

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125370

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO *Paul C Brown 3-3-11*

BLA: 125370

Submission date: 6/9/2010

Drug: Benlysta (belimumab)

Sponsor: Human Genome Sciences

Indication: systemic lupus erythematosus

Reviewing Division: Division of Pulmonary, Allergy and Rheumatology Products

Background Comments:

The pharmacology/toxicology reviewer and supervisor in the Division of Pulmonary, Allergy and Rheumatology Products have reviewed the nonclinical information for belimumab and found it adequate to support approval from a pharmacology/toxicology perspective for the indication listed above.

Carcinogenicity:

No carcinogenicity studies were conducted with belimumab. Studies in rodents do not appear feasible due to antigenicity. Mice with a nonfunctional B-cell receptor (BR3/BAFF-R) that results in B-cell deficiency similar to that induced by belimumab have not shown an increased rate of neoplasia. As with any immune modulating drug, the potential for an increased risk of malignancy may exist. Proposed labeling includes a warning about this possibility.

Reproductive and Developmental Toxicity:

Belimumab was not teratogenic in monkeys. Some infant and fetal death was observed in belimumab-treated monkeys although the cause is unknown. The reviewer and supervisor have recommended that belimumab be labeled with pregnancy category C.

Established Pharmacologic Class:

The proposed text phrase for the highlights section of the labeling for Established Pharmacologic Class is " B-lymphocyte stimulator (BLyS)-specific inhibitor". The pharm/tox reviewer and supervisor considered this acceptable based on the pharmacology of the drug and its mechanism of action. This pharmacologic class appears to be supported by the information submitted and reviewed. This is a new FDA Established Pharmacologic Class.

Conclusions:

I concur with the Division pharmacology/toxicology recommendation that this BLA can be approved from a pharm/tox perspective. I discussed draft labeling with the reviewer and supervisor. I agree with the labeling changes suggested in the supervisor's secondary review memo.

INTEROFFICE MEMO

TO: BLA 125370 Original submission
BENLYSTA® (Belimumab)

FROM: Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Pulmonary, Allergy and Rheumatology Products

DATE: February 14, 2010

Human Genome Sciences (HGS) submitted their BLA 125370 on June 9, 2010 for BENLYSTA (belimumab) as a chronic treatment of Systemic Lupus Erythematosus (SLE). The proposed treatment regimen is 10 mg/kg, intravenously at 2-week intervals for the first 3 doses and at 4-week intervals thereafter. Belimumab is a fully human IgG1 monoclonal antibody that binds to soluble human B-lymphocyte stimulator (BLyS, also known as B cell activating factor or BAFF) and inhibits its biological activity. Belimumab does not bind B-cells directly but by binding inhibits survival of B cells and reduces the differentiation of B cells into immunoglobulin-producing plasma cells.

Dr. Mamata De reviewed the nonclinical portions of the BLA. The belimumab application has completed adequate general toxicity and reproductive toxicity studies in cynomolgus monkeys. The sponsor has not evaluated belimumab's carcinogenic potential.

Belimumab was shown to bind to both human and cynomolgus monkey BLyS protein with similar affinity and activity demonstrating that the cynomolgus monkey was an appropriate species in which to characterize its pharmacological and toxicological profile. Belimumab neutralizes BLyS which results in a reduction of B cell numbers. In the repeat dose toxicity study, the drug product reduced the B-cell markers (CD20+ and CD 20+/21+) indicating that it can effectively bind to the target and achieve the desired result of reducing the B-cell population.

Toxicology studies to support the chronic use of belimumab included 4-week (0, 5, 15, and 50 mg/kg/week) and 6-month (0, 5, 15 and 50 mg/kg every two weeks) intravenous (IV) studies in cynomolgus monkeys. In the 4-week study, the target organs of toxicity were the injection site, lymph system, spleen and peripheral blood (B-cell depletion). In the 6-month IV study, the target organs of toxicity were the spleen (lymphoid depletion and hyperplasia), mesenteric lymph node (lymphoid depletion and hyperplasia), GI tract (lymphoid hyperplasia), kidney (regeneration of tubule and glomerular thickening), pancreas (mononuclear infiltration and fibrosis), and thyroid (mononuclear infiltration, follicular degeneration) and peripheral blood (B-cell decreased). Vasculitis was observed in a number of organs including the kidney, sciatic nerve, cervix, and heart with

low incidence in females in the high-dose group (50 mg/kg). Most of these findings were considered as exaggerated pharmacological effect of the drug product with the exception of the observed vasculitis.

The reproductive toxicology program showed that belimumab did not affect male or female reproductive organs or female menstrual cyclicity with treatment up to 6-months. In the embryo-fetal and peri- and post-natal development study in monkeys, monkeys were intravenously dosed with 0, 5 and 150 mg/kg of belimumab. There were 3 (fetal), 8 (6 fetus+ 2 infants), and 4 (3 fetal+ 1 infant) deaths in the 0, 5, and 150 mg/kg dose group, respectively. The total sum of fetal and infant death percentage in the belimumab treated animals was higher than the historical control levels. However, there was no dose response in the belimumab treatment groups. The cause of deaths of the fetuses and the infants was unknown; however, most of the deaths were associated with atelectasis of the lungs. Belimumab was shown to cross the placenta and was excreted in milk. The drug is recommended as Pregnancy Category C for labeling and will capture the reproductive study results.

A review of the nonclinical sections of the label is provided herein. Briefly, the sponsor's proposed Pharmaceutical Class as a "B-lymphocyte stimulator (BLyS)-specific inhibitor" is considered acceptable based on the pharmacology of the drug/mechanism of action. The sponsor's proposed labeling Sections 8.1 (Pregnancy), 8.3 (Nursing Mothers), 10 (Overdosage), 11(Description), 12.1 (Mechanism of Action), and 13.1(Carcinogenesis, Mutagenesis, Impairment of fertility) were reviewed. The recommended changes to the labeling are provided below as additions to the label highlighted in red and deletions as strikethroughs.



(b) (4)



Molly E. Topper 2/14/2011
Molly E. Topper, Ph.D.
Pharmacology/Toxicology Supervisor

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

PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION

Application number: 125,370
Supporting document/s: 0000, 0003
Applicant's letter date: 06-09-2010; 08-26-2010
CDER stamp date: 06-09-2010; 08-27-2010
Product: BENLYSTA® (belimumab)
Indication: (b) (4) adult patients with active, autoantibody positive, systemic lupus erythematosus.
Applicant: Human Genome Sciences (HGS), Rockville, MD
Review Division: Pulmonary, Allergy, and Rheumatology Products
Reviewer: Mamata De, Ph.D.
Supervisor/Team Leader: Molly Topper, Ph.D.
Division Director: Badrul Chowdhury, M.D., Ph.D.
Project Manager: Philantha Bowen

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 125,370 are owned by Human Genome Science INC. or are data for which Human Genome Science INC. has obtained a written right of reference. Any information or data necessary for approval of BLA 125,370 that Human Genome Science INC. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of 125,370.

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1 Executive Summary

1.1 Introduction

This application is a 505 (b)(1) BLA for belimumab (Benlysta). The proposed indication is for the treatment of systemic lupus erythematosus (SLE) in adults. Belimumab is a humanized recombinant, monoclonal antibody that inhibits BLYS, a B cell survival and differentiation factor. The sponsor proposes that inhibition of BLYS leads to a reduction in B-cells, which in turn, reduces the pathology observed in SLE patients. There are no approved drugs in this class. The drug is currently not approved outside of the US.

There are no animal models available in which to test belimumab's efficacy to treat SLE. The pharmacology of belimumab was well characterized using in vitro and in vivo test assays. The pharmacological and toxicological effects of belimumab were assessed in Cynomolgus monkeys. The Cynomolgus monkey was shown to be a relevant species in which to assess belimumab as belimumab binds to the BLYS protein from humans and Cynomolgus monkeys with high affinity and interrupts BLYS-BLYS receptor interaction. Belimumab was examined in a chronic intravenous toxicity study and in an intravenous combined embryofetal development and peri/postnatal development (ePPND) study. The results of these studies are provided herein. Fertility and carcinogenicity studies were not completed by the Applicant in this application. Labeling will capture the ePPND study results and summarize the lack of fertility and carcinogenicity information.

1.2 Brief Discussion of Nonclinical Findings

The specific pharmacologic activity of belimumab is its ability to neutralize BLYS and therefore reduce B cell numbers in vivo. It recognizes soluble BLYS from human and Cynomolgus monkey with nearly identical affinity, with equilibrium dissociation constant (K_D) values in the 250-350 pM range. There is no animal model of lupus; therefore, efficacy of the product could not be tested in an animal model. In the repeat dose toxicity study, the drug product reduces B-cell markers (CD20+ and CD 20+/21+) indicating that it can effectively bind to the target and achieve the desired result of reducing the B-cell population.

Toxicology studies were conducted in Cynomolgus monkeys (a proven relevant species) to support the chronic use of the product. The sponsor conducted a 4-week and a 6-month repeat dose IV toxicity study to complete their general toxicology program. In the 4-week IV study, monkeys were dosed with 0, 5, 15 and 50 mg/kg of belimumab IV bi-weekly. The main target organs of toxicity included the lymph, spleen and the gut associated lymphoid tissue (GALT). Changes in these tissues/systems were due to exaggerated pharmacology. No NOAEL was identified in this study. However, a LOAEL of 5 mg/kg was determined which has an associated AUC_{0-24} of 2868 mcg.h/mL.

In the chronic 6-month toxicity study, monkeys were intravenously dosed with 0, 5, 15, and 50 mg/kg bi-weekly. An interim evaluation was completed at 3-months and then at 6-months and finally at 8-months post-dose. The major histopathological observation

from the 3- and 6-month toxicity study consisted of lymphoid depletion from spleen, mesenteric, and mandibular lymph nodes and extramedullary hematopoiesis. The histology findings at recovery showed extramedullary hematopoiesis, hyperplasia of the mesenteric lymph nodes, and splenic lymphoid hyperplasia at Week 60 (recovery). Additional target organs of toxicity included the heart, thyroid, kidney, and lung. Ovarian hyperplasia was observed histopathologically. Menstrual cyclicity and hormone analysis were not assessed in this study. Due to males being sexually immature, the male reproductive assessment was considered inadequate in this study. Based on these findings a NOAEL of 15 mg/kg was identified.

The Applicant completed an intravenous combined embryo-fetal and peri- and post-natal development study in monkeys. Monkeys were dosed bi-weekly with 0, 5, 150 mg/kg from gestation days 20 to 150. There were 3 fetal, 8 (6 fetus+ 2 infants), and 4 (3 fetal+ 1 infant) deaths in the 0, 5, and 150 mg/kg dose group respectively. The percent of fetal losses were 14, 20%, respectively, from the control and test article treated animals (historical control for fetal death is 17.6%). The percent of infant deaths was 0 and 7% in control and test article treated animals, respectively (historical control for infant death is 10-12%). The total sum of fetal and infant death percentage in the belimumab treated animals was higher than the historical control levels. However, there was no dose response in the belimumab treatment groups and the individual treatment groups fetal deaths were 24% and 15% for the 5 and 150 mg/kg groups, respectively. Similarly, the individual treatment group infant deaths were 8% and 5% for the 5 and 150 mg/kg groups, respectively. The cause of deaths of the fetuses and the infants was unknown; however, most of the deaths were associated with atelectasis of the lungs. Belimumab was shown to cross the placenta and was excreted in milk. The drug is recommended as Pregnancy Category C for labeling and will capture the reproductive study results.

Carcinogenicity assessment was not conducted for belimumab due to high anti-drug-antibody formation and death in mice with the repeated administration of the test article. The A/WySNJ mouse which has a non-functional BR3/BAFF-R (BLyS) lacks neoplasia finding. No neoplasia was observed in the repeat dose toxicity study in the Cynomolgus monkeys administered with belimumab for 6-months, (2x/week) and a post dosing period of 8-month. Based on all these findings, the reviewer believes that the rodent carcinogenicity bioassays are not doable and the lack of carcinogenicity data should be addressed in the label.

1.3 Recommendations

1.3.1 Approvability

The Applicant has adequately characterized the pharmacology and toxicology of belimumab in their nonclinical program. From the nonclinical perspective, belimumab is recommended for approval with the appropriate changes to the nonclinical sections of the label.

1.3.2 Additional Non Clinical Recommendations

As the potential effects of belimumab were inadequately assessed for male and female fertility, labeling should capture the lack of evaluation and potential effects on fertility should be monitored clinically.

1.3.3 Labeling

The following nonclinical sections (8.1, 8.2, 10, 12.1, 13.1, and 13.2) were reviewed for format and content. Based on the nonclinical data submitted and reviewed in this BLA, changes are recommended for Sections 8.1, 12.1, and 13.1. Addition of Section 13.2 Animal Pharmacology and/or Toxicology was included to provide the overall study results of the enhanced peri/post-natal toxicity study. This section of the label should be added to the Label's Table of Contents.

The sponsor's submitted label is considered acceptable in terms of complying to the SPL and PLR labeling.

The following recommended changes to the label are shown below. The deletions to the sponsor's proposed labeling are represented by ~~strike-through~~ and the additions are highlighted in red.

(b) (4)



2 Drug Information

2.1 Drug

CAS Registry Number: 356547-88-1

Generic Name: Belimumab

Code Name: HGS 1006

Other Name: Lymphostat B (LSB), monoclonal anti BLyS, (b) (4)

Chemical Name: Immunoglobulin G₁, anti-(human cytokine BAFF) (human monoclonal LymphoStat-B heavy chain) disulfide with human monoclonal LymphoStat-B light-chain, dimer

Molecular Formula/Molecular Weight: C₆₃₅₈H₉₉₀₄N₁₇₂₈₀O₂₀₁₀S₄₄/147.0 kDa

Biochemical Description: Monoclonal antibody

Structure or Biochemical Description:

(b) (4)

Pharmacologic Class: Biologic; Human monoclonal antibody against BLYS (B lymphocyte stimulator)

2.2 Relevant INDs, NDAs, BLAs and DMFs

BB-INDs (b) (4) (b) (4), 9970

2.3 Drug Formulation

The belimumab drug product (DP) will be provided as a lyophilized powder for intravenous infusion in sterile single-use vials. Belimumab is filled and lyophilized in Type I glass vials, sealed with a latex-free, (b) (4) rubber stopper and a flip-off aluminum seal, and stored at 2-8°C protected from light. The belimumab DP will be available in two forms (to accommodate dosing based on patient weight): 120 mg in a 5 mL vial and 400 mg in a 20 mL vial. The DP is 80 mg/mL belimumab in a formulation containing citrate buffer, sucrose and polysorbate 80 at a pH of 6.5. The following is the composition of the belimumab drug product.

Table 1 Qualitative Composition of Belimumab Clinical Formulation

Ingredient	Amount Per Vial ¹		Function	Quality Standard
	400 mg/vial (5 mL Deliverable) Configuration	120 mg/vial (1.5 mL Deliverable) Configuration		
Belimumab	400 mg	120 mg	API	HGS Specification
Citric acid (b) (4)		(b) (4)		Multicompendial ²
Sodium citrate (b) (4)		Multicompendial ²		
Sucrose		Multicompendial ³		
Polysorbate 80		Multicompendial ³		

¹ Amount listed is deliverable amount.
² According to supplier's definition, multicompendial grade includes full compendial testing as appropriate to USP or NF, EP, BP, and JP.
³ According to supplier's definition, multicompendial grade includes full compendial testing as appropriate to USP/NF, EP, and JP.

2.4 Comments on Novel Excipients

The excipients in the belimumab DP include a (b) (4). The excipients were selected (b) (4). Sodium citrate (b) (4) at a concentration of (b) (4) and pH 6.5 (0.16 mg/mL citric acid (b) (4)) was selected to (b) (4) Sucrose, at a concentration of 80 mg/mL, (b) (4) The concentrations of sucrose also (b) (4) Polysorbate 80 was added (b) (4).

The reviewer verified that all of these excipients are qualified qualitatively and quantitatively.

Table 2 Excipients of Belimumab

Excipients	Amount	Function
Citric acid (b) (4)	(b) (4)	(b) (4)
Sodium citrate (b) (4)		
Sucrose		
Polysorbate 80		
Sterile WFI		

¹ 120 mg/vial configuration.
² 400 mg/vial configuration.

2.5 Comments on Impurities/Degradants of Concern

Belimumab drug product (DP) is manufactured by (b) (4). The toxicity studies conducted to characterize

impurities present in the DP focused on degradation products. Belimumab's degradants were assayed under different stress conditions such as storage at high temperatures, light, temperature cycling, elevated residual moisture content, and shipping-related mechanical stress. Belimumab's bioactivity was stable under these conditions. The acceptance criterion for its potency is established by its binding activity (75-133%). The purity of the commercial grade protein is >97.5%. The remaining 2.5% is aggregate.

2.6 Proposed Clinical Population and Dosing Regimen

Belimumab is BLYS specific inhibitor indicated for (b) (4) adult patients with active, autoantibody-positive, systemic lupus erythematosus who are receiving standard therapy. The Applicant recommended a dosage regimen of 10 mg/kg intravenously at 2-week intervals for first 3 doses and at 4-week intervals thereafter.

2.7 Regulatory Background

The original IND was submitted in CBER, 2003. In June 2006, the IND was transferred to CDER/Division of Anesthesia, Analgesia and Rheumatology. Finally, in March 2010, the IND and subsequent BLA were transferred to DPARP.

The following table summarizes the regulatory history and agreements between the Applicant and FDA for the nonclinical requirements.

Table 3 Summary of Regulatory Correspondence

Date of Meeting	Type of Correspondence
26 March 2003	Teleconference Minutes (end of Phase 1) <ul style="list-style-type: none"> • Agreed the nonclinical toxicology studies that had been completed at the time supported initiation of Phase 3 studies and these studies, in addition to a reproductive toxicology study (that had acceptable results), would support BLA filing. • Agreed necessary safety margins for reproductive toxicology study as well as the need to assess belimumab levels in maternal milk. • Agreed further evaluation of fertility in nonclinical studies would not be necessary if results of reproductive toxicology studies did not suggest a defect in this function. • Confirmed formulation changes made after the conduct of the reproductive toxicology study would not compromise the utility of the reproductive toxicology study as long as exposure to active agent is comparable and new excipients are not associated with an inherent toxicity concern. (FDA)
03 June, 2006	Agreed nonclinical genotoxicity and carcinogenicity studies were not needed; Other CHMP comments on nonclinical program addressed in Follow-Up Scientific Advice (30 May 2008, below). (European Agency) Suggested establishing a correlation between the potency estimation by binding of the antibody to BLYS and the biological activity based on the attribute of the product which is linked to the relevant biological properties, and to establish antibody-BLYS binding studies using Plasmon surface resonance (European Agency)
30 May, 2008	Agreed use of cynomolgus monkeys as single species for toxicological

Date of Meeting	Type of Correspondence
	<p>evaluation was justified and that safety pharmacology assessment in toxicology studies appeared adequate.</p> <p>Agreed that the reproductive toxicology study performed appears was well-designed and sufficiently informative to support MAA filing.</p> <p>Pregnancy language in SPC will depend on risk:benefit assessment of the data in the SLE disease population.</p> <p>Immuntotoxicological assessment of belimumab for MAA considered sufficient. Requested data demonstrating the binding profile of belimumab to cynomolgus monkey membrane and soluble isoforms.</p> <p>Agreed further nonclinical drug interaction studies not necessary. (European Agency)</p>
02 April, 2010	<p>Agreed that the nonclinical and clinical results appear sufficient to support the filing of a BLA for belimumab treatment of patients with systemic lupus erythematosus provided following statements from pre BLA Meeting are addressed appropriately.</p> <ol style="list-style-type: none"> 1. The potential for belimumab to alter male and female fertility parameters was not evaluated as per ICH S6 (RI) draft dated December 2009. Therefore, provide clear and adequate justification for not conducting male and female fertility studies with your product. Provide all of the existing data and published literature regarding these endpoints (this information was also requested as pre BLA meeting dated March 08, 2010). 2. The carcinogenic potential for belimumab has not been evaluated as per ICH S6 (RI) draft dated December 2009. Provide a clear rationale on how you intend to address the carcinogenicity section of your product labeling (this information was also requested at pre BLA meeting dated March 08, 2010). 3. The embryo-fetal development/postnatal development study does not appear to have functional characterization of the impact of belimumab on the developing immune system. Exposure of belimumab during development can alter the offspring's immune system. Provide information with regards to what is known and expected and how you intend to address this concern in the product labeling (this information was also requested at pre BLA meeting dated March 08, 2010). <p>The Applicant addressed all of the above concerns raised by the agency appropriately in their submission dated August 27, 2011.</p>

3 Studies Submitted

3.1 Studies Reviewed

Table 4 Studies Reviewed

STUDY NUMBER	STUDY TITLE
PHARMACOLOGY	
1	HG19300.CVID.0.019 Effect of Recombinant Human BLyS on Human

	STUDY NUMBER	STUDY TITLE
		Tonsillar B Cell Proliferation
2	HG19300.CVID.0.020	Effects of BLyS on Proliferation of Cynomolgus Splenocytes
3	HG19300.CVID.0.021	Effects of BlyS on Proliferation of Murine Splenocytes
4	HG19300.CVID.0.022	Effects of BLyS on <i>In Vitro</i> Secretion of Ig by Human Tonsillar B cells
5	HG19300.CVID.0.023	Analysis of BLyS binding to human PBMC Using Flow Cytometry
6	HG19300.CVID.0.026	Expression of BLyS and BLyS-R on Hematopoietic and Nonhematopoietic Cell Lines
7	HG19300.CVLD.0.028	Analysis of BLyS Receptor Expression on Normal Mouse Splenocytes Determined by Flow Cytometry.
8	HG19300.CVLD.0.029	Analysis of Human BLyS Binding to Cynomolgus Monkey PBMC Using Flow Cytometry
9	HG19300.CVLD.0.030	rhuBLyS Binding to Mouse Peripheral Blood Mononuclear Cells
10	HG19300.CVID.0.039	Northern Analysis of BLyS mRNA Expression in Human Tissues and Cells
11	HG19399.SLE.0.001	Generation of Monoclonal Antibodies Specific for B Lymphocyte Stimulator (BLyS)
12	HG19399.SLE.0.002	A01, a BLyS Antagonist, Binding ELISA
13	HG19399.SLE.0.003	Equilibrium Binding Determination of A01, a BLyS Antagonist
14	HG19399.SLE.0.004	A01 Bioassay Development
15	HG19399.SLE.0.006	Neutralization of the Effects of Subcutaneously Injected Recombinant Human B-Lymphocyte Stimulator (rhuBLyS) by Intravenous Administration of A01 in BALB/c Mice
16	HG19399.SLE.0.011	A01 Specificity for Soluble BLyS
17	HG19399.SLE.0.019	LymphoStat-B Inhibits <i>In Vitro</i> B Lymphocyte Proliferation Induced by Human and Cynomolgus Monkey BLyS
18	HG19399.SLE.0.026	The Binding of Flag Tagged BLyS on LymphoStat-B: (b) (4) Binding Kinetic Analysis
19	HG19399.SLE. 0.048	Binding of Human and Murine BLyS to Belimumab and to Murine BAFF Receptor: (b) (4) Binding Kinetic Analysis
20	HG19399.SLE.0.049	<i>In Vivo</i> Activity of Belimumab on Murine B Cells
21	HG19399.SLE.0.051	Binding Affinities of Human and Cynomolgus Monkey BLyS for Belimumab and BLyS Receptors and Proliferation Activity Induced by Human and Cynomolgus Monkey BLyS
22	HG19399.SLE.0.053	BLyS Receptor Profile on Human B Cells and Inhibition of BLyS Induced Proliferation by Belimumab
23	(b) (4) study #00-116	Cross Reactivity of anti-BLyS antibody with human and

STUDY NUMBER		STUDY TITLE
		Cynomolgus monkey Tissue Ex Vivo,
PHARMACOKINETICS		
24	HG19399.SLE. 0.008, HG10399-T03	A Single Dose Pharmacokinetic Study of BLYS Antagonist Administered by Intravenous Injection to Cynomolgus Monkeys
25	HG19399.SLE.0.014	Immunogenicity of A01, a BLYS antagonist, in cynomolgus monkeys following a single intravenous dose
26	HG19399.SLE.0.024	Qualification of the ELISA for Quantitating LymphoStat-B Monoclonal Antibody in Cynomolgus Serum (SOP 01ILS-PH-06-2199)
27	HG19399.SLE.0.029	Pharmacokinetics of LymphoStat-B. in Female Cynomolgus Monkeys Following a Single 150 mg/kg Intravenous Dose
28	HG19399.SLE.0.036	Comparability Assessment of 2 LymphoStat-B Formulations Following A Single Intravenous Administration to Monkeys
29	HG19399.SLE.0.038	Pharmacokinetics of LymphoStat-B in BALB/c Mice Following a Single Intravenous or Subcutaneous Administration
30	HG19399.SLE.0.028	Pharmacokinetics of LymphoStat-B. in Cynomolgus Monkeys Following a Single Intravenous and Subcutaneous Administration
31	HG19399.SLE.0.039	Pharmacokinetics of LymphoStat-B. in Cynomolgus Monkeys Following a Single Intravenous or Subcutaneous Administration of Liquid Formulation
32	HG19399.SLE.0.030	Qualification of the LymphoStat B Neutralization Assay in Cynomolgus Monkey Serum (SOP 01TRF-PH-06-2211);
33	HG19399.SLE.0.032	Qualification of a LymphoStat-B (LSB) Immunogenicity Assay (SOP 01ELM-PH-06-2269) as a Means of Detecting anti-LSB Antibodies in Cynomolgus Macaque Sera
34	HG19399.SLE.0.054	Prediction of Serum Belimumab Exposure in a 6-Month Toxicity Study of Belimumab Administered Bi-Weekly by Intravenous Injection to Cynomolgus Monkeys
LOCAL TOXICITY		
35	6962-157	Subcutaneous Local Tolerance Study with LymphoStat-B in Cynomolgus Monkeys
36	HGS19399.SLE.0.037	Serum Exposure Of LymphoStat B in (b) (4) Study no 6962-157
GENERAL TOXICITY		
37	HG10399-T04	A 4-Week Repeat Dose Toxicity Study of BLYS

STUDY NUMBER		STUDY TITLE
		Antagonist Administered by Intravenous Injection to Cynomolgus Monkeys, with a 4-Week Recovery Period
38	HG19300-T05	A 6-Month Toxicity Study of LymphoStat-B™ Administered Bi-Weekly by Intravenous Injection to Cynomolgus Monkeys, with an 8-Month Recovery Period
39	6962-161	22-Week Subcutaneous Injection Immunogenicity and Toxicokinetic Study with LymphoStat-B in Cynomolgus Monkeys
REPRODUCTIVE TOXICITY		
40	1736-95	A Dose-Tolerance Study of Lymphostat-B™ Administered by Intravenous Injection to Non-Pregnant Female Cynomolgus Monkeys Study number
41	1721-95	Maternal, Fetal and Neonatal Toxicity Study of LymphoStat-B™ Administered Bi-Weekly by Intravenous (Bolus) Injection to Pregnant Cynomolgus Monkeys, Including a One Year Postnatal Evaluation

3.2 Studies Not Reviewed

All of the nonclinical studies submitted with the original BLA submission have been reviewed.

3.3 Previous Reviews Referenced

There were no previous reviews for belimumab.

4 Pharmacology

Belimumab is a recombinant, fully humanized, monoclonal antibody, intended to inhibit BLYS, a B cell survival and differentiation factor. The Applicant submitted a series of In Vitro and In Vivo studies to characterize belimumab's pharmacological activity. The first studies characterized the BLYS protein expression patterns and binding abilities. Development of the biologic BLYS antagonist is summarized. Following its development and the drug candidate selection, studies (reviewed below) were conducted that showed that rantiBLYS antibody binds to BLYS protein from human and Cynomolgus monkey with high affinity and interrupts BLYS-BLYS receptor interaction. Belimumab was shown to be selective for BLYS protein with no other receptor binding observed under the conditions tested.

