/1
-follicular hyperplasia	0/1	1/1	0/1	1/1	1/1	0/1
Liver						
-nonsuppurative inflammation	1/1	1/1	1/1	1/1	1/1	1/1
Duodenum						
-lymphoid hyperplasia	0/1	1/1	0/1	1/1	0/1	0/1
Stomach						
-intraluminal yeasts	0/1	1/1	0/1	0/1	0/1	0/1
-nonsuppurative inflammation	1/1	1/1	1/1	1/1	1/1	1/1
-spirochaetosis	1/1	1/1	0/1	1/1	1/1	1/1
-lymphoid hyperplasia	1/1	0/1	0/1	1/1	1/1	1/1

* Numerator = Number of tissues with lesion in group; Denominator = Number of tissues examined in group.

Pathology Table 2 (Continued)
Incidence of Microscopic Lesions

Tissue/Lesion	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
Colon						
-balantidiasis	1/1	0/1	0/1	0/1	1/1	0/1
-submucosal granuloma	1/1	0/1	0/1	0/1	0/1	0/1
-spirochaetosis	1/1	1/1	1/1	1/1	1/1	1/1
Cecum						
-trematodiasis	0/1	0/1	1/1	0/1	0/1	0/1
-nonsuppurative inflammation	1/1	0/1	0/1	0/1	0/1	0/1
-spirochaetosis	1/1	1/1	1/1	1/1	1/1	1/1
-balantidiasis	1/1	0/1	0/1	0/1	1/1	0/1
Rectum						
-spirochaetosis	0/1	0/1	1/1	1/1	1/1	0/1
Spleen						
-lymphoid hyperplasia	1/1	1/1	1/1	1/1	1/1	1/1
Thyroid gland						
-nonsuppurative inflammation	0/1	0/1	0/1	0/1	0/1	1/1
Kidneys						
-nonsuppurative inflammation	0/1	1/1	0/1	0/1	1/1	1/1
Skin						
-nonsuppurative inflammation	0/1	0/1	1/1	0/1	0/1	0/1
Testes		NA		NA		NA
-tubular rupture	1/1		0/1		0/1	
-tubular ectasia	1/1		0/1		0/1	
Epididymides		NA		NA		NA
-immature spermatogonia	1/1		0/1		0/1	
Vagina	NA		NA		NA	
-lymphoid hyperplasia		1/1		0/1		0/1
-nonsuppurative inflammation		0/1		1/1		1/1

* Numerator = Number of tissues with lesion in group; Denominator = Number of tissues examined in group; NA = Not Applicable.

Pathology Table 2 (Concluded)
Incidence of Microscopic Lesions

Tissue/Lesion	Group 1		Group 2		Group 3	
	M	F	M	F	M	F
Admin Site						
-thrombosis	0/1	1/1	0/1	0/1	0/1	0/1
-acute hemorrhage	1/1	1/1	1/1	1/1	0/1	0/1
-intimal hyperplasia	1/1	1/1	1/1	1/1	1/1	1/1
-suppurative inflammation	1/1	1/1	0/1	1/1	0/1	0/1
-nonsuppurative inflammation	0/1	1/1	1/1	1/1	0/1	0/1
Pituitary Gland						
-vacuolization	0/1	0/1	0/1	0/1	1/1	0/1
Eyes						
-lenticular degeneration	1/1	1/1	1/1	1/1	1/1	1/1
Brain						
-vacuolization	0/1	0/1	0/1	0/1	1/1	0/1

* Numerator = Number of tissues with lesion in group; Denominator = Number of tissues examined in group.

Toxicokinetics: Data not provided

Saturation of Binding and Circulating Antibody: To monitor the saturation of surface antigen by LDP-02, lymphocytes present in whole blood were collected at various time points and were stained with an anti-human IgG-biotin using flow cytometry. The results indicated that LDP-02 saturated the surface antigen at the tested dose. Circulating levels of LDP-02 in these animals dropped to 900 ng/mL by Day 14 postdose. There were no free $\alpha 4\beta 7$ binding sites on lymphocytes.

Dosing Solution Analysis: Data not provided

Summary: In a 14-day IV infusion study in Cynomolgus monkeys, animals (n =1/sex) were treated with LDP-02 at 2.5 mg/kg once a day for 14 days. In this study, three groups received identical treatment and were sacrificed at different time points. There were no control animals and only one dose was tested using one animal per sex. There was no mortality. Ova and parasites (*Balantidium coli*, *Oesophagostomum* sp, *Trichuris* sp, etc.) were observed in the stool samples of several male monkeys. Parasitic infections are common in monkeys and the relation to the treatment is unclear. Histopathological findings included testes (focal seminiferous tubular ectasia and rupture, accompanied by focal aggregates of immature spermatogonia within the adjacent epididymal tubules of the Group 1 male), pituitary (focal vacuolization within the hippocampus and pars intermedia in the Group 3 male), and lens (mild lenticular degeneration at the posterior equator of the lens in all six animals). As per the Applicant, these findings in the testes, pituitary and lens were incidental and unrelated to the test article as there was no distinct difference in severity of these changes among

treatment groups. One Group 2 female (No. 2101) showed linear hemorrhage and necrosis within the myocardium which was attributed to the cardiac puncture procedure performed on this animal for blood collection. Moreover, moderate lymphoid hyperplasia was observed within the spleen, salivary gland, stomach, duodenum, and vaginal stroma as well as within mandibular and mesenteric lymph nodes in several animals. It is unclear whether this lymphoid hyperplasia is related to test article administration in the absence of a control. No conclusions could be made from this study, as this study used only one animal per sex, one dose and there was no control group. Overall, the results of this study are not interpretable.

13-Week Intravenous Injection Toxicity Study of MLN0002 in Cynomolgus Monkeys with a 12-Week Recovery Period (Report No. 502045)

The review of this study is incorporated below from the pharmacology review of IND 9125 dated July 11, 2007.

Study Title: 13-Week Intravenous Injection Study of MNL002 in Cynomolgus Monkeys

Key Study Findings: In a 13-week IV injection study in Cynomolgus monkeys with a 12-week treatment-free recovery, animals (n = 4/sex/group for main study; 2/sex/group for recovery) were treated once every 2 weeks at 0, 10, 30 and 100 mg/kg/day. The target organs of toxicity could not be identified in the absence of any significant organ toxicity. The NOAEL appeared to be 100 mg/kg/day. MLN0002 was immunogenic in most, but not all, animals. In general, the PAHA had an effect on the TK and PD of MLN0002, with the effect being most pronounced at the lowest dose (10 mg/kg).

Study No.: 502045

Volume # Page #: Vol. 2, page 1

Conducting Laboratory and Location:

(b) (4)

(b) (4)

Date of Study Initiation: May 11, 2006

Date of Study Completion: May 29, 2007

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: MLN0002, Lot No. MLN02-2006-1, 98%

Methods:

Doses: 0(0.9% NaCl for Injection, USP), 10, 30 and 100 mg/kg/day (once every 2 weeks)

Basis of Dose Selection: Not provided

Species/Strain: Cynomolgus monkeys (*Macaca fascicularis*)

Number/Sex/Group or Time Point (Main Study): 4/sex/group

Route, Formulation, Volume, and Infusion Rate: Intravenous slow injection, solution, 13.2 mL/kg. The test/control articles were administered by slow intravenous injection over 10 minutes using an infusion pump into the saphenous vein on Days 1, 15, 29, 43, 57, 71, and 85. Although the protocol stated that dose formulations would be administered over a 20-30 minute period, the formulations were administered over a 10-minute period. The sponsor stated that this protocol deviation did not appear to have any significant impact on the results of the study since the intent of the protocol was met (i.e., injection was slow and constant rather than being a fast manual push). However, the sponsor did not clarify the reason for this protocol deviation.

Satellite Groups Used for Recovery: 2/sex/group

Age: 3-3.5 years

Weight: Males: 1.8-2.5 kg; Females: 1.8-2.4 kg

Study Design or Methodology: The study design is shown in the following table (from page 15 of the study report)

Text Table 1	Study Design	Number of Animals			
		Main Study ^a		Recovery ^b	
		Males	Females	Males	Females
1/ Vehicle Control	0	101, 102, 104, 105	151, 152, 154, 155	103, 106	153, 156
2/ MLN0002	10	201, 202, 204, 205	251, 252, 254, 255	203, 206	253, 256
3/ MLN0002	30	301, 302, 304, 305	351, 352, 354, 355	303, 306	353, 356
4/ MLN0002	100	401, 402, 404, 405	451, 452, 454, 455	403, 406	453, 456

^a Main Study animals were euthanized on Day 92

^b Recovery animals were euthanized on Day 176

Observation and Times:

Clinical Signs: Clinical signs were observed twice daily.

Mortality: Mortality was observed twice daily.

Body Weights: Body weights were recorded twice weekly.

Food Consumption: Food consumption was not recorded and was observed qualitatively.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted once prior to the start of treatment (all animals) and, before dosing during Week 13. Funduscopy (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed by a board-certified veterinary ophthalmologist. No ophthalmology examinations were performed during the recovery period since no treatment-related findings were observed during the treatment.

Electrocardiography: Once during the pretreatment period and during Week 13 of the treatment period, electrocardiogram recordings were performed on all animals using limb leads I, II, III, aVR, aVL and aVF. Electrocardiograms were also obtained during Week 25 of the recovery. Since there were no treatment-related cardiologic changes seen after 13 weeks of treatment, the ECG tracings obtained during the recovery were retained and archived with the study but not evaluated.

Hematology: Hematology was conducted twice prior to the start of treatment, during Weeks 5 and 9, and at scheduled necropsy (Days 92 and 176).

Clinical Chemistry: Serum chemistry was conducted twice prior to the start of treatment, during Weeks 5 and 9, and at scheduled necropsy (Days 92 and 176).

Urinalysis: Urinalysis was performed once prior to treatment, on all surviving animals prior to necropsies (Day 92 for main study animals; Day 175 for recovery animals).

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed from all main study and recovery animals at necropsy: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries/testes, pituitary gland, politeal lymph nodes, prostate, spleen, thymus, thyroid and parathyroid, and uterus.

Histopathology: Histopathological examinations were conducted on the following tissues from all animals (main study and recovery animals) at the scheduled necropsies. The following (from page 36 and 37 of the study report) organs and tissues were examined for histopathology:

abnormalities
animal identification
adrenals
aorta (thoracic)
bone and marrow (sternum)
bone and marrow (distal femur including articular surface)
brain (cerebrum, cerebellum, midbrain and medulla oblongata)
cecum
colon
duodenum
epididymides
esophagus
eyes
gallbladder
heart (including section of aorta)
ileum
injection site /infusion site (from the last site of injection)
jejunum

kidneys
lacrimal glands
larynx (1 level)
liver (sample of 2 lobes)
lungs (including bronchi)
lymph nodes (mandibular, politeal –bilateral, mesenteric)
mammary gland (ventral thoracic)
nasal cavity/turbinates (1 level)
optic nerves
ovaries
oviducts
pancreas
pharynx
pituitary
prostate
rectum
salivary glands (mandibular)
sciatic nerve
seminal vesicles
skeletal muscle (thigh)
skin and subcutis(ventral thoracic)
spinal cord (cervical, thoracic, lumbar)
spleen
stomach
testes
thymus
thyroid lobes (and parathyroids)
tongue
trachea
urinary bladder
uterus (cervix and body)
vagina

Toxicokinetics and Immunogenicity: Blood samples were collected from the femoral vein of all animals for PK and primate anti-human antibody (PAHA) assays. Blood samples for PAHA were collected twice prior to treatment initiation, on Day 8 (168 hours postdose), during Weeks 5, and 9 (predose), on Day 92 (main study animals at necropsy), and on recovery Days 106, 120,

134, 148, 162, and 176. Samples for PK were collected as follows (from page 26 and 27 of the study report):

Day 1: Predose, 0.5, 1, 4, 12, 24 (Day 2), 48 (Day 3), 72 (Day 4), 120 (Day 6), 168 (Day 8), 240 (Day 11), and 336 hours (Day 15) postdose. The 336-hour postdose samples were collected before Day 15 dose administration.

Day 29 (Week 5): Predose

Day 57 (Week 9): Predose

Day 85: Predose, 0.5, 1, 4, 8, 12, 24 (Day 86), 48 (Day 87), 72 (Day88), 120 (Day 90), 168 (Day 92), 240 (day 95), and 336 (Day 99) hours postdose. The 240- and 336-hour postdose samples were collected from recovery animals only (main study animals euthanized on Day 92).

Recovery Days 106, 120, 134, 148, 162, and 176.

Pharmacodynamic (PD) Assay: Blood samples for PD assay were collected from the femoral vein of all animals. Samples were collected in the following order (from page 27 of the study report):

Day 1: Predose, 0.5, 1, 168 (Day 8), and 336 (Day 15) hours postdose. The 336-hour postdose samples were collected before Day 15 dose administration.

Day 29 (Week 5): Predose

Day 57 (Week 9): Predose

Day 85: Predose, 0.5, 1, 4, 8, 12, 24 (Day 86), 48 (Day 87), 72 (Day88), 120 (Day 90), 168 (Day 92), 240 (day 95), and 336 (Day 99) hours postdose. The 240- and 336-hour postdose samples were collected from recovery animals only (main study animals euthanized on Day 92).

Recovery Days 106, 120, 134, 148, 162, and 176.

Immunology:

Immunophenotyping

Lymphocyte phenotyping was conducted on all animals on Days -10, -7, 33 (Week 5), and 61 (Week 9) and prior to main necropsies (Days 92 and 176). Immunophenotyping results were reported as relative proportions and absolute numbers of total T lymphocytes (CD3+), Helper T lymphocytes (CD3+/CD4+), cytotoxic T lymphocytes (CD3+/CD8+), activated natural killer (NK) cell subset lymphocytes (CD3-/CD8+), B lymphocytes (CD20+) and natural killer (NK) lymphocyte (CD3-/CD16+).

Cerebrospinal Fluid (CSF) Analysis

Cerebrospinal fluid was collected for immunophenotyping of T cells and assessment of CD4:CD8 ratios. The analysis of CSF samples was considered exploratory in nature and was not conducted in compliance with 21 CFR Part 58.

Natural Killer Cell (NK) Assay

Natural killer cell activity assay was conducted on all animals once prior to the start of treatment and on all animals at scheduled euthanasia (Days 92 and 176). The NK cell activity results were reported as % lysis of target cells at four different effectors to target (E:T) cell ratios.

T-cell Dependent Antibody Response Assay

The T-cell dependent primary antibody response was evaluated by injecting subcutaneously a KLH solution (10 mg) to main study animals on Day 75 and to recovery animals on Day 159. The following (from page 34 of the study report) assays were conducted:

Main Study Animals:

Anti-KLH IgM: Pre-KLH administration (Day 75); and again 5, 6, and 7 days following KLH administration (Days 80, 81, and 82). Anti-KLH IgG: Pre-KLH administration (Day 75); and again 14 and 17 days following KLH administration (Days 89 and 92).

Recovery Animals:

Anti-KLH IgM: Pre-KLH administration (Day 159); and again 5, 6, and 7 days following KLH administration (Days 164, 165, and 166). Anti-KLH IgG: Pre-KLH administration (Day 159); and again 14 and 17 days following KLH administration (Days 173 and 176).

Anti-KLH IgM and anti-KLH IgG antibodies were determined by using a quasi-quantitative enzyme linked immunosorbent assay (ELISA). The parameters evaluated and reported included anti-KLH IgM antibody concentrations on Days 5, 6, and 7 post-KLH injection and anti-KLH IgG antibody concentrations on Days 14 and 17 post-KLH injection.

Results:

Mortality: No mortality occurred during the course of the treatment and recovery periods.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial (Day -1) and final (Day 91) weights of control males were 2.12 and 2.50 kg, respectively. The mean initial and final weights of control females were 2.10 and 2.23 kg, respectively. There were no significant treatment-related effects.

Food Consumption: The food consumption was not measured but examined qualitatively. There were no significant treatment-related effects.

Ophthalmoscopy: No significant treatment-related changes were observed.

Electrocardiography: No qualitative abnormalities attributable to the intravenous administration of MLN0002 were observed during the treatment period and, therefore, electrocardiograms obtained at the end of the recovery period were not evaluated.

Hematology: There were no changes in the hematological data attributable to treatment with MLN0002.

Clinical Chemistry: No significant treatment-related findings were observed in the serum chemistry.

Urinalysis: There were no changes in urinalysis attributable to the administration of MLN0002.

Gross Pathology: There were no macroscopic findings attributed to the administration of MLN0002 in the main study animals terminally euthanized on Day 92 and in recovery animals euthanized on Day 176. Pale material in the vaginal lumen was seen in all four females treated with 100 mg/kg/dose, one female treated with 10 mg/kg/dose MLN0002 but none of the control animals.

Organ Weights: There were no test article-related effects on organ weights (absolute and relative to body weight or brain weight) in the main study or the recovery animals.

Histopathology: There were no significant histopathological findings associated with MLN0002 treatment.

Toxicokinetics: After the first dose, the exposure to MLN0002 (either C_{max} or AUC) increased as the dose increased, with the increase tending to be approximately dose-proportional. After the administration of the last dose, the exposure to MLN0002 increased as the dose increased. However, the increase was more than dose-proportional. The exposure (C_{max} and AUC_{0-168hr}), appeared to be lower on Day 85 than on Day 1 at 10 mg/kg, and was similar on Day 1 and Day 85 at 30 mg/kg. At a dose of 100 mg/kg, the exposure on Day 85 was greater than the exposure

on Day 1. The following tables (from the sponsor's submission) show the mean TK parameters (gender combined) on Day 1 and Day 85.

Table 6 Mean Serum Toxicokinetic Parameters (Genders Combined; 10 mg/kg)

Toxicokinetic Parameter	Day 1		Day 85	
	Mean	SD	Mean	SD
T_{max} (hr)	0.5	0.1	0.6	0.2
C_{max} ($\mu\text{g/mL}$)	300	66.3	150	172
AUC_{0-10hr} ($\mu\text{g}\cdot\text{hr/mL}$)	22,500	1820	12,100	18,500
$AUC_{0-336hr}$ ($\mu\text{g}\cdot\text{hr/mL}$)	32,500	4690	28,400	35,800
$AUC_{0-2160hr}$ ($\mu\text{g}\cdot\text{hr/mL}$)	N/A ^a	N/A	55,600	73,400

SD = Standard deviation.

a: Not applicable, due to protocol-specified sample time points.

Table 7 Mean Serum Toxicokinetic Parameters (Genders Combined; 30 mg/kg)

Toxicokinetic Parameter	Day 1		Day 85	
	Mean	SD	Mean	SD
T_{max} (hr)	0.6	0.2	1.2	2.2
C_{max} ($\mu\text{g/mL}$)	911	450	902	377
AUC_{0-10hr} ($\mu\text{g}\cdot\text{hr/mL}$)	72,400	19,600	71,700	58,300
$AUC_{0-336hr}$ ($\mu\text{g}\cdot\text{hr/mL}$)	117,000	30,900	110,000	122,000
$AUC_{0-2160hr}$ ($\mu\text{g}\cdot\text{hr/mL}$)	N/A ^a	N/A	190,000	240,000

SD = Standard deviation.

a: Not applicable, due to protocol-specified sample time points.

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Table 8 Mean Serum Toxicokinetic Parameters (Genders Combined; 100 mg/kg)

Toxicokinetic Parameter	Day 1		Day 85	
	Mean	SD	Mean	SD
T_{max} (hr)	1.8	3.4	1.2	1.3
C_{max} ($\mu\text{g/mL}$)	3010	729	5070	875
$AUC_{0-108\text{hr}}$ ($\mu\text{g}\cdot\text{hr/mL}$)	255,000	39,300	490,000	89,300
$AUC_{0-252\text{hr}}$ ($\mu\text{g}\cdot\text{hr/mL}$)	406,000	60,700	834,000	142,000
$AUC_{0-216\text{hr}}$ ($\mu\text{g}\cdot\text{hr/mL}$)	N/A ^a	N/A	1,570,000	326,000

SD = Standard deviation.

a: Not applicable, due to protocol-specified sample time points.

Primate AntiHuman Antibody (PAHA) Response: MLN0002 was immunogenic in almost all animals. In general, PAHA appeared to have an effect on the TK and PD of MLN0002, with the effect being most pronounced at the lowest dose (10 mg/kg). For the purpose of data interpretation, a positive PAHA titer was considered to be ≥ 300 and the titer had to have been observed in 2 consecutive samples. At 10, 30, and 100 mg/kg, PAHA was observed in 12 of 12, 12 of 12, and 11 of 12 animals, respectively. There was no apparent relationship between dose and titer or between dose and day on which the highest titer was observed. The highest observed titers were 312,500, 312,500, and 62,500 at 10, 30, and 100 mg/kg, respectively. These titers were observed on Days 29 through 134, 92 through 106, and Day 29 at 10, 30, and 100 mg/kg, respectively. Titers greater than or equal to 300 were first observed as early as Day 8 at 10 mg/kg, and Day 29 at 30 and 100 mg/kg. Titers greater than or equal to 300 were observed as late as Day 176 at all doses.

Pharmacodynamic (PD) Assay: There was no apparent difference in PD response between the different cell types evaluated. At the highest dose of 100 mg/kg, the $\alpha 4\beta 7$ sites on peripheral lymphocytes appeared to be saturated (nearly 100%) up to Day 176 (the last day of sampling). There was a trend towards a dose dependent percent of cells staining positive for free $\alpha 4\beta 7$ sites or bound MLN0002 prior to the last dose and at the end of the recovery period.

Blood Immunophenotyping: There were no significant treatment-related effects on immunophenotyping.

Cerebrospinal Fluid (CSF) Immunophenotyping: In the CSF samples, the presence of red blood cells (RBC) was observed for all the samples collected at necropsy of main study (Day 92) and recovery (Day 176) animals. The sponsor stated that the presence of RBC in the CSF samples indicated peripheral blood contamination. Interpretation of this dataset was inconclusive.

Natural Killer (NK) Cell Activity: At the end of the treatment period (Day 92), the NK cell activity group mean values demonstrated no MLN0002-related changes in either sex in any of the groups.

T Cell Dependent Antibody Response (TDAR) - Anti-KLH IgG and Anti-KLH IgM: When compared to control group mean, there were no substantial changes in the anti-KLH IgG and Anti-KLH IgM response for any of the MLN0002-treated groups for either males or females.

Summary: In a 13-week IV injection study in Cynomolgus monkeys with a 12-week treatment-free recovery, animals (n = 4/sex/group for main study; 2/sex/group for recovery) were treated once every 2 weeks at 0, 10, 30 and 100 mg/kg/day. The target organs of toxicity could not be identified in the absence of any significant organ toxicity. The NOAEL (no-observed-adverse-effect-level) appeared to be 100 mg/kg/day. MLN0002 was immunogenic in most, but not all, animals. In general, the PAHA had an effect on the TK and PD of MLN0002, with the effect being most pronounced at the lowest dose (10 mg/kg). However, the sponsor did not address whether the anti-drug antibody was neutralizing or not. The sponsor should be asked to address this issue. It is to be noted here that the sponsor did not mention the basis of dose selection.

Addendum: Balantidium parasites were seen in the cecum and colon of one and two females at 30 and 100 mg/kg, respectively. The Applicant stated that ciliated protozoa of the genus Balantidium sp. are common inhabitants of the large intestine in nonhuman primates and are usually non-pathogenic and cited Toft JD et al, 1998 (Toft JD and M Eberhard. 1998. Parasitic Diseases. In: BT Bennett, CR Abee and R Henrickson, eds. Nonhuman Primates in Biomedical Research, Diseases, San Diego (CA): Academic Press). In the main study, they were increased number of Balantidium sp. in the colon of males (2 of 4) at 100 mg/kg. The following table shows the incidence of Balantidium sp. in the cecum and colon.

Male (n = 4)

Tissue	Dose (mg/kg)			
	0	10	30	100
Cecum	4	2	2	2
Colon	0	0	0	2

Female (n = 4)

Tissue	Dose (mg/kg)			
	0	10	30	100
Cecum	4	3	3	3
Colon	2	1	3	3

The protozoa findings were not associated with any clinical signs or pathological adverse effects. The presence of *B. coli* did not appear to be treatment-related due to lack of a dose response, the presence of this parasite in control animals and the background findings [Drevon-Gaillot E et al. 2006, A review of background findings in Cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins, Exp Toxicol Pathol, 58:77-88].

Study title: 3-Month Pharmacokinetic and Safety Study of MLN0002 in Cynomolgus Monkeys

Study no.: KLaw-111
Report No.: KLaw-111-01-607
Study report location: EDR 4.2.3.2
Conducting laboratory and location: (b) (4)
Date of study initiation: January 26, 2001
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: LDP-02, 22.1.1, 99%

Key Study Findings:

- In a 3-month IV study in Cynomolgus monkeys, animals were treated with LDP-02 at 0, 2.5 and 10 mg/kg.
- There was no treatment-related mortality.
- Clinical signs at 2.5 and 10 mg/kg included vomiting, facial swelling, erythema, and pale mucous membranes.
- LDP-02 was immunogenic, with anti-idiotypic PAHA responses in all animals and anti-isotype responses in some animals. Anti-isotype PAHA had a neutralizing effect that resulted in early, rapid clearance of LDP-02 from the serum and unsaturation of $\alpha 4\beta 7$ on T cells.
- *Balantidium coli* organisms were observed in 3 of 28, 1 of 18, and 1 of 30 fecal samples at 0, 2.5 and 10 mg/kg groups, respectively. The lack of a dose response and the presence of this parasite in control animals indicated that this was not related to LDP 02 treatment.
- There were no significant treatment-related histopathological findings.
- The no-observed-adverse-effect level (NOAEL) was considered as 10 mg/kg.

Methods:

Doses: 0, 2.5 and 10 mg/kg

Frequency of dosing: For 0 and 10 mg/kg, dosing was performed on Days 1 and 4 and then once weekly through Day 85 (14 administrations). For 2.5 mg/kg, dosing was performed daily on Days 1 through 8, twice weekly through Day 29 and once weekly through Day 85 (22 administrations).

Route of administration: IV infusion on Day 1, followed by IV injection on subsequent days

Dose volume: Dose volume for Group 2 (2.5 mg/kg) was 0.5 mL/kg; dose volume for Group 3 (10 mg/kg) was 2 mL/kg

Formulation/Vehicle: 0.9% sodium chloride

Species/Strain: Cynomolgus monkey

Number/Sex/Group: The study consisted of three groups of monkeys receiving 0 (saline; 5 male, 5 female), 2.5 (3 male, 3 female) or 10 (5 male, 5 female) mg/kg of LDP-02

Age: Young adult

Weight: Males: 2.7 to 3.3 kg; Females 2.4 to 3.1 kg

Satellite groups: Recovery (70-day, treatment-free, shown in the table below)

Unique study design: Study design is shown below

Deviation from study protocol: There were no protocol deviations that affected the study objectives, results or interpretations.

The following table (from page 23 of the report) shows the study design.

Text Table 3-1 Study Design

Group No.	No. Animals	Control/ Test Materials	Treatment			Euthanasia			
			Dose Level (mg/kg)	Dose Regimen (Days)	Route of Administration	Terminal (Day 86)		Recovery (Day 156)	
						M	F	M	F
1 (control)	5M/5F	Saline (Vehicle)	0	1, 4, 8; 15, 22, 29; 36, 43; 50, 57, 64, 71, 78, 85	Delivered as IV infusion in 30 mL over ~30 min on Day 1; Delivered as IV injection in 10 mL over ~1 min on subsequent days	1001	1101	1003*	1103
				1002		1102	1004	1104	
2 (low-dose)	3M/3F	LDP-02	2.5	25, 29; 36, 43, 50, 57, 64, 71, 78, 85		2001	2101		
				1, 4, 8; 15, 22, 29; 36, 43; 50, 57, 64, 71, 78, 85		2002	2102		
3 (high-dose)	5M/5F		10	1, 4, 8; 15, 22, 29; 36, 43; 50, 57, 64, 71, 78, 85		3002	3103	3001	3101
						3004	3104	3003	3102
								3005	3105

*Animal 1003 was euthanized moribund on Day 43.

Day 1 for females occurred two weeks after that for males.

Doses were based on the most recent weekly body weight.

Dose regimen for low-dose was once daily for 1 week, twice weekly for 3 weeks, then once weekly for 2 months. Dose regimen for high-dose and controls was twice weekly for 1 week, then once weekly for remainder of 3 months.

LDP-02 was supplied by the Sponsor at a concentration of 5 mg/mL; dose volume for Group 2 (2.5 mg/kg) was 0.5 mL/kg; dose volume for Group 3 (10 mg/kg) was 2 mL/kg.

Dose volumes were rounded to the nearest 0.1 mL. Final injection volume was brought up to 30 or 10 mL as indicated using sterile saline.

Two males and two females from Group 3 were selected for euthanasia on Day 86 after review of pharmacodynamic data (T cell $\alpha 4\beta 7$ saturation and free sites). Two males and two females from Group 1 were selected in animal order for euthanasia on Day 86 and all animals in Group 2 were euthanized on Day 86. Remaining animals were euthanized on Day 156.

IV = intravenous; M = male; F = female.

Observations:

Mortality: Mortality was observed twice daily.

Clinical Signs: Clinical signs were checked twice daily.

Body Weights: Body weights were recorded on Day 1 and weekly thereafter.

Food Consumption: Not recorded

Ophthalmoscopy: Ophthalmoscopy was conducted on Day 1, Day 16, and prior to terminal and recovery necropsy.

Electrocardiography (ECG): Body temperatures, heart and respiratory rates, and electrocardiograms (ECGs) were recorded prior to and following dosing on Days 1, 8, 29, 57, and 85, and on Days 86, 99, and 156.

Hematology: Blood samples were collected as per the schedule shown in the table below (from page 24 of the report).

Text Table 3-2 Study Procedures

Study Day	Tx Group (1, 2, 3)	PK/TK	PD; IMT	IMG				Safety			
		Sera mAb conc. (A)	Cell bound and free $\alpha 4\beta 7$ (B)	Anti-mAb Abs (C)	SC (D)	CBC (E)	Coag (F)	BW; PE; OE; UA, O+P	Clin-ical Obs.	BT, HR, RR, ECG	Nx
BL-1		X	X	X	X	X	X	BW,P E,OE	X		
BL-2		X	X	X	X	X		BW, UA	X		
1	1, 2, 3	X (pre, post, 1, 4, and 8 hrs)	X (pre, post, 1 and 4 hrs)	X				BW	X Day 1 (pre and post) then daily	X (pre, post)	
2	2	X (24 hrs)	X (24 hrs)			X					
3	2	X	X		X	X					
4	1, 2, 3	X (pre, post)	X				X				
5	2	X	X		X	X					
6, 7	2										
8	1, 2, 3	X (pre, post, 1, 4, and 8 hrs)	X (pre, post, 1 and 4 hrs)	X	X	X		BW, UA, O+P		X (pre, post)	
9		X (24 hrs)	X (24 hrs)								
11	2	X	X			X	X				
15	1, 2, 3	X (pre, post)	X	X	X	X		BW,P E,OE*			
18	2										
22	1, 2, 3	X (pre, post)	X	X	X	X		BW			
25	2										
29	1, 2, 3	X (pre, post, 1, 4, and 8 hrs)	X (pre, post, 1 and 4 hrs)	X	X	X	X	BW, UA, O+P		X (pre, post)	
30		X (24 hrs)	X (24 hrs)								
36	1, 2, 3	X (pre, post)	X					BW			
43	1, 2, 3	X (pre, post)	X	X	X	X		BW			
50	1, 2, 3							BW BW BW			
57	1, 2, 3	X (pre, post, 1, 4, and 8 hrs)	X (pre, post, 1 and 4 hrs)	X	X	X	X			X (pre, post)	

Clinical Chemistry: Blood samples were collected as per the schedule shown in the above table

Urinalysis: Urine samples were collected once prior to dosing, on Days 8 and 29, and prior to necropsy.

Stool: Fecal samples were collected on Days 8, 29, and 86 for the presence of parasites and ova.

Gross Pathology: Gross pathology was conducted at necropsy.

Organ Weights: The following organs were weighed: adrenal glands, brain, kidneys, liver, ovaries, spleen, testes and thymus.

Histopathology: The following (from page 32 of the report) tissues were collected for histopathology.

Text Table 3-8 **Tissues Observed, Collected for Fixation and Processed for Histopathology**

Adrenal glands
Aorta
Bone marrow (sternum)
Brain
Cerebellum
Cerebrum
Brain stem
Cervix
Epididymides
Esophagus
Eye, with optic nerve
Femur, with articular surface
Gallbladder
Heart
Injection site (last)
Intestine, large
Cecum
Colon
Rectum
Intestine, small
Duodenum
Jejunum
Ileum
Kidneys
Liver
Lungs, with bronchi
Lymph nodes
Mandibular
Mesenteric
Mammary gland
Ovaries
Pancreas
Parathyroid glands
Pituitary gland
Prostate gland
Salivary gland
Mandibular
Sciatic nerve
Seminal vesicles
Skeletal muscle
Skin, mammary
Spinal cord
Cervical
Thoracic
Lumbar
Spleen
Stomach
Cardiac
Fundic
Pyloric

Toxicokinetics: Blood samples were collected as per the above table for TK analysis using ELISA (enzyme-linked immunoabsorbent assay).

Immunogenicity [Primate Anti-Human Antibody (PAHA)]: Blood samples were collected as per the above table for immunogenicity analysis by using enzyme linked immunosorbent assay (ELISA) and Biacore assay.

Immunotoxicity (Lymphocyte Subset Immunophenotyping): Relative and absolute numbers of total (CD3⁺), helper (CD4⁺ [measured as CD3⁺/CD8⁻]), cytotoxic/suppressor (CD8⁺), and activated memory (CD4⁺/CD45⁺RO⁺ [measured as CD4⁺/CD45⁺RA⁻]) T cells; natural killer (NK) (CD3⁻/CD4⁻/CD8⁻) cells; and B cells (gated as non-T, non-NK cells) in the peripheral blood were measured using flow cytometry.

Immunohistochemistry (Free α 4 β 7 Sites in Colon Tissue): Only colon tissues were collected for staining for immunohistologic assessment of free α 4 β 7 sites.

Pharmacodynamics (PD) (Bound LDP-02 and Free α 4 β 7 Sites on T Cells): Blood samples were collected as per the above table for PD. Bound LDP-02 and free α 4 β 7 sites (receptor occupancy) on total (CD3⁺), helper (CD4⁺ [CD3⁺/CD8⁻]), cytotoxic/suppressor (CD8⁺) and activated memory (CD4⁺/CD45⁺RO⁺ [measured as CD4⁺/CD45⁺RA⁻]) T cells in the peripheral blood were measured using flow cytometry.

Dosing Solution Analysis: Data not provided

Results:

Mortality: There were no treatment-related mortalities.

Clinical Signs: Treatment related clinical signs included vomiting, facial swelling, erythema, and pale mucous membranes. The following table (from page 41 of the report) shows the summary of clinical signs.

Text Table 4-1 Summary of LDP-02-related Clinical Signs

Dose Group (mg/kg)	Animal Number	Study Days on which LDP-02 was Administered and Associated Clinical Signs Occurred												
		1	8	22	25	29	36	43	50	57	64	71	78	85
2.5	2002M			p,s,v	p,e,s,v		v		s,v	p,e,v				s
10	3001M			v										
	3002M			v		v			v	v	v	v	v	v
	3003M		s											
	3004M												v	
	3005M		p		e,s,v				v	v	v	v		v
	3102F			v					v					
	3104F								v		e,v			

e = erythema (facial); p = pale mucous membranes; s = swelling (facial); v = vomiting

The Applicant commented that the types of clinical signs observed and their pattern of occurrence (majority occurring on or after Day 22) suggested a possible relationship to the onset of anti-LDP-02 antibodies. All treated animals developed an increase in PAHA values by Day 15, with the exception of one animal (Female No. 3101) in which the increase occurred on Day 22.

Body Weights: The mean initial (Day 1) and final (Day 85) weight of control males were 2.94 and 3.25 kg, respectively. The mean initial (Day 1) and final (Day 85) weight of control females were 2.88 and 2.98 kg, respectively. In males, final body weights were 106% and 97% of control at 2.5 and 10 mg/kg, respectively. In females, final body weights were 96% and 93% of control at 2.5 and 10 mg/kg, respectively. There were no significant treatment-related effects.

Food Consumption: Not recorded

Ophthalmoscopy: There were no significant treatment-related effects.

Electrocardiography (ECG), Heart Rate, Respiratory Rate and Body Temperature: There were no significant treatment-related effects.

Hematology: There were no significant treatment-related effects.

Clinical Chemistry: There were no significant treatment-related effects.

Urinalysis: There were no significant treatment-related effects.

Stool: *Balantidium coli* organisms were observed in 3 of 28, 1 of 18, and 1 of 30 fecal samples at 0, 2.5 and 10 mg/kg groups, respectively. *B. coli* are common commensal intestinal parasite of macaques [Drevon-Gaillot E et al. 2006, A review of background findings in Cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins, Exp Toxicol Pathol, 58:77-88]. The presence of *B. coli* did not appear to be treatment-related due to lack of a dose response, the presence of this parasite in control animals and the background findings.

Gross Pathology: There were no LDP-02-related macroscopic findings.

Organ Weights: There were no significant treatment-related effects.

Histopathology: There were no significant treatment-related effects. *Balantidium coli* organisms were observed in gastrointestinal tissue sections from 2 of 10, 0 of 6, and 0 of 10 animals at 0, 2.5 and 10 mg/kg, respectively. The presence of *B. coli* did not appear to be treatment-related due to lack of a dose response, the presence of this parasite in control animals and the background findings.

Toxicokinetics: Exposure to LDP-02 was approximately dose-proportional. At 2.5 and 10 mg/kg, the mean exposure (AUC_{0-24h}) values on Day 85 were 4,960 and 13,300

µg.hr/mL, respectively. At 10 mg/kg on Day 85, the mean exposure (AUC_{0-1680h}) value was 310,000 µg.hr/mL and the terminal elimination half-life (t_{1/2}) for LDP-02 was 540 hours (22.5 days). The following tables (from page 956 and 957 of the report) show the mean exposure values at 2.5 and 10 mg/kg doses.

Mean Exposure Values (C_{max} and AUC) in Animals Receiving Repeated IV Administrations of 2.5 mg/kg LDP-02.

Study Day	C _{max} (µg/mL)		AUC _{0-8hr} (µg*hr/mL)		AUC _{0-24hr} (µg*hr/mL)		AUC _{0-72hr} (µg*hr/mL)		AUC _{0-168hr} (µg*hr/mL)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	126	40.8	826	191	1980	612	NA	NA	NA	NA
8	530	234	3120	552	8400	2010	18700	3930	NA	NA
29	181	59.4	996	308	2480	1030	NA	NA	11100	5210
57	197	53.0	1230	312	NA	NA	NA	NA	NA	NA
85	275	82.2	1610	391	4960	1460	NA	NA	NA	NA

Data from Animals 2002 and 2102 were excluded from calculation of mean AUCs

on Days 29, 57, and 85 due to the neutralizing effect of PAHA on the serum concentrations of LDP-02.

C_{max} = Maximum serum LDP-02 concentration.

AUC = Area under the serum LDP-02 concentration versus time curve.

Mean Exposure Values (C_{max} and AUC) in Animals Receiving Repeated IV Administrations of 10 mg/kg LDP-02.