The belimumab target, BLYS protein, is derived from the BLYS gene that encodes for the generation (b) (4) BLYS is a membrane bound protein expressed on cells of myeloid origin including monocytes, T-cells, dendritic cells, granulocytes, and activated neutrophils. Cleavage of the membrane bound BLYS

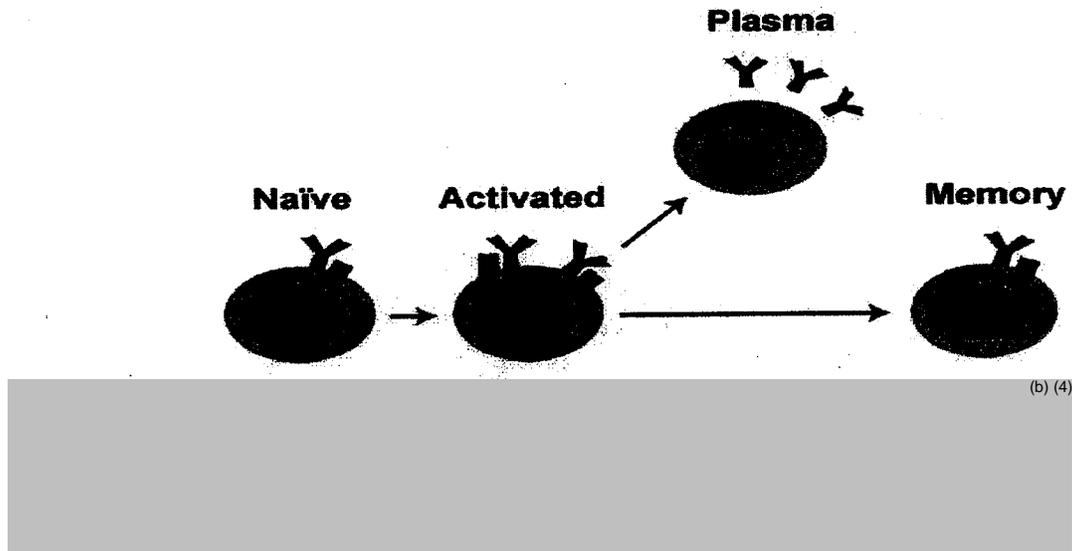
results in a soluble

(b) (4)

(b) (4)

(b) (4)

BLYS receptor expression of each of the three BLYS receptors, BR3, TACI and BCMA on naïve (IgD+, CD27-), activated (IgD+, CD27-), plasma (IgD-, CD38+) and memory (IgD-, CD27-) B cells through development was examined. Naïve B cells were shown to express BR3 at high levels, but not BCMA or TACI. Memory B cells express BCMA and TACI predominantly. However, naïve B cells also expressed BR3 and memory cells deemed to have different subsets for BR3 expression. Plasma B cells express BCMA and TACI, but usually do not express BR3. Murine antiBLYS antibody 10F6 raised in hamster did block primary B-cell function but not the B memory cell (Scholz et al 2008). The effects of belimumab on monkey and human memory cells have not been examined.

Figure 2 BLyS Receptor Expression during B Cell Development

An alignment of the amino acid sequence of soluble BLyS from human, Cynomolgus monkey, rat, and mouse showed that human soluble BLyS is 98% identical to Cynomolgus monkey BLyS and the region of BLyS involved in receptor binding is 100% conserved. In contrast, human and mouse or rat BLyS share 84% identity, and some of the differences in amino acid residues fall within the receptor binding region. Although the epitope/s for belimumab is not empirically known, the binding characteristics of belimumab, and the capability of belimumab to disrupt BLyS-BLyS receptor interaction indicate that belimumab binds to a conformational epitope at or near the receptor. The alignment of the 3 amino acid difference between human and Cynomolgus monkey is expected to minimally interfere with belimumab binding as they reside at the N terminus of BLyS in a region distinct from the receptor binding region of BLyS.

Figure 3 Sequences of BLyS from Human, Cynomolgus Monkey, Rat, and Mouse

Belimumab has nearly the same affinity for human and Cynomolgus monkey BLyS (approximately 270 pM), indicating that the binding epitope of belimumab may be conserved between these two species and that belimumab binds both human and Cynomolgus monkey soluble BLyS. There are, however, several differences in the amino acid sequence of mouse BLyS compared with human and monkey BLyS which may impact the way belimumab recognizes mouse BLyS (lower affinity for mouse BLyS). Belimumab binds with higher affinity to human and monkey BLyS and with a lesser affinity to mice BLyS. The Applicant showed that human and Cynomolgus monkey BLyS bind to human BR-3, TACI and BCMA with very similar kinetics.

In addition to binding activity, the applicant also demonstrated that belimumab was active on both human and monkey receptors using in vitro assays where belimumab inhibited BLyS induced proliferation of human PBMC and human and monkey BLyS induced proliferation of murine splenocytes. These data supported the selection of the Cynomolgus monkey as a relevant species in which to evaluate belimumab's potential toxicity.

The following section summarizes the In Vitro and In Vivo pharmacology studies submitted to the BLA.

4.1 Primary Pharmacology

Key Study Findings from the pharmacology studies submitted by the Applicant:

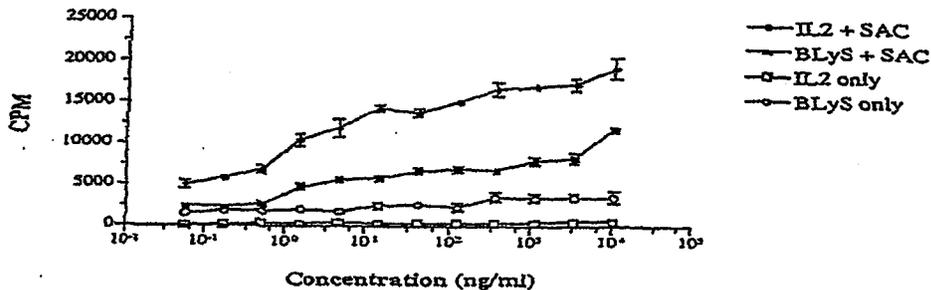
- In vitro primary pharmacodynamic:
 - Belimumab binds to human and Cynomolgus monkey BLyS with nearly identical affinity 274 and 264 pM, respectively. Belimumab's affinity for binding to murine BLyS is approximately 10-fold lower (9.98 nM).
 - BLyS mRNA expression was found to be in tissues containing cells from the myelogenic origin only.
 - Belimumab does not recognize the membrane bound BLyS isoform in a subpopulation of peripheral blood mononuclear cells (PBMCs) and myeloid K-562 cells.
 - Belimumab inhibits binding of BLyS to its receptors BR3, TACI, and BCMA with IC₅₀s of 69, 53, and 97 nM, respectively
 - Belimumab inhibits BLyS induced proliferation of human PBMC (IC₅₀ 8.9 ng/mL), human and monkey BLyS induced proliferation of murine splenocytes with EC₅₀=2 ng/mL chemiluminiscence assay and IC₅₀ =0.06 nM in a ³H-thymidine incorporation assay.

In vivo pharmacodynamic studies in mice showed that belimumab inhibits BLyS induced proliferation of splenocytes, binds specifically to B-cells, and is immunogenic to mice.

Study Title: Effect of Recombinant Human BLyS on Human Tonsillar B cell Proliferation; Study Number: HG19300.CVID.0.019

In order to assess the proliferative effects of BLyS, the human tonsillar B cells was isolated and cultured with BLyS in the presence of *Staphylococcus aureus* (SAC, formalin fixed) as the priming agent. Interleukin 2 (IL-2) plus SAC were used as a positive control. The cells were incubated for 72 hours (37°C, 5% CO₂) and 0.5 microCi/well of ³H-thymidine were added for an additional 20-24 hours. ³H-thymidine was used to measure cell proliferation.

Figure 4 Effect of BLyS on Proliferation of Human Tonsillar B Cells



BLyS induced a concentration-dependent proliferation of tonsillar B cells similar to that of recombinant IL-2 in presence of a co stimulant, staph bacteria. The negative controls (medium, IL-2 only, and BLyS only) induced suboptimal levels of proliferation.

Study Title: Effects of BLyS on Proliferation of Cynomolgus Splenocytes; Study Number: HG19300.CVID.0.020

To assess the effects of BLyS on the primate splenocytes, isolated spleen cells were incubated for 72 hrs (37°C, 5% CO₂) in the presence SAC (formalin fixed- the priming agent) and the purified recombinant human BLyS. Tritiated thymidine incorporation was used as a measure of the cell proliferation. The cell culture media, IL-2, BLyS, and SAC were used as negative controls and IL-2 plus SAC was used as a positive control. The binding of BLyS with the different subpopulations of immune cells from the isolated splenocytes were also examined by flowcytometry (FACS).

Figure 5 Effect of BLyS on Proliferation of Cynomolgus Splenocytes

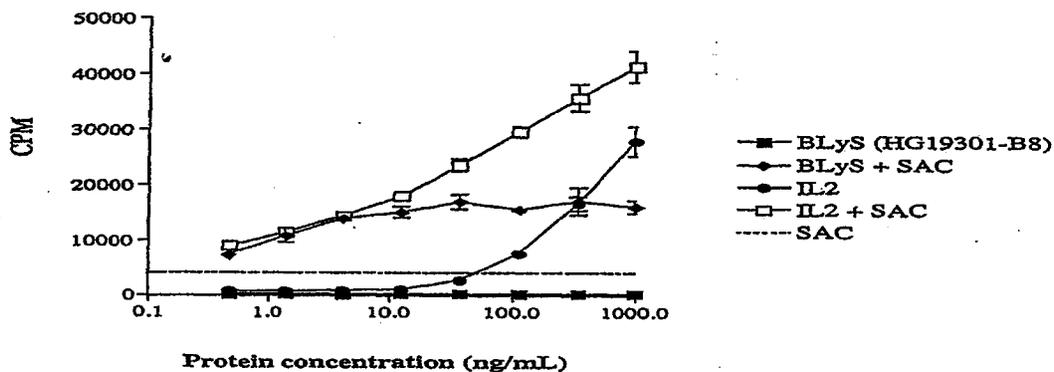
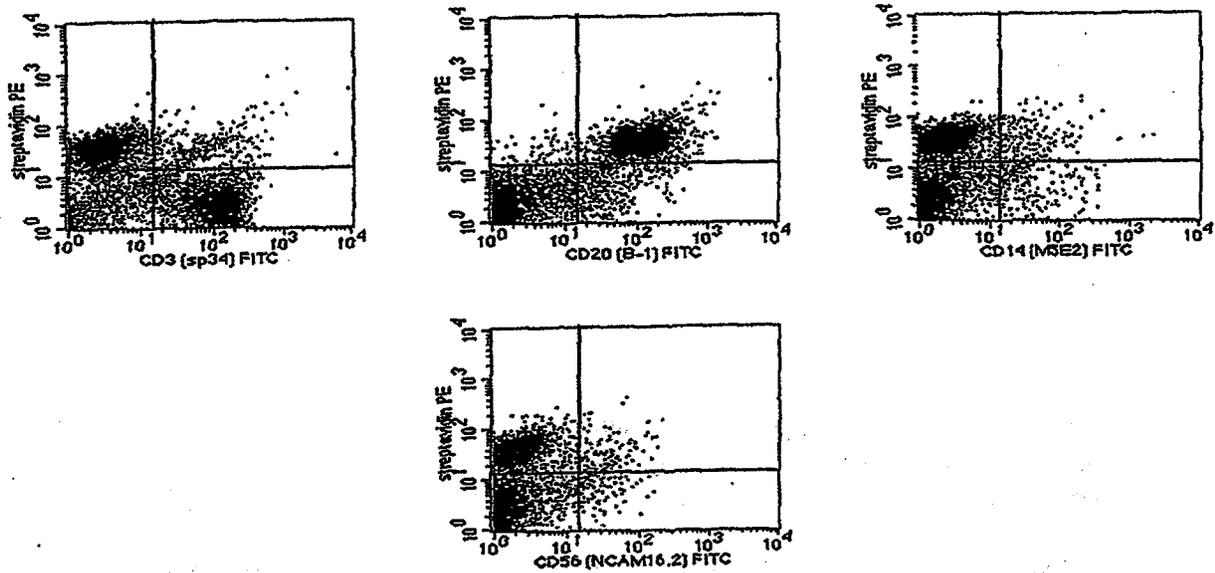


Figure 6 BLyS Expression on Splenocytes from Cynomolgus Monkey

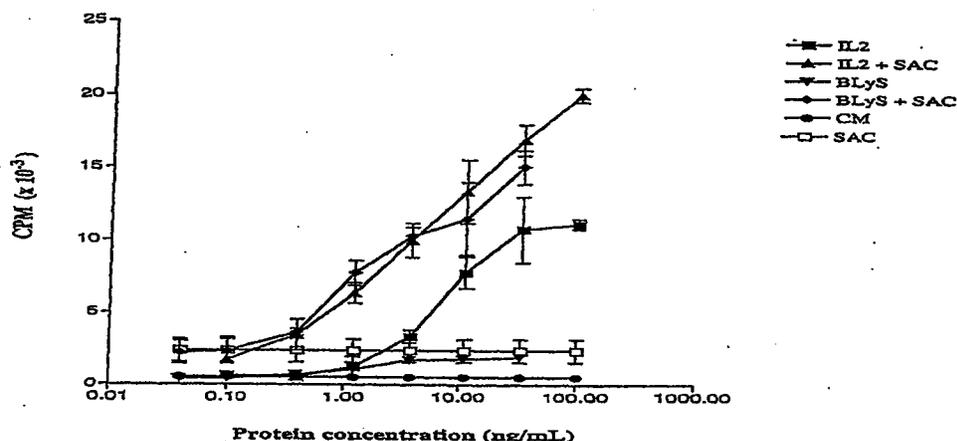
Human recombinant BLyS induced a concentration-dependent proliferation of splenocytes isolated from Cynomolgus monkeys. These findings are similar to the findings from the human tonsillar cells (HG19300.CVLD.0.019). The magnitude of this proliferation was only half of the proliferative responses caused by IL-2 plus SAC under the same experimental condition. Similar to the human tonsillar cell, BLyS was effective only when the co stimulant was present. Also, BLyS binds only with CD20⁺ splenocytes (B cell) and does not bind with CD14⁺ (monocytes), CD3⁺ (undifferentiated T cells), or CD56⁺ (NK cells) indicating its specificity for B cells.

Study Title: Effects of BlyS on Proliferation of Murine Splenocytes; Study Number: HG19300.CVID.0.021

To assess the effect of BLyS on murine splenocytes, cells were isolated from the inbred BALB/c mice and incubated for 72 hrs (37°C, 5% CO₂) with SAC (formalin fixed priming agent) and the purified recombinant human BLyS. Tritiated thymidine incorporation was used as a measure of cell proliferation. The cell culture media, IL-2, BLyS, and SAC were used as negative controls and IL2+SAC was used as a positive control.

Under this experimental condition, human BLyS induced a concentration-dependent proliferation of splenocytes isolated from BALB/c mice. The magnitude of this proliferation was similar to the proliferative response caused by IL-2 under the same experimental condition. Similar to the human tonsillar cell, and the splenocytes from the Cynomolgus monkeys, the BLyS was effective only when the co stimulant was present.

Figure 7 Effect of BLyS on Proliferation of Murine Splenocytes



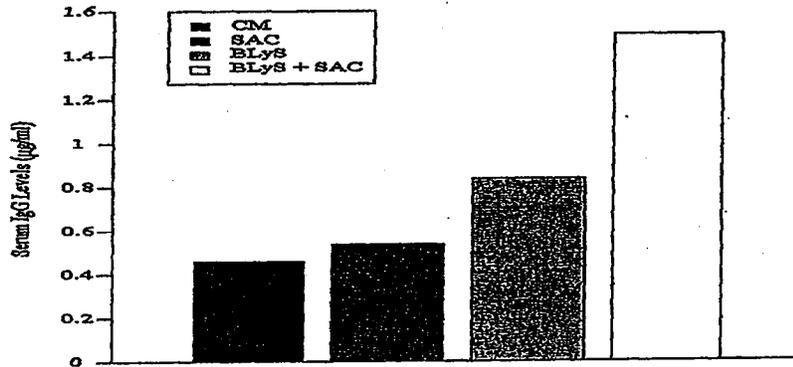
Study Title: Effects of BLyS on In Vitro Secretion of Ig by Human Tonsillar B cells;
Study Number: HG19300.CVID, 0.022

To assess the effect of BLyS on the consequences of B-cell proliferation, human tonsillar cells were isolated and incubated for 7 days (37°C, 5% CO₂) with SAC (formalin fixed priming agent) and the purified recombinant human BLyS. The effect of BLyS on immunoglobulin (IgG, IgA, and IgM) secretion was assessed by solid phase ELISA using affinity purified goat anti-human Ig capture antisera. The cell culture media and SAC were used as controls.

Table 5 Effect of BLyS on Ig Secretion (mcg/mL) from Human Tonsillar B cells

<u>Treatment</u>	<u>IgA</u>	<u>IgG</u>	<u>IgM</u>
CM	0.052	0.461	0.184
SAC	0.111	0.538	0.297
BLyS	0.205	0.836	0.319
BLyS + SAC	0.238	1.492	0.787

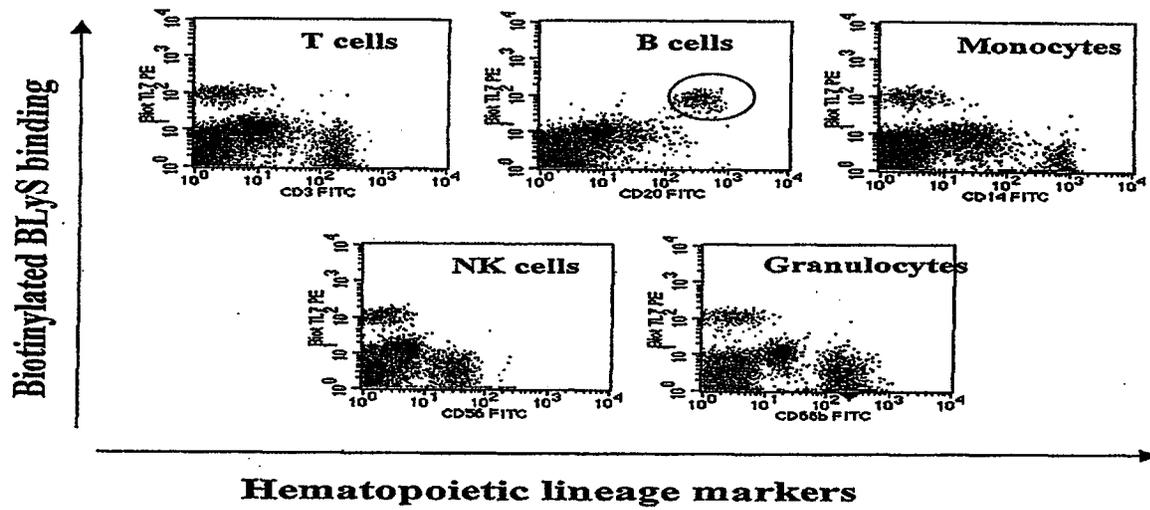
Figure 8 Effect of BLyS on Human Serum IgA Levels



Human tonsillar cells treated with BLyS together with SAC induced IgA, IgG, and IgM secretion. All of these Ig secretions were dose related. These data show that BLyS binding can effectively induce immunoglobulin secretions of the B cell proliferations.

Study Title: Analysis of BLyS Binding to Human PBMC Using Flow Cytometry;
Study Number: HG19300.CVID.0.023

For understanding the binding profile of BLyS to human peripheral blood cells (PBMC), cells were isolated from whole blood. The PBMCs were then stained with FITC-labeled lineage-specific markers (CD3, C020, CD14, CD56, and CD66b) and biotinylated BLyS. The ability of the BLyS to bind to the different subsets of PBMCs was assessed by flow cytometry.

Figure 9 BLYS Expression on Human PBMCs

BLYS binds specifically to the CD20⁺ B cells and not to T cells or cells from monocytic lineage within the PBMC.

Study Title: Expression of BLYS and BLYS-R on hematopoietic and non-hematopoietic cell lines; Study Number: HG19300.CVID.0.026

To identify the cell type that expresses BLYS or cells that are responsive to BLYS, a variety of different tumor cell lines were obtained from the American Tissue Culture Company (ATCC) and expanded *in vitro*. The cell suspensions were stained with anti-BLYS monoclonal antibody for the BLYS expression and BLYS-biotin for the BLYS receptor expression. These cells were subjected to the immunocytochemical analyses. Isotype matched antibodies were used as controls.

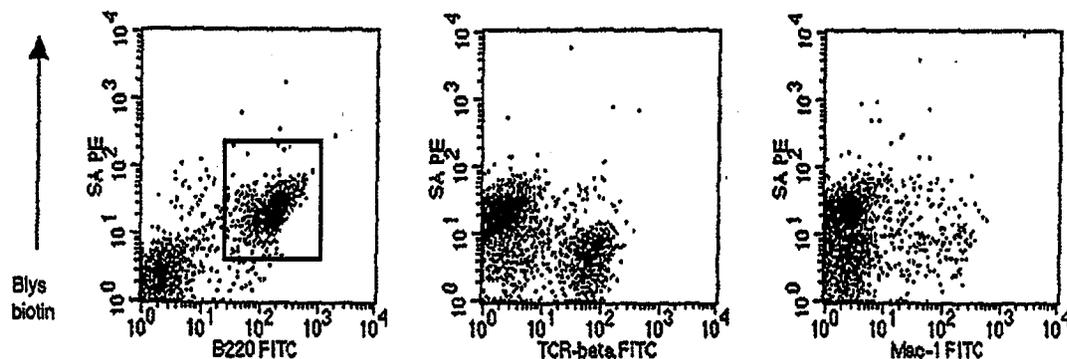
Table 6 Summary of BLYS and BLYS Receptor Expression on Cell Lines

Cell Line	Cell type	Data source	BLYS Expression	BLYS-R Expression
HL-60	AML/monocytic	Pages 4, 7	+	-
Jurkat	Acute T leukemia	Page 4	-	nd
DAUDI	Burkitt's lymphoma/B	Page 5	-	-
K562	CML	Page 5, 7	+	-
NAMALWA	Burkitt's lymphoma/B	Page 6	-	+
Raji	Burkitt's lymphoma/B	Page 6	-	+
KG1	AML	Page 8	-	-
THP 1	AML/monocytic	Pages 8, 10, 15, 19, 24	+	-
REH	ALL/non-B, non-T	Page 9	-	+
HCT 116	Colorectal carcinoma	Pages 10, 11, 12, 17, 19, 24, 26	-	-
MDA231	Mammary carcinoma	Pages 11, 12, 13, 14, 25	-	-
HT29	Colorectal adenocarcinoma	Pages 12, 13, 15, 25	-	-
SU86.86	Pancreatic carcinoma	Pages 14, 26, 28	-	-
SK-NEP-1	Wilm's tumor (kidney)	Pages 14, 25, 27	-	+
WI DR	Colorectal adenocarcinoma	Pages 14, 21, 23	-	-
SCaBER	Bladder carcinoma	Pages 15, 20, 23	-	-
MIA PaCa	Pancreatic carcinoma	Page 15	-	-
CaCo-2	Colorectal adnocarcinoma	Page s 16, 24, 26	-	-
Colo 201	Colorectal adenocarcinoma	Pages 16, 20, 22	-	-
Caki-1	Clear cell kidney carcinoma	Pages 19, 21	-	-
A498	Kidney carcinoma	Pages 16, 19	-	-
Caki-2	Clear cell kidney carcinoma	Pages 19, 22	-	-
HS766T	Pancreatic carcinoma	Pages 17, 20, 22	-	-
J82	Bladder carcinima	Pages 20, 22	-	-
HT-1197	Bladder carcinoma	Pages 24, 27	-	-
IM9	Multiple myeloma	Pages 12, 18, 21, 26	-	+
TF1	Erythroleukemia	Page 18	-	-

BLYS expression was restricted to the cell lines derived from the myeloid lineage such as K562, HL-60, and THP-1. BLYS- receptor expression was observed on B-lineage tumors (RAJI, RBH, IM9, and NAMALWA), but not on DAUDI cells (which is also a B-cell lymphoma derived cell line). BLYS receptor was not found on any of the various carcinomas. Interestingly, low levels of BLYS-R were detected on a Wilm's tumor (SK-NEP-1) derived from kidney. Under these experiment conditions BLYS or BLYS receptor expression was found mainly from the cells of monocyte-myeloid origin.

Study Title: Analysis of BLYS Receptor Expression on Normal Mouse Splenocytes Determined by Flow Cytometry; Study Number: HG19300.CVLD.0.028

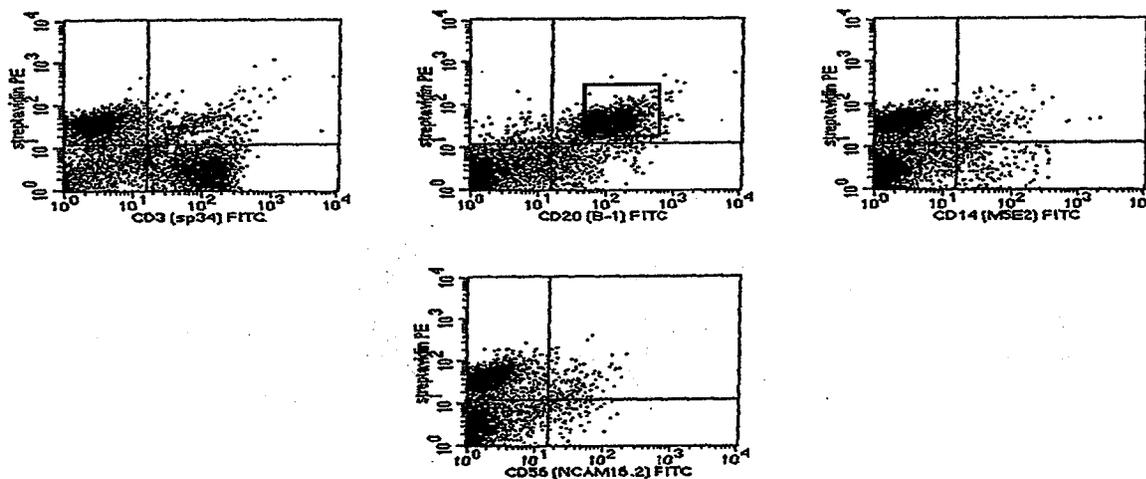
To find out which of the murine splenocyte subpopulations bind to human recombinant BLYS, splenocytes from the BALB/c mice were isolated and single cell suspensions were prepared. The splenocytes were then stained with FITC-labeled lineage-specific markers including CD45R (B220), TCR-alpha/beta, and CD11bMac along with the biotinylated BLYS and analyzed using a flowcytometer.

Figure 10 BLYS Receptor Expression on Murine Splenocytes

Recombinant human BLYS binds to mice B cells from spleen (stained with CD45) and not with monocytes (no staining observed with CD11Mac) or T cells (no staining observed with TCR alpha/beta). These findings are similar to those from the human PBMCs (Study number HG19300.CVLD.0.023).

Study Title: Analysis of Human BLYS Binding to Cynomolgus Monkey PBMC Using Flowcytometry; Study Number: HG19300.CVLD.0.029

To identify the cell population from the peripheral blood of Cynomolgus monkey that binds to human recombinant BLYS, PBMCs from Cynomolgus monkeys were isolated from whole blood. The ability of the human BLYS to bind with these PBMCs was then assessed from single cell suspensions of PBMCs staining with the FITC-labeled lineage-specific markers (CD3, CD20, and CD4, CD8, and CD14 along with the biotinylated BLYS. All analyses were conducted on a Becton Dickinson FACS scan using Cell Quest software.

Figure 11 BLYS Expression on PBMCs from Cynomolgus Monkey

Recombinant human BLYS binds to B cells from the PBMCs isolated from the Cynomolgus monkeys (stained with CD20) and not with monocytes (no staining observed with CD14) or T cells (no staining observed with CD 3, 4, and 8). These findings are similar to those from the human PBMCs and murine splenocytes (Study numbers HG19300.CVLD.0.023, 028).

Study Title: rhuBLYS Binding to Mouse Peripheral Blood Mononuclear Cells;
Study Number: HG19300.CVLD.0.030

To characterize the cell population from the peripheral blood of mice that binds to human recombinant BLYS, PBMCs from BALB/c mice were isolated from whole blood. The ability of the human BLYS to bind with these PBMCs was then assessed from the single cell suspensions of PBMCs by staining them with the FITC-labeled lineage-specific markers for mouse T cells (TCR alpha/beta), B cells [CD 45R (B220)], macrophage/ monocyte (CD14), NK cells [(pan NK), DX5], and granulocytes (CD 11b+GR-1⁺GR +). B lineage subsets [CD45R (B220+)] were distinguished based on the expression of CD5, BP-1(6C3), CD19, CD22, ThB (Ly6D), and anti-mouse IgM mAbs. All analyses were conducted on a Becton Dickinson FACS scan using Cell Quest software.