Study Day	C _{max} (µg/mL)		AUC _{0-8hr} (µg*hr/mL)		AUC _{0-24hr} (µg*hr/mL)		AUC _{0-72hr} (µg*hr/mL)		AUC _{0-168hr} (µg*hr/mL)		AUC _{0-1680hr} (µg*hr/mL)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	340	86.8	2240	274	5350	1060	12000	2510	NA	NA	NA	NA
8	570	96.1	3610	702	11570	2220	27300	7180	45200	12700	NA	NA
29	660	306	3420	1520	7570	3170	NA	NA	31700	16700	NA	NA
57	600	260	3770	1800	NA	NA	NA	NA	NA	NA	NA	NA
85	770	132	4810	760	13300	1680	41600	4710	86200	14200	310000	98600

Data from Animals 3002, 3004, 3005, 3103 and 3104 were excluded from calculation of mean AUCs

on Days 29, 57 and 85 due to the neutralizing effect of PAHA on the serum concentrations of LDP-02.

Data from Animal 3003 was excluded from calculation of mean AUCs on Day 85 due to the possible neutralizing effect of PAHA on the serum concentrations of LDP-02.

C_{max} = Maximum serum LDP-02 concentration.

AUC = Area under the serum LDP-02 concentration versus time curve.

Immunogenicity: LDP-02 was immunogenic in monkeys. Anti-drug antibody was detected in the serum of all animals when tested with ELISA and Biacore assays. In ELISA assay, PAHA were detected on Day 15 in 16 of 16 animals that received LDP-02, regardless of dose. In Biacore assay, PAHA responses were detected on Day 8 in 6 of 6 animals at 2.5 mg/kg and on Day 15 in 10 of 10 animals at 10 mg/kg. PAHA had variable effects on serum LDP-02 concentrations (PK) and T cell bound LDP-02 and free α4β7 (PD) parameters, however, there was no apparent gender effect on the incidence of PAHA or their effect on PK and/or PD parameters. PAHA had an apparent neutralizing effect on PK/PD parameters in 2 of 6 and 5 of 10 animals at 2.5 and 10 mg/kg, respectively. Continued administration of LDP-02 was generally associated with serum LDP-02 concentrations that were below the limit of quantitation (LOQ, 0.8 µg/mL) and with brief saturation of α4β7 on T cells followed by rapid unsaturation. PAHA had no apparent effect on serum LDP-02 concentrations or saturation of α4β7 on T cells in 4 of 6 and 4 of 10 animals at 2.5 and 10 mg/kg, respectively. When no neutralizing effect

was evident, serum LDP-02 concentrations generally declined steadily after the last dose and the LDP-02 concentrations continued to be detectable in serum through the last day of the study. As a result, in animals without a neutralizing effect from PAHA, saturation of $\alpha 4\beta 7$ on T cells was maintained until terminal sacrifice on Day 86 at 2.5 mg/kg and on Days 86 or 156 at 10 mg/kg.

Immunotoxicity: There were no LDP-02-related changes in absolute numbers for $CD3^+$, $CD4^+$, $CD8^+$ T cells or NK ($CD3^-/CD4^-/CD8^-$) cells.

Immunohistochemistry (Free $\alpha 4\beta 7$ Sites in the Colon Tissue): There was a positive correlation between the presence of free $\alpha 4\beta 7$ on the peripheral blood T cells and the presence and intensity of staining for free $\alpha 4\beta 7$ sites on mononuclear cells (lymphocytes) in the colon tissue, regardless of the dose. There was no apparent gender difference. In the colon of normal Cynomolgus monkeys, $\alpha 4\beta 7$ was distributed on numerous lymphocytes scattered within the lamina propria and occasionally within the colonic epithelium (intraepithelial leukocytes). In addition, the luminal surface of gut-associated lymphoid tissue (GALT) displayed a dense “cap-like” aggregation of $\alpha 4\beta 7^+$ lymphocytes. These staining patterns were consistent with the function of $\alpha 4\beta 7$ in the trafficking of gut-homing lymphocytes.

In control animals at necropsy (Day 86 or 156), about 74-99 % of the $\alpha 4\beta 7$ on the peripheral blood T cells was unsaturated. All of these animals had free $\alpha 4\beta 7$ present on lymphocytes in their colon tissue.

In the presence of PAHA, in 2 of 6 and 5 of 10 animals at 2.5 and 10 mg/kg, respectively, 78 to 98 % of the $\alpha 4\beta 7$ on peripheral blood T cells was unsaturated. All of these animals had free $\alpha 4\beta 7$ present on lymphocytes in their colon tissues. Thus, when free $\alpha 4\beta 7$ was available on peripheral blood T cells, it was generally also available on colonic lymphocytes.

In the absence of PAHA at necropsy, in 4 of 6 and 4 of 10 animals at 2.5 and 10 mg/kg, respectively, only 1 to 5 % of $\alpha 4\beta 7$ on peripheral blood T cells was unsaturated. All of these animals had little or no free $\alpha 4\beta 7$ present on lymphocytes in their colon tissue when there was no PAHA. Thus, when free $\alpha 4\beta 7$ was not available on peripheral blood T cells, it was generally not available on colonic lymphocytes (16 of 17 animals). Alternatively, when bound LDP-02 was present on peripheral blood T cells, free $\alpha 4\beta 7$ was not present on colonic lymphocytes (8 of 8 animals). Based on this, the Applicant stated that peripheral blood T cell $\alpha 4\beta 7$ saturation is a reasonable surrogate for colon tissue lymphocyte $\alpha 4\beta 7$ saturation, and that saturation of $\alpha 4\beta 7$ in the colon was achieved following administration of LDP-02 and was maintained through the recovery period.

Pharmacodynamics (PD) (Bound LDP-02 and Free $\alpha 4\beta 7$ Sites on T Cells): Initial saturation of $\alpha 4\beta 7$ on $CD4^+/CD45^+RO^+$ T cells by LDP-02 was independent of dose. Immediately after administration of 2.5 and 10 mg/kg LDP-02 on Day 1, 93% and 91% of $\alpha 4\beta 7$ receptors were saturated, respectively. There was no apparent gender difference on the saturation of $\alpha 4\beta 7$ on T cells.

Dosing Solution Analysis: LDP-02 was stable under the conditions of storage and use in this study.

Summary: In a 3-month IV study (KLAW-111) in Cynomolgus monkeys with a 70-day recovery period, animals were treated with LDP-02 at 0, 2.5 and 10 mg/kg. For 0 and 10 mg/kg, dosing was performed on Days 1 and 4 and then once weekly through Day 85 (14 administrations). For 2.5 mg/kg, dosing was performed daily on Days 1 through 8, twice weekly through Day 29 and once weekly through Day 85 (22 administrations). There was no treatment-related mortality. Clinical signs at 2.5 and 10 mg/kg included vomiting, facial swelling, erythema, and pale mucous membranes. These signs were resolved during the recovery period and were attributed to the presence of PAHA, and not to the direct effects of LDP-02. LDP-02 was immunogenic and PAHA developed in all animals that received LDP-02. PAHA had a neutralizing effect that resulted in rapid clearance of LDP-02 from the serum and unsaturation of $\alpha 4\beta 7$ on T cells. *Balantidium coli* organisms were observed in 3 of 28, 1 of 18, and 1 of 30 fecal samples at 0, 2.5 and 10 mg/kg groups, respectively. *Balantidium coli* are common commensal intestinal parasite of macaques [Drevon-Gaillot E et al. 2006, A review of background findings in Cynomolgus monkeys (*Macaca fascicularis*) from three different geographical origins, Exp Toxicol Pathol, 58:77-88]. The presence of *B. coli* did not appear to be treatment-related due to lack of a dose response, the presence of this parasite in control animals and the background findings. There were no significant treatment-related histopathological findings. The NOAEL was considered as 10 mg/kg.

26-Week Toxicology Study of MLN0002 Administered by Intravenous Infusion to Cynomolgus Monkeys, with a 12-Week Recovery Period (KLA00290)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated July 14, 2008.

Study Title: 26-Week Toxicology Study of MLN0002 Administered by Intravenous Infusion to Cynomolgus Monkeys, with a 12-Week Recovery Period

Key Study Findings: In a 26-week IV infusion study in Cynomolgus monkeys, animals were treated once every two weeks at 10, 30 and 100 mg/kg/day. There were no test article-related changes in the in-life parameters [clinical observations, food consumption, electrocardiography (ECG), ophthalmology, clinical pathology parameters (serum chemistry, hematology and urinalysis), and cerebrospinal fluid evaluations], and postmortem organ weight and macroscopic data evaluated through Day 183. Target organs appeared to be the gastrointestinal tract (minimal to mild lymphoid depletion in the Peyer's patches of the GIT in male animals at 10, 30, and 100 mg/kg/day) and the stomach (increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg/day). The no-observed-adverse-effect-level (NOAEL) could not be determined as treatment-related histopathological changes were observed at all doses.

Study No.: KLA00290

Volume # Page #: C375.1, 6

Conducting Laboratory and Location:

(b) (4)

Date of Study Initiation: October 5, 2006

GLP Compliance: A statement of compliance was included

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: MLN002, Lot No. 0002-807-GO

Methods:

Doses: 10, 30 and 100 mg/kg/day (Once every two weeks, total of 13 doses, 20 min infusion)

Basis of Dose Selection: The doses were selected based on the results of a previous 13-week toxicity study in Cynomolgus monkeys.

Species/Strain: Cynomolgus monkeys (*Macaca fascicularis*)

Number/Sex/Group (Main Study): 4/sex/dose

Route, Formulation, Volume, and Infusion Rate: Intravenous infusion, 11.8 mL/kg, 20 min infusion

Satellite Groups Used for Toxicokinetics or Recovery: 2/sex for control and high dose

Age: Males: 3.4 to 7.1 years; Females: 2.8 to 4.6 years

Weight: Males: 2.7-5.2 kg; Females: 2.4-2.8 kg

Study Design: The study design is shown in the following Table (from page 14 of the sponsor's submission).

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Group No.	No. of Males/ Females	Dose Level (mg/kg)	Dose Volume (mL/kg)	Dose Solution Conc. (mg/mL)	Number Necropsied:	
					Week 27	Week 38
1	6/6	0 (control)	11.8	0	4/4	2/2
2	4/4	10	11.8	0.85	4/3*	-/-
3	4/4	30	11.8	2.5	4/4	-/-
4	6/6	100	11.8	8.5	4/4	2/2

*One Group 2 animal was euthanized on Day 64 for reasons not related to the study.

Observation and Times:

Clinical Signs: Twice daily, from Day -7 to Day 183

Body Weights: Once weekly

Food Consumption: Once daily

Ophthalmoscopy: Pre-study and in Week 26

EKG: Pre-study and in Week 26

Hematology: Blood samples for evaluation of hematology parameters were collected from all animals during Weeks -2, -1, 5, 13, and 25 and from the recovery animals during Week 38.

Clinical Chemistry: As above

Urinalysis: Urine samples were obtained from all animals during Weeks -1 (pre-study) and 26 and from recovery animals during Week 38.

Gross Pathology: At necropsy

Organ Weights: The following (from page 42 of sponsor’s submission) organs were weighed from all animals at necropsy.

Organs Weighed	
Adrenals	Brain
Epididymides	Heart
Kidneys	Liver
Lungs	Ovaries
Pituitary Gland	Spleen
Testes	Thymus
Thyroid with parathyroids	Prostate Gland
Uterus	

Organ/body weight ratios were calculated (using the final body weight obtained prior to necropsy), as well as organ/brain weight ratios.

Histopathology: The following (from page 42 and 43 of sponsor’s submission) tissues were collected from all animals for histopathology.

eyes, which were preserved in formalin & stained for optimal pathology.

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate Gland
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenal Gland
Cecum	Pituitary Gland
Colon	Thyroid/Parathyroid Gland ^a
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (psoas and diaphragm)
Trachea	Nervous/Special Sense
Lung	Eyes with Optic Nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum)	Brain
Thymus	Spinal Cord (thoracic)

APPEARS THIS WAY ON ORIGINAL

Tissues Collected	
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Mandibular	Gross Lesions
Mesenteric	Injection Site(s) ^b

^a The occasional absence of the parathyroid gland from the routine tissue section did not require a recut of the section.

^b Cephalic, and/or saphenous vein(s)

Toxicokinetics: Blood samples were collected as per the following schedule for toxicokinetics (TK), primate anti-human antibody (PAHA) and pharmacodynamic (PD) analyses.

Days 1 (Week 1) and 169 (Week 25): Predose, 0.5, 4, 8, 12, 24, 32, 48, 72, 168, 336, and 333 hours post dose

Days 85 (Week 13) and 141 (Week 21): Predose and as soon as possible following the completion of infusion

Days 211 (Week 31), 239 (Week 35), and 262 (Week 38): No specified time of day

Primate Anti Human Antibody (PAHA):

Days 1 (Week 1), 85 (Week 13), 141 (Week 21), and 169 (Week 25): Predose

Days 183 (Week 27), 211 (Week 31), 239 (Week 35), 262 (Week 38): No specified time of day

Pharmacodynamic (PD) Assay:

Days (Week 1) and 169 (Week 25): Predose, 0.5, 4, 48, 168, 336, and 333 hours post dose

Days 85 (Week 13) and 141 (Week 21): Predose and as soon as possible following the completion of infusion

Days 211 (Week 31), 239 (Week 35), and 262 (Week 38): No specified time of day

Results:

Mortality: One Group 2 female (Animal No. 2503) was humanely euthanized on Day 64 due to an apparent positive reaction to the tuberculin skin test performed as part of a routine colony health screen. There was no evidence of mycobacterium infection on gross or histological examination, and acid-fast stains of relevant tissues in this animal were negative for Mycobacterium species.

Clinical Signs: There were no significant treatment-related clinical signs.

Body Weights: The mean initial (Week -1) and final (Week 26) weights (pooled) were 3.08 and 3.61 kg, respectively. There were no significant treatment-related changes.

Food Consumption: The sponsor did not record the food consumption. However, the sponsor stated that there were no significant treatment-related effects on food consumption.

Ophthalmoscopy: There were no significant treatment-related observations.

Electrocardiography (ECG): There were no abnormal ECG findings attributable to the administration of MLN0002.

Hematology: There were no significant treatment-related changes.

Clinical Chemistry: There were no significant treatment-related changes.

Urinalysis: There were no significant treatment-related changes.

Gross Pathology: No significant treatment-related changes were observed.

Organ Weights: There were no treatment-related changes.

Histopathology: Histological findings included minimal to mild lymphoid depletion in the Peyer's patches of the gastrointestinal tract in male animals at 10, 30, and 100 mg/kg/day and increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg/day. After an approximately 12-week dose-free period, lymphoid depletion in the Peyer's patches of the gastrointestinal tract was noted in 1 of 4 females at 100 mg/kg/day and histological changes in the stomach of treated animals were comparable to control animals. The following Table (page 1596 of the report) shows the histological changes in the gastrointestinal tract.

Text Table 4: Incidence and Severity of Lymphoid Depletion* in Peyer's Patches Day 183

Group (Dose Level)	Males	Females	Total	Animal # - Severity
1 (0 mg/kg)	0/4	0/4	0/8	none
2 (10 mg/kg)	1/4	0/3	1/7	2302 - minimal
3 (30 mg/kg)	2/4	0/4	2/8	3102 - minimal 3004 - minimal
4 (100 mg/kg)	2/4	0/4	2/8	4003 - minimal 4004 - mild

*Substantial variability in the appearance of Peyer's patches exists between animals and across sections of gut. For the purpose of this assessment, the variability within the controls was assigned a score of zero with the call of lymphoid depletion considered to be an overall depletion (compared to controls) within that individual when all sections of gut were considered together. No single section of gastrointestinal tract was considered abnormal.

The following Table (page 1597 of the report) shows the histological changes in the stomach.

Text Table 5: Severity Scores for Lymphoplasmacytic Gastritis and Regeneration of Superficial Mucosal Epithelium on Day 183

Group 1: Control Animal #	1003	1004	1105	1006	1503	1504	1605	1506	Group Average
Gender	M	M	M	M	F	F	F	F	
Lymphoplasmacytic Gastritis	0	2	1	2	2	2	3	3	1.9
Regeneration, Superficial Mucosa, stomach	0	2	0	1	2	3	1	1	1.3
Group 2: 10 mg/kg Animal #	2101	2302	2003	2004	2601	2602	2504		
Gender	M	M	M	M	F	F	F		
Lymphoplasmacytic Gastritis	2	3	1	2	2	3	2		2.1
Regeneration, Superficial Mucosa, stomach	3	2	1	3	2	3	2		2.3
Group 3: 30 mg/kg Animal #	3101	3102	3003	3004	3601	3602	3503	3504	
Gender	M	M	M	M	F	F	F	F	
Lymphoplasmacytic Gastritis	0	3	3	2	3	2	3	3	2.4
Regeneration, Superficial Mucosa, stomach	0	2	3	1	3	2	3	3	2.1
Group 4: 100 mg/kg Animal #	4003	4004	4105	4006	4503	4504	4605	4506	
Gender	M	M	M	M	F	F	F	F	
Lymphoplasmacytic Gastritis	3	2	3	2	3	0	2	2	2.1
Regeneration, Superficial Mucosa, stomach	3	1	3	2	4	0	2	3	2.3

M = Male, F = Female

Severity Score: 0 = not present, 1 = Minimal, 2 = Mild, 3 = Moderate, 4 = Marked

The following Table (from page 1598 of the report) shows the histopathological changes in the GIT of the recovery animals.

Text Table 6: Incidence and Severity of Lymphoid Depletion* in Peyer's Patches Day 264

Group (Dose Level)	Males	Females	Total	Animal # - Severity
1 (0 mg/kg)	0/2	0/2	0/4	none
4 (100 mg/kg)	0/2	1/2	1/4	4501 - minimal

*Substantial variability in the appearance of Peyer's patches exists between animals and across sections of gut. For the purpose of this assessment, the variability within the controls was assigned a score of zero with the call of lymphoid depletion considered to be an overall depletion (compared to controls) within that individual when all sections of gut were considered together. No single section of gastrointestinal tract was considered abnormal.

Toxicokinetics: Peak serum concentrations occurred at 0.5 hours post-infusion at each dose level. MLN0002 was detectable at 100 mg/kg on Day 169. The exposure to MLN0002 (as measured by either C_{max} or AUC) increased as the dose increased and was nearly dose-proportional. Steady-state serum concentrations of MLN0002 were obtained by Day 85 at all dose levels. The systemic exposure, as measured by C_{max} and AUC_{0-336hr} was greater on Day 169 than on Day 1 at all dose levels indicating drug accumulation after multiple doses (dose normalized accumulation ratios of 1.77, 1.97, and 2.30 at 10, 30 and 100 mg/kg/day, respectively). The following Table (from page 48 of sponsor's submission) shows the toxicokinetic (TK) parameters.

Group	Summary of Toxicokinetic Parameters Excluding Animals with Remarkable PAHA Neutralizing Effects (Sexes Combined)					
	Day 1			Day 169		
	C _{max} (µg/mL)	AUC _{0-336hr} (µg•hr/mL)	T _{max} (day)	C _{max} (µg/mL)	AUC _{0-333hr} (µg•hr/mL)	T _{max} (day)
1 (control)	NA	NA	NA	NA	NA	NA
2 (10 mg/kg)	296±59.3	38,200±12,400	1.4±1.6	503±60.4	82,500±17,200	3.0±4.3
3 (30 mg/kg)	946±228	113,000±22,100	0.9±1.2	1,400±324	232,000±63,800	0.5±0
4 (100 mg/kg)	2,720±278	376,000±38,100	0.5±0	5,260±1,070	876,000±266,000	0.5±0

NA = not applicable
AUC = area under the curve
C_{max} = maximum concentration
T_{max} = time to C_{max}

Immunogenicity and PD: MLN0002 was immunogenic in most, but not all animals. In general, PAHA appears to have an effect on the TK and PD profiles of MLN0002, with the effect being most pronounced at the lowest dose (10 mg/kg/day). The results of the PD assay demonstrated that the α4β7 sites on peripheral lymphocytes were saturated with MLN0002 shortly after dosing. There was no marked difference in target binding between the different cell types evaluated.

Cerebrospinal Fluid (CSF) Evaluation and Immunophenotyping: There were no changes in CSF parameters (white blood cell counts, red blood cell counts, and total protein concentrations) or T-lymphocyte populations that were considered related to the administration of MLN0002.

Lymphocytic pleocytosis was observed in a few animals from each group, including controls. One Group 4 animal (Animal No. 4004) had xanthochromic CSF with 279.5 RBC/ μ L at Week 27. The sponsor stated that this color along with the presence of RBC is suggestive of previous hemorrhage into the CSF. However, in the absence of relevant clinical observations or histopathological changes in routine sections from the brain and spinal cord, this finding was considered incidental. Xanthochromic CSF was not observed in any recovery animals.

Summary: In a 26-week IV infusion study in *Cynomolgus* monkeys, animals were treated once every two weeks at 10, 30 and 100 mg/kg/day. There were no test article-related changes in the in-life parameters (clinical observations, food consumption, ECG, ophthalmology, clinical pathology parameters (serum chemistry, hematology and urinalysis), and CSF evaluations, and postmortem organ weight and macroscopic data evaluated through Day 183. Target organs appeared to be the gastrointestinal tract (minimal to mild lymphoid depletion in the Peyer's patches of the gastrointestinal tract in male animals at 10, 30, and 100 mg/kg/day) and the stomach (increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg/day). The NOAEL could not be determined as treatment-related histopathological changes were observed at all doses.

Addendum: Histopathological changes (lymphoid depletion in Peyer's patches and stomach changes) were re-evaluated. Histopathological changes were seen in the gastrointestinal tract (minimal to mild lymphoid depletion in the Peyer's patches of the gastrointestinal tract in male animals at 10, 30, and 100 mg/kg/day and increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg). Minimal to mild lymphoid depletion in Peyer's patches of males and an analogous decrease in leukocytes expressing the $\alpha 4\beta 7$ integrin in crypt epithelium appeared to be due to the pharmacologic effect of MLN0002 (decreased trafficking of peripheral lymphocytes to the gut). However, the relation to the treatment is not clear in the absence of a dose response. MLN0002 treated monkeys had increased severity of regeneration of superficial mucosal epithelium in response to lymphoplasmacytic gastritis. Lymphoplasmacytic gastritis (lymphoplasmacytic infiltrates into the lamina propria of the stomach) is a common incidental finding in *Cynomolgus* monkeys and epithelial regeneration is an expected physiologic response to lymphoplasmacytic gastritis. The incidence of lymphoplasmacytic gastritis and epithelial regeneration was comparable in both MLN0002 treated and control monkeys. However, the severity of the epithelial response to the inflammation was slightly increased in MLN0002 treated monkeys when compared to control monkeys. The toxicological significance of this increase in the regenerative response of the epithelium is not clear. *Balantidium coli* (parasites) were observed in the cecum (male: 3 of 4 at 10 mg/kg; female: 1 of 4 at 100 mg/kg) and colon (male: 1 of 4 at 10 mg/kg; female: none) of both control and MNL0002 treated monkeys. *Balantidium coli* are common commensal intestinal parasite of macaques and are generally non-pathogenic [Drevon-Gaillot E et al. 2006, A review of background findings in *Cynomolgus* monkeys (*Macaca fascicularis*) from three different geographical origins, *Exp Toxicol Pathol*, 58:77-88]. The presence of *Balantidium coli* observed in the cecum and colon of monkeys did not appear to be treatment related due to lack of a dose response, presence of this parasite

in control animals and reported background incidences. Based on these, the NOAEL was considered as 100 mg/kg.

3-Month Intravenous Infusion Toxicology and Toxicokinetic Study of MLN0002 in New Zealand White Rabbits (Report No. 416055)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated February 5, 2009.

Study Title: 3-Month Intravenous Infusion Toxicology and Toxicokinetic Study of MLN0002 in New Zealand White Rabbits

Key Study Findings: In a 3-month IV infusion study in New Zealand white rabbits with a 4-week treatment-free recovery, animals were treated once every 2 weeks at 0, 30 and 100 mg/kg.

The target organs appeared to be the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) and ileum (hyperplasia of submucosal lymphoid nodules). The NOAEL (no-observed-adverse-effect-level) could not be determined as treatment-related histopathological changes were observed at all tested doses.

Study No.: DSD-00741 (b)(4)-416055

Volume # Page #: Vol. 1, page 1

Conducting Laboratory and Location: (b)(4)

Date of Study Initiation: November 8, 2006

Date of Study Completion: May 20, 2008

GLP Compliance: A statement of compliance was included.

QA Report: yes (X) no ()

Drug, Lot #, and % Purity: MLN0002, Lot No. 0002-807-GO, 99%

Methods:

Doses: 0 (saline), 30 and 100 mg/kg, once every 2 weeks (Days 0, 14, 28, 42, 56, 70, and 84)

Basis of Dose Selection: Dose levels were selected based on pharmacodynamic data from a 13-week study in cynomolgus monkeys (Study No. 502045) and data from a dose-range finding study in pregnant rabbits (Study No. (b)(4)-416036). In the dose range-finding study in pregnant rabbits, dose levels were 10, 30 and 100 mg/kg administered once on Gestation Day 7. Clinical signs included urination, labored respiration, body limp, vocalization, hyporesponsiveness, head tilt and/or gasping and were noted at 30 or 100 mg/kg, and mortality occurred at 10 and 30 mg/kg. Based on the sudden mortalities and immediate onset clinical findings, an investigative anaphylactic phase was added to study (b)(4)-416036 whereby selected females at 30 and 100 mg/kg groups were infused a second time (all at 10 mg/kg) on Gestation Day 23. During the second infusion, clinical signs consisted of increased respiration for all females and discolored (red) urine for 1 of the previously sensitized rabbits.

Species/Strain: New Zealand White rabbits

Number/Sex/Group or Time Point (Main Study): 5/sex/group

Route, Formulation, Volume, and Infusion Rate: Intravenous, solution, 11.8 mL/kg, 0.20 mL/kg/min over a period of 60 min

Satellite Groups Used for Recovery: 2/sex/group

Age: 5-5.5 months

Weight: Males: 3.004-3.752 kg; Females: 3.042-3.614 kg

Study Design or Methodology: The study design is shown in the following table (from page 22 of the study report)

<u>Group Number</u>	<u>Test Article</u>	<u>Dose Level^a (mg/kg)</u>	<u>Dose Concentration (mg/mL)</u>	<u>Dose Volume (mL/kg)</u>	<u>Infusion Rate over 60 minutes (mL/kg/minute)</u>	<u>Number of Animals</u>	
						<u>Males</u>	<u>Females</u>
1	Vehicle	0	0.0	11.8	0.20	5 ^{b,e}	5 ^{b,e}
2	MLN0002	30	2.5	11.8	0.20	5 ^b	5 ^b
3	MLN0002	100	8.5	11.8	0.20	5 ^{b,c}	5 ^{b,e}

^a = Dose levels were not corrected for purity.

^b = 3 animals/sex/group, when possible, were euthanized and necropsied on Day 98; the remaining 2 animals/sex/group were euthanized following a 4-week nondosing (recovery) period.

^c = Blood samples for toxicokinetic, rabbit anti-human antibody, and/or pharmacodynamic analysis were collected from all surviving rabbits on Days 0, 14, 28, 56, 84, and 126.

Observation and Times:

Clinical Signs: Clinical signs were observed twice daily.

Mortality: Mortality was observed twice daily.

Body Weights: Body weights were recorded twice weekly.

Food Consumption: Food consumption was recorded on a daily basis.

Ophthalmoscopy: Ocular examinations were conducted on all animals prior to the initiation of dose administration (Day -6/-7), on Day 18/19, and prior to the first scheduled necropsy (Day 96/97).

Hematology: At pretest and at scheduled necropsy

Clinical Chemistry: At pretest and at scheduled necropsy

Urinalysis: At necropsy (Day 98 and 126)

Gross Pathology: At necropsy.

Organ Weights: The following (from page 34 of the study report) organs were weighed from all animals at the scheduled necropsies.

Adrenals	Pituitary
Brain	Popliteal lymph nodes ^a
Epididymides	Prostate
Heart	Spleen
Kidneys	Testes
Liver	Thymus
Lungs	Thyroid with parathyroids
Ovaries	Uterus

^a - Popliteal lymph nodes from both sides (of animal) weighed due to size variability

Histopathology: The following (from page 33 of the study report) tissues were examined histopathologically.

Adrenals (2)	Lymph nodes
Aorta	Mandibular (2)
Bone with marrow	Mesenteric ^d
Femur with joint	Popliteal ^e
Sternum	Mammary gland (females only)
Bone marrow smear ^a	Nasal cavity/turbinates
Brain	Ovaries with oviducts (2) ^f
Forebrain	Pancreas
Midbrain	Peripheral nerve (sciatic)
Hindbrain	Pharynx
Epididymides (2) ^b	Pituitary
Eyes with optic nerve (2) ^c	Prostate
Gastrointestinal tract	Salivary glands [mandibular (2)]
Esophagus	Seminal vesicles (2)
Stomach	Skeletal muscle (rectus femoris)
Duodenum	Skin
Jejunum	Spinal cord (cervical, midthoracic, lumbar)
Ileum	Spleen
Cecum	Testes (2) ^b
Colon	Thymus
Rectum	Thyroid [with parathyroids, if present (2)] ^f
Gallbladder	Tongue
Harderian glands (2)	Trachea
Heart	Urinary bladder
Kidneys (2)	Uterus with cervix
Lacrimal gland	Vagina
Larynx	Gross lesions (when possible)
Liver (sections of 2 lobes)	
Lungs (including bronchi, fixed by inflation with fixative)	

^a - Bone marrow smears were obtained at scheduled necropsies only, but not placed in formalin; slides were examined only if scientifically warranted.

^b - Fixed in Bouin's solution

^c - Fixed in Davidson's solution

^d - Additional tissue embedded in Optimal Cutting Temperature medium for potential immunohistochemistry

^e - As many popliteal lymph nodes (per side) as could be found were retained for weight evaluation.

^f - Parathyroids and oviducts were examined microscopically if in the plane of section and in all cases were a gross lesion of the parathyroid was present.

Toxicokinetics and Pharmacodynamics and Rabbit Anti-Human Antibody (RAHA) Analyses:
Blood samples were collected as per the following schedule (from page 31 of the study report).

Day	Time Point	Sample Type	No. Of Animals, when possible
0	Prior to dosing (time zero)	TK, RAHA, PD	5/sex
	1 hour post-infusion	TK, PD	5/sex
14	Prior to dosing (time zero)	TK, RAHA, PD	5/sex
	1 hour post-infusion	TK, PD	5/sex
28	Prior to dosing (time zero)	TK, RAHA, PD	5/sex
	1 hour post-infusion	TK, PD	5/sex
56	Prior to dosing (time zero)	TK, RAHA, PD	5/sex
	1 hour post-infusion	TK, PD	5/sex
84	Prior to dosing (time zero)	TK, RAHA, PD	5/sex
	1 hour post-infusion	TK, PD	5/sex
	4 hours post-infusion	TK, PD	5/sex
	8 hours post-infusion	TK	5/sex
	24 hours post-infusion (Day 85)	TK, PD	5/sex
	120 hours post-infusion (Day 89)	TK, PD	5/sex
	168 hours post-infusion (Day 91)	TK, PD	5/sex
	336 hours post-infusion (Day 98)	TK, PD, RAHA	5/sex
126	Recovery phase	TK, PD, RAHA	2/sex

Results:

Mortality: Female No. 48024 at 30 mg/kg was found dead approximately 3 minutes after the start of the second infusion (Day 14). In addition, Female No. 48031 at 100 mg/kg was found dead approximately 20 minutes after the start of the fourth infusion (Day 42). This animal had clear material around eyes and was struggling in the restrainer.

Clinical Signs: Treatment-related clinical signs included clonic convulsions, increased respirations, lacrimation, and clear material around the eyes.

Body Weights: The mean initial (Day 0) and final (Day 91) weights of control males were 3.256 and 3.9132.50 kg, respectively. The mean initial and final weights of control females were 3.328 and 4.478 kg, respectively. There were no significant meaningful treatment-related effects.

Food Consumption: The mean initial and final food consumption in control males were 148 and 122 g/animal/day, respectively. The mean initial and final food consumption in control females were 177 and 143 g/animal/day, respectively. There were no significant treatment-related changes.

Ophthalmoscopy: No significant treatment-related changes were observed.

Hematology: Test article-related higher mean white cell counts and mean percent and absolute lymphocyte counts were observed in the 30 and 100 mg/kg groups. These changes are presented in the following Table (from page 43 of the study report; bold and underlined values were considered test article-related).

Text Table 1.
Selected Mean Hematology Findings, Primary and Recovery Necropsies

Group (mg/kg):	Males			Females		
	0	30	100	0	30	100
Analysis:						
White Cells (thous/uL)						
Day -2 ^a	6.38	5.51	4.98	5.5	4.95	5.61
% Difference		-13.6	-21.9		-10.0	2.0
Day 98 ^b	4.85	<u>7.76</u>	<u>11.07</u>	3.94	4.56	5.27
% Difference		60.0	128.2		15.7	33.8
Day 126 ^c	4.49	NA	<u>12.21</u>	4.40	NA	<u>10.75</u>
% Difference			171.9			144.3
Percent Lymphocytes						
Day -2 ^a	74.5	75.5	73.2	68.9	67.2	71.5
% Difference		1.3	-1.7		-2.5	3.8
Day 98 ^b	72.6	70.3	<u>87.1</u>	66.8	68.5	<u>77.5</u>
% Difference		-3.2	20.0		2.5	16.0
Day 126 ^c	76.4	NA	81.9	59.7	NA	<u>77.7</u>
% Difference			7.2			30.2
Absolute Lymphocytes (thous/uL)						
Day -2 ^a	4.76	4.16	3.64	3.76	3.31	4.00
% Difference		-12.6	-23.5		-12.0	6.4
Day 98 ^b	3.48	<u>6.15</u>	<u>9.63</u>	2.64	3.31	<u>4.26</u>
% Difference		76.7	176.7		25.4	61.4
Day 126 ^c	3.42	NA	<u>9.99</u>	2.63	NA	<u>8.36</u>
% Difference			192.1			217.9

^a - Day -2, n = 5/group

^b - Day 98, n = 3, 5, 3 for males and 3, 4, 2 for females in Groups 1-3, respectively

^c - Day 126, n = 2, 1 for males and 1, 1 for females in Groups 1 and 3, respectively

NA = Not Applicable

Clinical Chemistry: Higher mean globulin and higher mean total protein and lower mean albumin/globulin (A/G) ratio were observed in the 30 and 100 mg/kg group females at the primary necropsy (Day 98) and in the 100 mg/kg group female (No. 48031) that had blood collected on Day 30 and was later found dead during infusion on Day 42. Higher globulin and lower A/G ratio were also observed in the 30 and 100 mg/kg group males. These results are presented in the following Table (from page 45 of the study report; bold and underlined values were considered test article-related).

Text Table 2.
Selected Mean Serum Chemistry Findings, Primary and Recovery Necropsies

Group (mg/kg):	Males			Females		
	0	30	100	0	30	100
Analysis:						
Total Protein (grams/dL)						
Day -2 ^a	6.0	6.2	6.0	5.9	6.1	6.0
% Difference		3.3	0.0		3.4	1.7
Day 98 ^b	6.4	6.5	6.4	6.1	<u>6.5</u>	<u>6.8</u>
% Difference		1.6	0.0		<u>6.6</u>	<u>11.5</u>
Day 126 ^c	6.5	NA	6.4	6.1	NA	<u>6.4</u>
% Difference			-1.5			<u>4.9</u>
Globulin (grams/dL)						
Day -2 ^a	1.5	1.5	1.5	1.5	1.6	1.5
% Difference		0.0	0.0		6.7	0.0
Day 98 ^b	1.5	<u>1.8</u>	<u>1.8</u>	1.5	<u>2.0**</u>	<u>2.0</u>
% Difference		<u>20.0</u>	<u>20.0</u>		<u>33.3</u>	<u>33.3</u>
Day 126 ^c	1.6	NA	<u>1.8</u>	1.7	NA	<u>1.9</u>
% Difference			<u>12.5</u>			<u>11.8</u>
A/G Ratio						
Day -2 ^a	3.20	3.12	3.03	3.09	2.74	2.94
% Difference		-2.5	-5.3		-11.3	-4.9
Day 98 ^b	3.22	<u>2.70</u>	<u>2.63</u>	3.19	<u>2.22**</u>	<u>2.50</u>
% Difference		<u>-16.1</u>	<u>-18.3</u>		<u>-30.4</u>	<u>-21.6</u>
Day 126 ^c	3.12	NA	<u>2.61</u>	2.48	NA	<u>2.39</u>
% Difference			<u>-16.3</u>			<u>-3.6</u>

** - Significantly different from the control group at 0.01 using Dunnett's test

^a - Day -2, n = 5/group

^b - Day 98, n = 3, 5, 3 for males and 3, 4, 2 for females in Groups 1-3, respectively

^c - Day 126, n = 2, 1 for males and 1, 1 for females in Groups 1 and 3, respectively

NA = Not Applicable

Urinalysis: In the 100 mg/kg group males and females, and the 30 mg/kg females, mean urine pH was higher than the respective control group. Urine pH values are shown in the following Table (from page 47 of the study report; test article-related changes were bold and underlined).

Text Table 3.
Selected Mean Urinalysis Findings, Primary and Recovery Necropsies

	Group (mg/kg):	Males			Females		
		0	30	100	0	30	100
Analysis:							
pH							
Day 98 ^a		6.8	6.4	<u>7.2</u>	6.7	<u>7.0</u>	<u>7.5</u>
Day 126 ^b		7.0	NA	7.0	7.5	NA	7.5

^a - Day 98, n = 3, 5, 3 for males and 3, 4, 2 for females in Groups 1-3, respectively

^b - Day 126, n = 2, 1 for males and 1, 1 for females in Groups 1 and 3, respectively

NA - not applicable

Gross Pathology: There were no test article-related macroscopic observations.

Organ Weights: Test article-related organ weight changes were observed in the spleen at 30 and 100 mg/kg. The organ weight data for the spleen are presented in the following Table (from page 48 of the study report).

Table 4.
Test Article-Related Mean Organ Weight Changes At The Primary Necropsy

Organ	Direction and magnitude of change	Dose Level(s) (mg/kg)	Histologic Correlate
Spleen	↑70%, 142%** M ↑50%, 119% F	30 & 100 30 & 100	Lymphoid hyperplasia; amyloidosis
Spleen/final body weight	↑54%, 139%** M ↑68%, 161% F	30 & 100 30 & 100	Lymphoid hyperplasia; amyloidosis
Spleen/brain weight	↑78%*, 135%** M ↑59%, 100% F	30 & 100 30 & 100	Lymphoid hyperplasia; amyloidosis

* - Significantly (p<0.05) different from the control group using Dunnett's test.

** - Significantly (p<0.01) different from the control group using Dunnett's test.

Histopathology: Treatment-related microscopic changes were observed in the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) at 30 and 100 mg/kg males and females, and in the submucosal lymphoid nodules of the ileum (hyperplasia). The incidences and severity of lymphoid hyperplasia are presented in the following Table (from page 50 of the study report).

Text Table 5.
Incidence (percent) of Selected Histopathologic Findings,
Study Day 98 Primary Necropsy and Unscheduled Deaths

Dose (mg/kg):	Males			Females		
	0	30	100	0	30	100
Spleen ^a	3	5	3	3	5^b	3^b
Lymphoid Hyperplasia	0	4 (80)	3 (100)	0	5 (100)	2 (67)
minimal	0	2	0	0	3	1
mild	0	2	3	0	2	1
Amyloidosis	0	4 (80)	2 (67)	2 (67)	3 (60)	3 (100)
minimal	0	3	1	2	3	2
mild	0	1	1	0	0	1
Ileum (sacculus rotundus) ^a	3	5	3	3	5^b	3^b
Small lymphoid follicles	0	3 (60)	1 (33)	0	1 (20)	2 (67)
minimal	0	1	1	0	0	2
mild	0	2	0	0	1	0

^a - Number of tissues examined from each group.