Figure 12 BLYS Expression on Murine PBMCs

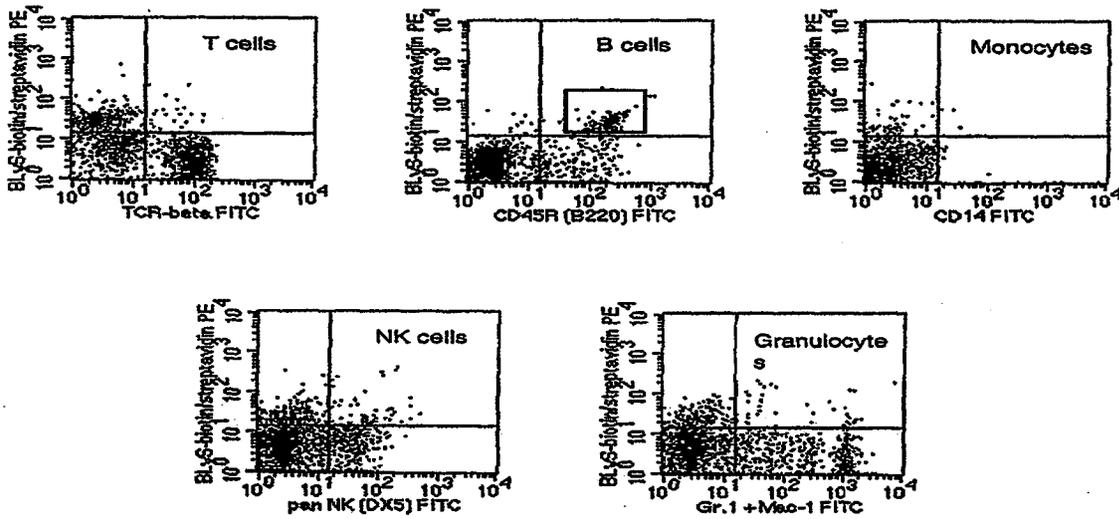
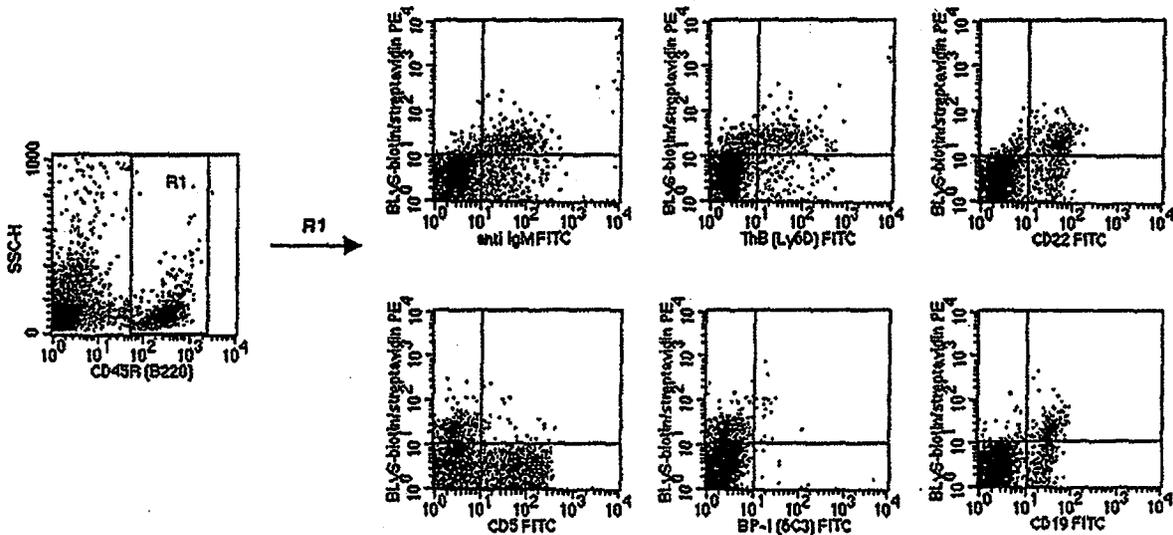


Figure 13 Cell Surface Phenotyping of CD45R (B220)⁺ B cells



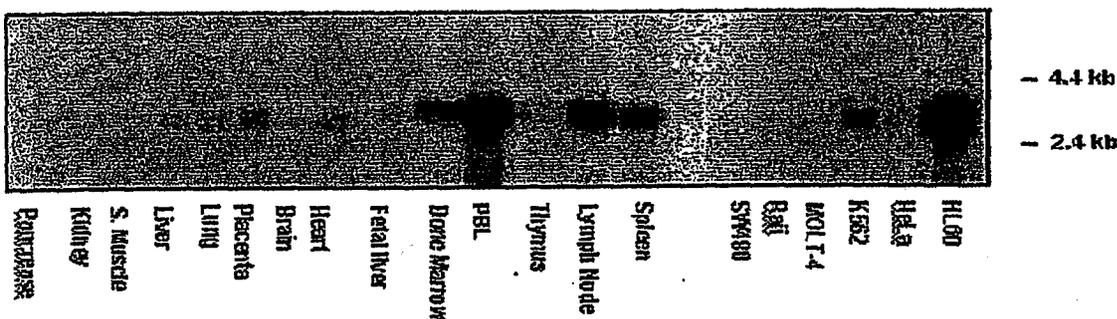
rhBLYS binds only to the B cells and not to T cells or macrophages. Among B cell subsets as defined by CD 45R(B220, BLYS-receptor was identified on cells expressing

CD5, CD19, CD22, ThB (Ly6D), and anti-mouse IgM. The population of responsive cells was phenotypically shown to correspond to mature B cells, as identified by a panel of markers (Ig54+, ThB+).

Study Title: Northern Analysis of BLYS mRNA Expression in Human Tissues and Cells; Study Number: HG19300.CVID.0.039

To determine the tissue distribution of BLYS mRNA, northern blot analysis was performed on a panel of 'Northern Blots' obtained from (b) (4). Three Northern Blots including Human Multiple Tissue Northern Blot, Human Immune System Multiple Tissue Northern Blot, and Human Cancer Cell Line Multiple Tissue Northern Blot were probed with a ³²P radiolabeled BLYS cDNA probe, using the complete BLYS open reading frame as the probe.

Figure 14 Northern Analysis of BLYS mRNA



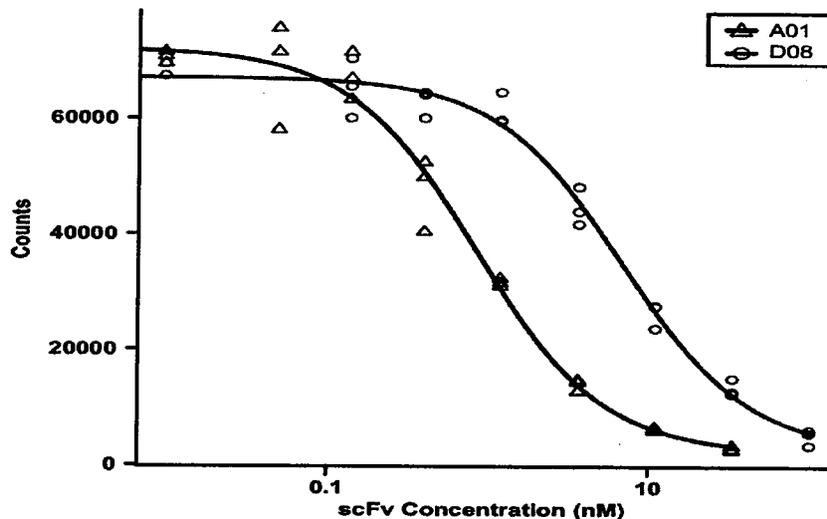
BLYS mRNA was detected as a single species of approximately 2.6 kb. Analysis of the Human Multiple Tissue Northern Blot revealed detectable expression in placenta, heart, lung and liver. Analysis of the Immune System Northern Blot revealed the highest expression of BLYS in PBMC (Peripheral Blood Mononuclear Cells), followed by lymph node, spleen, and bone marrow, with considerably lower expression seen in thymus and fetal liver. Analysis of the Human Cancer Cell Line Multiple Tissue Northern blot revealed very high expression in the promyelocytic leukemia cell line HL60 and detectable expression in the chronic myelogenous leukemia cell line K562. HeLa cells, the lymphoblastic MOLT4 leukemia cell line, the Burkitt's lymphoma Raji cell line, and the colorectal adenocarcinoma SW480 cell line failed to exhibit a detectable transcript. These results showed that BLYS mRNA was predominantly expressed in the hematopoietic cell types suggesting a potential immunomodulatory role of BLYS.

Study Title: Generation of Monoclonal Antibodies Specific for B Lymphocyte Stimulator (BLYS); Study Number: HG19399.SLE.0.001

In the process of generating a fully humanized anti BLYS antibody, a lead single-chain fragment chain variant (scFv) called D08 was isolated from a library of antibody derived from B-cells (tonsillar, PBL, and bone marrow) displayed in bacteriophage. An affinity matured variant of D08 was then isolated from a randomized library by DNA manipulation [that is by isolating the DNA by PCR using oligos mutated at 5' end and

ligating it into a phagemid vector and ultimately introducing it into *E. coli* by electrophoresis and growing the variants in the bacteria]. The therapeutic candidate developed was called scFvA01. The relative potencies of D08 and A01 were tested in a binding assay with BLYS which is expressed naturally in IM9 cells (ATCC).

Figure 15 Inhibition of BLYS Binding by Different Antibodies (D08 & A01)



BLYS was cloned and characterized for its biological activity. BLYS was then used to isolate a human scFv from bacteriophage displaying the human antibody fragment. This scFv (D08, IC_{50} 6.9) was then reformatted as an IgG (A01). The antiBLYS antibody is a genetically engineered fully humanized antibody which is capable of neutralizing BLYS. The binding affinities of these monoclonal anti BLYS antibodies (D08 and A01) were tested in a binding assay with BLYS isolated from a multiple myeloma (IM9) cell line. The mabA01 was observed (refer to Figure) to have much higher affinity (IC_{50} 0.85) than that of the D08 (IC_{50} 6.9).

Study Title: A01, a BLYS Antagonist, Binding ELISA; Study Number: HG19399.SLE.0.002

To characterize the BLYS binding to its antibodies (D08 and A01), a direct binding assay (ELISA) of the single chain (D08) and IgG based (A01) antibody of BLYS was established with the recombinant human BLYS protein. The direct binding of D08 and A01 to BLYS was demonstrated by binding of the antibodies to the BLYS coated on the ELISA plate at different concentrations. These were then incubated with biotinylated BLYS captured on streptavidin coated ELISA plates, which were detected by antihuman IgG - peroxidase conjugate (refer to Figure). Next, to find out the relative potencies of D08 and A01 to bind to BLYS the antibodies were incubated with immobilized BLYS at concentrations equivalent to their EC_{50} values (generated as in Fig 1). Different concentrations of BLYS in solution were used. The binding of the antibodies to immobilized BLYS was detected by antihuman IgG - peroxidase conjugate. Biotinylated D08 was then bound to immobilized BLYS directly on the ELISA plate with an EC_{50} value of 21.1 nM. Finally, the biotinylated D08 was assessed for binding directly to the

immobilized BLyS in the presence of unlabelled D08 or A01 in a competitive ELISA to show that both of these antibodies bind to the same epitope. A01 strongly inhibited the binding of biotinylated D08 to the immobilized BLyS, suggesting that these two antibodies recognize the same epitope. A01 competes with biotinylated D08 more efficiently than D08 itself, reflecting the fact that A01 has a higher affinity for BLyS.

Figure 16 Binding of D08 and A01 to Human BlyS

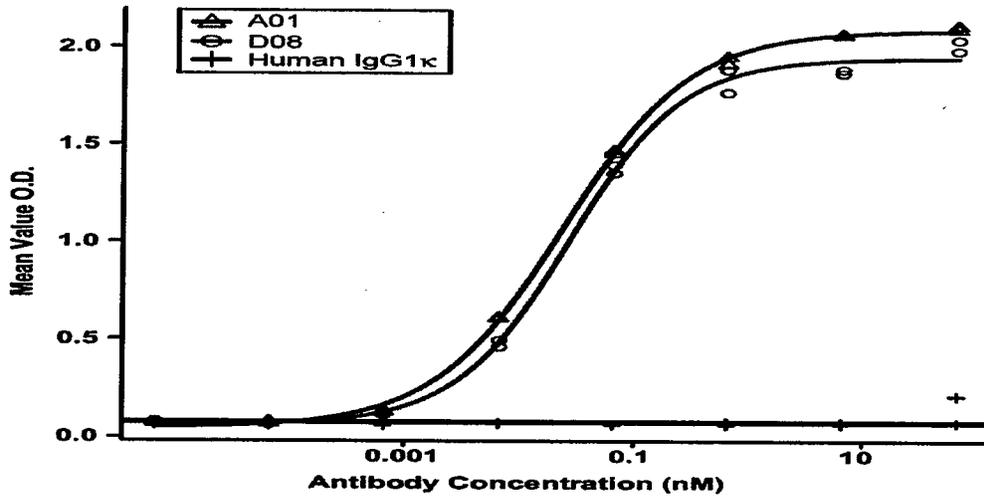


Figure 17 Inhibition of Binding of D08 and A01 to Immobilized BLYS

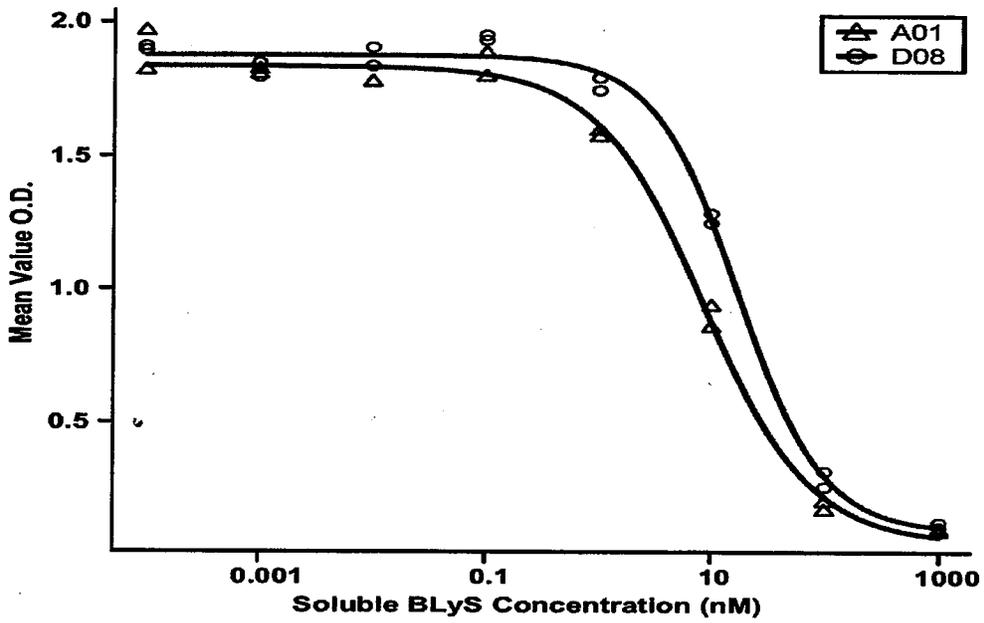
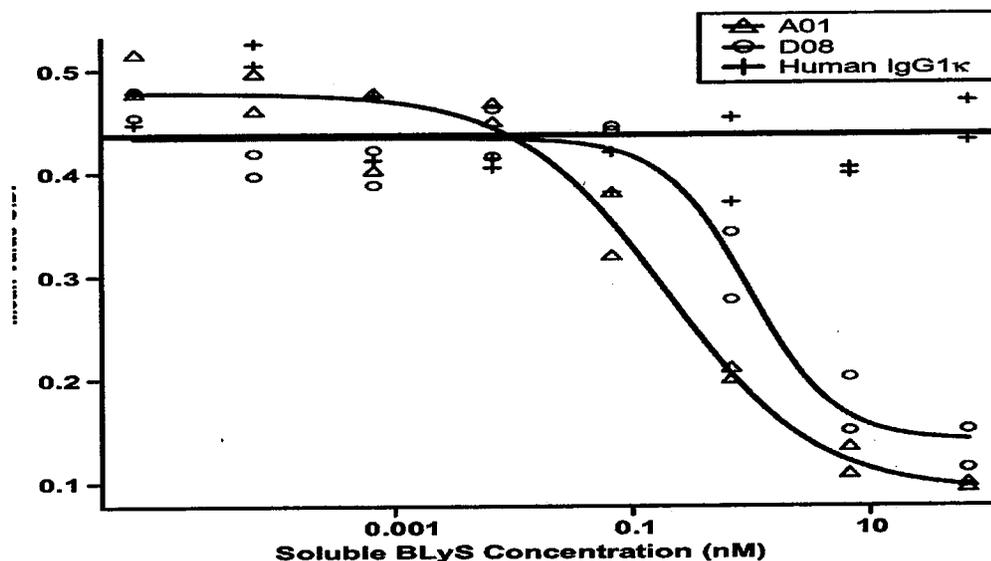


Figure 18 Competition of D08 and A01 for Binding to BLYS

A series of non cell based binding assays (ELISA) showed that D08 and A01 bind directly to BLYS with an EC_{50} of 0.03. Both A01 and D08 inhibited BLYS with IC_{50} s of 8.53 and 17.22, respectively. Finally, D08 and A01 bound to the same epitope. In a competitive ELISA, D08 and A01 binds to BLYS with an IC_{50} of 0.93 and 0.19, respectively, showing that A01 is more potent in binding with BLYS.

Study Title: Equilibrium Binding Determination of A01, a BLYS Antagonist;
Study Number: HG19399.SLE.0.003

In order to characterize the binding of both of the anti BLYS antibodies (D08 and A01) with BLYS, a method for affinity binding of BLYS with its antibodies in a BIA core 3000 instrument was developed. As per the principle of the ^{(b) (4)} technology a Protein A-antibody complex was first prepared on the flow column and then different concentrations of BLYS were flowed through the column and the kinetics of the binding were analyzed.

Table 7 Kinetics of BLyS Antagonists: A01 and D08

<u>Mab</u>	<u>Lot</u>	<i>k_a</i> , 1/Ms	<i>k_d</i> , 1/s	<i>K_d</i> , (pM)
		<u>Mean ± SD (10⁶)</u>	<u>Mean ± SD (10⁻⁴)</u>	<u>Mean ± SD</u>
A01	M3	1.26 ± 0.136	3.32 ± 0.631	267 ± 70
D08	M2	1.43 ± 0.130	5.8 ± 2.42	498 ± 151

A ^{(b) (4)} based BLyS-antiBLyS binding assay was developed and the K_D of D08 and A01 for binding to BLyS was calculated. The values are 498 and 267 pM, respectively, indicating that both of these antibodies bind to the target with a high affinity and possess a low dissociation constant.

Study Title: A01 Bioassay Development; Study Number: HG19399.SLE.0.004

In an effort to develop a bioassay for quantifying the effects of antiBLyS induced inhibition of BLyS mediated proliferation of splenocytes, splenocytes were isolated from BALB/c mice. The cells were incubated in the presence of different concentrations of BLyS for 72 hrs at 37°C, 5% CO₂ in a cell culture incubator. The proliferation of the splenocytes containing B-cells was then measured by thymidine incorporation. The optimal concentration of BLyS for the proliferation of splenocytes was $EC_{50} = 0.06$ nM. The splenocytes were then cultured in the presence of 0.06 nM BLyS together with the different concentrations of either D08 or A01 to determine the antibody induced inhibition of the BLyS induced proliferation of the splenocytes by thymidine incorporation method.

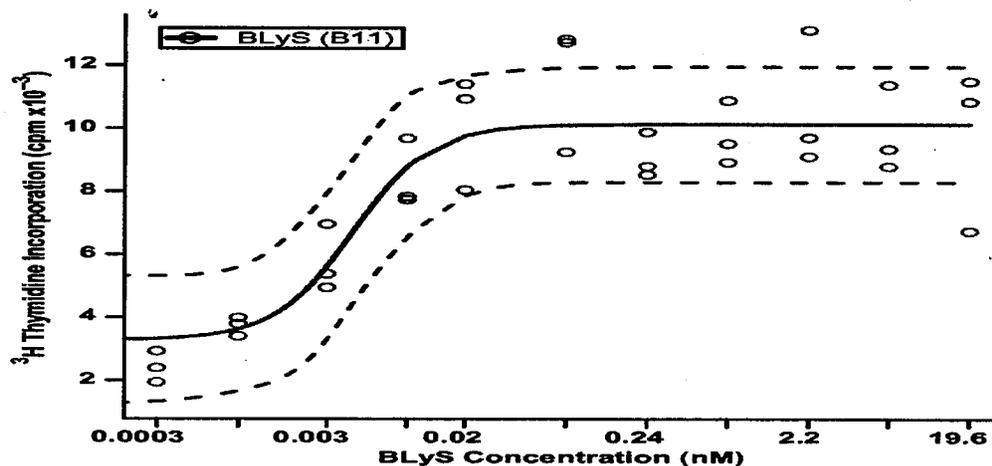
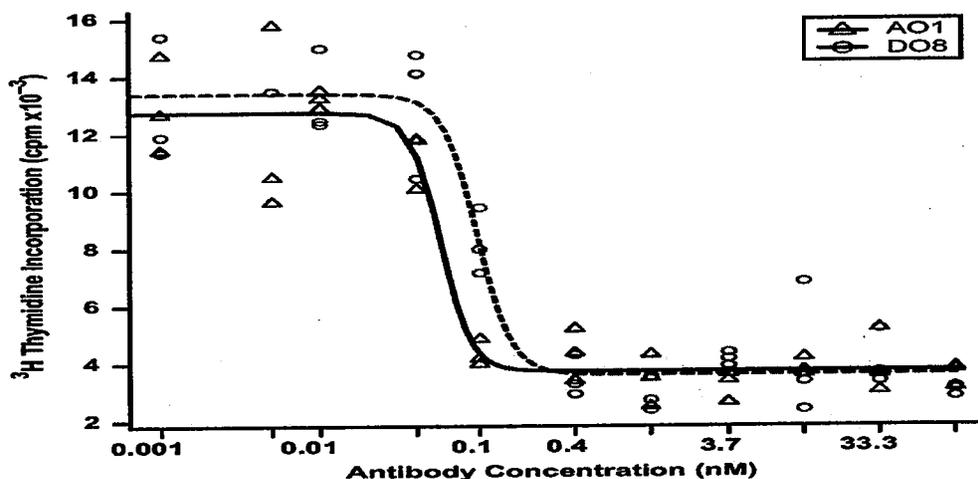
Figure 19 Induction of Murine Splenocytes Proliferation by BLyS

Figure 20 Inhibition of BLYS Induced Proliferation of Splenocytes by Antibodies

An ex vivo bioassay using murine splenocytes to determine the antiBLYS antibody induced inhibition of BLYS mediated cell proliferation. In this assay the IC_{50} of the antiBLYS antibodies D08 and A01 were observed to be 0.09 and 0.06, respectively.

Study Title: Neutralization of the Effects of Subcutaneously Injected Recombinant Human B-Lymphocyte Stimulator (rhuBLYS) by Intravenous Administration of A01 in BALB/C mice; Study Number: HG19399.SLE.0.006

The study was conducted to verify the ability of the fully human antiBLYS monoclonal antibody to neutralize the effects rhuBLYS on splenic wet weight, B lymphocyte population, and serum IgA concentration in mice. The mice were then injected intravenously with one of the five doses (0.05, 0.15, 0.5, 1.5, 5.0 mg/kg) of antiBLYS antibody (A01) or IgG_1 one hour prior to the subcutaneous administration of BLYS (0.53mg/kg) for four consecutive days. The effect of rhBLYS and anti BLYS on the proliferation of splenic B-cell was examined by isolating the splenocytes from the BALB/c mice, then by staining them with $\text{B220}^+/\text{ThB}^+$ splenic B lymphocytes, and analyzing them by flow cytometry. The wet weights of the spleen were measured and the serum IgA levels were analyzed by ELISA.

Figure 21 Effect of BLyS and anti BLyS on Murine Spleen Derived B-Cells

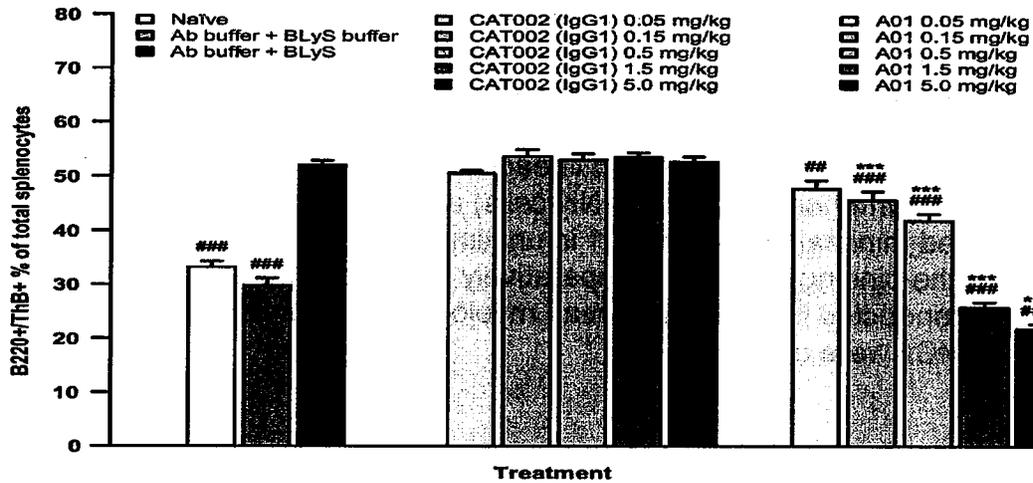
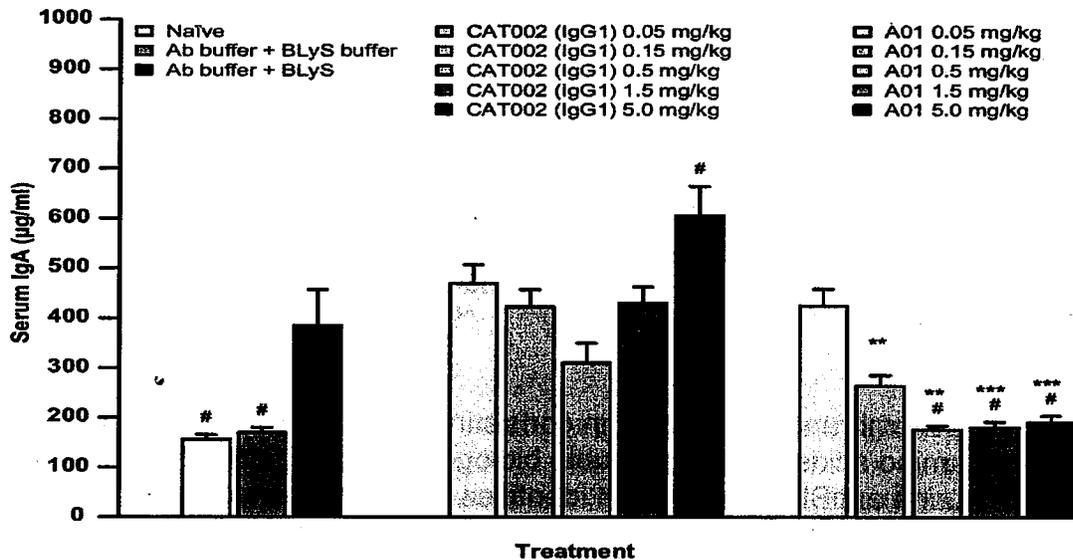


Figure 22 Effect of BLyS and anti BLyS on Murine Serum IgA Level

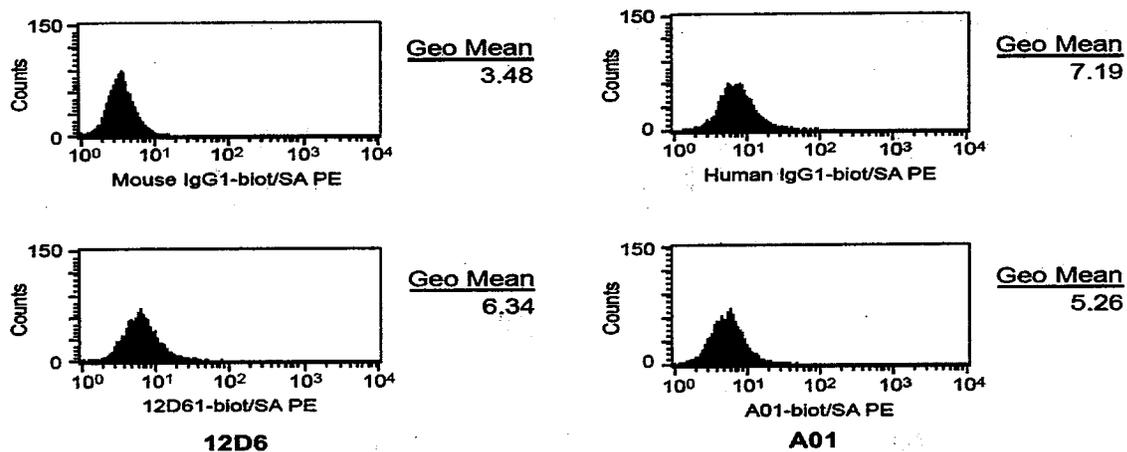


rhBLyS increased the weight of the spleen in the BALB/c mice. B-cell numbers in the spleen and the serum IgA levels increased following the treatment of rhBLyS. AntiBLyS monoclonal antibody reversed all of the above mentioned effects of the rhBLyS suggesting that humanized BLyS and antiBLyS antibody were functionally active in mice.