^b - Includes found dead animal.

After a 4-week treatment-free recovery period, lymphoid hyperplasia of the periarteriolar lymphoid sheaths in the spleen persisted in all animals at 100 mg/kg group, and amyloid-like deposits were observed at higher incidence in the 100 mg/kg group males. However, the size of the submucosal lymphoid nodules in the ileum in the treated animals was comparable to that of the control group in all animals except 1 male at 100 mg/kg group (No. 48015).

Toxicokinetics: Generally, MLN0002 was immunogenic in most (8 of 10 animals at 30 mg/kg and 10 of 10 animals at 100 mg/kg) animals. In general, RAHA appeared to have an effect on the TK and PD of MLN0002, with the effect being most pronounced at the lowest dose (30 mg/kg). The highest observed titers for both doses (30 and 100 mg/kg) were 93,750. These titers were observed on Days 14 through 98 (Day 84, Hour 337) for both doses.

There was no apparent gender difference in TK parameters. The exposure to MLN0002 (C_{max} or AUC) increased as the dose increased, with the increase tending to be greater than dose-proportional. The mean TK parameters are shown in the following Table (from page 893 of the study report).

Table 4 Mean Serum Toxicokinetic Parameters (Genders Combined) on Day 84

MLN0002 Dose (mg/kg)	T_{max} (hr)		C_{max} ($\mu\text{g/mL}$)		$AUC_{0-337hr}$ ($\text{hr} \cdot \mu\text{g/mL}$)	
	Mean ^a	SD	Mean	SD	Mean	SD
30	4.7	7.6	645	608	82,900	122,000
100	6.7	7.4	9890	4970	1,230,000	644,000

SD = standard deviation.

a: Mean values were calculated from rounded toxicokinetic parameters for individual animals (N=9 for 30 and 100 mg/kg dose groups).

The PD effect of MLN0002 was evaluated by determining the percentage of CD4+ cells that stained positive for bound MLN0002 sites and free $\alpha 4\beta 7$. At 100 mg/kg, saturation of $\alpha 4\beta 7$ (as measured by the bound MLN0002 assay) was maintained throughout the dosing interval and for the duration of the dosing phase of the study in the majority of animals. At 30 mg/kg, the effect of RAHA on the PD appeared to be more pronounced (apparent reduced PD response). The mean percentages of CD4+ cells that stained positive for bound MLN0002 sites and free $\alpha 4\beta 7$ are summarized in the following Tables (from page 895 and 896 of the study report).

Table 6 Mean Percentages of CD4⁺ Cells Staining Positive for Bound MLN0002 (Genders Combined)

Protocol-Specified Time Point		Percentage of CD4 ⁺ Cells Positive (%)					
Day	Hour	30 mg/kg			100 mg/kg		
		Mean	SD	n	Mean	SD	n
0	0	30.1	30.5	10	33.2	33.3	10
0	2	70.0	5.64	10	41.8	6.29	10
14	0	71.4	32.3	10	77.2	8.57	10
14	2	71.3	5.57	9	38.9	22.6	10
28	0	19.3	33.0	8	56.7	29.8	10
28	2	56.1	14.9	9	32.7	12.6	10
56	0	9.66	27.9	9	26.0	38.8	9
56	2	31.1	38.0	9	22.0	26.5	9
84	0	25.8	37.1	9	51.6	20.6	9
84	2	59.4	13.2	9	32.5	6.82	9
84	5	51.3	19.1	9	34.0	6.68	9
84	25	33.7	38.2	9	55.5	10.4	9
84	121	27.6	33.6	9	40.4	24.3	9
84	169	23.3	34.0	9	52.0	22.2	9
84	337	26.3	38.4	9	56.5	21.4	9
126	0	N/S ^a	N/A ^b	0	92.2	1.76	3

N/A = not applicable; N/S = no sample; SD = standard deviation.

a: Per protocol, no animals from the 30 mg/kg dose group were included in the recovery period.

b: Not applicable, no samples were collected.

Table 7 Mean Percentages of CD4⁺ Cells Staining Positive for Free Alpha 4 Beta 7 Sites (Genders Combined)

Protocol-Specified Time Point		Percentage of CD4 ⁺ Cells Positive (%)					
		30 mg/kg			100 mg/kg		
Day	Hour	Mean	SD	n	Mean	SD	n
0	0	48.0	7.91	10	47.7	14.4	10
0	2	0.0700	0.0675	10	0.0600	0.0516	10
14	0	0.610	0.794	10	2.27	7.04	10
14	2	0.222	0.519	9	0.0400	0.135	10
28	0	3.39	5.84	8	0.260	0.366	10
28	2	0.111	0.176	9	0.0200	0.0422	10
56	0	3.89	7.65	9	0.0667	0.0866	9
56	2	0.0667	0.0866	9	0.144	0.255	9
84	0	3.02	4.62	9	0.178	0.427	9
84	2	0.0778	0.0972	9	0.0333	0.0866	9
84	5	0.0667	0.0500	9	-0.0111	0.0928	9
84	25	0.0222	0.109	9	0.0778	0.0833	9
84	121	11.2	22.3	9	0.211	0.494	9
84	169	8.73	17.7	9	0.0222	0.0972	9
84	337	3.51	6.67	9	0.189	0.242	9
126	0	N/S ^a	N/A ^b	0	0.0333	0.0577	3

N/A = Not applicable; N/S = No sample; SD = standard deviation.

a: Per protocol, no animals from the 30 mg/kg dose group were included in the recovery period.

b: Not applicable, no samples were collected.

Summary: In a 3-month IV infusion study in New Zealand white rabbits with a 4-week treatment-free recovery, animals (n = 5/sex/group for main study; 2/sex/group for recovery) were treated once every 2 weeks at 0, 30 and 100 mg/kg. The target organs appeared to be the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) and ileum (hyperplasia of submucosal lymphoid nodules). The NOAEL (no-observed-adverse-effect-level) could not be determined as treatment-related histopathological changes were observed at all tested doses. MLN0002 was immunogenic in most animals. In general, the rabbit anti-human antibody (RAHA) appeared to have an effect on the TK and pharmacodynamic (PD) parameters of MLN0002, with the effect being most pronounced at the lowest dose (30 mg/kg). It is to be noted here that the sponsor tested two doses. The sponsor should have tested at least 3 dose levels.

Addendum: Histopathological changes were seen in the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) and ileum (hyperplasia of submucosal lymphoid nodules) of treated and control animals. However, the incidences and severity were not dose related and these changes were also seen in control animals and the relation to the treatment is uncertain.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

N/A

7.2 *In Vitro* Assays in Mammalian Cells

N/A

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

N/A

7.4 Other Genetic Toxicity Studies

N/A

8 Carcinogenicity

In accordance with ICH Guideline S6(R1), conventional carcinogenicity studies (i.e., rodent bioassays) have not been conducted with MLN0002 to assess its carcinogenic potential as it lacks pharmacological activity in mice and rats. MLN0002 bound with similar affinity to leukocytes from rabbits, monkeys, and humans, but not from mice, rats, or guinea pigs. Rodents are not considered pharmacologically relevant species for MLN0002. However, carcinogenic potential of Act-1 (murine homologue of MLN0002) was assessed in an *in vitro* study (Report RPT-01335) using human tumor cells (RPMI 8866 cell line derived from a human B-cell lymphoma) that expressed $\alpha 4\beta 7$ integrin. In this study, Act-1 did not stimulate the growth or cellular proliferation of RPMI 8866 human B-cell lymphoma cell that express the $\alpha 4\beta 7$ integrin. MLN0002 also did not affect other factors that could affect oncogenesis, such as cytokine production, activation, cell proliferation of primary human leukocytes expressing the $\alpha 4\beta 7$ integrin. Lymphoid hyperplasia was observed in the 13- and 26-week toxicology studies in Cynomolgus monkeys and in a 3-month toxicology study in rabbits. These findings most likely resulted from the immunogenicity (i.e., antigenic stimulation) associated with infusing nonhuman species with a humanized monoclonal antibody. In addition, there was no evidence of systemic immunosuppression in toxicology studies. Overall, MLN0002 does not appear to have a carcinogenic potential based on the following: a) $\alpha 4\beta 7$ integrin expression has only been observed on the surface of mature leukocytes and malignant lymphoid cell lines, and the $\alpha 4\beta 7$ integrin is not expressed by solid tumors, b) there were no effects of Act-1 on cell proliferation or growth of an $\alpha 4\beta 7$ integrin-positive human tumor cell line *in vitro*, and c) there was no evidence of systemic immunosuppression in chronic toxicology studies with MLN0002 in Cynomolgus monkeys.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

N/A

9.2 Embryonic Fetal Development

A Segment II Intravenous Infusion Dose Ranging Study in Rabbits (416036/DSD-00661)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated April 11, 2008.

A Segment II Intravenous Infusion Dose Ranging Study in Rabbits (416036/DSD-00661)

Methods: The objective of the study was to determine the dose levels of MLN0002 for the Segment II teratology study in rabbits. In this study, timed pregnant New Zealand white rabbits (n = 5/group) were administered as 15 min IV infusion of MLN0002 at 10, 30, and 100 mg/kg on Gestation Day 7. The control animals were treated with saline. All animals were observed twice daily for mortality and morbidity. Clinical signs, body weights, and food consumption were recorded at appropriate intervals. On Gestation Day 29, a laparohysterectomy was performed on controls (n = 5), and 10 mg/kg (n = 5), 30 mg/kg (n = 3), and 100 mg/kg (n = 4) dose groups.

The uterus, placenta, and ovary were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were also recorded, and net body weights and net body weight changes were determined. The fetuses were weighed and examined for external malformations and developmental variations. For the toxicokinetic (TK) evaluation, an additional 3 rabbits/group were administered the vehicle or test article on a comparable regimen as main study rabbits. Blood samples were collected from these rabbits at approximately 0, 0.5, 48, 168 and 312 hours post-dose for pharmacokinetic analysis (PK), rabbit anti-human antibody (RA) analysis and pharmacodynamic analysis (PD). Toxicokinetic phase animals were euthanized on Gestation Day 20 and pregnancy status was determined for each female.

Results: One animal at 10 mg/kg and one animal at 30 mg/kg died within minutes following drug administration. There were no treatment-related adverse effects on body weights or food consumption. No late resorptions or dead fetuses were noted at GD 29 at any dose level. Intrauterine growth and survival were unaffected by treatment. There were no external fetal malformations or developmental variations in any fetuses in the test article-treated groups. MLN0002 was not teratogenic in this study.

The TK data showed that MLN0002 was detected at all time points through 312 hours post-dose (Gestation Day 20) in all treated animals. The AUC_{0-312hr} values were 20400, 72800, and 279000 µg*hr/mL at 10, 30, and 100 mg/kg, respectively. Exposure to MLN0002 (either AUC or Cmax) increased as the dose increased in an approximately linear dose proportional manner. MLN0002 was immunogenic at all doses, with RAHA (rabbit antihuman antibody) detected through GD20.

For PD analysis, mean pre-study values (all doses combined) of percent CD4+ cells staining positive for α4β7 free sites was 48.1 % and the mean percentage of cells with bound MLN0002 was negligible (1.6%). After IV administration of MLN0002, the mean percentage of CD4+ cells with α4β7 free sites decreased to values of 0.3% to 0.4%, while bound site rapidly increased to values of 86% to 89% at 0.5 hours postdose. The PD response was dose independent. The percentage of CD4+ cells with free α4β7 sites gradually increased with time, reaching 0.6%, 1.7%, and 3.8% at 312 hours postdose at doses of 10, 30, and 100 mg/kg. The mean percentage of CD4+ cells staining positive for bound MLN0002 did not appear to change through Gestation Day 14 (range: 84.2% - 95.2%). On Gestation Day 20 (312 hours postdose), the mean percentage of CD4+ cells staining positive for bound MLN0002 were 39.4%, 69.3%, and 76.3% at doses of 10, 30, and 100 mg/kg, respectively. Overall, rapid saturation of α4β7 sites (as measured by the bound MLN0002 assay) was observed and was maintained through GD20.

Intravenous Infusion Study of the Effects of MLN0002 on Embryofetal Development (Segment II Teratology) in Rabbits ((b) (4) -416044)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated July 14, 2008.

Study title: Intravenous Infusion Study of the Effects of MLN0002 on Embryofetal Development (Segment II Teratology) in Rabbits

Key study findings: In a Segment II teratology study in New Zealand White rabbits, pregnant animals were treated with MLN0002 at 10, 30, and 100 mg/kg/day by IV infusion from GD7 through GD28. All females survived to the scheduled laparohysterectomy. There were no test article-related clinical signs at any dose level. There were no significant treatment-related effects on any of the C-section parameters (postimplantation loss, live litter size, mean fetal body weights, and fetal sex ratios, mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss). There were no meaningful significant treatment-related fetal external, visceral or skeletal anomalies. MLN0002 was not teratogenic in rabbits under tested conditions.

Study no.: (b)(4)-416044

Volume # and page #: C146.7, 1

Conducting laboratory and location: (b)(4)

Date of study initiation: February 21, 2007

GLP compliance: A statement of compliance was included

QA reports: yes (X) no ()

Drug, lot #, and % purity: MLN0002, Lot No. 0002-807-HO, 98%

Methods:

Doses: 0 (Saline), 10, 30 and 100 mg/kg/day

Dose Selection: Dose levels were selected based on the results of a previous dose range-finding study in pregnant rabbits. In that study, there were no adverse effects on maternal body weights and food consumption or external fetal malformations or developmental variations at dose levels of 10, 30, and 100 mg/kg/day. Additionally, intrauterine growth and survival were unaffected by test article administration at the same dose levels. The sponsor should have administered higher doses, as the highest tested dose of 100 mg/kg/day did not produce any toxicity in the dose ranging study.

Species/strain: New Zealand White rabbit

Number/sex/group: 25/group

Route, formulation, volume, and infusion rate: intravenous, solution, 10.2 mL/kg, 0.17 mL/kg/minute

Satellite groups used for toxicokinetics (TK), rabbit anti-human antibody (RAHA) and pharmacodynamic (PD): For the TK, RAHA and PD evaluations, an additional 3 rabbits/group were administered the vehicle or test article on a comparable regimen as main study rabbits. Blood samples were collected from these rabbits at approximately 0, 1 (Gestation Day 7, TK and PD only), 48 (Gestation Day 9, TK and PD only), 168 (Gestation Day 14, TK and PD only) and 312 hours (Gestation Day 20; at approximately the same time of day as time zero on Gestation Day 7) after the end of infusion on Gestation Day 7. Toxicokinetic phase animals were sacrificed on GD 20 and pregnancy status was determined for each rabbit.

Study design: The following Table (from page 18 of the sponsor's submission) shows the study design.

Embryo/Fetal Development Phase (b)(4)-416044)

Group Number	Test Article	Dose Level ^a (mg/kg)	Dose Volume (mL/kg)	Infusion Rate over 60 minutes (mL/kg/minute)	Number of Females
1	Vehicle	0	10.2	0.17	25
2	MLN0002	10	10.2	0.17	25
3	MLN0002	30	10.2	0.17	25
4	MLN0002	100	10.2	0.17	25

^a = Dose levels were not corrected for purity.

Toxicokinetic Phase (b)(4)-416044T)

Group Number	Test Article	Dose Level ^a (mg/kg)	Dose Volume (mL/kg)	Infusion Rate over 60 minutes (mL/kg/minute)	Number of Females
1	Vehicle	0	10.2	0.17	3
2	MLN0002	10	10.2	0.17	3
3	MLN0002	30	10.2	0.17	3
4	MLN0002	100	10.2	0.17	3

^a = Dose levels were not corrected for purity.

Parameters and endpoints evaluated: All animals were observed twice daily for mortality and moribundity. Clinical observations, body weights, and food consumption were recorded at specific intervals. On Gestation Day 29, a laparohysterectomy was performed on each female. The uteri, placentae, and ovaries were examined, and the numbers of fetuses, early and late resorptions, total implantations, and corpora lutea were recorded. Gravid uterine weights were recorded, and net body weights and net body weight changes were calculated. The fetuses were weighed and examined for external, visceral and skeletal malformations and developmental variations.

Results:

Mortality (dams): There were no mortalities in this study.

Clinical signs (dams): There were no test article-related clinical findings at any dose level.

Body weight (dams): The mean initial (Day 0) and final (Day 29) body weights of control animals were 3259 and 3757 g, respectively. There were no significant treatment-related changes.

Food consumption (dams): The mean initial (Day 4-6) and final (Day 24-29) were 131 and 99 g/animal/day, respectively. There were no significant treatment-related changes.

Toxicokinetics: The mean Cmax of MLN0002 were 244, 818 and 3020 µg/mL at 10, 30 and 100 mg/kg, respectively. The AUC_{0-337hr} values were 28,300, 102,000 and 366,000 µg.hr/mL at 10, 30 and 100 mg/kg, respectively. Exposure to MLN0002 (either Cmax or AUC) showed trends towards increasing in a greater than dose-proportional manner.

The following Table shows the mean TK parameters.

Dose (mg/kg/day)	Tmax (hr)	Cmax (µg/mL)	AUC _{0-336hr} (µg.hr/mL)
10	2.0	244±31.8	28,300±4480
30	2.0	818±24.3	102,000±8260
100	2.0	3020±159	366,000±26,800

The immunogenicity of MLN0002 at 30 and 100 mg/kg was minimal, with no animal showing a positive RAHA response. Two of 3 animals at 10 mg/kg group developed a high RAHA titer (~18,750) on gestation day 20 (2 weeks post-infusion). The RAHA response in these animals appeared to be neutralizing, as it affected both serum concentrations of MLN0002 and PD parameters.

There was no marked difference in the percentage of cells staining positive for bound or free $\alpha 4\beta 7$ site between the different peripheral lymphocyte populations evaluated. The results demonstrated that the $\alpha 4\beta 7$ sites were saturated with MLN0002 shortly after dosing and continuous $\alpha 4\beta 7$ saturation was achieved throughout the study in animals dosed at 100 mg/kg.

Terminal and necroscopic evaluations:C-section data (implantation sites, pre- and post-implantation loss, etc.): There were no significant treatment-related effects on any of the C-section parameters (postimplantation loss, live litter size, mean fetal body weights, and fetal sex ratios, mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss). Intrauterine growth and survival were unaffected by test article administration at dosage levels of 10, 30, and 100 mg/kg. The following Table (from page 74 of the report) shows the C-section data.

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MLN0002

IND 9,125 SN 00/3
Information Amendment

TABLE 11
RABBIT IV INFUSION STUDY OF MLN0002 ON EMBRYO/FETAL DEVELOPMENT
SUMMARY OF FETAL DATA AT SCHEDULED NECROPSY

PROJECT NO. (b) (4) 15044
SPONSOR: MILLENNIUM PHARM.
SPONSOR NO.: DSD-00730

PAGE 1

GROUP	SEX		VIABLE FETUSES	DEAD FETUSES	RESORPTIONS		POST IMPLANTATION LOSS		CORPORA LUTEA	PRE IMPLANTATION LOSS	FETAL WEIGHTS IN GRAMS	NO. OF GRAVID FEMALES
	N	F			EARLY	LATE	IMPLANTATION SITES	IMPLANTATION SITES				
1	TOTAL 95	99	194	0	12	3	15	209	222	13	NA	24
	MEAN 4.0	4.1	8.1	0.0	0.5	0.1	0.5	8.7	9.3	0.5	39.0	
	S.D. 1.88	1.45	2.22	0.00	1.47	0.34	1.47	1.68	1.51	0.98	4.81	
	S.E. 0.38	0.30	0.45	0.00	0.30	0.07	0.30	0.34	0.31	0.20	0.98	
2	TOTAL 98	102	200	0	13	0	13	213	223	10	NA	24
	MEAN 4.1	4.3	8.3	0.0	0.5	0.0	0.5	8.9	9.3	0.4	41.2	
	S.D. 1.69	1.67	2.43	0.00	1.10	0.00	1.10	2.07	2.18	0.78	3.87	
	S.E. 0.35	0.34	0.50	0.00	0.23	0.00	0.23	0.42	0.44	0.16	0.79	
3	TOTAL 102	99	201	0	0	5	5	206	221	15	NA	22
	MEAN 4.6	4.5	9.1	0.0	0.0	0.2	0.2	9.4	10.0	0.7	40.3	
	S.D. 1.56	1.77	1.49	0.00	0.00	0.53	0.53	1.68	1.86	0.72	4.66	
	S.E. 0.33	0.38	0.32	0.00	0.00	0.11	0.11	0.36	0.40	0.15	0.99	
4	TOTAL 86	85	171	0	6	4	10	181	203	22	NA	22
	MEAN 3.3	3.9	7.8	0.0	0.3	0.2	0.5	8.2	9.2	1.0	41.4	
	S.D. 1.93	1.83	2.22	0.00	0.46	0.39	0.53	2.07	1.77	1.63	3.79	
	S.E. 0.41	0.39	0.47	0.00	0.10	0.08	0.11	0.44	0.38	0.35	0.81	

None significantly different from control group
NA = NOT APPLICABLE
MEAN NUMBER OF VIABLE FETUSES, MEAN NUMBER OF IMPLANTATION SITES, MEAN NUMBER OF CORPORA LUTEA, FETAL WEIGHTS COMPARED USING DUNNETT'S TEST

1- 0 MG/KG 2- 10 MG/KG 3- 30 MG/KG 4- 100 MG/KG

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Offspring (malformations, variations, etc.): There were no meaningful significant treatment-related fetal external, visceral or skeletal anomalies. The following Table (from page 78 of the study report) shows the summary of fetal malformations.

Millennium Pharmaceuticals, Inc.
MLN0002

IND 9,125 SN 00/3
Information Amendment

TABLE 12
RABBIT IV INFUSION STUDY OF MLN0002 ON EMBRYO/FETAL DEVELOPMENT
SUMMARY OF FETUSES AND LITTERS WITH MALFORMATIONS [ABSOLUTE NO.]

PROJECT NO. (b) (4) 416044
SPONSOR: MILLENNIUM PHARM.
SPONSOR NO.: DSD-00730

PAGE 1
DAY 29

	DOSE GROUP:				LITTERS			
	1	2	3	4	1	2	3	4
NUMBER EXAMINED EXTERNALLY	194	200	201	171	24	24	22	22
OPEN EYELID	0	0	1	0	0	0	1	0
SHORT TAIL	1	1	0	0	1	1	0	0
GASTROSCCHISIS	0	1	0	0	0	1	0	0
NUMBER EXAMINED VISCERALLY	194	200	201	171	24	24	22	22
PERSISTENT TRUNCUS ARTERIOSUS	1	1	0	1	1	1	0	1
HYDROCEPHALY	0	0	0	1	0	0	0	1
KIDNEY(S) - MALPOSITIONED	0	1	0	0	0	1	0	0
NUMBER EXAMINED SKELETALLY	194	200	201	171	24	24	22	22
RIB ANOMALY	0	1	1	0	0	1	1	0
VERTEBRAL ANOMALY WITH OR WITHOUT ASSOCIATED RIB ANOMALY	1	0	0	0	1	0	0	0
STERNEBRAS FUSED	1	2	0	0	1	2	0	0
SKULL ANOMALY	1	0	0	0	1	0	0	0
TOTAL NUMBER WITH MALFORMATIONS								
EXTERNAL :	1	1	1	0	1	1	1	0
SOFT TISSUE :	1	2	0	2	1	2	0	2
SKELETAL :	2	2	1	0	2	2	1	0
COMBINED :	4	3	1	2	3	3	1	2

1- 0 MG/KG 2- 10 MG/KG 3- 30 MG/KG 4- 100 MG/KG

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Summary: In a Segment II teratology study in pregnant New Zealand White rabbits, animals were treated with MLN002 at 10, 30, and 100 mg/kg/day by IV infusion from GD7 through GD28. All females survived to the scheduled laparohysterectomy. There were no test article-related clinical signs at any dose level. There were no significant treatment-related effects on any of the C-section parameters (postimplantation loss, live litter size, mean fetal body weights, and fetal sex ratios, mean numbers of corpora lutea and implantation sites and the mean litter proportions of pre-implantation loss). There were no meaningful significant treatment-related fetal external, visceral or skeletal anomalies. MLN002 was not teratogenic in rabbits under tested conditions.

Addendum: This addendum is to correct the previous statement on the dosing schedule for the above embryofetal development study in rabbits. In the above review, it was stated that the doses were administered from GD 7 through GD 28, which is incorrect. The doses were administered once as a 60-minute IV infusion on GD 7.

9.3 Prenatal and Postnatal Development

Intravenous Infusion Study for Effects on Pre- and Postnatal Development in Cynomolgus Monkeys (2091-003)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated May 13, 2011.

Study title: Intravenous Infusion Study for Effects on Pre- and Postnatal Development in Cynomolgus Monkeys

Study no.:	2091-003
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 7, 2007
Date of study completion:	July 28, 2008
GLP compliance:	A statement of compliance was included
QA statement:	A QAU statement was included
Drug, lot #, and % purity:	MLN0002, MLN-0002-807-HO, 98%

Key Study Findings:Maternal:

- MLN0002 was administered at 10, and 100 mg/kg/day IV via a 20 to 30 minute infusion (dosed every two weeks from GD20 to GD140).
- Two F0 high dose animals were sacrificed early due to humane reasons. These decedent animals had diarrhea, weight loss and dehydration. Deaths were considered due to bacterial infection and were not considered treatment-related.
- MLN0002 did not increase the incidence of prenatal loss/death or stillbirth.
- No MLN0002-related changes were observed in clinical signs, body weight development, or estimated food consumption.
- There were significant treatment-related macroscopic or microscopic changes.

Fetal:

- There were no MLN0002-related effects on number of live birth, clinical signs, and infant development assessed by body weight gain, neurobehavioral, and morphological examination. At the end of the study (Day 181 post-partum), the survival rate of the infants was similar in all study groups.
- There were no MLN0002-related effects on hematology and clinical chemistry values.
- There were no MLN0002-related effects on infant organ weights.
- There were no gross or histopathological evidence of MLN0002-related organ toxicity in the infants.

PAHA/TK/PD:

- MLN0002 was immunogenic in some, but not all, dams. In general, PAHA had an effect on TK and PD of MLN0002 at the low dose (10 mg/kg) in dams. In the 10- and 100-mg/kg dose groups, PAHA was observed in 9 of 12, and 9 of 12 dams, respectively.
- MLN0002 was detected in the fetal blood at 100 mg/kg. MLN0002 was not detected in the breast milk at low dose.
- MLN0002 was mildly immunogenic in infants whose mothers were treated with MLN0002, with PAHA primarily present at the low dose (10 mg/kg). Positive titers were found in 2 of 7 infants at 10 mg/kg and in 1 of 9 infants at 100 mg/kg.
- Pharmacodynamic response was seen in infants whose mothers were treated with MLN0002. On Day 28 post partum, target saturation was noted in all infants at 100 mg/kg. On Day 120 post partum, target saturation was noted in only 3 of 9 infants at 100 mg/kg. On Day 181 post partum, no target saturation was noted in any of the infant (0 of 9 animals) at 100 mg/kg.

Methods:

Doses:	0, 10 and 100 mg/kg/day
Frequency of dosing:	Every two weeks
Dose volume:	10.2 mL/kg
Route of administration:	Intravenous infusion (20 to 30 min)
Formulation/Vehicle:	0.9% saline
Species/Strain:	Cynomolgus monkeys (<i>Macaca fascicularis</i>). Wt./Age: 2.7-4.0 kg on GD19/3 Years old
Number/Sex/Group:	12 females/dose
Satellite groups:	None
Study design:	As shown in the following table (from page 13 of the report)
Deviation from study protocol:	The sponsor stated that protocol deviations were minor and these deviations neither affected the overall interpretation of study findings nor compromised the integrity of the study.

Observations and Results:**F0 Dams:**

Mortality: Mortality was observed on a daily basis. Two dams at the high-dose (Group 3, 100 mg/kg) had diarrhea that resulted in weight loss, dehydration, and morbidity requiring euthanasia for humane purposes. The onset of diarrhea occurred prior to parturition in both dams and both delivered healthy infants. One Group 3 dam (13531F) was killed moribund on Day 35 post-partum. The other Group 3 dam (13507F) had chronic intermittent diarrhea over 2 months before experiencing weight loss and dehydration and was killed moribund on Day 53 post-partum. The infant remained healthy. At necropsy, one (13531F) had liquid contents of the large intestine and pale, dry skeletal muscle, while the other (13507F) had moderately enlarged, dark-brown adrenals and also moderately dry skeletal muscle as key findings. Deaths were attributed to bacterial infection and were not considered treatment-related.

Clinical Signs: Clinical signs were observed twice daily. Clinical signs included emesis, lesions, and/or hair thinning which were occasionally observed in several animals. The pattern and the frequency of these observations did not show any dose relationship and hence were not considered to be related to the treatment.

Body Weight: Body weights were recorded on a weekly basis. The mean initial (Day 19) and final (Day 166) body weights of control animals were 3.1 and 5.5 kg, respectively. There were no significant treatment-related effects.

Food Consumption: Food consumption was estimated twice daily. There were no significant treatment-related changes.

Uterine Data: Pregnancy status was checked on Days 30, 44, 58, 72, 86, 100, 114, 128, 142, and 156 of gestation. Vaginal smears were examined daily from GD20 until delivery, where appropriate. MLN0002 did not increase the incidence of prenatal loss/death or stillbirth. The incidence of prenatal loss/death was 1 of 12 (8.3%), 1 of 12 (8.3%), 0 of 12 (0%) in Group 1 (C), 2 (Low), and 3 (High), respectively. There were no significant treatment-related effects on pregnancy status.

Necropsy: There were no significant treatment-related changes.

Histopathology: No significant treatment-related changes were observed.

Toxicokinetics: Blood samples were collected on GD20 at 0.5, 1, 4, 12, 24, 72, 168 and 336 hours after dosing. Exposure to MLN0002 (C_{max} or AUC) increased in a dose-proportional manner. Exposure in dams, as measured by C_{max} and AUC_{0-336hr}, was higher on GD132 than on GD20 at 10 and 100 mg/kg. MLN0002 was secreted at low levels through 28 days postpartum in the breast milk of dams dosed with MLN0002 between GD20 and GD140. MLN0002 was found to be present at low levels in the serum of infants whose mothers were treated with MLN0002. MLN0002 was detected in infants on Day 28 post-partum. In all of the infants (11 out of 11) at 100 mg/kg, MLN0002 was detected in the infant serum (approximately 10-100 µg/mL). MLN0002 was also detected in the infant serum (< 10 µg/mL) on Day 28 post-partum in 3 of 7 infants at 10 mg/kg. In addition, MLN0002 was detected in the infant serum (0.166 µg/mL) on post-partum day 120 in one animal (#13481) at 100 mg/kg.

Dosing Formulation Analysis: Dosing solutions were analyzed on Day 20, 76, and 132 and the mean concentration of MLN0002 ranged from 96.8% to 99.9% of nominal concentration. Administered dosing solutions of this study contained MLN0002 concentrations within the range from 90 to 110% of nominal concentration.

Primate Anti-Human Antibody (PAHA) Analysis: PAHA was analyzed at GD20 at predose and 4 hours postdose. MLN0002 was immunogenic in some, but not all, dams. In general, PAHA had an effect on the TK and PD of MLN0002 at 10 mg/kg in dams. In the 10 and 100 mg/kg, PAHA was observed in 9 of 12, and 9 of 12 dams, respectively. MLN0002 appeared to be mildly immunogenic in infants whose mothers were treated with MLN0002, with PAHA primarily present at the low dose (10 mg/kg). Positive titers were found in 2 out of 7 infants at 10 mg/kg; and there was 1 of 9 infants had detectable titer (Day 120 sample) at 100 mg/kg.

Pharmacodynamic (PD) assay: Pharmacodynamics was examined at GD20 and GD132 at 0.5, 4, 168, 336 hours postdose. There was no marked difference in PD response in dams among the peripheral blood B and T cells examined.

F₁ Generation

Mortality: Mortality was observed daily. The infant of mother 13531 (unscheduled termination) was also sacrificed moribund along with its mother on Day 34 post-partum. The moribund condition of the infant was considered related to poor condition of the mother and was not considered related to treatment with MLN0002.

Clinical Signs: Clinical signs were observed daily. There were no significant treatment-related clinical signs.

Body Weight: Body weight was recorded and examined for external abnormalities on Days 1, 7, 14, 21, and 28 post-partum and then at monthly intervals for up to 6 months. There were no significant treatment-related effects on F1 body weights.

Physical Development: Morphological examinations were performed on Days 1, 21, 50, 88, and 180 post-partum. There were no significant treatment-related developmental changes.

Neurological Assessment: Neurobehavioral test battery was performed on Days 1 and 7 post-partum. No significant treatment-related effects were observed including grip strength.

Other: There were no significant treatment-related changes in hematology, organ weight, macroscopy or microscopy.

Overall, administration of MLN0002 at dose levels of 0, 10, and 100 mg/kg/day (dosed every two weeks from GD20 to GD140) did not produce any significant overt maternal toxicity. No significant treatment-related adverse effects were observed on growth and development and neurobehavioral parameters of F1 offspring.

10 Special Toxicology Studies

A Single-Dose Local Toxicity Study of MLN0002 Administered Either Subcutaneously or Intramuscularly to Male New Zealand White Rabbits (Study No. 416096)

Methods: The objective of this study was to examine the local irritation potential of MLN0002 when administered by subcutaneous (SC) or intramuscular (IM) injection to New Zealand White rabbits. In this study, male New Zealand White rabbits were administered MLN0002 via SC injection (back) and IM injection (thigh) once as shown in the table below (from page 14 of the report).

Table T1 Study Design

<u>Group Number</u>	<u>Test Article</u>	<u>Dose (mg)</u>	<u>Dose Concentration (mg/mL)</u>	<u>Route</u>	<u>Number of Males</u>
1	Vehicle ^a	0 and 0	0	IM	4
2	Vehicle ^b	0 and 0	0	SQ	4
3	MLN0002 ^c	69 and 138	30 and 60	SQ	6
4	MLN0002 ^d	17.4 and 34.8	30 and 60	IM	6

^a = Two concentrations (low and high) were administered intramuscularly (*M. vastus lateralis*) on the right and left leg of each animal.

^b = Two concentrations (low and high) were administered subcutaneously at 2 separate sites on the back of each animal.

^c = Two concentrations (30 and 60 mg/mL) were administered subcutaneously at 2 separate sites on the back of each animal.

^d = Two concentrations (30 and 60 mg/mL) were administered intramuscularly (*M.vastus lateralis*) on the right and left leg of each animal.

IM= Intramuscular; SQ = Subcutaneous

Group 3 rabbits received single SC injections (2.3 mL) of MLN0002 to sites on the back at two concentrations (30 and 60 mg/mL). Group 4 animals received single IM injections (0.58 mL) of MLN0002 to sites on the thigh at two concentrations (30 and 60 mg/mL). Concurrent control groups (Groups 1 and 2) received low (25 mM Histidine/Histidine-HCl, 62.5 mM Arginine-HCl, 0.03% Polysorbate 80, 5% Sucrose, pH 6.3, 315 mOsm/kg) and high (50 mM Histidine/Histidine-HCl, 125 mM Arginine-HCl, 0.06% Polysorbate 80, 10% Sucrose, pH 6.3, 655 mOsm/kg) concentrations of the vehicle control article on a comparable regimen. Groups 1 and 2 each consisted of four males and Groups 3 and 4 each consisted of six males. All animals were observed twice daily for mortality and moribundity. Detailed physical examinations were performed and body weights were recorded on the day of dosing and at necropsy. Clinical signs were observed approximately 1, 24, 48 and 72 hours post-dose. Following the 72 hour post-dose, all surviving animals were euthanized and the injection sites were examined macroscopically and microscopically.

Results: There was no mortality. There was no significant treatment-related effect on body weight. Clinical signs at the SC injection sites at 1 hour post-dosing included swelling within the injection site in 4 of 6 and 2 of 6 sites at 30 mg/mL and 60 mg/mL of MLN0002, respectively. Swelling was also observed, at 1 hour post-dose, for 1 of 4 SC injection sites with the low concentration of the vehicle control and for 2 of 4 SC injection sites with the high concentration of the vehicle control article. Clinical signs observed at the IM injection sites at 48 hours post-dose included a reddened area within the injection site in 2 of 6 sites at 30 mg/mL of MLN0002. Macroscopic observations included slight to moderate hyperemia and discoloration (scores of 1 and 2) at the SC injection site in 1 of 4, 1 of 6, and 2 of 6 sites injected with the high concentration of vehicle control, 30 mg/mL, and 60 mg/mL of MLN0002, respectively. Moderate hyperemia and discoloration was also observed at the IM injection sites in 2 of 4 sites at the high concentration of the vehicle control article. Minimal to mild hemorrhage and/or inflammation were observed microscopically for 1 of 4, 1 of 4, 1 of 6, and 3 of 6 of the

SC injection sites at the low or high concentrations of the vehicle control or the 30 mg/mL or 60 mg/mL of MLN0002, respectively. Minimal to mild hemorrhage and/or inflammation were also observed microscopically for 2 of 4, 3 of 4, 4 of 6, and 3 of 6 of the IM injection sites at the low or high concentrations of the vehicle control or the 30 mg/mL or 60 mg/mL of MLN0002, respectively. The incidence and severity of the observations were similar between the vehicle and the test article-injected sites. The changes were attributed to mechanical trauma related to the injection procedure.

Overall, it was concluded that MLN0002 administered via a single SC or IM injection to New Zealand White rabbits did not result in any significant irritation at the tested concentrations (30 or 60 mg/mL). The incidence and severity of the findings were similar between vehicle- and MLN0002-injected sites. The changes observed at the vehicle and MLN0002 injection sites were attributed to the injection site trauma.

3-Week Comparative Immunotoxicity Study of Natalizumab (Tysabri®) and Vedolizumab (MLN0002) Administered by Intravenous Infusion to Cynomolgus Monkeys (20002458)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated May 13, 2011.

3-Week Comparative Immunotoxicity Study of Natalizumab (Tysabri®) and Vedolizumab (MLN0002) Administered by Intravenous Infusion to Cynomolgus Monkeys (20002485)

Methods: The objectives of this study were to compare potential immunotoxicity, toxicokinetics, and pharmacodynamics of Natalizumab (Tysabri®) and Vedolizumab (MLN0002) when given by IV infusion once weekly for 3 weeks to Cynomolgus monkeys at 30 mg/kg. In this study, Cynomolgus monkeys (5/sex/dose, Males: 2.8-4.4 years, 2.4-3.1 kg; Females: 2.7-5.8 years, 2.4-2.9 kg) were assigned to dose groups as shown in the Table below (from page 9 of the report).

Group No.	No. of Animals		Test Material	Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)	Dose Volume (mL/kg/dose)
	Male	Female				
1	5	5	Control Article (saline)	0	0	10
2	5	5	Natalizumab	30	3	10
3	5	5	Vedolizumab (MLN0002)	30	3	10

On Day -14 and 1 (prior to administration of test or control article), all animals were immunized with Tetanus Toxoid (TT) injected intramuscularly. On Day 1 (prior to administration of TT, test or control article), all animals were immunized with 1000 µL of a 1:1 emulsion of Keyhole Limpet Hemocyanin (KLH) and Incomplete Freund's Adjuvant injected intramuscularly to achieve a dose of 750 µg/animal. Animals were administered test or control article via a 30 minute IV infusion on Days 1, 8, and 15 and were examined for clinical signs, food consumption, and body weight. Blood samples were collected for hematology, toxicokinetic, immunogenicity, pharmacodynamic, flow cytometry, anti-KLH antibody, and anti-TT antibody analyses at various time points.