Study Title: A01 Specificity for soluble BLyS; Study Number: HG19399.SLE.0011

In order to determine the isoform specificity of the antiBlyS antibody, its binding potential was assessed on PBMCs which are known to express both membrane-bound and soluble BlyS. 12D6, a murine antibody known to recognize BlyS, expressed on the cell surface was used as a positive control. PBMCs were cultured in the cell culture incubator in the presence and absence of interferon gamma (IFN-gamma) which is known to induce B-cell expression. The freshly prepared PBMCs and the PBMCs cultured with IFN-gamma were then subjected to two color FACS analysis using biotinylated A01 (rhantiBlyS) and FITC labeled lineage specific antibodies: CD3 (T-lymphocytes), CD20 (B-lymphocytes), CD56 (NK-cells) and CD14 (monocytes). The FACS analysis showed minimal binding of A01 in unstimulated freshly prepared monocytes subsets, the binding of A01 is comparatively less than that of 12D6. FACS analyses were also conducted on K-562-a human myelogenic leukemia cell line and results similar to PBMCs were observed.

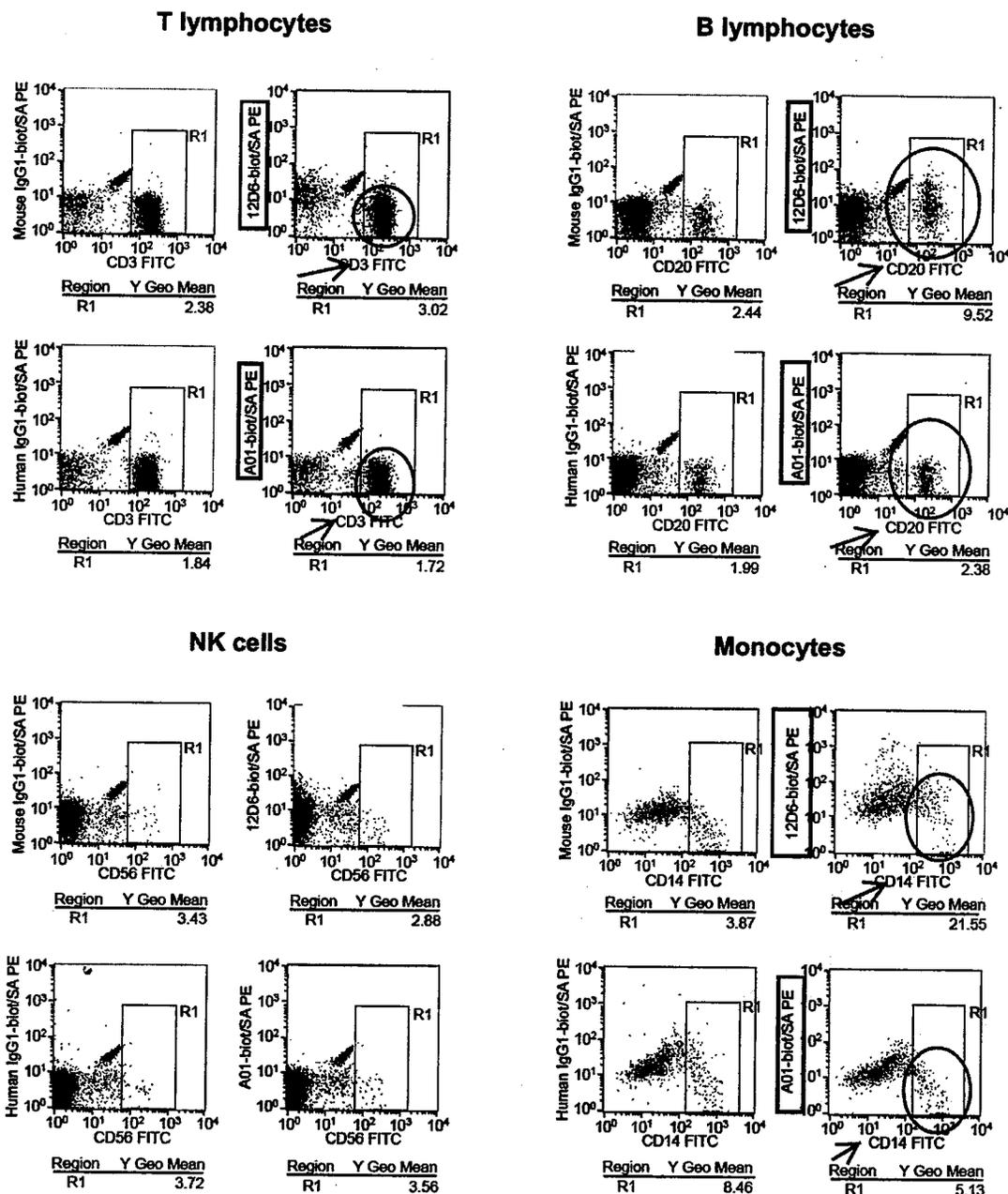
Figure 23 Binding of A01 to K562 cells



(b) (4)

(b) (4) Murine antiBlyS mAb 12D6 identifies both of these isoforms. In an effort to understand whether A01 recognizes both of these isoforms or only the soluble form of BlyS, the human PBMCs (stimulated with interferon gamma and unstimulated) were stained either with A01 together with CD56-NK cell), CD3 (T-cell), and CD14 (monocytes) or 12D6 together with CD56-NK cell), CD3 (T-cell), and CD14 (monocytes). The A01 antibody has been demonstrated to bind and inhibit the biological effects of soluble BlyS. The results from the current experiment showed that 12D6 bound to human PBMCs. As with unstimulated PBMC, lesser binding of A01 was detected in this experiment, while the 12D6 antibodies showed and an increase binding to CD14 and CD20 positive cells. In addition, K562 cells, which naturally express BlyS, were observed to recognize 12D6 better than A01. All of these data suggest that A01 might have better affinity for the soluble BlyS.

Figure 24 Binding of rantiBlyS (A01) Antibodies to Human PBMCs

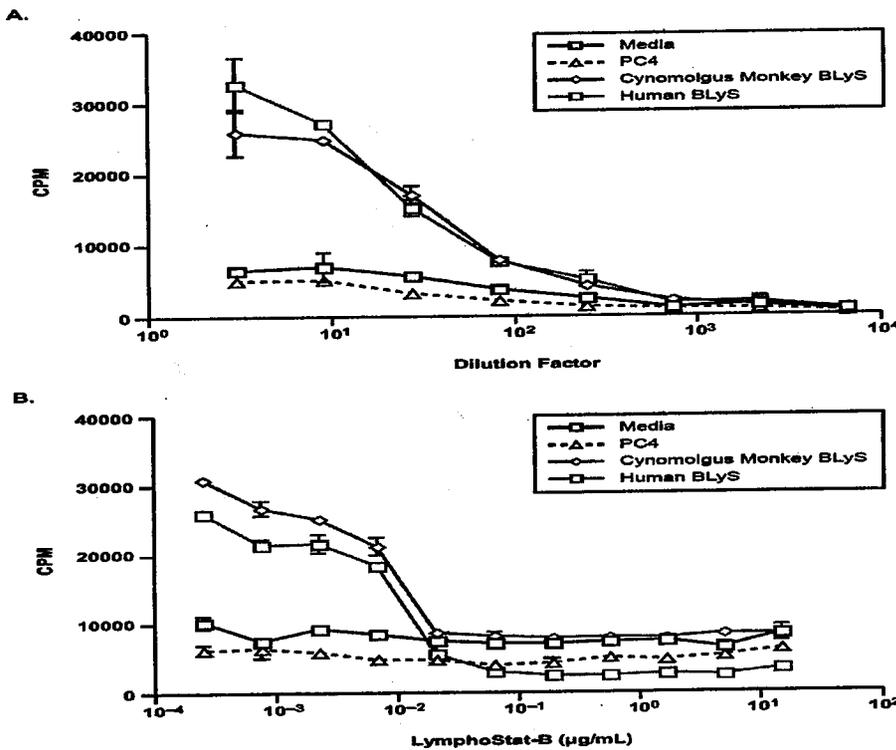


Study Title: LymphoStat-B Inhibits In Vitro B Lymphocyte Proliferation Induced by Human and Cynomolgus Monkey BlyS; Study Number HG19399.SLE.019

To determine whether the rantiBlyS antibody (LymphoStat B, A01) inhibits BlyS induced splenocyte proliferation in BALB/c mice, splenocytes were isolated and cultured in the presence of rantiBlyS, BlyS, and the co stimulator (SAC). BlyS used in this study was from transient supernatants of either human or Cynomolgus monkeys (cloned

and transfected) expressing BLyS in 293 cells. The supernatants from these cells were isolated and added at different dilutions to the freshly prepared murine splenocytes. The splenocyte proliferation was examined by ³Hthymidine incorporation.

Figure 25 Splenocyte Proliferation in Human and Cynomolgus Monkeys



A dose related proliferation of the splenocytes was noted with BLyS derived from human as well as Cynomolgus monkey and the rantiBlyS antibody inhibited splenocyte proliferation. The data showed that rantiBlyS can cross react with BLyS derived from Cynomolgus monkey suggesting that monkey might be an appropriate species for conducting toxicity studies with rantiBlyS.

Study Title: The Binding of Flag-tagged BLyS on LymphoStat-B: (b) (4) Binding Kinetic Analysis; Study Number: HG19399.SLE.0.026

To determine and compare the dissociation constants of rantiBlyS to BLyS derived from the human and Cynomolgus monkey, the affinity binding analysis was conducted using the (b) (4) 3000 instrument. Soluble Protein-A was covalently immobilized to 2 (b) (4) CM5 flow cells. The test and control antibodies were then injected over separate Protein-A derived flow cells. After washing off any unbound antibody serial dilutions of Flag-tagged BLyS, rantiBlyS were flowed over the antibody-Protein-A-complex. The off rate of bound antigen was determined by washing the antibody-Protein A-complex in the presence of HBS-EP buffer for an appropriate time. The

binding data were analyzed using the BIA evaluation software and used to determine the association (k_a) and dissociation (k_d) rate constants.

Table 8 Kinetics of Belimumab Binding

Table 1 Mean kinetic rate constants for LSB binding to BLYS antigen (no mass transfer)

<u>Antigen</u>	<u>$k_a \pm 1.96 * SE$</u> (1/Ms)	<u>$k_d \pm 1.96 * SE$</u> (1/s)	<u>$KD = k_d/k_a$</u> (M)
Cynomolgus Flag-BLYS ¹	1.75 e + 06 ± 1.14 e 06	3.27 e - 04 ± 1.82 e - 04	2.64 e - 10
Human Flag-BLYS ²	1.22 e + 06 ± 0.34 e 06	3.12 e - 04 ± 1.41 e - 04	2.74 e - 10

Table 2 Mean Kinetic rate constants for LSB binding to BLYS antigen (with mass transfer)

<u>Antigen</u>	<u>$k_a \pm 1.96 * SE$</u> (1/Ms)	<u>$k_d \pm 1.96 * SE$</u> (1/s)	<u>$KD = k_d/k_a$</u> (M)
Cynomolgus Flag-BLYS ¹	1.75 e + 06 ± 1.13 e 06	3.42 e - 04 ± 1.76 e - 04	2.73 e - 10
Human Flag-BLYS ²	1.23 e + 06 ± 0.34 e 06	2.98 e - 04 ± 1.30 e - 04	2.61 e - 10

¹Values represent mean ± 1.96 * SE (n = 4).

²Values represent mean ± 1.96 * SE (n = 5).

The bindings of BLYS derived from Cynomolgus monkeys and human with rhantiBlyS were determined to be 264 and 274 pM, respectively. No mass transfer limiting effects were noted as there were no significant changes in binding kinetics with addition of a mass transfer parameter. Under these experimental conditions, LymphoStat B binds with the BLYS derived from human and monkey in a picomolar concentration.

Study Title: Binding of Human and Murine BLYS to Belimumab and to Murine BAFF Receptor: (b) (4) Binding Kinetic Analysis; Study Number: HG19399.SLE.0.048

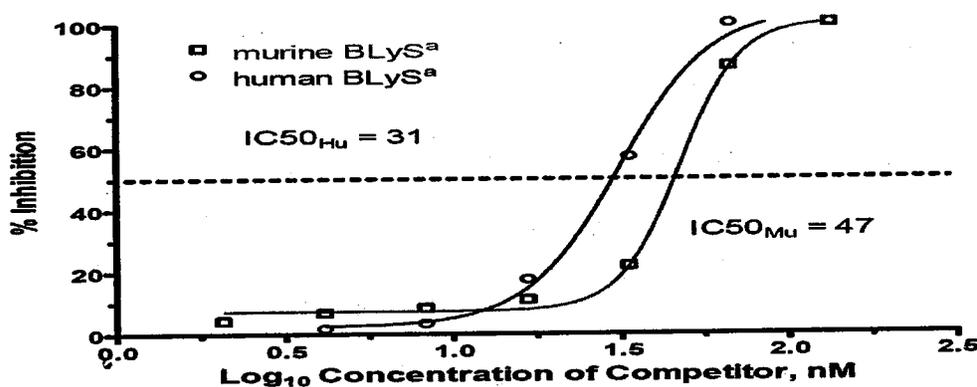
To evaluate the binding of belimumab to BLYS derived from human and murine BAFF receptor, the affinity binding analysis was performed with belimumab using the (b) (4) 3000 instrument. Soluble Protein-A was covalently immobilized to 2 (b) (4) CM5 flow cells. Belimumab was then injected over the Protein-A derived flow cells. After washing off any unbound antibody for 2.5 minutes BLYS was flowed over the belimumab-Protein A complex. In another experiment, human or murine BAFF-R-Fc was directly immobilized to separate (b) (4) CM5 flow cell to determine the BAFF-R-Fc affinity for human and murine BLYS. Competitive binding of BLYS on BAFF-R-Fc by belimumab was then analyzed in the murine system by adding a constant concentration of BLYS to six or more concentrations of belimumab to determine the IC₅₀ for belimumab inhibition of BLYS Binding to BAFF-Receptor.

Table 9 Belimumab Inhibition to BLYS Binding from Human & Mice

Exp. System ¹	ka (1/Ms)	kd (1/s)	Rmax (RU)	KD (M)
huBLYS→ belimumab				
Set 1	1.94E+06	2.02E-03	34.5	1.04E-09
Set 2	1.65E+06	6.73E-04	34.1	4.09E-10
Set 3	2.04E+06	3.66E-03	35.2	1.79E-09
Set 4	1.60E+06	1.17E-03	38.6	7.31E-10
Mean ² :				9.93E-10
± 95% CI ³ :				5.79E-10
muBLYS→ belimumab				
Set 1	8.73E+05	9.54E-03	25.9	1.09E-08
Set 2	5.85E+05	7.37E-03	30.6	1.26E-08
Set 3	6.45E+05	3.18E-03	56.8	4.9E-09
Mean ² :				9.48E-09
± 95% CI ³ :				4.55E-09

¹ Belimumab bound to immobilized Protein A at a density of ~120 RU.
² Mean KD is calculated as the avg of mean KD for n = number of sets.
³ 95% CI of the mean is the mean ± 1.96*SE.

Figure 26 Binding of Human and Murine BLYS to BAFF Receptor



The binding of murine and human BLYS to belimumab was determined to be 9.48 ± 4.55 nM and 0.99 ± 0.58 nM, respectively. These results indicate that there is an approximately 10-fold lower affinity of binding of murine BLYS to belimumab. Both human and murine forms of BLYS bound to the murine BAFF-R with comparable affinity (611 ± 83 pM and 492 ± 115 pM, respectively), thus suggesting similar binding epitopes. Belimumab competed for BLYS binding to BAFF-R in both systems.

Study Title: Binding Affinities of Human and Cynomolgus Monkey BLYS for Belimumab and BLYS Receptors and Proliferation Activity Induced by Human and Cynomolgus Monkey BLYS; Study Number: HG19399.SLE.0.051

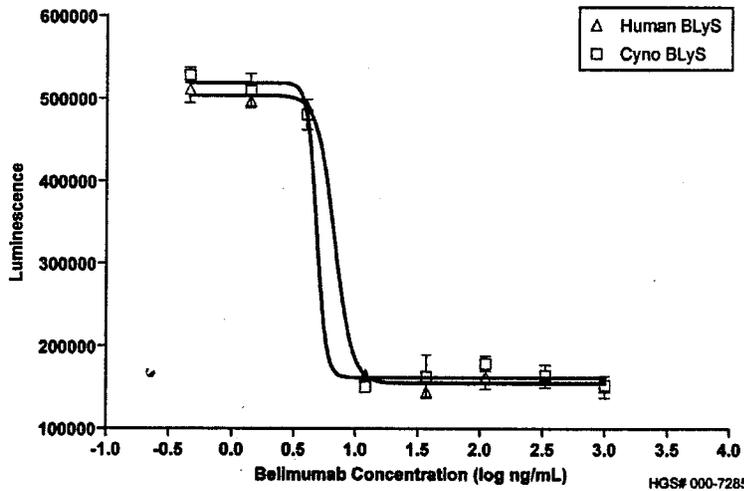
The primary objective of this study was to compare the binding of the BLYS derived from human and cynomolgus monkey to their cognate receptors including BCMAFc, TACIFc, and BR3Fc and belimumab using ^{(b) (4)} analysis. To achieve this purpose, soluble

Protein-A was covalently immobilized to two (b) (4) CM5 (either belimumab or the receptors) was then injected over the Protein-A derivatized flow cells and serial dilutions of BLYS were flowed over the belimumab-Protein A complex. The ability of belimumab to inhibit purified BlyS (source: human and monkey) induced proliferation of freshly prepared murine splenocytes was tested.

Table 10 Kinetics of BLYS Binding to BR3-Fc, TACI-Fc and BCMA-Fc

Experimental System	K _D (nM)
Human BLYS---belimumab	0.250
Cyno BLYS---belimumab	0.388
Human BLYS---human BR3-Fc	2.24
Cyno BLYS---human BR3-Fc	3.02
Human BLYS---human TACI-Fc	1.57
Cyno BLYS---human TACI-Fc	1.26
HumanBLYS.humanBCMA-Fc	2.64
CynoBLYS.humanBCMA-Fc	2.54

Figure 27 Inhibition of Murine Splenocytes Proliferation by Belimumab



BLYS derived from human and Cynomolgus monkey bound to belimumab and three of its receptors (BR3, TACI and BCMA) with comparable affinities. Also, BLYS from the two different sources were able to induce proliferation of murine splenocytes with nearly identical EC₅₀ values. Under these experimental conditions, belimumab demonstrated the comparable inhibitory activity on splenocyte proliferation induced by human and cynomolgus monkey BLYS. This study supports the choice of Cynomolgus monkey as a relevant species to conduct the toxicology and the pharmacokinetic/pharmacodynamic (PK/PD) studies.

Study Title: BLYS Receptor Profile on Human B cells and Inhibition of BLYS-Induced Proliferation by Belimumab; Study Number: HG19399.SLE.0.053

To identify the expression profiles of the BLYS receptors including TAC1, BCMA, and BAFFR in human PBMCs, the cells were obtained from 4 donors. B cell subsets were identified based on their membrane protein expression pattern. The cells were then stained with the receptor specific antibodies and analyzed by flow cytometry. Additionally, the ability of belimumab to inhibit BLYS induced proliferation of PBMCs was investigated. The cells were stimulated by incubating with rhBLYS and SAC and the inhibitory effect of belimumab was assessed by adding it in the culture for 72 hrs at 37°C. The Cell Titer Glo (Promega Inc) assay kit was used to measure the cell proliferation.

The 4 B cell subsets, naïve, Ag-activated, memory, and plasma cells were identified by their membrane protein expression patterns. While all normal B cells express the CD19 marker, B cell subsets were identified as follows:

Figure 28 Strategy to identify B-cell Subsets

Naïve: membrane IgD ^{positive} / CD27 ^{negative}

Ag-activated: membrane IgD ^{positive} / CD27 ^{positive}

Memory: membrane IgD ^{negative} / CD27 ^{positive}

Plasma cell: membrane IgD ^{negative} / CD38 ^{positive}

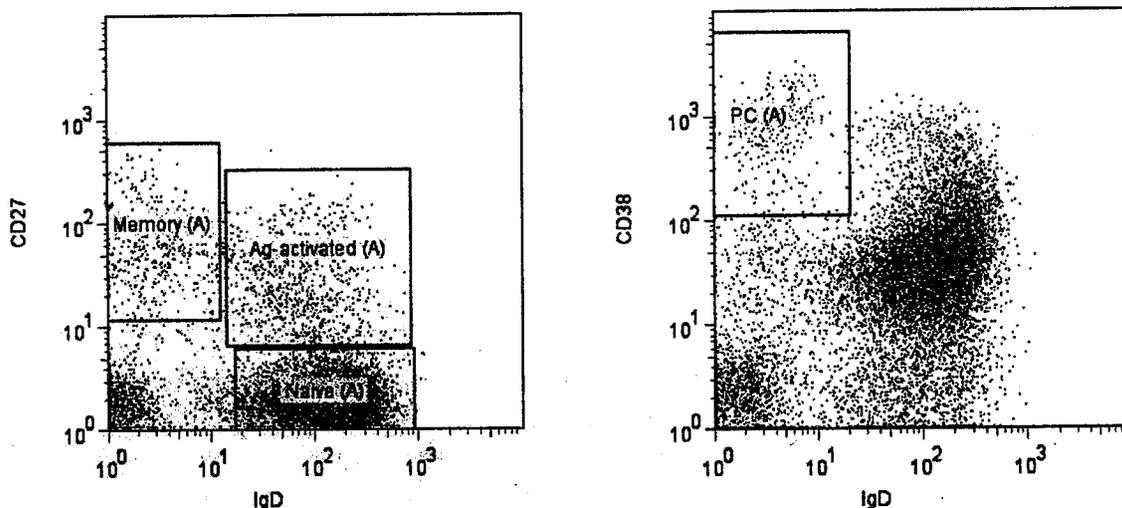


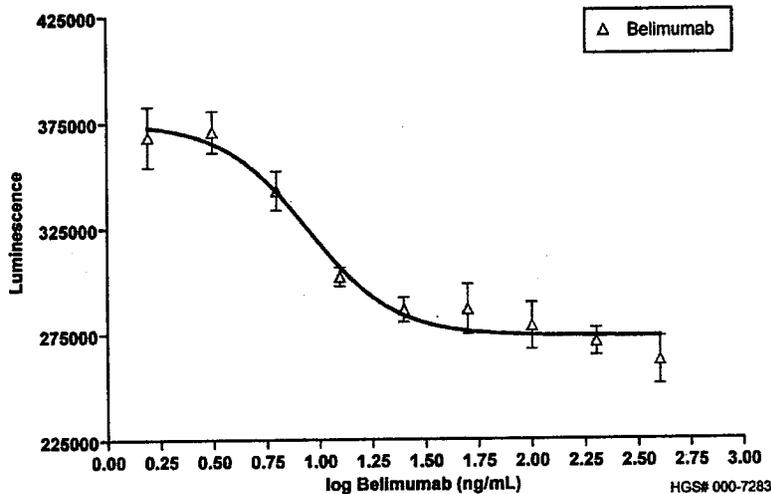
Figure 29 Human B-Cell Subset BLyS Receptor Expression:

- | | |
|---|--------------|
| — | Plasma |
| — | Memory |
| — | Ag-activated |
| — | Naive |



The intensity of labeling for each subset was evaluated qualitatively by comparing each subset's BLYS receptor expression level within each donor. In the figures, the relative intensity on the x-axis corresponds to receptor expression level. The background was set using the matched isotype control to be in the low end of the log scale

Figure 30 Inhibition of BLYS-Induced Proliferation by Belimumab



This is a pivotal pharmacology study which showed that the B cells derived from human PBMCs from different donors expressed BLYS. Among the donors tested, the BLYS receptor expression pattern follows a consistent pattern. Generally, naïve cells expressed low levels of BCMA and TACI, and high levels of BR3. Antigen activated cells highly expressed all 3 receptors. Memory cells express BCMA, and TACI, but vary in their BR3 expression. There are two populations of memory cells that express BR3 at lower and higher levels. Plasma cells express BCMA, high levels of TACI, and appear to lose BR3 expression. Plasma cells are rare in blood compared to the other subsets; therefore the graphed peak height for plasma cells may be more variable between donors than for the more frequent subsets.

Study Title: *In vivo* Activity of Belimumab on Murine B cells; Study Number: HG19399.SLE.0.049

The objective of this study was to evaluate the effect of belimumab (anti human BLYS antibody) and 10F4 (anti mice BLYS antibody raised in hamster) on the B-cell proliferation, immunogenicity, and pharmacokinetic in mice. Two experiments were conducted.

In the first experiment, mice (15/group) were administered IV at Day 0, 14, and 28 with 10 mg/kg of vehicle, or belimumab, or 10F4. The animals were observed for mortality and clinical signs. The blood samples were collected at Days 0, 3, 6, 9, 13, 21, 28, 35, 42, 49, and 57 for counting WBCs a FACS analyses. Peripheral blood was

stained with the following antibodies: CD45R/B220 PE, CD4 FITC, CD8 PE, AA4.1 FITC, anti-IgM FITC, and CD45 Cychrome. Antibodies were combined for 3 color analyses: AA4.1 FITC/B220 PE/CD45; anti-IgM FITC/B220 PE/CD45 Cychrome and CD4 FITC/CD8 PE/CD45 Cychrome.

Table 11 Study Design: Assessing Effect of Belimumab on Murine B Cells

Group	No. of Animals	Dose Level (mg/kg)
1 Vehicle (PBS)	15	0
2 10F4	15	10
3 belimumab	15	10

After the 2nd injection, 6/15 Belimumab treated animal died with 30 mins of the treatment administration. After the 3rd injection all of the belimumab and 10F4 treated animals appeared distressed with labored breathing and recumbency for two hours after the drug administrations. Most of the animals recovered, however, 5/9 remaining animals died at different days prior to the study termination at Day 15 from the belimumab treated group.

Figure 31 Belimumab's Effect on Murine WBC Counts

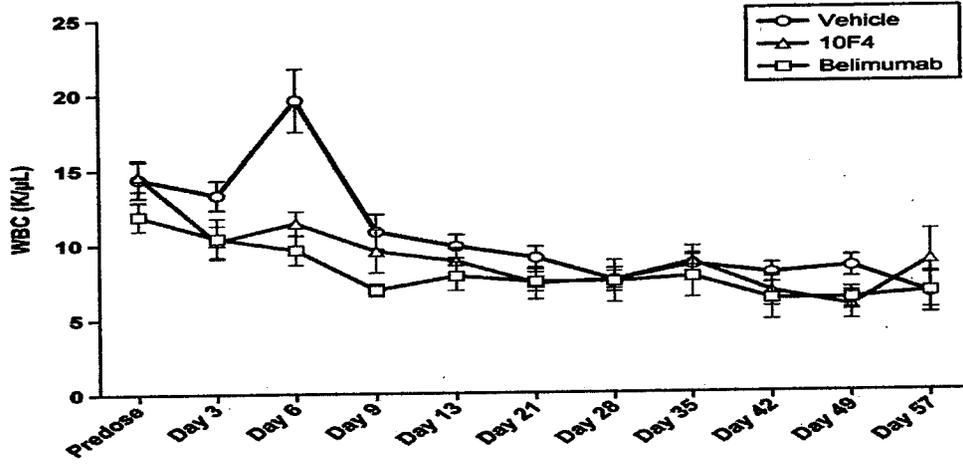


Figure 32 Belimumab's Effect on B220+ B cells (Percentage total WBC)

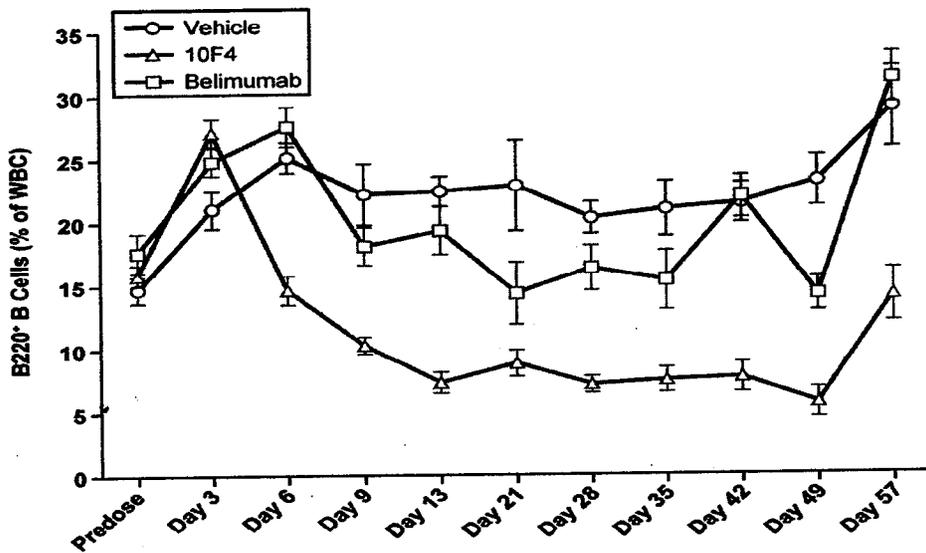


Table 12 Immunogenicity of Belimumab in Mice

Treatment	Animal #	Mean ECL Count
Vehicle	262	228
Vehicle	263	177
Vehicle	264	201
Vehicle	265	173
Vehicle	266	323
Vehicle	280	351
Vehicle	293	277
Vehicle	294	302
Vehicle	296	180
Vehicle	1950	510
belimumab	232	26855
belimumab	234	83622
belimumab	237	81258
belimumab	241	2627
10F4	247	256
10F4	248	185
10F4	249	391
10F4	251	193
10F4	272	177
10F4	274	217
10F4	276	190

The data showed that the WBC counts were similar between control, 10F4, and belimumab-treated mice, with the exception of Day 6 when the control group means WBC counts were more than 47% higher than at Day 3 (19.6 at Day 6 vs 13.3 at Day 3). The percent of B cells from blood in belimumab-treated mice was decreased relative to that of the control group at Day 9 through Day 35. The percent of blood B cells from blood of 10F4-treated mice decreased earlier than in the belimumab mice, with a reduction apparent at Day 6. The percent of blood B cells remained low for the 10F4-treated mice through the end of the study at Day 57. The percent of blood CD4⁺ and CD8⁺ T cells was similar for all groups throughout the duration of the study. The belimumab treated animals (4/15 surviving) had generated a potential anti-belimumab response (as observed by the high electroluminescence, no confirmatory test was conducted). None of the control or 10F4-treated mice had elevated counts in the assay.