Results: There were no mortalities. No significant treatment-related effects were observed on clinical signs, food consumption or body weight. Natalizumab caused a significant increase in leukocyte counts (white blood cells, lymphocytes, monocytes, eosinophils, and basophils), B-lymphocytes, total T-lymphocytes, T-helper lymphocytes, T-cytotoxic lymphocytes, memory T-cytotoxic, and memory T-helper cell populations at Days 8, 15, and 22. There were no MLN0002-related changes in lymphocyte populations. There were no Natalizumab or MLN0002-related changes in anti-KLH or TT IgM and IgG values as compared to the control group.

Exposure (C_{max} and AUC_{0-168hr}), was higher on Day 15 than on Day 1 in both the treatment groups indicating accumulation in the serum. The AUC_{0-168hr} ratio (Day 15:Day 1) in the MLN0002 and Natalizumab dose groups was 2.00 and 1.37, respectively. There were no apparent sex-related differences in serum concentrations or derived TK parameters after exposure to either Natalizumab or MLN0002.

Natalizumab was immunogenic in all animals and PAHA had a moderate effect on TK and PD. MLN0002 was immunogenic in some animals. Overall, PAHA did not appear to have significant effect on the TK and PD of MLN0002.

Pharmacodynamic responses were observed for both MLN0002 and Natalizumab. Natalizumab increased the level of CD4+/CD45RA+ and CD4+/CD45RA- lymphocytes in peripheral blood by approximately 2-fold. MLN0002 increased the level of CD4+/CD45RA+ and CD4+/CD45RA- lymphocytes by approximately 3-fold.

Single Dose TDAR Study in Cynomolgus Monkeys with Natalizumab (Tysabri®)(KLA00441)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated May 13, 2011.

**A Single Dose TDAR Study in Cynomolgus Monkeys with Natalizumab (Tysabri®)
Report No. KLA00441**

Methods: The objective of this study was to determine the potential toxicity and acquired immunity of Natalizumab (Tysabri®) when administered by single IV infusion to Cynomolgus monkeys. In this study, Cynomolgus monkeys (n = 4/sex/dose, 2.9 to 3.2 years of age for the males and 2.6 to 3.4 years of age for the females, and weighing 2.3 to 2.6 kg for the males and 2.0 to 2.3 kg for the females) were assigned to dose groups as shown in the Table (from page 22 of the study report) below. Group 1, was treated with the control article (0.9% saline). Groups 2 and 3 were dosed with Natalizumab at 10 mg/kg and 30 mg/kg, respectively, and Group 4 was dosed with the reference article, FK506 (Prograf®) at 1.5 mg/kg (twice daily at 0.75 mg/kg). All animals were necropsied on Day 17.

Group No.	No. of Males/Females	Dose Level (mg/kg/day)	Test Material	No. Necropsied: Day 17
1	4/4	0 (control)	0.9% Saline	4/4
2	4/4	10	Tysabri®	4/4
3	4/4	30	Tysabri®	4/4
4	2/2	1.50 ^a	Prograf®	2/2

^a Prograf® was administered at a dose level of 0.75 mg/kg twice daily (12 hours [± 30 minutes] apart) for 16 consecutive days.

All animals in Groups 1-3 were dosed once via IV infusion. Animals in Group 4 were dosed twice daily (12 hours apart) via nasogastric gavage for 16 consecutive days. Animals were examined for clinical signs, mortality and/or morbidity (twice daily), food consumption (once daily), body weight (twice prior to the first dose [Weeks -2 and -1], Day 7, and Day 14), clinical pathology parameters, including serum chemistry (Days -6, 3, 9, and 16), hematology and flow cytometry parameters (Day -13, -6, 3, 9, and 16). The following (from page 33 of the report) cellular antigens and cell populations were quantified using specific antibodies against marker antigens.

Antigen Marker(s)	Cell Population Identified
CD20+	B-lymphocytes
CD3+	T-lymphocytes
CD3+/CD4+	T-helper lymphocytes
CD3+/CD8+	T-cytotoxic/suppressor lymphocytes
CD3-/CD8+	NK cells
CD3-/CD16+	NK cells
CD34+	Hematopoietic precursors

^a A lymphocyte purity estimate (CD2+/CD20+) was calculated but not reported.

In addition, natural killer (NK) and keyhole limpet hemocyanin (KLH) analyses were performed at various time points. Twenty-eight animals were euthanized on Day 17. At termination, a full necropsy was conducted on all animals, and the liver, spleen, and

ileum (containing the gut-associated lymphoid tissue [GALT]) was examined for histopathology. The following (from page 37 of the report) organs were weighed.

Organs Weighed	
Adrenals	Ovaries
Brain	Pituitary
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Lungs	

Results: There was no mortality or treatment-related clinical signs. There were no significant treatment-related changes in body weight or food consumption. There were no Natalizumab-related serum chemistry changes, but there were Prograf-related minimal increases in mean potassium (18% increase over prestudy) and triglycerides (74% increase over prestudy) beginning on Day 3 and continued through Day 16. There were Natalizumab-related increases in total WBC, lymphocytes, monocytes, basophils at 10 and 30 mg/kg, and there were Prograf-related increases in total WBC, lymphocytes, RBC, hemoglobin, hematocrit, and reticulocytes.

Natalizumab-related alterations in peripheral blood mononuclear cells (PBMCs) consisted of significant dose-related increases in all lymphocyte subsets including: CD20+ B-lymphocytes, CD3+ total T-lymphocytes, CD3+/CD4+ T-helper lymphocytes, and CD3+/CD8+ T-cytotoxic lymphocytes beginning on Day 3 and persisted through Day 16. Prograf also caused marginal increases in PBMCs of all lymphocyte subsets on Day 3, which subsequently returned to prestudy values by Day 16.

Animals dosed with Natalizumab exhibited decreases in NK cell cytolytic activity at Day 16 when compared to Group 1 control animals and to Day -6 prestudy values. Group 4 animals (Prograf-treated) did not exhibit definitive alterations in NK cell cytolytic activity. Alterations in the humoral immune response attributed to Natalizumab included a reduced primary immune response demonstrated by a reduction in anti-KLH immunoglobulin M (IgM) and immunoglobulin G (IgG) values measured 6 and 13 days post KLH immunization when compared to control values. Inhibition of the IgM and IgG T-cell dependant antibody response (TDAR) response was not observed with Prograf; however, there was a trend towards reduction of IgM and IgG in male animals. A subsequent in-house study (report not provided) was conducted with the same and a higher dose of Prograf. Results from this study indicated complete inhibition of the anti-KLH IgG response in the high-dose group (3 mg/kg) and in some animals in the low-dose group (0.75 mg/kg). Same trend to a lesser degree was seen with the anti-KLH IgM responses. The data from this internal study strengthened the marginal, slight decrease in the anti-KLH IgM immune response observed in the two males after administration of twice daily Prograf in this study.

Spleen weight (absolute and relative) was increased in animals treated with Natalizumab. Histopathology findings in the Natalizumab-treated animals included lymphoid hyperplasia in the spleen (increased size/number of germinal centers and

hypercellularity of the peri-arteriolar lymphoid sheaths) at 10 and 30 mg/kg. In addition, sinusoidal leukocytosis in the liver was seen 10 and 30 mg/kg. There were no gross, organ weight, or histological findings that were associated with Prograf.

Overall, Natalizumab when administered as a single IV infusion in Cynomolgus monkeys at 10 and 30 mg/kg did not cause any mortality. The target organ appeared to be the spleen (lymphoid hyperplasia) and liver (sinusoidal leukocytosis). Natalizumab also caused hematology changes which included increase in total WBC, lymphocytes, monocytes, basophils at 10 and 30 mg/kg. In addition, Natalizumab caused significant increases in PBMCs for all lymphocyte subsets and decreased NK cell cytolytic activity and decreased TDAR.

Delayed-Type Hypersensitivity Responses, Anti-Tetanus Antibody Titers, and Laboratory Assay Results in Act-1 Treated Rhesus Monkeys (RPT-02275)

Methods: The objective of this study was to examine whether $\alpha 4\beta 7$ blockade inhibited cell-mediated immunity in the skin. This study was conducted with Act-1 (murine homologue of MLN0002) in tetanus-sensitized Rhesus monkeys (*Macaca mulatta*) challenged intradermally (ID) with tetanus toxoid (TT) in a cutaneous delayed-type hypersensitivity (DTH) model using IV/IM routes. Act-1 was administered repeatedly to three Rhesus monkeys (2.0 mg/kg for 10 days, the first dose IV and subsequent doses IM). Skin biopsies were conducted at 72 hours after each ID challenge to serve as baseline controls for comparison of responses to a third ID challenge after $\alpha 4\beta 7$ blockade. Beginning one day before the third ID challenge with TT, three animals received either Act-1 or an irrelevant IgG1 isotype control antibody. At 72 hours after ID challenge, the skin sites were removed by biopsy. The number of leukocytes in the dermis of each of the three sequential skin biopsies was determined following staining for T cells (CD3⁺) or monocytes/macrophages (HAM-56⁺). Results (percentage of area examined with positive immunostaining) from post-dose biopsies were normalized to results for pre dose biopsies, and were compared between groups.

Results: Act-1 treated animals had no significant difference in CD3⁺T cell or HAM-56⁺ monocyte/macrophage cell density within the inflammatory foci when compared to controls. The following table (from page 6 of the report) shows the results of this study.

Table 1 Lack of Act-1–Induced Reduction in Dermal Leukocyte Density in Skin DTH Sites in Rhesus Monkeys

Test Article	Dermal Cell Density Relative to Predose Values (%) (Mean [SEM])	
	CD3 ⁺ T cells	HAM-56 ⁺ Monocytes/Macrophages
Act-1	154.1 (44.5)	134.1 (38.7)
Irrelevant IgG1	133.6 (38.6)	126.2 (36.4)

DTH = delayed-type hypersensitivity; Ig = immunoglobulin; SEM = standard error of the mean.

Based on these, it was concluded that there was no evidence that $\alpha 4\beta 7$ blockade inhibited cell-mediated immunity in the skin.

Cross-Reactivity Study of MLN0002 with Normal Human Tissues (Study No. IM1493)

Methods: The objective of this study was to evaluate the potential cross-reactivity of MLN0002 with a panel of normal human tissues. In this study, MLN0002 was applied to cryosections of normal human tissues (from at least three separate donors per tissue) at two concentrations (2 and 20 $\mu\text{g}/\text{mL}$). The following (from page 12 of the report) tissues were examined.

8.2.2.2. Normal Human Tissue from at Least Three Separate Donors

Adrenal Lung		Spinal Cord
Blood Cells ^f Lym	ph Node	Spleen
Blood vessels (endothelium)	Ovary	Striated (skeletal) Muscle
Bone Marrow	Fallopian Tube (oviduct)	Testis
Brain – cerebrum (cortex)	Pancreas	Thymus
Brain – cerebellum	Parathyroid	Thyroid
Breast (mammary gland)	Peripheral Nerve	Tonsil
Eye Pituitary		Ureter
Gastrointestinal Tract ^g Placenta		Urinary Bladder
Heart	Prostate	Uterus- body (endometrium)
Kidney (glomerulus, tubule)	Salivary Gland	Uterus – cervix
Liver Skin		

Results: MLN0002 stained the positive control tissue [human tonsil (mononuclear cells)] as expected. Moderate to intense MLN0002 staining of the membrane of mononuclear cells was observed in interfollicular and submucosal regions of the tonsil at the high concentration of MLN0002. MLN0002 did not react with the negative control [human striated (skeletal) muscle (myocytes)]. The negative control antibody, HuIgG1, did not react with either the positive or negative control tissues or tissue elements. The specific reactions of MLN0002 with the positive control tissue and the lack of specific reactivity with the negative control tissue, as well as the lack of reactivity of the negative control antibody, indicated that the assay was valid.

MLN0002 stained mononuclear cells in multiple human tissues as follows: adrenal (1 of 3 donors), blood cells (2 of 3 donors), bone marrow (1 of 3 donors), breast [mammary gland (1 of 3 donors)], gastrointestinal tract [colon (3 of 3 donors)], esophagus (2 of 3 donors), small intestine (2 of 3 donors), stomach (3 of 3 donors), kidney (1 of 3 donors), liver (2 of 4 donors), lymph node (3 of 3 donors), fallopian tube (2 of 4 donors), prostate (3 of 3 donors), salivary gland (2 of 3 donors), skin (1 of 3 donors), spleen (3 of 3 donors), thymus (3 of 3 donors), tonsil (3 of 3 donors), urinary bladder (2 of 3 donors), and uterus-cervix (1 of 3 donors). In all tissues positive staining was observed at the membrane of the MLN0002-stained cells. In gastrointestinal tract tissues, MLN0002 stained mononuclear cells were organized in submucosal lymphoid nodules and/or

scattered in lamina propria. In the lymphoid tissues (tonsil, submucosal lymphoid nodules in gastrointestinal tract), the staining was observed at the membrane of mononuclear cells in interfollicular and submucosal regions. In one thymus sample, MNL0002 staining predominated in the medulla. Overall, the human tissue cross-reactivity profiles were consistent with the patterns of $\alpha 4\beta 7$ integrin expression.

A Cross-Reactivity Study of MLN0002 with Normal Cynomolgus Monkey Tissues (RPT-IM1741)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated April 29, 2011.

A Cross-Reactivity Study of MLN0002 with Normal Cynomolgus Monkey Tissues (RPT-1M1741)

The objective of this study was to evaluate the potential cross-reactivity of MLN0002 in Cynomolgus monkey tissues. MLN0002 was applied to cryosections of Cynomolgus monkey tissues (2 donors per tissue) at two concentrations (2 and 20 µg/mL). MLN0002 produced moderate to intense plasma membrane and plasma membrane granule staining of mononuclear cells in the interfollicular (T-cell) area in the positive control human tonsil tissue at 20 µg/mL. MLN0002 did not specifically react with the negative control tissue [cryosections of human striated (skeletal) muscle (myocytes)]. The negative control antibody, HulgG1 (human IgG1), did not specifically react with either the positive or negative control tissue elements.

MLN0002 reacted with the membrane of lymphocytes in the blood and mononuclear cells in the colon, small intestine, stomach, heart, lung, lymph node, salivary gland, spleen, thymus, tonsil, and urinary bladder. In the colon and stomach, MLN0002-stained mononuclear cells were scattered within the lamina propria, and/or in mucosal or submucosal lymphoid nodules. In the small intestine, MLN0002-stained mononuclear cells were located intraepithelial, scattered within the lamina propria, and in submucosal lymphoid nodules. In the heart, MLN0002-stained mononuclear cells were located within the myocardial interstitium. In the lung, MLN0002-stained mononuclear cells were located within nodules adjacent to bronchi/bronchioles (BALT). In the salivary gland, stained mononuclear cells were located in small aggregates within the interstitium. In the spleen, stained mononuclear cells were most prominent in periarteriolar lymphoid sheath (PALS). In the thymus, stained mononuclear cells were more prominent in the medulla than in the cortex. In the tonsil, test article-stained mononuclear cells were located primarily in interfollicular and submucosal regions of the tonsil. In the urinary bladder, MLN0002-stained mononuclear cells were located primarily in small aggregates within the propria-submucosa. The $\alpha 4\beta 7$ integrin is known to be expressed on the membrane of mononuclear cells. Therefore, the observed staining of mononuclear cells and lymphocytes of different tissues was expected. There was no other unexpected staining or cross-reactivity observed with MLN0002 in the Cynomolgus monkey tissues examined.

In conclusion, the MLN0002 staining pattern observed in the Cynomolgus monkey tissues was similar to that observed in the human tissues in the companion human tissue cross-reactivity study (Study No. IM1493) and was consistent with the known pattern of expression of $\alpha 4\beta 7$ integrin.

A Study of Binding Specificity of ACT-1 in Malignant Human Tumors (RPT-1M1806)

The review of the above study is incorporated below from the pharmacology review of IND 9125 dated April 29, 2011.

A Study of Binding Specificity of ACT-1 in Malignant Human Tumors (RPT-1M 1806)

This study was conducted to determine the cross-reactivity of ACT-1, a monoclonal mouse IgG1 antibody, with stromally-derived tumor tumors of the gastrointestinal tract. ACT-1 directed against $\alpha 4\beta 7$ was tested at 2 and 20 $\mu\text{g}/\text{mL}$ concentrations on 10

samples of human colon malignant adenocarcinoma. ACT-1 produced strong to intense staining of the positive control material (mononuclear cells in cryosections of normal human tonsil). ACT-1 did not react with the negative control material (cryosections of normal human skeletal muscle). The negative control antibody, MslgG1 (mouse IgG1), did not specifically react with either the positive or negative control.

All 10 samples demonstrated ACT-1-specific staining of cytoplasmic granules and membranes of mononuclear cells (representing the resident or infiltrating lymphocyte population) with a lack of staining in surrounding neoplastic tissue. This pattern of staining was consistent with the expression pattern of $\alpha 4\beta 7$ integrin; which is expressed on the surface of lymphocytes.

Preliminary Studies of Cross Reactivity of a Humanized Monoclonal Antibody LDP-02 (Study No. IM353)

Methods: This exploratory study was conducted to determine the appropriate positive controls, concentrations of antibody, and the fixation and staining conditions to be used in a subsequent full human tissue cross reactivity study. In this study, several different forms of LDP-02 (biotinylated, unconjugated, fluoresceinated) and its murine homologue ACT-1 were tested. Multiple human and monkey tissues were examined as potential positive controls including tonsil, lymph node, small intestine, spleen, and cultured lymphoblastoid cells. In addition, multiple fixation procedures were evaluated; and different fixatives were tested, which included paraformaldehyde, formalin, acetone, and citrate buffered acetone. Multiple immunoperoxidase staining procedures were also examined including direct immunoperoxidase, indirect immunoperoxidase, precomplexing indirect immunoperoxidase, anti-id indirect immunoperoxidase. Two amplification systems were examined, one avidin/biotin complex-based peroxidase stain (tyramide signal amplification) and the other based on a peroxidase-labeled polymer.

Results: For LDP-02, the strongest signal and the best signal-to-noise ratio was demonstrated for an anti-id indirect immunoperoxidase system using a post fixation step and a peroxidase-labeled polymer amplification system. For the ACT-1, the strongest signal and the best signal-to-noise ratio was demonstrated for an indirect immunoperoxidase system and a peroxidase-labeled polymer amplification system. In addition, several potential positive control tissues were identified. The tonsil showed the most consistent positive signal. The results indicated that LDP-02 should be titrated over a narrow range (approximately 5-20 $\mu\text{g}/\text{mL}$) as reasonable signal was evident at 10 $\mu\text{g}/\text{mL}$ but the intensity of the signal often dropped at 1 $\mu\text{g}/\text{ml}$.

Cross Reactivity of Mouse Monoclonal Antibody ACT-1 with Normal Human Tissues (IM433)

Methods: This study examined the potential tissue cross-reactivity of ACT-1 (murine homologue of MLN0002) with cryosections of 37 normal human tissues from each of three donors. In this study, ACT-1 was applied to human tissues (three sources per tissue) at two concentrations (5 and 20 µg/mL) and incubated for 120 minutes. The concentrations of the test article, positive and negative controls were selected based on the previous study (IM353) described above.

Results: ACT-1 specifically stained the cell membranes of positive control, $\alpha 4\beta 7$ expressing lymphocytes in the mantle and interfollicular regions of human tonsil. There was no specific staining of the negative control follicular germinal center lymphocytes at either concentration of the test article. ACT-1 reacted with lymphocytes in the lymphoid tissues, within the lumens of blood vessels, or as low-grade inflammatory infiltrates in various nonlymphoid tissues. ACT-1 specifically stained lymphocytes of lymph nodes (paracortex and mantle), tonsil (interfollicular/mantle regions), positive control tissue, and spleen (periarteriolar lymphoid sheath). ACT-1-reactive lymphocytes were found in the central regions of less fully developed lymphoid follicles in the lymph node or gut-associated lymphoid tissues (GALT, e.g., submucosal lymphoid nodules in the esophagus, large intestine, small intestine, and stomach). ACT-1 also reacted with lymphocytes in the thymic medulla and rare mononuclear cells in the bone marrow. ACT-1 specifically stained lymphocytes found within vessel lumens in the brain (cerebrum), liver, and lymph node. Within the lymph nodes, ACT-1 reactive lymphocytes were particularly observed within the lumens of efferent lymphatics at the node hilus. ACT-1 also reacted specifically with perivenular lymphocytes in the liver of one donor. This report commented that although the liver is not considered a primary or secondary lymphoid tissue, activated T-lymphocytes are known to circulate to the liver and enter the parenchyma via a perivenular portal and undergo apoptosis. As per the Applicant, as ACT-1 reacts with $\alpha 4\beta 7$ integrin, the reactivity of ACT-1 with occasional perivenular lymphocytes in the liver was not unexpected.

Cross Reactivity of Humanized Monoclonal IgG1 LDP-02/3A9-8E3.C1 Antibody with Normal Human Tissues (Study No. IM434)

Methods: This study examined the tissue cross reactivity of LDP-02 with 37 normal human tissues from each of three donors. LDP-02 was applied to human tissues (three sources per tissue) at two concentrations (5 and 20 µg/mL). Cryosections of $\alpha 4\beta 7$ expressing lymphocytes in the mantle and interfollicular regions of the tonsil were used as positive control tissues. The lymphocytes in the follicular germinal centers of normal human tonsil were selected as the negative control.

Results: The positive and negative control showed expected results. Reactivity of LDP-02 was restricted to lymphoid tissues or rare to occasional lymphocytes within the lumens of blood vessels or as low grade inflammatory infiltrates in various tissues.

Lymphoid Tissues: LDP-02 specifically stained lymphocytes in the T-dependent regions of lymph nodes (paracortex and mantle), tonsil (interfollicular/mantle regions) and positive control tissue and spleen (periarteriolar lymphoid sheath). Lymphocytes were not stained in the fully developed germinal centers of the secondary follicles in the tonsil, spleen or lymph node. However, LDP-02-reactive lymphocytes were also found in the central regions of less fully developed lymphoid follicles found in the lymph node or GALT (submucosal lymphoid nodules in the esophagus, large intestine, small intestine, and stomach). LDP-02 also reacted with lymphocytes in the medulla of the thymus.

Intravascular Lymphocytes: LDP-02 specifically stained lymphocytes found within the vessel lumens in the liver, lung and lymph node. Within the lymph nodes, LDP-02-reactive lymphocytes were particularly observed within the lumens of efferent lymphatics at the node hilus. LDP-02 also reacted with rare to occasional lymphocytes in the peripheral blood smears from two donors.

Nonlymphoid Tissues (Liver, Kidney, Prostate, and Thyroid): Like ACT-1, LDP-02 reacted specifically with perivenular lymphocytes in the liver of one donor. As per the Applicant, as LDP-02 reacts with $\alpha 4\beta 7$ integrin, the reactivity of LDP-02 with occasional perivenular lymphocytes in the liver was not unexpected. LDP-02 also specifically stained lymphocytes within the kidney from one donor, within the prostate from one donor, within the thyroids of two donors, and within the thyroid tissue associated with parathyroids. In those tissues, multifocal, relatively low-grade, infiltrates of lymphocytes were organized into nodules. In nodules with well-developed follicular germinal centers, LDP-02 reacted with the interfollicular regions but not with the follicular germinal center regions.

Overall, no unanticipated tissue cross reactivity was observed in this study. LDP-02 shares its complementarity-determining-regions (CDRs) with its murine homologue, ACT-1. Therefore, it was expected that the tissue cross reactivity of LDP-02 observed in the present study was very similar to that observed in the previous tissue cross reactivity (TCR) study with ACT-1 (Study No. IM433).

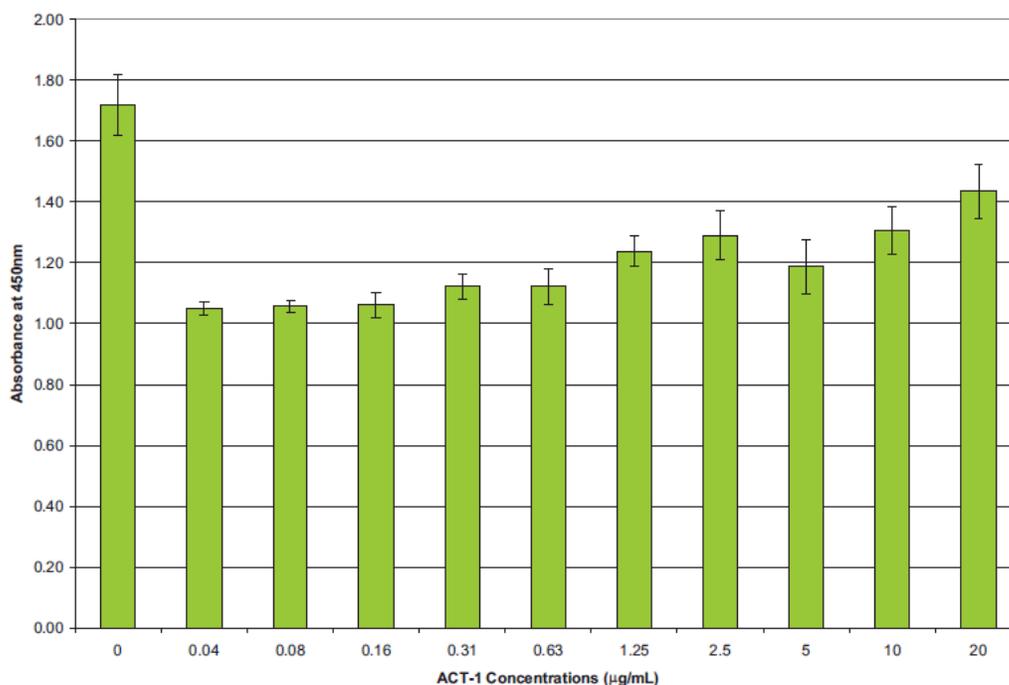
Effect of ACT-1 Antibody on the Growth of the Alpha 4 Beta 7-Expressing RPMI 8866 Human B-Cell Lymphoma Cell Line (RPT-01335)

Methods: This *in vitro* study examined the carcinogenic potential of Act-1 (murine homologue of MLN0002) using human tumor cells (RPMI 8866 cell line derived from a human B-cell lymphoma) that expressed $\alpha 4\beta 7$ integrin. The objective of this study was to investigate the effect of ACT-1 on the growth rate of a human chronic myeloid B cell line that expresses $\alpha 4\beta 7$ integrin. The Roswell Park Memorial Institute (RPMI) 8866 cell line was derived from a human B cell lymphoma that expresses the $\alpha 4\beta 7$ integrin. Thus, this cell line was used as a model for the effect of MLN0002 on tumors that express the $\alpha 4\beta 7$ integrin. A change in the growth rate of this cell line following treatment with ACT-1 was considered to indicate the carcinogenicity potential of ACT-1. This study was conducted using cell counting kit-8 (CCK-8) cell proliferation assay kit. The WST-8 tetrazolium salt-based growth assays were performed on RPMI 8866 cells cultured in

96-well plates. Cells were incubated with CCK-8 reagent for 1 to 5 hours. The assay measured the activity of cellular dehydrogenases that reduce the WST-8 compound into a water-soluble formazan dye. The amount of the formazan dye generated by the activity of dehydrogenases in cells is directly proportional to the number of living cells. The reaction was stopped by adding 1% sodium dodecyl sulfate (SDS) to each well. Absorbance of the formazan dye was measured at 450 nm. In this study, cells were plated into 96-well plates at 30,000 cells per well and were treated either with the IgG1 control or the ACT-1 antibodies at concentrations ranging from 0.039 to 20 $\mu\text{g}/\text{mL}$. The IgG1 antibody (mouse mAb) was used as the control. The first cell growth assay was performed 24 hours after antibodies were added to the cells, then once every consecutive day for a total of 4 measurements. A confirmatory assay was conducted using ACT-1 at concentrations ranging from 0.039 to 20 $\mu\text{g}/\text{mL}$ for 4 consecutive days and the CCK-8 assay was performed on Day 4 only.

Results: Both the control and ACT-1 antibodies had minimal inhibitory effect on cell growth. Statistical analysis of the data showed that both (control and Act-1) antibodies inhibited cell growth, although the degree of cell growth inhibition in the ACT-1 groups was lower than in the corresponding IgG1 groups, which was most visible at Days 3 and 4. In the confirmatory assay on Day 4, Act-1 inhibited cell growth as shown in the following figure (from page 12 of the report).

Figure 3 Effect of ACT-1 Antibody on the Growth of RPMI 8866 Cells on Day 4



In conclusion, ACT-1, the murine analog of MLN0002 with an identical CDR, did not stimulate the growth of the RPMI 8866 human B-cell lymphoma cell line, which expresses $\alpha 4\beta 7$ the integrin.

11 Integrated Summary and Safety Evaluation

Vedolizumab (MLN0002) is a novel humanized IgG1 monoclonal antibody indicated for the treatment of adult patients with ulcerative colitis (UC) and Crohn's disease (CD). Alpha 4 beta 7 integrin, a key mediator of gastrointestinal (GI) inflammation, is expressed on the surface of a discrete subset of memory T-lymphocytes that preferentially migrates into the gastrointestinal tract and can cause inflammation, characteristic of UC and CD. Vedolizumab binds selectively to $\alpha 4\beta 7$ integrin on the gut-homing lymphocytes and selectively inhibits adhesion of $\alpha 4\beta 7$ integrin to MAdCAM-1 and to the extracellular matrix glycoprotein fibronectin but not to VCAM-1. MAdCAM-1 is preferentially expressed on endothelial venules at sites of lymphocyte extravasation in the GI mucosa and gut associated lymphoid tissue (GALT). Binding of MAdCAM-1 by $\alpha 4\beta 7$ mediates migration of leukocytes into the GI mucosa and GALT. Vedolizumab antagonizes the $\alpha 4\beta 7$ /MAdCAM-1 interaction, inhibits associated migration of leukocytes into the GI mucosa, and thereby reduces inflammation in the GI tract.

Vedolizumab has been evaluated in a comprehensive program of nonclinical studies which included pharmacology, pharmacokinetics (PK), acute IV toxicology (monkey), repeated dose IV toxicology (14-day, 13-week, 26-week) in Cynomolgus monkeys and rabbits (3-month), reproductive toxicology (single dose IV embryofetal development in rabbits and pre- and postnatal development in monkeys), local tolerance and special toxicology studies.

In pharmacology studies, MLN0002 has been shown to bind selectively to $\alpha 4\beta 7$ integrin; it did not bind to the $\alpha 4\beta 1$ integrin or the $\alpha E\beta 7$ integrin, and appears to be selective for $\alpha 4\beta 7$ integrin. This specificity for the $\alpha 4\beta 7$ integrin seems to differentiate MLN0002 from other integrin antagonists, such as the pleiotropic integrin antagonist natalizumab, which binds to $\alpha 4\beta 1$ and $\alpha 4\beta 7$ and etrolizumab, which binds to $\alpha E\beta 7$ and $\alpha 4\beta 7$. MLN0002 selectively inhibited adhesion interaction of $\alpha 4\beta 7$ to MAdCAM-1 and fibronectin, but not VCAM-1. In an *in vivo* animal efficacy study with ACT-1 (murine homologue of MLN0002) in cotton-tap Tamarin monkeys (*Sanguinus Oedipus*) with naturally occurring chronic colitis, ACT-1 showed efficacy (resolution of diarrhea, reduction of inflammatory activity, e.g., leukocyte infiltration of the gut lamina propria and associated degenerative structural changes). Immune surveillance of the CNS was tested in experimental autoimmune encephalitis (EAE, a primate model of multiple sclerosis) model in Rhesus monkeys. MLN0002 did not appear to inhibit immune surveillance of the CNS as assessed in this model of EAE in Rhesus monkeys. In contrast, natalizumab blocked immune surveillance of the CNS in this model. However, since EAE is not an animal model of PML; the results of this study do not directly demonstrate that vedolizumab has no potential to cause PML.

In a cardiovascular safety pharmacology study in telemetered Cynomolgus monkeys, MLN0002 did not show any effect on ECG parameters, arterial blood pressure or heart rate.

The pharmacokinetics (PK) of MLN0002 has been studied in Cynomolgus monkeys. In a single dose intravenous PK/PD (pharmacodynamic) study of MLN0002 in Cynomolgus Monkeys at 10 and 100 mg/kg, C_{max} of 214 and 2090 $\mu\text{g/mL}$, respectively, was observed at a T_{max} of 0.5 hour postdose, and the $AUC_{0-16\text{hr}}$ values were 32,700 and 54,2000 $\mu\text{g}\cdot\text{hr/mL}$ at 10 and 100 mg/kg, respectively. The clearance of MLN0002 was 0.266 and 0.180 mL/h/kg at 10 and 100 mg/kg, respectively, and the volume of distribution was 80.7 and 88.3 mL/kg, respectively. The terminal elimination half-life ($t_{1/2}$) was 336 and 362 hours (14-15 days), respectively. The PK appeared to be approximately dose proportional between 10 and 100 mg/kg.

The immunogenicity of MLN0002 was assessed by measuring RAHA (rabbit antihuman antibody) and PAHA (primate antihuman antibody) in rabbits and monkeys, respectively. The incidence of RAHA and PAHA was variable across single- and repeat-dose studies and across doses. In some animals, RAHA/PAHA had a neutralizing effect on the PK and PD of MLN0002 at the low and mid doses (10 and 30 mg/kg); however, saturation of receptors was achieved in animals that did not develop a neutralizing RAHA/PAHA effect. At the highest tested dose (100 mg/kg), RAHA/PAHA appeared to have little effect on the PK or PD of MLN0002 and receptor saturation was observed at this dose.

Acute, 14-day, 3-month (10, 30 and 100 mg/kg) and 6-month (10, 30 and 100 mg/kg) toxicity studies were conducted in Cynomolgus monkeys using the IV route. Histopathological changes were seen in the gastrointestinal tract (minimal to mild lymphoid depletion in the Peyer's patches of the gastrointestinal tract in male animals at 10, 30, and 100 mg/kg/day and increased epithelial regeneration in stomachs of male and female animals with lymphoplasmacytic gastritis at 10, 30, and 100 mg/kg). Minimal to mild lymphoid depletion in Peyer's patches of males and an analogous decrease in leukocytes expressing the $\alpha 4\beta 7$ integrin in crypt epithelium appeared to be due to the pharmacologic effect of MLN0002 (decreased trafficking of peripheral lymphocytes to the gut). The relation to the treatment is not clear in the absence of a dose response. MLN0002 treated monkeys had increased severity of regeneration of superficial mucosal epithelium in response to lymphoplasmacytic gastritis. Lymphoplasmacytic gastritis (lymphoplasmacytic infiltrates into the lamina propria of the stomach) is a common incidental finding in Cynomolgus monkeys and epithelial regeneration is an expected physiologic response to lymphoplasmacytic gastritis. The incidence of lymphoplasmacytic gastritis and epithelial regeneration was comparable in MLN0002 treated and control monkeys in the 26-week study. However, the severity of the epithelial response to the inflammation was slightly increased in MLN0002 treated monkeys when compared to control monkeys. The toxicological significance of this increase in the regenerative response of the epithelium is not clear. *Balantidium coli* (parasites) were observed in the cecum and colon from both control and vedolizumab treated monkeys. *Balantidium coli* are common commensal intestinal parasite of

macaques and are generally non-pathogenic. The presence of *Balantidium coli* observed in the cecum and colon did not appear to be treatment related due to lack of a dose response, presence of this parasite in control animals and reported background incidences.

A 3-month IV toxicity study was conducted in New Zealand white rabbits at 30 and 100 mg/kg doses. Histopathological changes were seen in the spleen (minimal to mild lymphoid hyperplasia in the periarteriolar lymphoid sheaths and was characterized by increased width of the sheath of lymphocytes surrounding the central arteries of the spleen) and ileum (hyperplasia of submucosal lymphoid nodules) of treated and control animals. However, the incidences and severity were not dose related and these changes were also seen in control animals and the relation to the treatment is uncertain.

In a reproduction study in pregnant New Zealand white rabbits, vedolizumab administered on gestation day 7 at single IV doses up to 100 mg/kg (about 6.5 times the recommended human dose based on body surface area) revealed no evidence of impaired fertility or harm to the fetus. A pre and postnatal development study with vedolizumab in monkeys showed no evidence of any adverse effect on pre and postnatal development at IV doses up to 100 mg/kg (about 6.5 times the recommended human dose based on the body surface area).

Tissue cross-reactivity (TCR) studies were conducted using a panel of monkey and human tissues. Binding was restricted to leukocytes in lymphoid tissues, within the lumens of blood vessels, or as low-grade inflammatory infiltrates in various non-lymphoid tissues (liver, kidney, prostate, thyroid, etc.). Vedolizumab staining pattern observed in Cynomolgus monkey tissues appeared to be similar to that observed in the human tissues and results were stated to be consistent with the pattern of expression of $\alpha 4\beta 7$ integrin. No unanticipated tissue cross-reactivity or off-target staining was observed in TCR studies.

MLN0002 administered via a single SC or IM injection to New Zealand White rabbits did not result in any significant irritation at the tested concentrations (30 and 60 mg/mL). The incidence and severity of the findings were similar between vehicle- and MLN0002-injected sites. The changes observed at the vehicle and MLN0002 injection sites were attributed to the injection site trauma.

Immunotoxicity of natalizumab and vedolizumab were compared head-to-head in Cynomolgus monkeys. Test articles were administered by IV infusion once weekly for three weeks at a dose of 30 mg/kg. Natalizumab caused a significant increase in leukocyte counts (WBCs, lymphocytes, monocytes, eosinophils, and basophils), B-lymphocytes, total T-lymphocytes, T-helper lymphocytes, T cytotoxic lymphocytes, memory T-cytotoxic, and memory T-helper cell populations at Days 8, 15, and 22. There were no vedolizumab-related changes in lymphocyte populations.

The recommended human dose for MLN0002 is 300 mg (5 mg/kg or 185 mg/m²) administered by IV infusion at zero, two and six weeks and every eight weeks thereafter. The NOAEL in monkeys (100 mg/kg) in the 6-month IV toxicity study was

approximately 6.5 times the proposed human dose (185 mg/m²) based on body surface area. The exposure (AUC) at the NOAEL dose of 100 mg/kg in monkeys is approximately 18 times higher than the mean AUC at the recommended human dose of 300 mg. MLN0002 did not cause any adverse effect on embryofetal development in rabbits or on pre- and postnatal development in monkeys. In conclusion, this submission contains adequate nonclinical studies, meets the guidelines and satisfies the criteria for marketing authorization of vedolizumab. From a nonclinical perspective, this application is recommended for approval for its proposed use as indicated in the label.

12 Appendix/Attachments

None

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TAMAL K CHAKRABORTI
11/20/2013

SUSHANTA K CHAKDER
11/20/2013

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125476

**Applicant: Takeda Pharmaceuticals
U.S.A., Inc.**

Stamp Date: 6/20/13

Drug Name: Vedolizumab BLA Type: New BLA

Submit Date: 6/20/13

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	√		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	√		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	√		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	√		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).			N/A
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	√		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	√		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement
010908

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

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	√		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	√		The proposed labeling sections relevant to nonclinical studies may need to be revised during the labeling review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	√		
11	Has the applicant addressed any abuse potential issues in the submission?			N/A
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			N/A

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

If the NDA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. **N/A**

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter. **None**

Tamal K. Chakrabortit, Ph.D. July 16, 2013

 Reviewing Pharmacologist Date

Sushanta K. Chakder, Ph.D. July 16, 2013

 Supervisor Date

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

TAMAL K CHAKRABORTI
07/16/2013

SUSHANTA K CHAKDER
07/16/2013