In the second experiment, mice (10/group) were administered either IV or IP at Day 0, 14, and 28 with 5 mg/kg of CAT002 (control human antibody), belimumab, or 10F4. The animals were observed for mortality and clinical signs. The blood samples were collected at Days -4, 0, 14, 28, 35, 42, 49, 56, 63, and 70 for counting WBCs a FACS analyses. Peripheral blood was stained with the following antibodies: CD45R/B220 PE, CD4 FITC, CD8 PE, AA4.1 FITC, anti-IgM FITC, and CD45. Antibodies were combined for 3 color analyses: AA4.1 FITC/B220 PE/CD45; anti-IgM FITC/B220 PE/CD45 Cychrome; and CD4 FITC/CD8 PE/CD45 Cychrome.

Table 13 Study Design: Murine Immunogenicity Assessment

Group	No. of Animals	Dose Level (mg/kg)	Route
1 Control (CAT002)	10	5	IV
2 10F4	10	5	IV
3 belimumab	10	5	IV
4 Control (CAT002)	10	5	IP
5 10F4	10	5	IP
6 belimumab	10	5	IP

The cage-side observations following the 2nd injection at Day 14 for belimumab and 10F4-treated mice included labored respiration beginning 20-25 minutes after treatment. Two out of 10 mice in the belimumab IV group died 25-30 minutes after injection and all others recovered. Following the 3rd injection at Day 28, all of the belimumab and 10F4-treated mice had labored respiration and one of the remaining 8 mice in the belimumab-IV group died approximately 4 hours after injection. All others recovered and survived to the end of the study. Mice treated with CAT002 were unremarkable after each treatment.

Flow cytometry analysis of blood B cells at Days 42 and 56 showed that the mean WBC counts in the IV treated belimumab and 10F4 groups were lower relative to the control group. However, in the IP treated group the following trends were observed: at Days 14 and 28, the mean WBC of the 10F4 mice trended higher than the belimumab and control mice, and all 3 IP groups were similar from Day 42 through Day 70. There were no clear differences between treatment groups for either percentage of blood CD4+ or CD8+ T cells. Note that at Days 14 and 28, the percent of blood B cells of belimumab and 10F4 treated mice was lower than control mice in both the IV and IP arms. However, at Day 42 and through the end of the study at Day 70, the percent of blood B cells in belimumab-treated mice trended upward to levels similar to the control mice, while the 10F4 percent of blood B cells remained lower relative to the controls. This may be considered as evidence of loss of belimumab activity due to the emergence of anti-belimumab antibodies resulting in clearance of belimumab.

Table 14 Effect of Belimumab on B cell in Murine Immunogenicity Assay

Treatment Group		B220+ Cells (% of WBC)					
		Day 0	Day 14	Day 28	Day 42	Day 56	Day 70
1 CAT002 (IV)	mean	13.73	27.03	20.66	24.10	16.97	23.67
	SD	6.77	5.48	3.99	3.46	3.28	6.52
2 10F4 (IV)	mean	16.91	14.69	13.05	14.61	16.04	20.20
	SD	5.48	1.54	2.40	4.25	4.09	6.52
3 belimumab (IV)	mean	17.51	21.01	15.09	22.48	17.64	18.47
	SD	2.38	5.49	2.96	3.85	5.69	5.45
4 CAT002 (IP)	mean	15.59	21.81	19.17	27.37	17.94	22.45
	SD	4.36	4.69	0.95	5.33	3.32	8.04
5 10F4 (IP)	mean	15.59	13.76	9.15	11.09	10.78	14.10
	SD	5.24	4.07	3.06	4.74	7.26	8.23
6 belimumab (IP)	mean	20.67	14.40	16.07	22.43	20.01	23.46
	SD	4.27	6.20	2.98	3.30	4.14	5.93

PK analyses were conducted for belimumab and 10F4. In this study, only 1 sample from the belimumab IP arm had measurable belimumab at Day 35. All other samples (IV and IP, all sampling time points from Day 35 through Day 70) were below the assay limit of quantitation (BLOQ). In the 10F4 IP arm, 9/10 mice had measurable 10F4 at least 1 time point after treatment, but only 4 of those 9 mice had 10F4 present at values similar to the predicted PK values. In the IV arm, 7/10 mice had measurable 10F4 at least 1 time point after treatment; however, the measured serum concentrations were generally lower than in the IP arm and only 2 of those 7 mice had 10F4 concentrations similar to that predicted.

High ECL count in all sera was observed from samples collected post-treatment from belimumab mice indicating potential anti-belimumab antibodies in the immunogenicity assay. The confirmatory assay was not performed due to the lack of available serum volume. However, the high counts among the belimumab-treated mice strongly suggest a specific anti-belimumab response.

The incidence of anti-10F4 antibodies in IV treated mice was twice the incidence in IP treated mice. Among IP treated mice, 3/10 had 2 or more time points that were potentially positive in the immunogenicity assay, while among IV treated mice 7/10 had 2 or more time points that were potentially positive.

Table 15 Kinetics of Belimumab induced Immunogenicity Assessment in Mice

Animal Number	Time Point (Day)	Group	Mean ECL Count	Log ECL	SD	%CV	Ratio of Log Sample: Log NC	Result
883	-4	belimumab IP	309	2.49	36.77	11.90	0.99	Negative
	35	-	112424	5.05	18249.01	16.23	2.01	Potentially Positive
	49	-	111330	5.05	7499.57	6.74	2.00	Potentially Positive
	63	-	120494	5.08	25727.37	21.35	2.02	Potentially Positive
	70	-	138223	5.14	15738.08	11.39	2.04	Potentially Positive
884	-4	belimumab IP	368	2.57	32.53	8.84	1.02	Negative
	35	-	12472	4.10	539.52	4.33	1.63	Potentially Positive
	49	-	21164	4.33	1598.77	7.55	1.72	Potentially Positive
	63	-	24500	4.39	1649.68	6.73	1.74	Potentially Positive
	70	-	19219	4.28	283.55	1.48	1.70	Potentially Positive
882	-4	belimumab IP	490	2.69	9.90	2.02	0.61	Negative
	35	-	112359	5.05	3507.96	3.12	2.01	Potentially Positive
	49	-	108264	5.03	15550.69	14.36	2.00	Potentially Positive
	63	-	89334	4.95	6119.30	6.85	1.97	Potentially Positive
	70	-	63165	4.80	NA ¹	NA	1.91	Potentially Positive
886	35	belimumab IP	128243	5.11	7734.33	6.03	2.03	Potentially Positive
	49	-	129706	5.11	34206.29	26.37	2.03	Potentially Positive
	63	-	83142	4.92	8779.44	10.56	1.95	Potentially Positive
	70	-	112359	5.05	22802.07	20.29	2.01	Potentially Positive
885	-4	belimumab IP	387	2.59	28.99	7.50	1.03	Negative
	49	-	8660	3.94	480.83	5.55	1.56	Potentially Positive
	63	-	11375	4.06	268.70	2.36	1.61	Potentially Positive
	70	-	10405	4.02	346.48	3.33	1.60	Potentially Positive
863	35	belimumab IP	31816	4.50	5977.17	18.79	1.79	Potentially Positive
	49	-	131706	5.12	5619.38	4.27	2.03	Potentially Positive
	63	-	59374	4.77	2928.13	4.93	1.90	Potentially Positive
	70	-	60739	4.78	692.26	1.14	1.90	Potentially Positive
862	-4	belimumab IP	242	2.38	1.41	0.58	0.95	Negative
	35	-	173851	5.24	29815.86	17.15	2.08	Potentially Positive
	49	-	167847	5.22	29243.11	17.42	2.07	Potentially Positive
	63	-	135912	5.13	866.21	0.64	2.04	Potentially Positive
	70	-	131706	5.12	2930.25	2.22	2.03	Potentially Positive
866	-4	belimumab IP	318	2.50	9.90	3.11	0.99	Negative
	35	-	9131	3.96	683.07	7.48	1.57	Potentially Positive
	49	-	9219	3.96	NA	NA	1.57	Potentially Positive
	63	-	19468	4.29	1286.93	6.61	1.70	Potentially Positive
	70	-	18492	4.27	187.38	1.01	1.69	Potentially Positive

Animal Number	Time Point (Day)	Group	Mean ECL Count	Log ECL	SD	%CV	Ratio of Log Sample: Log NC	Result
865	-4	belimumab IP	653	2.81	28.99	4.44	1.12	Negative
	35	-	3075	3.49	144.25	4.69	1.38	Potentially Positive
	49	-	39833	4.60	795.50	2.00	1.83	Potentially Positive
	63	-	16172	4.21	7672.11	47.44	1.67	Potentially Positive
	70	-	6042	3.78	50.91	0.84	1.50	Potentially Positive
864	-4	belimumab IP	279	2.45	NA	NA	0.97	Negative
	35	-	265507	5.42	23960.31	9.02	2.15	Potentially Positive
	49	-	253890	5.40	3157.23	1.24	2.15	Potentially Positive
	63	-	208876	5.32	9047.43	4.33	2.11	Potentially Positive
	70	belimumab IP	248215	5.39	78348.14	31.56	2.14	Potentially Positive
887	-4	belimumab IV	506	2.70	29.70	5.87	1.10	Negative
	35	-	75154	4.88	28847.13	38.38	1.99	Potentially Positive
	49	-	66621	4.82	1122.89	1.69	1.97	Potentially Positive
	63	-	54361	4.74	11824.24	21.75	1.93	Potentially Positive
	70	-	60885	4.78	7516.55	12.35	1.95	Potentially Positive
888	-4	belimumab IV	667	2.82	159.10	23.87	1.15	Potentially Positive
	35	-	186480	5.27	18734.79	10.05	2.15	Potentially Positive
	49	-	167060	5.22	17831.11	10.67	2.13	Potentially Positive
	63	-	151943	5.18	9608.17	6.32	2.11	Potentially Positive
	70	-	105705	5.02	6248.70	5.91	2.05	Potentially Positive
889	-4	belimumab IV	246	2.39	46.67	18.97	0.98	Negative
	35	-	286762	5.46	86023.08	30.00	2.23	Potentially Positive
	49	-	433345	5.84	6450.94	1.49	2.30	Potentially Positive
	63	-	226934	5.36	84478.75	37.23	2.19	Potentially Positive
	70	-	354122	5.55	59899.72	16.92	2.26	Potentially Positive
891	-4	belimumab IV	389	2.59	16.26	4.19	1.06	Negative
	35	-	7973	3.90	1504.02	18.87	1.59	Potentially Positive
	49	-	8533	3.93	835.09	9.79	1.60	Potentially Positive
	63	-	354122	5.55	1019.65	0.29	2.26	Potentially Positive
	70	-	8906	3.95	584.78	6.57	1.61	Potentially Positive
903	-4	belimumab IV	474	2.68	116.67	24.64	1.09	Negative
904	-4	belimumab IV	786	2.90	8.49	1.08	1.18	Potentially Positive
	35	-	8059	3.91	660.44	8.20	1.59	Potentially Positive
	49	-	9194	3.96	1678.67	18.26	1.62	Potentially Positive
	63	-	12243	4.09	3687.56	30.12	1.67	Potentially Positive
	70	-	8542	3.93	310.42	3.63	1.60	Potentially Positive
905	-4	belimumab IV	468	2.67	2.12	0.45	1.09	Negative
	35	-	187247	5.27	6038.69	3.22	2.15	Potentially Positive
	49	-	240357	5.38	63283.23	26.33	2.20	Potentially Positive
	63	-	199118	5.30	63283.23	31.78	2.16	Potentially Positive
	70	-	168119	5.23	3218.04	1.91	2.13	Potentially Positive

Table 16 Kinetics of 10F4 (murine anti BLYS) in Mice

Animal #	Time Point (Day)	Group	Mean 10F4 (µg/mL)	%CV *
867	-4	10F4-IP	BLOQ	NA
	35	-	BLOQ	NA
	49	-	18.89	NA*
	63	-	BLOQ	NA
	70	-	BLOQ	NA
868	-4	10F4-IP	BLOQ	NA
	35	-	35.95	22.3
	49	-	9.92	13.3
	63	-	8.56	1.6
	70	-	4.35	4.3
869	-4	10F4-IP	BLOQ	NA
	35	-	3.41	11.7
	49	-	0.82	11.2
	63	-	0.08	2.3
	70	-	0.05	3.6
870	-4	10F4-IP	0.04	11.6
	35	-	31.89	NA*
	49	-	BLOQ	NA
	63	-	6.11	NA*
	70	-	2.72	24.7
871	-4	10F4-IP	0.08	0.4
	35	-	14.34	22.6
	49	-	2.26	1.2
	63	-	0.15	0.35
	70	-	0.04	7.7
872	-4	10F4-IP	0.06	13.1
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	BLOQ	NA
873	-4	10F4-IP	0.06	0.1
	35	-	32.64	7.7
	49	-	11.24	9.1
	63	-	8.08	6.7
	70	-	2.90	78.5
874	-4	10F4-IP	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	0.30	46
	70	-	BLOQ	NA

Animal #	Time Point (Day)	Group	Mean 10F4 (µg/mL)	%CV *
875	-4	10F4-IP	BLOQ	NA
	35	-	32.93	11.7
	49	-	14.62	13.5
	63	-	3.05	10.8
	70	-	0.71	25.3
876	-4	10F4-IP	BLOQ	NA
876	35	10F4-IP	31.76	28.2
	49	-	7.46	3.8
	63	-	BLOQ	NA
	70	-	0.04	17.4
877	-4	10F4-IV	BLOQ	NA
	35	-	6.03	NA*
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	0.03	9.6
878	-4	10F4-IV	0.05	4.1
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	BLOQ	NA
879	-4	10F4-IV	BLOQ	NA
	35	-	10.16	12.9
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	BLOQ	NA
880	-4	10F4-IV	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	0.06	14.9
	70	-	BLOQ	NA
881	-4	10F4-IV	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	0.03	5
907	-4	10F4-IV	BLOQ	NA
	35	-	32.78	24
	49	-	9.64	13.8
	63	-	1.40	45.9
	70	-	0.18	16.8
908	-4	10F4-IV	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	0.06	7.5
	70	-	BLOQ	NA

Animal #	Time Point (Day)	Group	Mean 10F4 (µg/mL)	%CV *
909	-4	10F4-IV	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
	63	-	BLOQ	NA
	70	-	BLOQ	NA
910	-4	10F4-IV	BLOQ	NA
	35	-	BLOQ	NA
	49	-	BLOQ	NA
910	63	10F4-IV	BLOQ	NA
911	-4	10F4-IV	BLOQ	NA
	35	-	63.56	NA*
	49	-	26.21	NA*
	63	-	8.90	NA*
	70	-	3.66	NA*

* Samples that were dissimilar to predicted PK values on initial testing were re-tested; thus some reported values are the result of 2 tests and no %CV is reported.

Table 17 Kinetics of 10F4 Induced Immunogenicity in Mice

Animal Number	Time Point (Day)	Group	ECL	Log (ECL)	Ratio of Log Sample: Log NC	Result
867	-4	10F4 IP	210	2.32	0.95	Negative
	35	-	9378	3.97	1.62	Potentially Positive
	49	-	532	2.73	1.11	Negative
	63	-	34633	4.54	1.85	Potentially Positive
	70	-	33790	4.53	1.85	Potentially Positive
868	-4	10F4 IP	149	2.17	0.89	Negative
	35	-	245	2.39	0.98	Negative
	49	-	505	2.70	1.10	Negative
	63	-	264	2.42	0.99	Negative
	70	-	349	2.54	1.04	Negative
869	-4	10F4 IP	283	2.45	1.00	Negative
	35	-	283	2.45	1.00	Negative
	49	-	490	2.69	1.10	Negative
	63	-	361	2.56	1.04	Negative
	70	-	396	2.60	1.06	Negative
870	-4	10F4 IP	326	2.51	1.03	Negative
	35	-	349	2.54	1.04	Negative
	49	-	39078	4.59	1.87	Potentially Positive
	63	-	472	2.67	1.09	Negative
	70	-	281	2.45	1.00	Negative
871	-4	10F4 IP	280	2.45	1.00	Negative
	35	-	449	2.65	1.08	Negative
	49	-	454	2.66	1.08	Negative
	63	-	490	2.69	1.10	Negative
	70	-	452	2.66	1.08	Negative
872	-4	10F4 IP	251	2.40	0.98	Negative
	35	-	76859	4.89	1.99	Potentially Positive
	49	-	62770	4.80	1.96	Potentially Positive
	63	-	56079	4.75	1.94	Potentially Positive
	70	-	52120	4.72	1.93	Potentially Positive
873	-4	10F4 IP	304	2.48	1.01	Negative
	35	-	269	2.43	0.99	Negative
	49	-	100	2.00	0.82	Negative
	63	-	267	2.43	0.99	Negative
	70	-	275	2.44	1.00	Negative
874	-4	10F4 IP	369	2.57	1.05	Negative
	35	-	157249	5.20	2.12	Potentially Positive
	49	-	171091	5.23	2.14	Potentially Positive
	63	-	210	2.32	0.95	Negative
	70	-	87809	4.94	2.02	Potentially Positive

Animal Number	Time Point (Day)	Group	ECL	Log (ECL)	Ratio of Log Sample: Log NC	Result
875	-4	10F4 IP	331	2.52	1.03	Negative
	35	-	255	2.41	0.98	Negative
	49	-	254	2.40	0.98	Negative
	63	-	302	2.48	1.01	Negative
	70	-	331	2.52	1.03	Negative
876	-4	10F4 IP	423	2.63	1.07	Negative
876	35	10F4 IP	424	2.63	1.07	Negative
	49	-	274	2.44	1.00	Negative
	63	-	109052	5.04	2.06	Potentially Positive
	70	-	427	2.63	1.07	Negative
877	-4	10F4 IV	393	2.59	1.06	Negative
	35	-	229	2.36	0.96	Negative
	49	-	13061	4.12	1.68	Potentially Positive
	63	-	12328	4.09	1.67	Potentially Positive
	70	-	2725	3.44	1.40	Potentially Positive
878	-4	10F4 IV	No Sample	No Sample	No Sample	No Sample
	35	-	267	2.43	0.99	Negative
	49	-	137830	5.14	2.10	Potentially Positive
	63	-	118071	5.07	2.07	Potentially Positive
	70	-	110577	5.04	2.06	Potentially Positive
879	-4	10F4 IV	No Sample	No Sample	No Sample	No Sample
	35	-	171548	5.23	2.14	Potentially Positive
	49	-	886	2.95	1.20	Negative
	63	-	439	2.64	1.08	Negative
	70	-	443	2.65	1.08	Negative
880	-4	10F4 IV	277	2.44	1.00	Negative
	35	-	75735	4.88	1.99	Potentially Positive
	49	-	61500	4.79	1.95	Potentially Positive
	63	-	67575	4.83	1.97	Potentially Positive
	70	-	47602	4.68	1.91	Potentially Positive
881	-4	10F4 IV	274	2.44	1.00	Negative
	35	-	5198	3.72	1.52	Potentially Positive
	49	-	7739	3.89	1.59	Potentially Positive
	63	-	7473	3.87	1.58	Potentially Positive
	70	-	12184	4.09	1.67	Potentially Positive
907	-4	10F4 IV	185	2.27	0.93	Negative
	35	-	262	2.42	0.99	Negative
	49	-	270	2.43	0.99	Negative
	63	-	241	2.38	0.97	Negative
	70	-	362	2.56	1.04	Negative

Animal Number	Time Point (Day)	Group	ECL	Log (ECL)	Ratio of Log Sample: Log NC	Result
908	-4	10F4 IV	212	2.33	0.95	Negative
	35	-	118333	5.07	2.07	Potentially Positive
	49	-	152675	5.18	2.12	Potentially Positive
	63	-	116957	5.07	2.07	Potentially Positive
	70	-	110409	5.04	2.06	Potentially Positive
909	-4	10F4 IV	294	2.47	1.01	Negative
	35	-	28899	4.46	1.82	Potentially Positive
	49	-	48511	4.69	1.91	Potentially Positive
	63	-	30859	4.49	1.83	Potentially Positive
	70	-	25072	4.40	1.80	Potentially Positive
910	-4	10F4 IV	273	2.44	0.99	Negative
	35	-	155824	5.19	2.12	Potentially Positive
	49	-	131694	5.12	2.09	Potentially Positive
910	63	10F4 IV	119157	5.08	2.07	Potentially Positive
	70	-	No Sample	No Sample	No Sample	No Sample
911	-4	10F4 IV	295	2.47	1.01	Negative
	35	-	170	2.23	0.91	Negative
	49	-	295	2.47	1.01	Negative
	63	-	246	2.39	0.98	Negative
	70	-	295	2.47	1.01	Negative

The following conclusions were made from the two *In vivo* pharmacology experiments:

- Belimumab administered IV or IP every 14 days for 4 weeks reduces the number of peripheral blood B-cells in mice. There was a similar reduction in B cell percentage in 10F4 treated mice; however, the reduction persisted longer compared with that observed in belimumab treated mice.
- Total WBC, CD4+ T cell percentage, and CD8+ T cell percentage were not significantly different between belimumab-treated animals and control animals.
- Belimumab induced a neutralizing antibody response in treated mice as evidenced by very high ECL counts in the belimumab immunogenicity assay accompanied by the absence of measurable belimumab concentrations in the PK assay. The rapid reversal of B cell reductions after belimumab treatment was stopped, as compared with a delayed reversal of B cell reductions in 10F4-treated mice to further support this conclusion.
- 10F4 also induces an anti-10F4 antibody response, as evidenced by altered PK and high ECL counts in the 10F4 immunogenicity assay in some mice. However, the rate of immunogenicity in mice administered 10F4 was lower than that observed after administration of belimumab.
- Belimumab causes death in some mice that may be the result of an anaphylactoid-like response since following repeated administration many mice appeared to be in respiratory distress. In Study 1, 6/10 mice died after a 2nd IV dose of belimumab (10 mg/kg) and in Study 2, where less belimumab was administered (5 mg/kg) 2/10 mice died after the 2nd IV dose of belimumab at Day 14, and 1/8 died after the 3rd dose at Day 28, while the rest recovered and survived to Day 70. No animals in the belimumab IP group of Study 2 died. In Study 1, two 10F4 treated animals died after the 2nd injection and in Study 2, none of the 10F4 treated animals died although these animals also developed anti-10F4 antibody responses. However, the immunogenic response induced by 10F4 was less robust than that observed after administration of belimumab, which is likely to be the reason for the difference in death rate between mice administered 10F4 and those administered belimumab.
- Given the strong neutralizing immunogenic response observed in mice after administration of belimumab resulting in clearance of belimumab and in some cases death, the mouse is not an appropriate species for repeat-dose pharmacology and/or toxicology studies of belimumab.

4.2 Secondary Pharmacology

Study Title: Cross Reactivity of anti-BLyS antibody with human and Cynomolgus monkey Tissue Ex Vivo, (b) (4) study #00-116

Key study findings:

- No specific staining was observed with the antiBLyS antibody in any of the human tissues. Minimal tissue staining was observed with human IgG in some tissues, similar staining was obtained in controls, this staining was considered as background stain.
- No specific staining was observed with the antiBLyS antibody in any of the Cynomolgus monkey tissues except the zymogen granules in the acinar cells. These findings were note with both concentrations of the antibody (2 and 10 µg/mL).
- Tissues cross reactivity studies in nonclinical species are considered to have limited value and therefore are not generally recommended. However, tissue cross-reactivity data with human tissues can provide useful information to supplement knowledge of target distribution and can provide information on unexpected epitope binding. [Addendum to ICH S6: Preclinical Safety Evaluation of biotechnology-derived pharmaceuticals S6 (R1) \\cdsnas\pharmtox2\files\guidances\ichs6r1.pdf]

Study no.: 00-116

Volume # and page #: 1, page 1-117

Conducting laboratory and location: (b) (4)

Date of study initiation: December 5, 2000

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: LymphoStat-B (belimumab), Lot # 00A06019, 99% pure; placebo Lot #01P06020 and #01P06040; supplied as liquid 5.1 mL/vial. Each test article vial contained the test article LymphoStat-B (belimumab), 22 mg/mL in 1.9% glycerin, 0.5% sucrose, 10 mM sodium citrate, and 0.01% Tween 80, pH 7.1

Methods: Tissues from human and Cynomolgus monkeys were tested for cross reactivity, in addition HEK cells transfected with full length human BLyS, mutant BLyS (positive control to block cell surface cleavage), and without BLyS expressed (negative control were also tested).

Human tissues listed below (3 donors) were collected from (b) (4)

(b) (4) Tissues (listed below) from Cynomolgus monkey (2 animals) obtained from the (b) (4) tissue bank were also tested.

The tissues from human and Cynomolgus monkeys were collected as surgical or autopsy specimens and frozen in liquid N₂ until processed. Unfixed tissues were

sectioned in the cryostat (7 μ m), mounted in slides, subjected to immunohistology using streptavidin-peroxidase labeling, and then stained by DAB. The slides were observed under light microscope and graded for positive binding using a scale from 1-4 (1 indicating rare staining, 4 indicating dense staining). For human tissues antiBLYS antibody concentrations used were 2, 10, 50, and 225 mcg/mL. All tissues from the Cynomolgus monkeys were tested with the antibody concentrations of 2 and 10 mcg/mL. The thyroid tissue from the monkeys was also stained with the antibody concentrations of 50 and 225 mcg/mL. Human lymphoid tissue was stained with the alkaline phosphatase system to alleviate macrophage staining.

Results: Validation of the integrity of the tissue sample was conducted by using CD71 (transferrin receptor which is expressed in all tissues). All tissues stained for CD71 indicating that the quality of the tissues was acceptable. The antiBLYS antibody was tested in the cell lines by immunohistology. The positive control cells were stained with the dark brown staining indicating the integrity of the antibody. No specific staining was observed with the antiBLYS antibody in any of the human tissue. Minimal tissue staining was observed with human IgG in some tissues. Similar staining was obtained in controls. This staining was considered as background stain.

No specific staining was observed with the antiBLYS antibody in any of the Cynomolgus monkey tissues except the zymogen granules in the acinar cells. These findings were observed with both concentrations of the antibody (2 and 10 mcg/mL). The gradation of dark staining was 1+ for low concentration and 4+ in the high concentration. Cervical epithelium of the monkeys was also found positive for anti BLYS (2+) at higher concentration. Minimal tissue staining was observed with human IgG in some tissues. Similar staining was obtained in controls. This staining was considered as background stain. The biological significance of the positive tissue cross reactivity in monkey is not known.

4.3 Safety Pharmacology

There was no safety pharmacology study report with this submission. Safety pharmacology assessments, however, were included in the two pivotal GLP toxicology studies in Cynomolgus monkeys: a 4-week (study report 1044-95) and a 3 and 6-month study (with 8-month recovery, study report 1177-95). The safety pharmacology parameters evaluated in these studies included electrocardiograms (ECGs, leads I, II, III, aVR, aVL, and aVF) and urinalysis. No abnormal findings were noted in the ECG and urinalysis parameters. No formal neurobehavioral or respiratory endpoints were monitored in the toxicology studies, however, no belimumab related changes in general behavior, clinical observations, or respiration was noted in any of the three monkey GLP studies during the cage side observations.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Several pharmacokinetics/toxicokinetics analyses of belimumab and antiproduct antibody analyses were conducted in the single and repeat dose toxicity studies. Table # 18 (reproduced from the Applicant) showed a list all of the non clinical PK studies conducted with belimumab.

Table 18 List of Nonclinical Pharmacokinetic Studies

Study Type and Duration	Formulation ¹	Route of Administration	Species
Single-dose PK			
Report HG19399.SLE.0.008 (Report 1065-95, in life portion only Report HG19399.SLE.0.014, immunogenicity)	06-A	IV	Cynomolgus Monkey
Report HG19399.SLE.0.029 Formulation Comparability	06-A	IV	Cynomolgus Monkey
Report HG19399.SLE.0.036 SC Bioavailability	06-A, 06-B	IV	Cynomolgus Monkey
Report HG19399.SLE.0.038	06-B, 06-C	IV, SC	Balb/c mice
Report HG19399.SLE.0.028	06-A	IV, SC	Cynomolgus Monkey
Report HG19399.SLE.0.039	06-C	IV, SC	Cynomolgus Monkey
Repeated-dose PK in Toxicology Studies			
Report 1044-95 Weekly dosing for 4 weeks	06-A	IV	Cynomolgus Monkey
Report 1177-95 Every other week dosing for 26 weeks HG19399.SLE.0.054 (PK simulation)	06-A	IV	Cynomolgus Monkey
Report 1721-95 Reproductive Toxicology Every other week dosing during gestation after confirmed pregnancy	06-A	IV	Cynomolgus Monkey
Other Studies			
Report 6962-161 22-week immunogenicity 2 or 4 injections/week for 13 weeks	06-B	SC	Cynomolgus Monkey
Report 6962-157 Local Tolerance Single dose or 4 doses every other day	06-B	SC	Cynomolgus Monkey

NA – Not Applicable

¹ Formulations 06-A and 06-B of lyophilized belimumab FDP are described briefly below and in Section 2.4. The PK study demonstrating the PK comparability of formulations 06-A and 06-B is presented in Section 2.6.4.3.1.3. Formulation 06-C is a liquid formulation of belimumab developed for SC administration.

(b) (4)

(b) (4). The new formulation was tested in Cynomolgus monkey and compared with the previous formulation. No changes in the serum concentration of the belimumab were noted. The Applicant is using the new formulation in the clinical studies. The table #19 (reproduced from Applicant's submission) showed a list of different formulations used in non clinical studies.

Table 19 Belimumab Formulations Used in Nonclinical Studies

¹ Except for the PK bioavailability study in cynomolgus monkeys (HG19399.SLE.0.036), nonclinical studies using formulation (b) (4)

² Figures in parentheses reflect the formulation proposed for marketing; both result in 10 mM total sodium citrate.

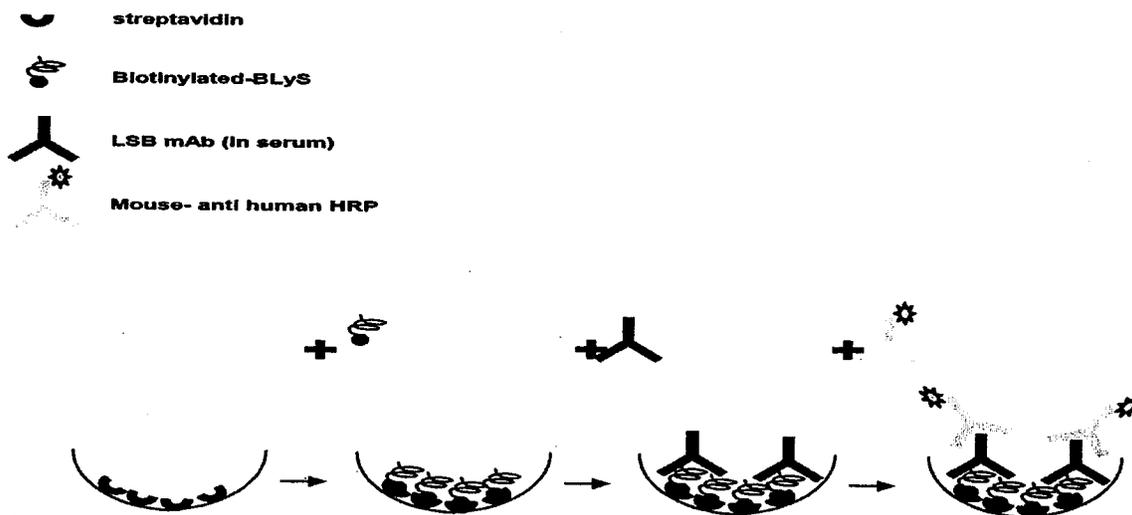
ELISAs were developed to quantify belimumab and its antibodies from the serum of the Cynomolgus monkeys. A brief description of the ELISA developed for the PK/TK analyses of the belimumab from the general toxicity study are described herein.

Study Title: Qualification of the ELISA for Quantization LymphoStat-B Monoclonal Antibody in Cynomolgus Serum (SOP 01ILS-PH-06-2199); Study Number: HG19399.SLE.0.024

Belimumab plasma levels from Cynomolgus monkey were assayed in the PK/TK assay. A micro titer plate was coated with streptavidin and biotinylated BLYS and then added to the plate. The belimumab mAb in Cynomolgus serum reactive with BLYS was captured from the diluted serum onto the BLYS-coated plate. The captured mAb was detected by the addition of a peroxidase-conjugated secondary mouse monoclonal anti-human IgG (Fc) antibody. The peroxidase activity was then quantitated by the color conversion of the 3,3', 5,5' tetramethylbenzidine (TMB) substrate in the presence of hydrogen peroxide. The absorbance was measured at 450 nm after stopping the reaction with

dilute acid. A schematic representation of the assay is shown as follows (Applicant's table):

Figure 33 Schematic Representation of the Assay



The assay was qualified for LOD, linearity, precision and range. The summary of qualification parameters and acceptance criteria, set up based on the qualification results, are presented in the summary table.

Table 20 Summary of PK Qualification Parameters

<u>Parameter</u>	<u>Section of the Report Standard Curve</u>	<u>Qualification Result</u>
LOD	7.2.1	<0.137 ng/mL
LLOQ on the plate	7.2.2	0.250 ng/mL
HLOQ on the plate	7.2.2	20 ng/mL
Linearity	7.2.3	(R ² of 0.9991 over the linear range)
Range	7.2.4	0.250 - 20 ng/mL
Matrix Effects		
Maximum serum concentration	7.3.2	1.0%
Theoretical LLOQ in undiluted serum ^a	7.4	25 ng/mL
Tested LLOQ in undiluted serum ^b	7.5	200 ng/mL
Background variation	7.4	72/72 samples were below LLOQ
Sample Analysis		
Precision: ^c	7.5.1	
Repeatability		10.21%
Intermediate, day-to-day		11.88%
Intermediate, analyst-to-analyst		16.94%
Range of the assay (in neat serum)	7.5	0.2 - 4000 ug/mL
Accuracy	7.5.2	99.12 to 115.11%
Linearity (spiked serum)	7.5.3	R ² value of 1.000 over the linear range

^aTheoretical LLOQ in undiluted serum was determined by multiplying the LLOQ on the plate by the lowest dilution factor (0.250 ng/mL x 100).
^bTested LLOQ in undiluted serum was based on the lowest concentration of LSB spiked into cynomolgus serum for which acceptable precision and accuracy were obtained.
^cMaximum CV observed.

Note an ELISA methodology was also provided (Study reports # tr-06-05-029, 002, 005,009) which had been modified to accommodate the estimation of belimumab from the placental fluid, or different concentrations of belimumab in serum. All of the methodology was based on the same principle described with study #024. Therefore, the detail of the studies is not described herewith.

Study Title: Qualification of the LymphoStat B Neutralization Assay in Cynomolgus Monkey Serum (SOP 01TRF-PH-06-2211); Study Number: HG19399.SLE.0.030

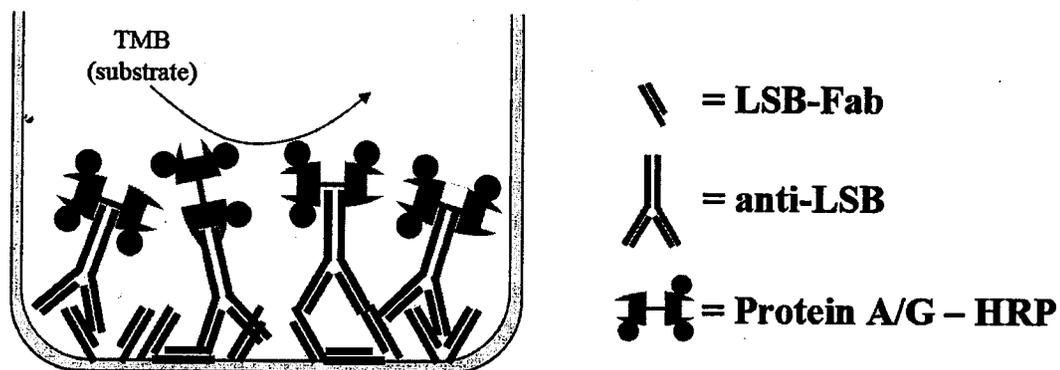
The reviewer observed that the Applicant tried to establish a methodology following the SOP mentioned above. However, the methodology could not be used due to higher than 20% artifact formed in this method. Therefore, the study is not documented in this review.

Study Title: Qualification of a LymphoStat-B (LSB) Immunogenicity Assay (SOP 01ELM-PH-06-2269) as a Means of Detecting anti-LSB Antibodies in Cynomolgus Macaque Sera, Study Number: HG19399.SLE.0.032

The binding assay was performed in individual wells of 96-well plates coated with 100 μL of a 0.5 $\mu\text{g}/\text{mL}$ solution of LSB-Fab in PBS for 14-24 hours at 2-8°C. The coating solution was removed and residual non-specific binding sites blocked by addition of 200 μL of blocking solution (PBS containing 2.0% Casein and 3% BSA) for 2-4 hours at 22°C. Plates were washed with wash buffer (PBS containing 0.1% Tween 20). Duplicate 100 μL aliquots of sample diluent (PBS containing 0.1% Tween 20, 1% Casein and 1.5% BSA), negative control (pooled normal Cynomolgus macaque sera, NCMS), positive control (affinity purified rabbit anti-LSB polyclonal antibody), or diluted individual serum samples were added to each plate. After a 2-hour incubation at 37°C, plates were washed 4 times with wash buffer. One hundred microliters of Horseradish Peroxidase-Protein A/G conjugate (Protein A/G-HRP) (1:10000 dilution) was then added to each well and plates were incubated at 22°C for 1 hour. After removal of conjugate and a total of 4 washes with wash buffer, 100 μL of 3,3',5,5' - tetramethylbenzidine (TMB) substrate solution (KPL) was added to each well. Plates were incubated for 12 minutes at room temperature and the reaction was stopped by addition of 50 μL per well of 1N H₂SO₄. The absorption at 450 nm was determined using a SpectraMax spectrophotometer (Molecular Devices).

The assessment of the ability of LSB-Fab-binding sera to bind LSB-Fab in the presence of excess soluble LSB, was performed exactly like the binding assay described above, with the exception that controls and serum samples were added to LSB-Fab coated plates in the presence of 100 $\mu\text{g}/\text{mL}$ of LSB.

Figure 34 Schematic Representation of Immunogenicity Assay A (direct binding)



The experimental and statistical data provided here qualifies the belimumab immunogenicity assay (SOP 01ELM-PH-06-2269) as a means of detecting anti-LSB antibodies in Cynomolgus serum. A summary of the critical parameters is presented (refer to Applicant's table).

Table 21 Summary of Qualification Parameters from Immunogenicity Assay

<u>Parameter</u>		<u>Report Section</u>	<u>Qualification Result</u>
LOD	Actual LOD	5.1.1	0.125 µg/mL
Precision ^a	Overall ^b	5.1.2	12.0% - 17.5%
	Plate-to-plate ^b	5.1.2	8.8% - 16.6%
	Day-to-day ^b	5.1.2	3.6% - 9.9%
	Analyst-to-analyst ^b	5.1.2	0% - 7.7%
Linearity		5.1.3	Linear 0 – 2.0 µg/mL
Specificity of positive control antisera		5.1.4	Tested up to 2.0 µg/mL of irrelevant antibody with no binding
Drug ^a interference	Actual LOD	5.1.5	Optimal up to 0.1 µg/mL LSB; marginal effect up to 1.0 µg/mL
Specificity of drug interference ^a		5.1.6	Tested up to 300 µg/mL of irrelevant inhibitor; inhibition relative to the control plate was within the plate-to-plate precision of the assay.
Cut-point titer	Normal Cynomolgous macaque serum	5.3	54

^aIn pooled serum^bRange

A brief documentation and analyses of each of the single dose pharmacokinetic studies submitted by the Applicant are provided as follows.

A Single Dose Pharmacokinetic Study of BLyS Antagonist Administered by Intravenous Injection to Cynomolgus Monkeys; Study Number: HG19399 SLE.0.008, HG19399 SLE.0.014, HG19399-T03

Cynomolgus monkeys (2.6-7.7 years of age and 2.3-3.6 kg in weight) 2/sex/group were administered with 5 and 50 mcg/kg of belimumab (22 mg/mL) formulated in 06-A via slow bolus administration for 30 mins (non GLP study). Anti product antibody formation was noted in 3/8 animals from the high dose group. Serum samples for PK and immunogenicity analysis were obtained prior to dose administration on Day 1 and at approximately 5 minutes and 4 and 8 hours after dosing, and on Days 2, 3, 5, 8, 15, 22, 29, 36, 50, and 64. PK parameters were calculated using a 2-compartment PK model. Two of the monkeys were weakly positive with anti product antibody formation; one monkey was strongly positive for anti-A01 antibodies, and A01 was undetectable by ELISA in the serum of this monkey after Day 15. The PK of belimumab was biphasic with the β elimination phase contributing approximately 95% of the total area under the curve (AUC). CL, V_i , V_{ss} , half-life of the alpha and beta phases ($t_{1/2\alpha}$, $t_{1/2\beta}$), and mean residence time (MRT) were independent of dose, whereas AUC and maximum serum drug concentration (C_{max}) were proportional to dose. No gender differences

were observed. The clearance of belimumab was observed to be slow, 5.6 mL/day/kg, with a small V_{ss} of 85-108 mL/kg. The terminal half-life of belimumab in cynomolgus monkeys was long, between 11 and 14 days, and the MRT was 15-19 days, demonstrating that the effective half-life was similar to the terminal elimination half-life.

Table 22 PK of Belimumab in Cynomolgus Monkeys (Single IV 5, 50 mg/kg)

<u>Parameter</u>	<u>5 mg/kg</u>	<u>50 mg/kg^a</u>	<u>P value</u>
n	4	3	
AUC (day*µg/ml)	901 ± 40	8970 ± 320	< 0.0001 *
C _{max} (µg/ml)	111 ± 1.8	1230 ± 180	0.0007 *
t _{1/2α} (day)	0.522 ± 0.13	0.436 ± 0.2	0.7235
t _{1/2β} (day)	11.2 ± 0.7	14 ± 1.8	0.1528
CL (ml/day/kg)	5.6 ± 0.2	5.6 ± 0.2	0.9753
V _i (ml/kg)	45.0 ± 0.7	42.4 ± 5.9	0.6286
V _{ss} (ml/kg)	85.0 ± 2.1	108 ± 14	0.1260
MRT (day)	15.4 ± 1.0	19.1 ± 2.1	0.1387

* denotes a significant p value at the 5% level of significance.

^a Animal number FN11208F was not included in the pharmacokinetic analysis due to a significant drop in serum levels of A01 after Day 8

Study Title: Pharmacokinetics of LymphoStat-B in Female Cynomolgus Monkeys Following a Single 150 mg/kg Intravenous Dose; Study number: HG19399.SLE. 0.029

Cynomolgus monkeys (non GLP study) 3 females/group (weighing 2.7-3.3 kg) were administered 150 mcg/kg of belimumab (22 mg/mL) formulated in 06-A via slow bolus IV administration for 5 mins (formulation 06-A). Serum samples for PK analysis were obtained prior to dose administration and approximately 5 minutes and 4, 8, and 24 hours after dosing on Day 1 and on Days 2, 4, 7, 14, 21, 28, 35, 49 and 63. Following IV administrations, serum belimumab concentrations declined in a multi-exponential manner with a mean t_{1/2} terminal of 11 days. CL was 6.8 mL/day/kg and V_{ss} was 90 mL/kg. The MRT was 14 days.

Table 23 Belimumab's PK in Cynomolgus Monkey (Single IV, 150 mg/kg)

Parameters	Animal ID			Mean ± SD
	C03936 ^a	C04734 ^b	C04743 ^c	
C _{max} (µg/mL)	4152	3628	3555	3778 ± 326
AUC _{0-∞} (day•µg/mL)	24621	18104	25352	22693 ± 3990
%AUC _{term}	86.19	49.90	87.01	74.36 ± 21.19
V ₁ (mL/kg)	35.93	41.09	42.17	39.73 ± 3.33
V _{ss} (mL/kg)	102.92	82.78	83.75	89.82 ± 11.36
CL (mL/day/kg)	6.09	8.29	5.92	6.76 ± 1.32
t _{1/2, 1} (day)	0.11	0.08	1.05	0.41 ± 0.55
t _{1/2, 2} (day)	1.83	3.89	N/A	2.86
t _{1/2, term} (day)	13.32	10.04	11.12	11.49 ± 1.67
MRT (day)	16.89	9.99	14.16	13.68 ± 3.48

^aSerum concentration data were fitted using a 3-compartment IV infusion model and a weighting scheme of $1/p$.

^bSerum concentration data were fitted using a 3-compartment IV infusion model and a weighting scheme of $1/p^2$.

^cSerum concentration data were fitted using a 2-compartment IV infusion model and a weighting scheme of $1/p^2$.

Study Title: Comparability Assessment of 2 LymphoStat-B Formulations Following a Single Intravenous Administration to Monkeys; Study Number: HG19399.SLE.0.036

Cynomolgus monkeys (8/sex/group) were administered (non GLP) intravenously 10 mg/kg of formulation 06-A and 06-B. Serum samples for PK analysis were collected prior to dosing and at 5 minutes, 4 hours, and 8 hours post-dose as well as at 1, 2, 4, 7, 14, 21, 28, 35, 49 and 63 days post-dose. Samples collected pre-dose and at 28 and 63 days post-dose were designated to be assessed for immunogenicity. There were no differences in the PK parameters using different formulations. The 90% confidence intervals for the ratios of the geometric means for formulation 06-A/06-B for C_{max} and AUC₀₋₈ fell within 80% to 125% demonstrating equivalent PK for the 2 formulations.

In this experiment, approximately 50% of samples from Day 63 could be evaluated for anti-belimumab antibodies. One monkey that received formulation 06-A tested positive for treatment emergent anti-belimumab antibodies at Day 63, this response was associated with reduced belimumab concentrations.

Table 24 PK of Belimumab in Cynomolgus Monkeys (single 10 mg/kg IV)

<u>Parameters</u>	<u>Phase 2 Formulation (n = 17)^{a,c}</u>	<u>Phase 3 Formulation (n = 18)^a</u>	<u>Ratio of Mean (90% Confidence Interval)^b</u>	<u>P Value^{b,d}</u>
C _{max} (µg/mL)	284.8	269.8	94.8% (86.4%-104.0%)	0.3289 (NS)
AUC _{0-∞} (day•µg /mL)	1641	1670	101.7% (90.1%-114.8%)	0.8118 (NS)
V _{ss} (mL/kg)	66.2	65.7	99.3% (92.2%-107.2%)	0.8803 (NS)
V _z (mL/kg)	56.2	61.7	109.7% (99.4%-121.1%)	0.1310 (NS)
CL (mL/day/kg)	6.13	5.97	97.3% (86.1%-109.9%)	0.7048 (NS)
t _{1/2, term} (day)	6.36	7.17	112.8% (97.8%-130.0%)	0.1614 (NS)
MRT (day)	10.8	11.0	102.1% (93.6%-111.2%)	0.6867 (NS)

^aData from Appendix 6 and Appendix 7.

^bData from Attachment 4.

^cMonkey #C13185 was anti-LymphoStat-B antibody positive on Day 63 post dose and had apparently altered PK. Therefore, it was excluded from the statistical analysis.

^dP value for the test that the difference between the 2 groups equals 0.

NS Not statistically significant.

Study Title: Pharmacokinetics of LymphoStat-B in BALB/c Mice Following a Single Intravenous or Subcutaneous Administration; Study Number HG19399.SLE.0.038

BALB/c mice 2-3 males/group were administered with a single intravenous (bolus) or subcutaneous administration of 10 mg/kg belimumab formulation 06-B or 06-C (non GLP study). The serum concentrations and absolute bioavailability of belimumab were similar after the administration of the two different formulations. The t_{1/2} terminal of belimumab in mice was about 6 days, CL was 10 to 12 mL/day/kg, and V_{ss} was 90 to 100 mL/kg. C_{max} was reached 1.1 days after dose administration with 06-C (liquid) and the absolute bioavailability was 89%. C_{max} was reached at 1.8 days after dose administration (06-B-lyophilised) formulation) and the absolute bioavailability was 96%. The study design and summary data are provided in the following tables.

Table 25 Study Design: Murine PK

Group No.	Treatment	Formulation	Route	No. Animals/ Timepoint	Dose Level mg/kg	Sampling Schedule
1	Belimumab	Lyophilized (06-B)	IV	3	10	5 min, 6h, 1, 2, 4, 7, 10, 14, 21 days
2	Belimumab	Lyophilized (06-B)	SC	3	10	30 min, 6h, 1, 2, 3, 4, 7, 10, 14, 21 days
3	Belimumab	Liquid (06-C)	IV	3	10	5 min, 6h, 1, 2, 4, 7, 10, 14, 21 days
4	Belimumab	Liquid (06-C)	SC	3	10	30 min, 6h, 1, 2, 3, 4, 7, 10, 14, 21 days
5	Vehicle Control	Lyophilized (06-B)	IV	2	0	5 min
6	Vehicle Control	Lyophilized (06-B)	SC	2	0	30 min
7	Vehicle Control	Liquid (06-C)	IV	2	0	5 min
8	Vehicle Control	Liquid (06-C)	SC	2	0	30 min

Table 26 Pharmacokinetics of Belimumab in Mice

Parameters	Phase 3 Liquid Formulation ^a		Phase 3 Lyophilized Formulation ^b	
	IV ^c	SC ^d	IV ^c	SC ^d
C _{max} (µg/mL)	196 ± 11	108 ± 4	226 ± 12	128 ± 5
t _{max} (day)	0	1.10 ± 0.11	0	1.82 ± 0.16
AUC _{0-∞} (day•µg/mL)	1085 ± 92	970 ± 54	1289 ± 100	1236 ± 78
CL or CL/F (mL/day/kg)	11.8 ± 1.0	13.2 ± 0.7	10.3 ± 0.8	10.8 ± 0.7
V ₁ (mL/kg)	65.4 ± 3.8	NA	59.1 ± 3.2	NA
V _{ss} or V _{ss} /F (mL/kg)	100 ± 8	103 ± 5	89.9 ± 6.2	81.7 ± 5
t _{1/2,abs} (day)	NA	0.231 ± 0.033	NA	0.478 ± 0.074
t _{1/2,α} (day)	0.277 ± 0.148	NA	0.268 ± 0.134	NA
t _{1/2,term} (day)	6.05 ± 0.83	5.42 ± 0.44	6.17 ± 0.77	5.25 ± 0.54
MRT (day)	8.50 ± 1.10	NA	8.69 ± 1.03	NA
F (%)	NA	89.4	NA	95.9

^aActual dose was 12.80 mg/kg and determined by ELISA assay.

^bActual dose was 13.34 mg/kg and determined by ELISA assay.

^cSerum concentration data were fitted using a 2-compartment model and a weighting scheme of 1/p^{0.5}.

^dSerum concentration data were fitted using a 1-compartment model and a weighting scheme of 1/p^{0.5}.

NA: Not applicable.

Study Title: Pharmacokinetics of LymphoStat-B in Cynomolgus Monkeys Following a Single Intravenous or Subcutaneous Administration of Liquid Formulation; Study Number: HG19399.SLE.0.039

Study Title: Pharmacokinetics of LymphoStat-B in Cynomolgus Monkeys Following a Single Intravenous and Subcutaneous Administration; Study Number HG19399.SLE.0.028

Both of the studies under the above mentioned study titles were conducted to evaluate lyophilized (06-A) or liquid (06-C) formulation of belimumab in monkeys (non GLP study). Cynomolgus monkeys, 4 sex/group, were administered a single dose of belimumab IV or SC in a parallel design, at either 10 or 30 mg/kg in Study HG19399.SLE.0.039. Serum samples for PK analysis were collected prior to dosing and post-dose at 5 minutes (IV only), 30 minutes (SC only), 1 hour (SC only), 2 hours (SC only), 4 hours, and 8 hours post-dose as well as at 1, 2, 3 (SC only for the liquid formulation), 4, 7, 14, 21, 28, 35, 49 and 63 days post-dose. Immunogenicity was not assessed in this study because the serum samples at designated time-points had belimumab concentrations higher than 400 ng/mL.

The IV administration of 06-A (lyophilized formulation) resulted in the mean $t_{1/2}$, term of belimumab of 7.76 days, CL of 6.25 mL/day/kg and V_{ss} of 70.0 mL/kg. The C_{max} after SC administration was reached at 2 days after dose administration and the absolute bioavailability was 79.0%.

The IV administration of 06-C (liquid formulation) resulted in the mean $t_{1/2}$, term of LymphoStat-B in cynomolgus monkeys of 8.51 days, CL of 6.35 mL/day/kg and V_{ss} of 72.9 mL/kg. Following SC administration, the C_{max} was reached at 3 days after dose administration and the absolute bioavailability was 92.12%. The mean serum concentrations of belimumab at all time points following IV administration were generally similar to or higher than those at the corresponding time point observed following SC administration in both of these studies.

Table 27 PK of Belimumab in Cynomolgus Monkeys (Single IV or SC, 30 mg/kg)

<u>Parameters</u>	<u>IV (n = 4)</u>	<u>SC (n = 3)^a</u>
t _{max} (day)	0.00347 ± 0.00000	3.33 ± 0.58
C _{max} (µg/mL)	852.2 ± 140.3	280.9 ± 55.4
AUC _{0-∞} (day•µg/mL)	4807 ± 505	4421 ± 595
AUC _{0-∞} /D (day•kg/mL)	0.1587 ± 0.0166	0.1462 ± 0.0194
V _{ss} (mL/kg)	72.9 ± 6.0	NA
V _z or V _z /F (mL/kg)	77.2 ± 13.9	108.7 ± 20.2
CL or CL/F (mL/day/kg)	6.35 ± 0.64	6.92 ± 0.86
t _{1/2, term} (day)	8.51 ± 1.93	10.85 ± 1.02
MRT _{IV} or MRT _{SC} (day)	11.53 ± 1.21	14.53 ± 0.90
F (%)	NA	92.12

^aMonkey #C09330 had a positive neutralizing anti-LymphoStat-B antibody response on Day 63 post dose, with apparently altered PK. Therefore, it was excluded from the descriptive statistic calculations.

Table 28 PK of Belimumab in Cynomolgus Monkeys (Single IV or SC, 10 mg/kg)

<u>IV Administration Parameters</u>	<u>Animal ID</u>				<u>Mean ± SD</u>
	<u>C01488</u>	<u>C01551</u>	<u>C02951</u>	<u>C03178</u>	
C _{max} (µg/mL) ^a	249.6	235.1	318.5	333.7	284.2 ± 49.1
T _{max} (day) ^a	0.00347	0.00347	0.00347	0.00347	0.00347 ± 0
AUC _{0-∞} (day•µg/mL)	1591	1531	1592	1689	1601 ± 65
V _{ss} (mL/kg)	72.7	64.8	77.7	64.8	70.0 ± 6.3
V _z (mL/kg)	71.6	65.6	81.3	61.7	70.0 ± 8.5
CL (mL/day/kg)	6.29	6.53	6.28	5.92	6.25 ± 0.25
t _{1/2, term} (day)	7.90	6.96	8.97	7.22	7.76 ± 0.90
MRT _{IV} (day)	11.6	9.93	12.4	10.9	11.2 ± 1.0

^aC_{max} and T_{max} for IV dosing were the value at the 1st sampling point.

<u>SC Administration Parameters</u>	<u>Animal ID</u>				<u>Mean ± SD</u>
	<u>00A002</u>	<u>C01285</u>	<u>C01311</u>	<u>C03199</u>	
C _{max} (µg/mL)	96.6	87.8	74.8	105.0	91.1 ± 12.9
T _{max} (day)	1.00	4.00	2.01	2.01	2.26 ± 1.26
AUC _{0-∞} (day•µg/mL)	1327	1363	1022	1344	1264 ± 162
V _z /F (mL/kg)	83.2	88.7	98.5	96.7	91.8 ± 7.1
CL/F (mL/day/kg)	7.54	7.34	9.79	7.44	8.03 ± 1.18
t _{1/2, term} (day)	7.65	8.38	6.98	9.01	8.00 ± 0.88
MRT _{SC} (day)	12.1	13.5	11.5	14.2	12.8 ± 1.3
F (%)	-	-	-	-	79.0

5.2 Toxicokinetics

The toxicokinetics analysis of belimumab was conducted with the repeat dose toxicity studies, therefore the studies were reviewed under the general toxicity section

The PK parameters for all single dose PK studies are dose proportional across the 5 to 150 mg/kg dose range after IV administration (refer to Applicant's summary table). The Applicant showed that the mean V_i in these studies ranged from 40-45 mL/kg, which is close to the plasma volume (45 mL/kg, Davies and Morris, 1993). The value for V_{ss} , ranging from 67-108 mL/kg, compared to the extracellular fluid volume (~170-210 mL/kg including plasma; Levine, 1990; Davies and Morris, 1993) may suggest that belimumab distribution is restricted to a space smaller than the extracellular fluid volume. Mean clearance values (ranging from 5.6-6.8 mL/day/kg of belimumab) are substantially less than the glomerular filtration rate for cynomolgus monkeys (~3000 mL/day/kg; Schaer et al, 1990; Davies and Morris, 1993), as expected for a large molecule such as an antibody (Gobburu et al, 1998). Based on these studies, the mean terminal half-life of belimumab is approximately 7-14 days.

Table 29 PK Parameters for Belimumab in Cynomolgus Monkeys (Single IV)

Dose (mg/kg)		C _{max} /Dose (kg/mL)	AUC/Dose (kg·day/mL)	V _i (mL/kg)	V _{ss} (mL/kg)	CL (mL/day/kg)	t _{1/2 term} (day)	MRT (day)
5 ¹	Mean	0.0222	0.180	45.00	85.05	5.58	11.16	15.36
	SD	0.0007	0.016	1.43	4.21	0.48	1.36	2.03
	95% CI Lower	0.0211	0.155	42.72	78.34	4.82	9.00	12.14
	95% CI Upper	0.0234	0.206	47.27	91.75	6.35	13.32	18.57
10 ²	Mean	0.0280	0.168	-	66.84	6.19	7.05	11.04
	SD	0.0043	0.033	-	8.71	1.28	1.65	1.53
	95% CI Lower	0.0267	0.157	-	64.02	5.77	6.52	10.54
	95% CI Upper	0.0294	0.179	-	69.66	6.60	7.59	11.54
30 ³	Mean	0.0281	0.159	-	72.90	6.35	8.51	11.53
	SD	0.0047	0.017	-	5.97	0.65	1.93	1.21
	95% CI Lower	0.0207	0.132	-	63.39	5.33	5.44	9.61
	95% CI Upper	0.0356	0.185	-	82.41	7.38	11.58	13.45
50 ¹	Mean	0.0246	0.179	42.41	107.55	5.59	14.01	19.14
	SD	0.0063	0.011	10.25	24.86	0.36	3.08	3.69
	95% CI Lower	0.0090	0.152	16.95	45.80	4.70	6.35	9.97
	95% CI Upper	0.0402	0.207	67.87	169.30	6.48	21.66	28.31
150 ⁴	Mean	0.0252	0.152	39.73	89.82	6.76	11.49	13.68
	SD	0.0022	0.025	3.33	11.36	1.32	1.67	3.48
	95% CI Lower	0.0198	0.091	31.45	61.60	3.49	7.35	5.05
	95% CI Upper	0.0306	0.214	48.01	118.04	10.04	15.64	22.31

¹ Report 1065-95/ HG19399.SLE.0.008.

² Reports HG19399.SLE.0.028 and HG19399.SLE.0.036.

³ Report HG19399.SLE.0.039.

⁴ Report HG19399.SLE.0.029.

No distribution studies have been conducted with belimumab. The volume of distribution of belimumab calculated in the single dose PK and 4-week repeat dose TK studies indicates that belimumab distribution is restricted to a space smaller than the extracellular fluid volume. This may explain the restricted distribution of belimumab to tissues where the target antigen, BLYS, is not present.

No metabolism or excretion studies have been conducted with belimumab. The elimination of monoclonal antibody drugs is generally through cellular catabolism following nonspecific uptake by pinocytosis (Lobo et al, 2004).

Three toxicology studies were conducted in the Cynomolgus monkey in which they completed a full toxicokinetic evaluation. Two of these studies administered Belimumab intravenously (1 for 4 weeks and 1 for 6-months) and one study administered Belimumab subcutaneously (13-weeks). After 4-week IV administration, Belimumab serum concentrations increased with increased doses. However, these increases were not strictly dose-dependent and the drug accumulated from Day 1 to the end of the dosing phase. In the 6-month IV study, serum concentrations also increased with increased doses. There were no significant gender differences in toxicokinetic

parameters after IV administration. Belimumab is considered to be minimally immunogenic in Cynomolgus monkeys. Across the nonclinical program, 12 belimumab-treated monkeys (out of 124 monkeys who had at least 1 post-treatment sample analyzed) were identified as having detectable, treatment-emergent, anti-belimumab antibodies; 6 of these also had reduced belimumab serum concentrations. The immunogenicity could not always be assessed because the sensitivity of the assays was impacted by the presence of belimumab in serum. Therefore, the low belimumab concentrations were also used as an indirect measure of the presence of neutralizing antibodies. However, the reviewer agreed with the Applicant's conclusion that the likelihood of a significant number of animals with undetected anti-belimumab antibodies is low. Because animals receiving belimumab showed expected pharmacological effects (reduction in B-lymphocytes), and there were no animals with belimumab exposures lower than expected, little anti-drug antibody formation occurred.

The current proposed clinical formulation of belimumab is a lyophilized IV formulation. The lyophilized and liquid formulations were tested in the monkeys after SC administration. There was similar exposure with SC administration of the 25 mg/kg, 2x/week or 3x/week (the only dose studied) in the Cynomolgus monkeys at day 7 and day 8. The PK bridging study with the different formulations was not done beyond Day-8. Following SC administration to cynomolgus monkeys the bioavailability was high, ranging from 79 to 92% and the mean time for drug absorption (MRTSC-MRTIV) was approximately 1.6 and 3 days for the lyophilized and liquid formulations, respectively. The PK profile in humans is similar to that of cynomolgus monkeys and supports the use of cynomolgus monkeys in the toxicology program.

6 General Toxicology

6.1 Single-Dose Toxicity

No single dose toxicity study was submitted.

6.2 Repeat-Dose Toxicity

Study title: Study title: A 4-Week Toxicity Study of BLYS Antagonist Administered by Intravenous Injection to Cynomolgus Monkeys, with a 4-Week Recovery Period

Study no.: (b) (4) Study #-1044-95, HG10399-T04
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: December 12, 2000
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: LymphoStat-B (belimumab), Lot # 00A06019, 99% pure; placebo Lot #01P06020 and #01P06040; supplied as liquid 5.1 mL/vial

Key Study Findings

- The Cynomolgus monkey (5/sex/group) was administered the lyophilized formulation of belimumab IV for 4 weeks (0, 5, 15, 50 mg/kg/week). 3/sex/group were sacrificed Day 29 and 2/sex/group were allowed to recover for an additional 4 weeks and sacrificed Day 57. There was no NOEL for this study due to histopathological lesions.
- The target organs of toxicity included the lymph (mesenteric lymph and GALT in the gastrointestinal tract), and thyroid (follicular degeneration). These findings appear to be associated with the exaggerated pharmacology of the drug product. In addition to these observations there were sporadic observations with increased mononuclear cell infiltration in the kidney, urinary bladder, skin and injection site. The biological significance of these findings is not known.
- B-cell depletion was observed in the peripheral blood and in the spleen and mesenteric lymph node at Day 29. This finding was observed to be similar at recovery (Day 57). There was an increase (statistically significant) in the T-cell subsets in spleen and mesenteric lymph node at Days 29 and 57.
- The serum concentration analyses of the belimumab showed that the concentration of the compound increased with dose, however, the increase was more or less dose proportional [3-fold-increase in dose (5-15 mg/kg) resulted in 4-fold increase in the serum concentration (27 vs 113 µg/mL); similarly a 10-fold increase in dose (5-50 mg/kg) resulted in > 11-fold (27 vs 302 µg/mL increase in the serum concentration)]. Exposure in females was comparable to those in males.
- The toxicokinetics were observed to be biphasic, the majority of the exposure occurred at the beta phase (elimination phase). Clearance, volume of distribution at the initial as well as in the steady state, half life of the alpha and beta phase, and the mean residence time were independent of dose. The terminal half life of the compound after 4-weekly administrations was found to be 14 days and the clearance is approximately 5-7 mL/day/kg. There were about 2-fold accumulation of the compound at Day 29 compared to Day 1 with all doses.

- The drug product showed minimal immunogenicity 1/40 animals had anti-drug antibody (ADA) production.
- The drug product showed statistically significant decreases in IgA and IgG production but not IgM production during the recovery period which appears to be associated with the decrease in B lymphocyte population.
- Due to the low incidence of the findings, the lower degree of the severity of the findings, and that the majority of these observations are due to the expected pharmacological effects of belimumab, the low dose of 5 mg/kg is considered the LOAEL. No NOAEL could be determined from this study. This LOAEL is associated with an AUC of 2868 mcg*day/mL.

Methods

Doses: 0, 5, 15, and 50 mg/kg
 Frequency of dosing: 1x/week for 4-weeks
 Route of administration: IV, slow bolus over 30 secs
 Dose volume: 2.5 mL/ injection
 Formulation/Vehicle: LymphoStat-B (belimumab), 22 mg/mL in 1.9% glycerin, 0.5% sucrose, 10 mM sodium citrate, and 0.01% Tween 80, pH 7.1
 Species/Strain: Cynomolgus monkeys
 Number/Sex/Group: 5/sex/group
 Age: 2.6-7.4 years
 Weight: 1.4-5.7 kg; Females: 2-3.5 kg
 Satellite groups: 2 animals/sex/group were maintained for recovery for 4 weeks, post treatment
 Deviation from study protocol: There was no study deviation that impacted the study outcome.

Table 30 Study Design: 4-Week Repeat Dose in Monkeys

Group No.	Treatment	Number of Animals (M/F)	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Vol. (mL/kg)	Number of Animals Sacrificed at	
						Day 29 (M / F)	Day 57 (M / F)
1	Control (Vehicle / Diluent)	5 / 5	0	0	2.5	3 / 3	2 / 2
2	BLyS Antagonist – Low Dose	5 / 5	5	2	2.5	3 / 3	2 / 2
3	BLyS Antagonist – Mid Dose	5 / 5	15	6	2.5	3 / 3	2 / 2
4	BLyS Antagonist – High Dose	5 / 5	50	20	2.5	3 / 3	2 / 2

Observations and Results

Mortality

Mortality and morbidity checks were performed once a day during all phases of the study. There was no mortality in this study.

Clinical Signs

Cage-side clinical signs (ill health, behavioral changes etc.) were recorded twice daily during the quarantine and pretreatment periods and twice daily during the treatment and recovery periods. A detailed clinical examination of each monkey was performed once at pretreatment, weekly during the treatment and recovery periods and before necropsy. All animals (treated and control) showed low food consumption and watery stools. There were no test article related clinical signs.

Body Weights

Body weights were recorded for all animals approximately two weeks prior to initiation of treatment and on the day prior to treatment (Day - 15). During the treatment and observation periods, body weights were recorded for all animals weekly, as well as terminally prior to necropsy (fasted). All animals gained weight; there were no treatment related differences in the body weight gain.

Feed Consumption

Individual weekly food intake was recorded for all animals during the last week of the pretreatment period and throughout the treatment and recovery periods. There were no treatment related changes in the feed consumption.

Ophthalmoscopy

Fundoscopy (indirect ophthalmoscopy) and bio microscopic (slit lamp) examinations were performed on all animals once during the pre-treatment period, on all animals once at the end of the treatment period, and at the end of the recovery period. There were no treatment related changes in the ophthalmoscopy.

ECG

ECGs were recorded (using Leads I, II, III, aVR, aVL, and aVF) from all monkeys prior to dosing and prior to termination at Week 4. There were no test article related abnormalities in ECG (the ECG reports were peer reviewed by (b) (4))

Hematology

Laboratory investigations (hematology, coagulation) were conducted on all animals at termination. Blood samples were collected by femoral vein puncture at termination. The full battery (refer to table) of the hematological parameters was analyzed. There were no statistically significant meaningful changes in the hematological parameters.

Table 31 Hematological Parameters Assayed in 4-Week Monkey Study

Red blood cell counts	Hemoglobin concentration	Mean corpuscular hemoglobin concentration
White blood cell counts	Mean cell hemoglobin	
Reticulocyte counts	Hematocrit	Abnormal blood cell morphology
Platelet counts	Mean corpuscular volume	
Coagulation Factor		
Activated partial thromboplastin time	Prothrombin time	Fibrinogen

Clinical Chemistry

Laboratory investigations (clinical chemistry) were conducted on all animals at termination. Blood samples were collected by femoral vein puncture at termination. The full batteries (refer to table) of the clinical chemistry parameters were analyzed. There were no statistically significant treatment related changes in the clinical chemistry parameter.

Table 32 Clinical Chemistry Parameters Assayed in 4-Week Monkey Study

Sodium	Phosphorus	Carbon dioxide	Albumin (A)
Potassium	Alkaline Phosphatase	Urea Nitrogen	Globulin (G)
Chloride	Aspartate aminotransferase	Creatinine	A/G ratio
Calcium	Alanine aminotransferase	Total protein	Cholesterol
Glucose	Gamma glutamyltransferase	Calcium	Triglyceride

Urinalysis

Laboratory investigations of urinalysis were conducted on all animals at termination. Urine samples were from bladder puncture during necropsy. A complete urinalysis was analyzed. There were significant changes in the urinalysis parameters.

Table 33 Urinalysis Parameters Assayed in 4-Week Monkey Study

Color/character	Nitrite	Glucose	Microscopic
Specific Gravity	pH	Ketone	
Leukocyte esterase	Protein	Bilirubin	Occult blood

Gross Pathology

Gross pathological observations were made at termination on Weeks 4 for the main study animals and at Weeks 8 for the recovery animals. A complete gross necropsy was conducted on all animals sacrificed during the study. The necropsy included examination of carcass and muscular/skeletal system, all external surfaces and orifices, cranial cavity and external surface of the brain, neck with associated organs and tissues, thoracic, abdominal and pelvic cavities with their associated organs and tissues.

Gross lesions identified at Week-4 (refer to table) necropsy were limited to enlarged thyroid from a single female at high dose, nodules in the cecum and colon (1/6 each at mid and high dose), abscess in the spleen (1/6) at high dose, and increase in the size of the ovary (1/3 each at mid and high dose). The thyroid finding was associated with the increased organ weight and histology correlated with colloid distention of follicular acini. This incidence was considered test article related; however, at recovery no thyroid finding was noted. There were no ovarian findings at the recovery sacrifice, indicating that discontinuation of the drug might have resolved this adverse finding. One high dose male had multiple splenic abscesses which had rare gram positive bacteria. The chronicity (as evidenced by extensive fibrosis) of the incidence may indicate that this

might be a preexisting incidence in this particular animal. The abscess might have been worsened in the presence of the test article by its plausible immunosuppressive activity; the abscess promoting activity of the test article in such a preexisting condition can not be excluded. One high dose female at recovery also showed abscess in the mandibular lymph node. There were nodules (single) in the cecum and colon 1/6 each at the mid and high dose animals from the schedule necropsy (Week 4). Similar findings were noted at recovery, 1/6 animal each. From the mid and high dose animals with nodules, the number of nodules increased in animals from the recovery animal (10 and 5 in the mid and high dose respectively). The Applicant mentioned that the nodules are probably *Oesophagostomum* a helminth species (free-living nematodes of the family *Strongyloidae*). *Oesophagostomum*, especially *O. bifurcum*, are common parasites of livestock and animals like goats, pigs and non-human primates. The increase in number of the nodules may suggest belimumab induced immunosuppression which might be responsible for the proliferation of the worms in colon.

Table 34 Findings from Gross Necropsy: 4-Week Monkey Study

Tissue/s	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
Schedule Necropsy Day 29, n=3								
Splenic abscess	0	0	0	0	0	0	1	
Thyroid, size increased, mild	0	0	0	0	0	0	0	1
Cecum, mucosa, single nodule,	0	0	0	0	1	0	1	0
Ovary, enlarged, discoloration	NA	0	NA	0	NA	1	NA	1
Recovery Necropsy, Day 57, n=2								
Mandibular lymph node, abscess	0	0	0	0	0	0	0	1
Cecum, mucosa, nodules, #s = 5-10	0	0	0	0	0	1	0	1
Liver, 2 foci 1mm-rt medial lobe	0	0	0	0	0	0	0	1

NA- Not applicable

Organ Weights

The Applicant weighed the organs (listed in the attached table) before fixation, paired organs were weighed together. An adequate assessment was completed.

Table 35 List of Organs Weighed: 4-Week Monkey Study

Adrenal	Liver	Thyroids w/ parathyroids	Epididymes
Brain	Lungs	Thymus	Testes
Kidney	Pituitary		ovaries
Heart	Spleen		

There were changes in the following tissue weights [relative (brain to tissue) and absolute]. The table refers to the relative tissue weight changes. An increase in the thyroid weight (relative to brain) at Week 4 in high dose animals (42%↑) was noted. No such changes were noted at recovery. The weight of the ovaries (relative to brain) in the high dose doubled at terminal necropsy. However, at recovery necropsy only a 30% increase in organ weight was noted indicating recovery. The weight of the heart (relative to brain) was reduced approximately 20% at Week 4 with all dosages, no such findings were noted at Week 57 suggesting recovery. This study included 3 males/ group, (age 2.6-7.4 years), the male monkey in the high dose group did not show an increase in the weight of the testes. The monkeys in the low and mid dose group showed a 100% increase in the weight of the testes compared to those of the controls. The high dose males did not show any changes in the weight of the testes, since the finding is not dose related, the reviewer believes that the finding is not test article related but might be due to the inclusion of immature males in the high dose group.

Table 36 Organ Weights (Percent Control) from 4-week Monkey Study

Tissue/s	Low Dose	Mid Dose	High Dose
Schedule Necropsy Day 29, n=6			
Heart	20 ↓	18 ↓	18 ↓
Thyroid	15 ↓	29 ↓	42 ↑
Ovary	NC*	NC	100 ↑
Testes	48 ↓	38 ↓	62 ↓
Epididymes	50 ↓	37 ↓	45 ↓
Recovery Necropsy, Day 57, n=2			
Thymus	30 ↓	30 ↓	38 ↑
Ovary	NC	NC	30 ↑
Testes	100 ↑	100 ↑	NC

*NC-no change

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

The tissues (full battery, refer to attached table) from all of the animals at terminal and recovery necropsy were collected, and stained in hematoxylin–eosin and subjected to histopathological evaluation. All of the tissues from all dose groups were evaluated in histopathology. In addition, the tissues were also stained with Acid Fast Brown and Brenn's solution for identifying the bacteria.

Table 37 List of Tissues Collected from 4-Week Monkey Study

Aorta	Rectum	Kidney	Pituitary
Heart	Pancreas	Urinary Bladder	Thyroid/parathyroids
Salivary Glands	Liver	Testes	Skin/Mammary Gland
Tongue	Gall Bladder	Epididymes	Skeletal Muscle (thigh)
Esophagus	Trachea	Prostrate	Bone (femoral)
Stomach	Lungs	Seminal Vesicles	Bones (7 th rib)
Duodenum	Bone Marrow	ovaries	Eyes
Jejunum	Thymus	Uterus	Sciatic Nerves
Ileum	Spleen	Cervix	Brain
Cecum	Mandibular Lymph Node	Vagina	Spinal cord (thoracic)
Colon	Mesenteric Lymph nodes	Adrenal	Injection Sites

The histopathological findings are grouped as the findings from the lymphoid organs (which are believed to be the primary target organ for the product), the tissues of the digestive system and then other tissues to analyze the microscopic lesions.

The histological lesions in the lymphoid tissues consisted of the following findings (refer to table):

1. Dose dependent lymphoid depletion (minimal) from the mesenteric lymph nodes was observed in both males and females at Day 29. The lymphoid depletion was

characterized by a decrease in the number of lymphocytes surrounding the germinal centers within the lymphoid follicles. In mild cases, the cellular density of medullary cords also was decreased. The cellular density of the paracortex of the lymph node, a T-cell specific region, was not noticeably altered. This decrease in the number of lymphocytes in B-lymphocyte specific regions is congruent with the known pharmacological action of BLyS-antagonism and was consistent with decreases (not statistically significant) observed in B-lymphocyte subsets in peripheral blood on Day 29. These findings were observed to be partially recovered at Day 57.

2. Dose related mesenteric sinus histiocytosis, mesenteric leukocytosis and mandibular leukocytosis were observed at Day 29. No such changes were noted at Day 57, indicating recovery.
3. The histopathological changes in the spleen consisted of treatment related increase in protein deposition, and abscess in one male at high dose. One high dose animal had multiple coalescing abscesses within the splenic parenchyma. This abscess was of chronic duration, as evidenced by extensive fibrosis. Rare gram-positive particles suggestive of bacteria were present on a gram stain of the tissue. This animal also had mild lymphoid depletion of the mesenteric lymph node. It is unusual to see abscesses of this type as a spontaneous lesion in these animals.
4. The mandibular gland findings from Day 29 consisted of hyperplasia in males. No such findings were noted in control animals. This finding was, however, not found at Day 57 indicating recovery. In addition, one HD female had necrotizing granuloma in the mandibular lymph node at Day 57.

Table 38 Histopathological Findings: Incidence from Lymphoid Organs

Tissue/s*	Dose (mg/kg)							
	0		5		15		50	
	M	F	M	F	M	F	M	F
Day 29, n=3								
Mesenteric lymph node/sinus leukocytosis	0	0	0	0	0	0	0	1
Mesenteric lymph node/sinus histiocytosis	1	0	1	0	2	0	2	0
Mesenteric lymph node/lymphoid depletion	0	0	1, minimal	0	2 1minimal 1 mild	0	2 mild	3 1 minimal 2 mild
Mandibular lymph node/leukocytosis	0	0	1	0	0	0	1	1
Mandibular lymph node hyperplasia	0	0	1	0	0	0	1	0
Mandibular lymph node/extra medullary hematopoiesis	0	0	0	0	0	0	1	0
Spleen-abscess/chronic inflammation	0	0	0	0	0	0	1	0
Spleen/ protein deposition	0	0	1	0	2	1	1	1
Bone marrow/myeloid hyperplasia	0	0	0	1	0	1	1	3
Day 57, n=2								
Mesenteric gland/lymphoid depletion	0	0	0	0	0	0	0	1
Mesenteric lymph node/mineralized granuloma	0	0	0	0	0	0	0	1
Mandibular lymph node /fibrosis(capsular)	0	0	0	0	0	0	0	1
Mandibular lymph node /necrotizing granuloma	0	0	0	0	0	0	0	1
Bone marrow/lymphoid cell aggregates	0	0	0	0	0	0	0	1

* Severity index were reported only for the mesenteric lymphoid depletion; all other findings were mentioned by the Applicant as minimal

The histological lesions in the gastrointestinal tissues and glands consisted of the following findings (refer to table):

1. Dose dependent lymphoid depletion in GALT from ileum at Day 29; similar incidence of findings except for the findings from the mid dose were noted at Day 57. The severity of the findings was minimal indicating recovery.
2. Mononuclear cell infiltration in pancreas and liver was observed. The incidence increased with the increase in dose. The mononuclear cell infiltration increased in liver at recovery, the biological significance of this finding is, however, unknown.
3. Minimal inflammation was noted in different tissues such as pancreas, gall bladder at Day 29 and 57. Note the pancreatic inflammation was associated with mononuclear cell infiltration.
4. At Day 57, parasitic granuloma was noted in one female, this female also showed lesion at gross pathology examination indicating parasitic infection.

5. At Day 57, granulomas were noted in liver and pancreas most probably due to parasitic infection.

The depletion in the lymphoid cells in GALT may be due to the exaggerated pharmacology of the drug product. The occurrences of granulomas are most probably due to the increase in the infection in the animals suggesting immunosuppressive effect from BLYS-Antagonist.

Table 39 Histopathological Findings: Incidence from GI Tract:

Tissue/s	Dose (mg/kg)							
	0		5		15		50	
	M	F	M	F	M	F	M	F
Day 29, n=3								
Ileum/lymphoid depletion, GALT	0	0	1 minimal	0	1, minimal	2 1 minimal 1 moderate	1 mild	2 1 minimal 1 moderate
Jejunum/blunting of villi	0	0	0	0	0	0	1	0
Colon/ mononuclear cell infiltration	0	0	0	0	0	0	1	0
Liver/hepatocyte vacuolation	0	0	0	0	0	0	0	1
Liver/ mononuclear cell infiltration	0	0	1	0	1	0	1	0
Gallbladder/ inflammation	0	0	0	0	0	0	1	0
Pancreas/fibrosis	0	0	0	0	0	0	1	0
Pancreas/inflammation	0	0	0	0	0	0	1	0
Pancreas/ mononuclear cell infiltration	0	0	1	0	2	0	2	0
Day 57, n=2								
Ileum/GALT	0	0	1	0	0	0	1	2
Ileum/herniation	0	0	0	1	0	0	0	1
Stomach/lymphoid hyperplasia/GALT	0	0	0	0	0	0	1	1
Colon/ parasitic granuloma	0	0	0	0	0	0	0	1
Colon/herniation/GALT	0	0	0	0	0	0	1	0
Rectum/herniation/GALT	0	0	0	0	0	0	1	0
Duodenum/mineralized granuloma	0	0	0	0	0	0	0	1
Pancreas/ fibrosis	0	0	0	0	0	0	0	1
Pancreas/parasitic granuloma	0	0	0	0	0	0	0	1
Liver/biliary hyperplasia	0	0	0	0	1	0	0	1
Liver/eosinophilic granuloma	0	0	0	0	0	0	0	1
Liver/ mononuclear cell infiltration	0	1	0	2	0	2	0	2

** Severity index were reported only for ileum; all other findings were mentioned by the Applicant as minimal

The histological lesions in different tissues and glands consisted of the following findings (refer to table):

1. The treatment microscopic lesion in the thyroid consisted of follicular epithelial degeneration at high dose females (minimal) and males (mild). The lesion consists of sloughing of follicular epithelium into the lumen of the acini, forming large foamy cells within the colloid. This histology finding was associated with the changes in the organ weights. The thyroid lesion persisted in male at Day 57, however, the severity index reduced to minimal indicating partial recovery.
2. Mononuclear cell infiltrations were observed in several tissues such as heart, kidney, urinary bladder, injection sites, lung, trachea, and adrenal gland. No such changes were noted in control, therefore, the findings may be treatment related. The severity indexes of the findings were reported to be minimal. At Day 57, the incidence of the mononuclear cell infiltration in kidney was higher than those at Day 29. The mononuclear cell infiltration in heart, urinary bladder, and adrenal gland reduced at Day 57. The significance of these findings is not known. However, in tissues like trachea mononuclear cell infiltration was associated with inflammation at Day 57 suggesting a correlation between mononuclear cells mediated inflammation in this tissue.
3. Mineralization was noted in several tissues such as kidney, brain, and spinal cord in high dose group animals at Day 29. The biological significance of the findings is not known.
4. Fibrosis is noted in several tissues such as adrenal, injection site, and pancreas. The B-cell depletion was occasionally associated with the modulation of the collagen synthesis resulting in the proliferation of the connective tissues causing fibrosis which has specifically been observed at the injection sites.

The mononuclear cell infiltrations, mineralization, and fibrosis were also observed in tissues from the reproductive and gastrointestinal tissues, the biological significance of the findings are not known. However, the severity index of such findings was minimum and the incidence of such findings at low doses was rare. Therefore, a LOAEL could be established based on the analyses of the incidence.

Table 40 Histopathological Findings: Incidences from Different Tissues

Tissue/s	Dose (mg/kg)							
	0		5		15		50	
	M	F	M	F	M	F	M	F
Day 29, n=3								
Brain/mineralization	0	0	0	0	0	0	1	0
Brain/neuronal degeneration	0	0	0	0	0	0	0	1
Lung/interstitial inflammation	0	1	0	0	0	0	1	1
Trachea/Inflammation	0	0	0	0	0	0	1	0
Thyroid/follicular degeneration	0	0	0	0	0	0	2 min, mild	2 min
Adrenal gland fibrosis	0	0	0	0	0	0	1	0
Adrenal gland/mononuclear cell infiltration	0	0	0	0	0	0	0	1
Kidney/mineralization	1	0	0	0	0	0	2	0
Kidney/ mononuclear cell infiltration	0	0	0	0	2	0	2	0
Kidney/ectopic adrenal gland	0	0	0	0	0	0	0	1
Kidney/cyst	0	0	0	0	0	0	0	1
Urinary bladder/ mononuclear cell infiltration	0	0	1	0	0	0	1	1
Heart/ mononuclear cell infiltration	0	0	0	3	0	1	0	2
Spinal cord/ mineralization	0	0	0	0	0	0	0	1
Skin/ mononuclear cell infiltration	0	0	0	0	0	0	0	1
Injection site/ mononuclear cell infiltration	0	0	0	1	2	2	1	2
Injection site/fibrosis	0	0	0	0	0	0	0	1
Injection site/collagen degeneration	0	0	0	1	0	1	0	1
Injection site/muscle degeneration	0	0	0	1	0	0	0	1
Day 57, n=2								
Thyroid/follicular degeneration	0	0	1	0	0	0	1	0
Mammary gland/fibroplasia	0	0	0	0	0	0	0	1
Kidney/ degeneration of tubules	0	0	0	0	0	1	1	0
Kidney/ mononuclear cell infiltration	1	1	1	2	2	1	2	2
Urinary bladder/ mononuclear cell infiltration	0	0	1	0	0	0	1	0
Trachea/ mononuclear cell infiltration	0	0	0	0	0	0	1	0
Trachea/inflammation	0	0	0	0	0	0	1	0

** Severity index were reported only for thyroid; all other findings were mentioned by the Applicant as minimal (min)

The histological lesions in different reproductive tissues and glands consisted of the following findings (refer to table):

1. The ovarian weight at Day 29 and Day 57 was observed to be increased which might be related to these histology findings.
2. Dose related increase in mononuclear cell infiltrations was noted in ovary and cervix at Day 29, no such findings were observed in ovary at Day 57. However, mononuclear cell infiltrations were observed in cervix, uterus, and vagina at Day 57. The severity indexes of all of these findings were minimal. The biological significance of the findings is not known. No hormonal or menstrual cyclicity was systematically evaluated in this study.

3. Dose related mineralization was noted in ovary at Days 29 and 57. The findings are considered treatment related because no such findings were noted in control animals. The biological significance of the findings is unknown.
4. The male reproductive organ findings showed incomplete spermatogenesis, immature testes in 2/3 animals, distended seminal vesicles at Day 29. The weights of the testes were observed to be much lower compared to those of the controls. The data confirms that the monkeys used in this study were not suitable for evaluating male fertility related changes. There were no test article related male reproductive organ findings at Day 57 except the mononuclear cell infiltrate in prostate in 1/3 animals. The weight of the testes from the low and mid dose group animals and not the high dose group animal were doubled at Day 57 compared to those of the controls. The data are unclear to conclude that there was any affect of the test article on the male reproductive organs.

Table 41 Histopathological Findings: Incidences from Reproductive Organs

Parameters	Dose (mg/kg)			
	0	5	15	50
Day 29, n=3, Female				
Uterus/hemosiderosis	0	0	0	1
Uterus/distension	0	0	0	1
Ovary/ interstitial cell hyperplasia	1	1	1	2
Ovary/mineralization	1	1	2	1
Cervix/ mononuclear cell infiltration	0	0	1	2
Cervix/dilated glands	0	0	1	1
Day 57, n=2, Female				
Ovary/ serosal hyperplasia	0	0	0	1
Ovary mineralization	0	0	1	2
Cervix/ mononuclear cell infiltration	0	0	1	2
Uterus/ mononuclear cell infiltration	0	0	0	1
Vagina/ mononuclear cell infiltration	1	0	1	2
Day 29, n=3, Male				
Urethritis/leukocytic	0	0	0	1
Testes, incomplete spermatogenesis, immature	1	2	2	2
Seminal vesicle, distension	0	0	0	1
Day 57, n=2, Male				
Prostrate/ mononuclear cell infiltration	0	2	0	1

** Severity index were reported only for thyroid; all other findings were mentioned by the Applicant as minimal (min)

Special Evaluation

Peripheral Blood Mononuclear Cell Populations:

The blood samples were collected from all animals on predose period (Days -14, -7, and 1), treatment period (Days 14 and 28), and recovery period. At each time point, the samples were quantitatively analyzed for the following (refer to table) lymphocyte

subpopulation and monocytes. There were no sex-related differences and thus, the data are presented as pooled results.

Table 42 List of Cell Markers Analyzed from PBMCs

Cell Markers	Cell Identification
CD2/CD20	Total Lymphocytes (B, T, and NK cells)
CD21/CD20	Mature B-cells
CD3/CD4	T-helper lymphocytes
CD3/CD8	T-suppressor/cytotoxic lymphocytes
CD3/CD14	Monocytes

The result shows the following (refer to table)

1. There were no differences in CD3⁺/CD8⁺, CD3⁺/CD8⁺, CD2⁺/CD20⁺, and CD3⁺/CD14⁺ positive lymphocytes quantitatively. The magnitude of change from the baseline at each time point was similar between the belimumab treated animals and the control animals. These results indicate that belimumab is not affecting the number of total lymphocytes, T-helper/cytotoxic cells, and monocytes in the peripheral circulation.
2. There were no changes in the mean CD20⁺ and CD20⁺/CD21⁺ B lymphocytes at Day 28 in the belimumab treated animals compared to those of the control animals. At Day 56, there was a reduction ($\geq 20\%$) in both CD20⁺ and CD20⁺/CD21⁺ B lymphocytes. The reduction in the B lymphocytes populations were, however, not dose dependent and not statistically significant.

Table 43 Effect of Belimumab on Cell Markers: 4-Weeks Study in Monkeys

Time of Observation/Dosages (mg/kg)	CD20 ⁺			CD20 ⁺ /CD21 ⁺		
	5	15	50	5	15	50
Day 28	8	NC	5	30	10	10
Day 56	52	22	33	62	61	27

Lymphoid Tissue Analysis for Mononuclear Cell Populations:

Sections of spleens and mesenteric glands were collected from all animals from different dose groups at terminal and recovery necropsy. Single cell suspensions were prepared from these tissues and different subpopulations of the lymphocytes were identified by the flowcytometric analyses.

The results show the following (refer to table)

1. The CD20⁺ and CD20⁺/CD21⁺ B lymphocytes decreased at Days 29 and 57 ($\geq 30\%$ compared to control) in both spleen and mesenteric lymph nodes. The decrease of the B-cell subpopulation was not dose dependent (except CD20⁺/CD21⁺ cell population at Day 57). The decrease was statistically

- significant ($p < 0.05$) in most dose groups (except CD20⁺/CD21⁺ cells at Day 29 in spleen and mesenteric gland).
2. There was a decrease in the mononuclear (CD3⁺/CD14⁺) cells at Days 29 and 57; however, this decrease was not dose dependent.
 3. There was an increase in CD3⁺ and CD3⁺/CD4⁺ positive T-lymphocytes at Days 29 and 57 in both spleen and mesenteric lymph nodes. The increase was not dose dependent and similar increase was observed at all dosages. An increase in CD3⁺/CD8⁺ positive T-lymphocytes at Days 29 and 57 in spleen but not in mesenteric lymph nodes. The increase in the different subpopulation of the T-lymphocytes were mostly statistically significant ($p < 0.05$).

Table 44 Summary of Findings: Spleen and Mesenteric Lymph Nodes

Immune Cell Markers/ Dosages (mg/kg)	Spleen			Mesenteric Lymph Node		
	5	15	50	5	15	50
CD20 ⁺						
Day 29	57 ↓*	37 ↓	43 ↓	29 ↓	45 ↓*	37 ↓
Day 57	54 ↓*	33 ↓*	37 ↓*	41 ↓*	57 ↓*	58 ↓*
CD20 ⁺ /CD21 ⁺						
Day 29	42 ↓	44 ↓	40 ↓	39 ↓	35 ↓	54 ↓
Day 57	62 ↓*	41 ↓*	58 ↓*	50 ↓*	63 ↓*	74 ↓*
CD3 ⁺						
Day 29	44 ↑*	47 ↑*	42 ↑	14 ↑*	19 ↑*	16 ↑*
Day 56	25 ↑	20 ↑	39 ↑	12 ↑	16 ↑*	20 ↑*
CD3 ⁺ /CD4 ⁺						
Day 29	50 ↑*	42 ↑*	48 ↑	20 ↑*	22 ↑*	21 ↑*
Day 57	30 ↑	17 ↑	34 ↑	20 ↑	22 ↑*	20 ↑
CD3 ⁺ /CD8 ⁺						
Day 28	57 ↑	37 ↑	43 ↑	NC	NC	NC
Day 57	33 ↑	35 ↑	45 ↑*	NC	15 ↑	24 ↑
CD3 ⁺ /CD14 ⁺						
Day 28	57 ↓	37 ↓	33 ↑	44 ↓	64 ↓	NC
Day 57	14 ↑	23 ↑	54 ↑	53 ↓	NC	54 ↓

* Statistically significant ($p < 0.05$)

Toxicokinetics

The blood samples for the TK analyses of belimumab were collected prior to dosing and after dosing at Day 1, Day 8, Day 15, and Day 22. The blood samples were also collected at Days, 2, 3, 5, 24, 26, and 29. Additionally, blood samples were also collected from the recovery group at Days 36, 43, 50, and 57.

The concentrations in serum samples were determined with a sandwich type ELISA that utilized BLYS for capture and biotinylated anti-human antibody for detection. A standard curve was prepared in sample diluent beginning at a top concentration of 40 ng/mL and including seven additional 2-fold dilutions and zero. Serum samples were diluted in

sample diluent until the concentration of anti-BLyS antibody fell within the range of the standard curve.

The serum concentration analyses the belimumab showed that the concentration of the compound increased with dose, however, the increase was slightly more than dose proportional in certain instances like in females a 3-fold-increase in dose (5-15 mg/kg) resulted in a 4-fold increase in the serum concentration (27 vs 113 µg/mL). Similarly a 10-fold increase in dose (5-50 mg/kg) resulted in > 11-fold (27 vs 302 µg/mL) increase in the serum concentration. Exposure in females was comparable to those in males at 5 and 50 mg/kg dosages.

The PK was biphasic, the majority of the exposure occurred at the beta phase (elimination phase). Clearance, volume of distribution at initial as well as in the steady state, half life of the alpha and beta phase, and the mean residence time are independent of dose. The terminal half life of the compound after four weekly administrations was found to be 14 days and the clearance is approximately 5-7 mL/day/kg. There were about 2-fold accumulation of the compound at Day 29 compared to Day1 with all doses. Data between males and females were similar and thus averaged in the following table.

Table 45 Summary of Toxicokinetics: 4-Week Monkey Study

Table 4 Two-compartment pharmacokinetic parameters (+/- SEM) for A01 in the recovery group following four i.v injections of 5, 15, or 50 mg/kg

Parameter	5 mg/kg	15 mg/kg	50 mg/kg	P value
n	4	4	4	
AUC (day*µg/ml)	2868 ± 259	9459 ± 1000	37145 ± 3617	<0.0001 *
C _{max} (µg/ml)	153 ± 9.0	472 ± 21.8	1713 ± 142	<0.0001 *
t _{1/2α} (day)	0.71 ± 0.13	1.3 ± 0.31	0.77 ± 0.08	0.1314
t _{1/2β} (day)	13.5 ± 0.9	14.0 ± 1.6	14.6 ± 1.1	0.8415
CL (ml/day/kg)	7.17 ± 0.73	6.53 ± 0.59	5.54 ± 0.54	0.2343
V _i (ml/kg)	49.7 ± 3.4	48.4 ± 3.1	48.0 ± 3.9	0.9373
V _{ss} (ml/kg)	126 ± 8.8	108 ± 4.8	106 ± 8.8	0.1965
MRT (day)	17.8 ± 1.2	17.0 ± 2.1	19.4 ± 1.8	0.6209

* denotes a significant P value at the 5% level of significance. Abbreviation: AUC Area Under the Curve, C_{max} Maximum Concentration, t_{1/2α} Half-life of alpha-phase, t_{1/2β} Half-life of Beta-phase, CL Clearance, V_i Initial Volume of Distribution, V_{ss} Steady-State Volume of Distribution, MRT Mean Residence Time, and SEM Standard error of the Mean

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Immunogenicity Determination:

The blood samples for the immunogenicity analyses of belimumab were collected prior to dosing Day 1, and Days 15, 22, 43, and 57. The presence of belimumab specific antibodies was determined by standard ELISA techniques with detection using anti-IgA,

anti-IgM, anti-IgG-Fc and anti-kappa-light-chain antibodies. The presence of monkey antibodies is defined as a 2-fold increase in absorbance compared to the zero time (i.e uninjected) monkeys. Based on this criterion, 3 monkeys out of a total of 40 were positive for the generation of anti-product antibodies: FN16034F of the 15 mg/kg dose group and FN16043M and FN16036M of the 50 mg/kg dose group. Monkeys FN16034F and FN16036M were positive with the anti-kappa reagent. This suggests that these monkeys were generating only an anti-Fc response. Monkey FN16036M was borderline positive, barely reaching a two-fold increase in signal. Monkey FN16043M was strongly positive with all methods of detection, indicating that this monkey generated antibodies to both Fc- and Fab portions of the belimumab molecule. Peak absorbance was around Day 15 for the IgA and probably IgM and the peak for IgG response was around Day 29.

Table 46 Summary of Immunogenicity Findings: 4-Week Monkey Study

Monkey #s	Treatment	Study Days	AntiIgA+IgM	Anti Kappa	Anti Fc
FN16044M*	0	1	0.23	0.1	0.05
		15	0.26	0.1	0.05
		29	0.22	0.1	0.05
		43	0.20	0.1	0.05
		57	0.19	0.1	0.05
FN16034F	15 mg/kg	1	0.19	0.09	0.04
		15	0.31	0.31	0.05
		29	0.25	0.57	0.05
FN16036M	50mg/kg	1	0.998	0.15	0.05
		15	1.036	0.29	0.05
		29	0.737	0.21	0.05
FN16043M	50mg/kg	43	0.24	0.08	0.04
		57	0.87	0.58	0.06
		29	0.42	1.09	1.37
		43	0.26	0.93	0.57
		57	0.18	0.63	0.14

Immune suppression Determination:

The blood samples were collected at predose and Days 1, 15, 29, 43, and 57. Monkey IgA, IgM, and IgG were measured in a solid-phase ELISA in which affinity purified goat anti-human Ig capture antisera was adsorbed to individual wells of a 96 well micro titer plate and probed with various dilution of the serum from the belimumab treated monkeys.

The results show that the administration of belimumab (5, 15, and 50 mg/kg) produced different effects on the serum levels of different immunoglobulins in monkeys. The IgM level remained unchanged throughout the experimental period. The IgA level although remained unchanged during the treatment period, decreased at Days 43 and 57 (recovery period, refer to figure) with 15 and 50 mg/kg dose compared to its level at

predose as well as the levels compared to those of the control animals. This change was statistically significant ($P=0.004$) at the high dose. The IgG level at the high dose showed an increase at Day 14 of the treatment compared to the predose level. However, at recovery Day 57, the IgG level at the high dose decreased compared to its predose level. Both of these changes were statistically significant ($p=0.003$, 0.04 respectively at Days 14 and 57 respectively). The biological significance of these changes is not known.

Figure 35 Serum IgA Level

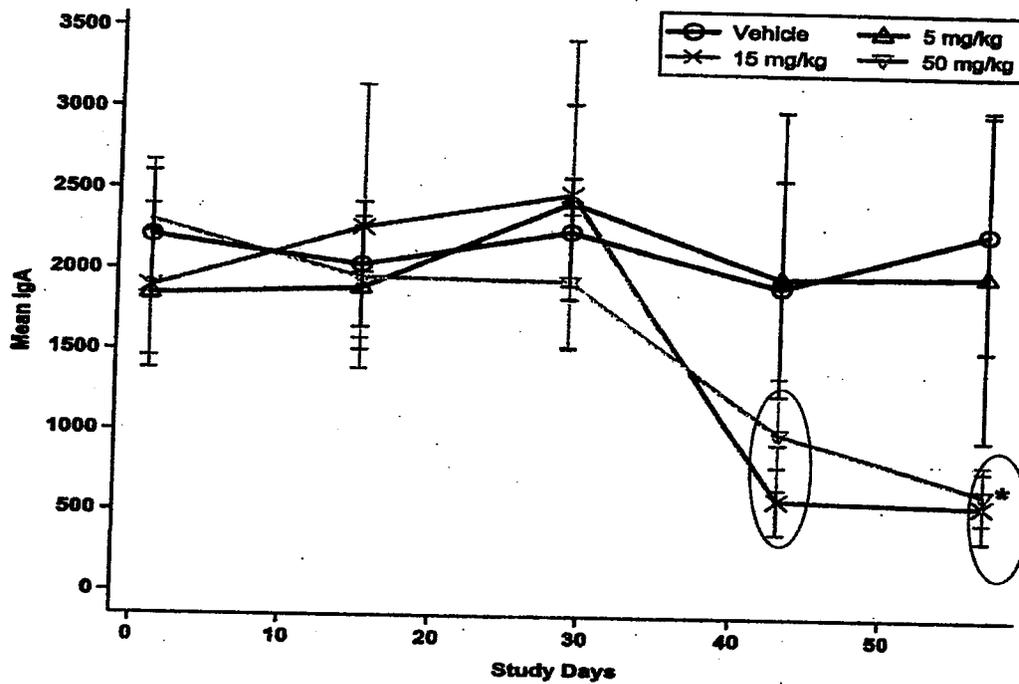
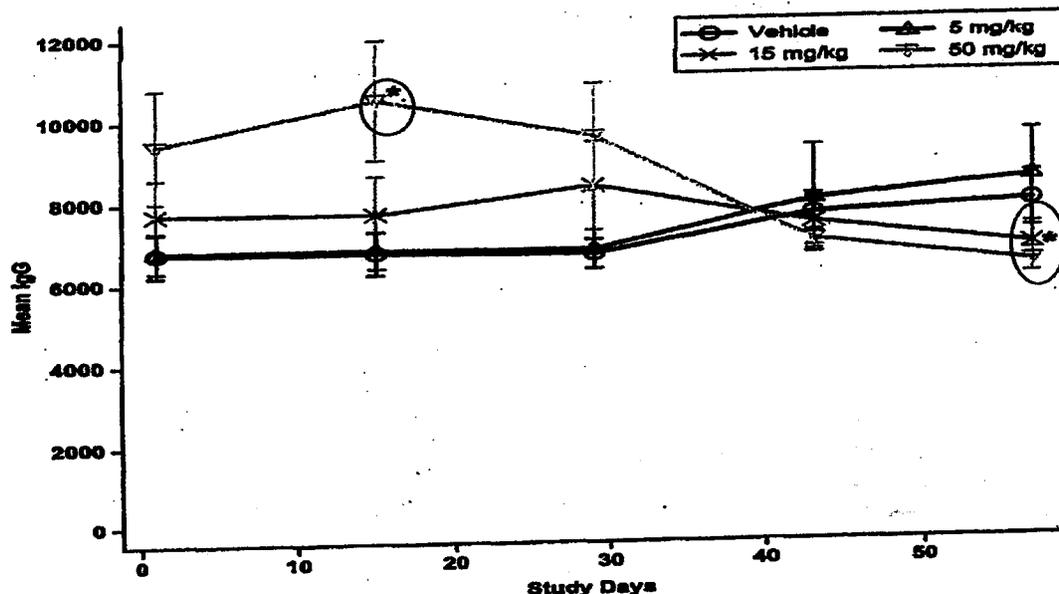


Figure 36 Serum IgG Level



Dosing Solution Analysis

Analysis of the dosing solutions indicated that all solutions ranged between 90% and 100% of the target concentration.

Study title: A 6-Month Toxicity Study of Lymphostat-B™ Administered Bi-Weekly by Intravenous Injection to Cynomolgus Monkeys, with an 8-Month Recovery Period

Study no.: (b) (4) 1177-95 ; HG10399-T05
 Study report location: FDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: November 14, 2001
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: LymphoStat-B (belimumab), Lot # 01A06041, 99% pure; placebo Lot #01P06039 and #01P06040; supplied as lyophilized powder; reconstituted in sterile water for injection

Key Study Findings

- The Cynomolgus monkey (8/sex/group) was administered a lyophilized formulation of belimumab via IV administration for 26 weeks (0, 5, 15, 50 mg/kg/every 2 week). 3 monkeys/sex/group were sacrificed at Week 13 and Week 26 and 2/sex/group were allowed to recover for an additional 32 weeks (sacrificed at Week 60).
- The B-cell (total and mature) population from the peripheral blood, spleen, and mesenteric lymph nodes decreased substantially at Weeks 13 and 26. The finding was observed to be recovered at Week 60. The changes were not dose related but statistically significant. The finding is considered as the pharmacological effect of the drug product.
- There was a decrease in IgG, IgM, and IgE levels in the belimumab treated animals until Week 26 (throughout the treatment period) compared to the vehicle treated animals.
- The major histopathological observation consisted of lymphoid depletion from spleen, mesenteric, and mandibular lymph nodes and extramedullary hematopoiesis. The histology findings at Week 60 (recovery) showed extramedullary hematopoiesis hyperplasia of the mesenteric lymph nodes and lymphoid hyperplasia in spleen at Week 60. These findings are likely a result of rebound effects to B-cell depletion.
- The serum concentration of the compound increased with the increase in dose. The steady state for the serum level was observed to be reached around Day 71. The accumulation of the compound was approximately 2-fold. During recovery, the serum concentration decline half lives ranged from 9-10 days. No appreciable level of belimumab was noted with low and mid dose at Days 309 and 351 respectively. In high dose animals only ¼ animals showed Belimumab level at 8-month recovery period. Two animals (1/3 males from low dose, 1/3 females from high dose) showed ADA.
- The effect of the belimumab on the male or female reproductive organs could not be appropriately evaluated due to male sexual immaturity and lack of adequate endpoint assessments in females. The histological changes in the ovary consisted of interstitial and germinal cell hyperplasia at Weeks 13 and 26. The incidences of these findings were higher than those of the controls and persisted at all doses in Week 60 suggesting no recovery. No sex hormones were evaluated or menstrual cyclicity in this study.
- Other major histological changes consisted of increased infection in the GI tract, pneumoconiosis was observed in lungs, mononuclear cell infiltration in different tissues. The findings at recovery were mainly restricted to the high dose (50 mg/kg) and consisted of hyperplasia in stomach, vasculitis in heart, and arterial hyperplasia in kidney. Based on these findings 15 mg/kg were considered the NOAEL by the reviewer.

Methods

Doses:	0, 5, 15, and 50 mg/kg
Frequency of dosing:	1x/biweekly for 3 and 6 months
Route of administration:	IV, slow bolus over 30 secs
Dose volume:	2.5 mL/ kg
Formulation/Vehicle:	LymphoStat-B (belimumab), 22 mg/mL in 1.9% glycerin, 0.5% sucrose, 10 mM sodium citrate, and 0.01% Tween 80, pH 6.5
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	3/sex/treatment group, 2/sex/control group
Age:	M: 2.5-5.3 years; F: 2.1-7.4 years
Weight:	Males: 2.0-4.7 kg; Females: 1.7-3.5 kg
Satellite groups:	2 animals/sex/group were maintained for recovery for 33 weeks, post treatment
Deviation from study protocol:	Refer to Applicant's study design table There were protocol deviations such as one blood sample was not collected or one sample was not analyzed, however, the reviewer believes that none of the above deviations affected the integrity of the overall toxicity findings

Table 47 Study Design (Applicant's table): 3, 6-month Monkey Study

Group No.	Number of Animals (Males/Females)	Dose Level (mg/kg)	Dose Vol. (mL/kg)	Dose Conc. (mg/mL)	Number Euthanized on:		
					Day 92 (M/F)	Day 183 (M/F)	Day 419 (M/F)
1	6/6	0 (control)	2.5	0	2/2	2/2	2/2
2	8/8	5	2.5	2	3/3	3/3	2/2
3	8/8	15	2.5	6	3/3	3/3	2/2
4	8/8	50	2.5	20	3/3	3/3	2/2

M = male; F = female

Observations and Results

Mortality and morbidity checks were performed twice a day throughout the study. There was no mortality in this study.

Clinical Signs

Cage-side clinical signs (general health and behavior) were recorded twice daily. Recordings of cage side observations began seven days prior to the initiation of the treatment and continued throughout to examined for changes in general appearance and behavior. There were no test article related findings.

Body Weights

Body weights were recorded for all animals approximately one week prior to the initiation of treatment and on the day prior to treatment (Day - 1). During the treatment

and observation periods, body weights were also recorded for all animals weekly, as well as terminally prior to necropsy (fasted). All animals gained weight; there were no treatment related differences in the body weight gain.

Feed Consumption

Individual weekly feed intake was recorded qualitatively for all animals at pre dose, weekly throughout the treatment period, and weekly during the recovery period. There were no treatment related changes in the food consumption.

Ophthalmoscopy

The anterior and the posterior chamber of eyes from all animals were examined by using a direct ophthalmoscope. The eye examination was also conducted by dilating the eyes with mydriatic solution. The examination was conducted once during the pre-treatment period and once at Weeks 13, 26, and 39. There were no treatment related ophthalmological findings.

EKG

EKGs were recorded (using Leads I, II, III, aVR, aVL, and aVF) from all monkeys prior to dosing and prior to termination at Weeks 13, 26, and 60. There were no test article related abnormalities in EKG. The monkeys were temporarily restrained in primate chairs for the evaluation which consisted of a qualitative examination of each tracing for abnormalities. The EKG recordings were evaluated by a veterinarian, cardiologist (consultant), [REDACTED] (b) (4). There were no treatment related EKG findings.

Hematology

Laboratory investigations (hematology, coagulation) were conducted on all animals at termination. Blood samples were collected by femoral vein puncture at termination. The full battery of the hematological parameters (refer to table 29) was analyzed. There were no significant changes in the hematological parameters. No test article related changes in the coagulation parameters were noted.

Clinical Chemistry

Laboratory investigations (clinical chemistry) were conducted on all animals at termination. Blood samples were collected by femoral vein puncture at termination. The full battery of the clinical chemistry parameters (refer to table 30) was analyzed. There were, however, sporadic increases in LDH, ALT, and AST in animals from all dose groups (including control), therefore the finding is not considered treatment related. One female at mid dose showed mottled liver (tan foci) at Week 13, higher levels of enzymes (LDH, AST, and ALT) were also observed in this animal. The increase in the liver enzyme levels in this female (FN18815F) might be related to the histopathological changes in the liver.

Urinalysis

Urine samples were obtained from the bladder during necropsy. The full battery of the urinalysis parameters (refer to table 31) was analyzed. There were no statistically significant changes in the urinalysis parameters.

Gross Pathology

Gross pathological observations were recorded at scheduled necropsies on Weeks 13, 26, and 60. The gross pathology observations included carcass, muscular and skeletal system; all external surfaces and orifices, cranial cavity and external surface of brain; neck with associated organ and tissues; thoracic, abdominal, and pelvic cavities with their associated organs and tissues.

Gross lesions identified at Week 4 (refer to table) that consisted of treatment related increases in nodules in pancreas, foci in lungs, nodules in spleen, stomach, cecum, and ileocecal valves. The nodules in GI tract were associated with bacteria and were not dose related. Because these findings were not observed in the control animals, the findings may be test article related and might be due to opportunistic infection associated with the immunosuppressive properties of the drug product. The pancreatic nodules were red in color and of 1-2 mm in diameter, the findings were observed in 1/3 males each at low and mid dose; similar findings were observed in recovery animals 2/4 animals and 1/4 animals respectively at low and mid dose respectively. These findings were associated with the histological findings of ectopic spleen associated with pancreas. There were increased incidences of lung findings in the test article treated animals. These findings consisted of gray foci on diaphragmatic lobe 1-4 mm which were sometimes black in color. The findings were observed in test article treated animals at Week-13 and 26, the findings were not dose related but higher in incidence than those of the controls. Similar type of lung findings was observed in recovery animals also. Histologically, the lung findings were associated with pneumoconiosis with/without bronchiectasis.

Table 48 Summary of Incidence Findings from Gross Necropsy

Tissue/s	Control		Low Dose		Mid Dose		High Dose	
	M	F	M	F	M	F	M	F
Schedule Necropsy Week 13, n=3/sex								
Pancreas: nodules, red, 1-2 mm	0	0	1	0	1	0	0	0
Lungs: foci, tan –gray, 1-3 mm at different lobes	0	0	0	0	1 mild	2 mild	0	1 min
Splenic nodule 1-2 mm	0	0	0	1 min	0	3 mild	0	0
Thymus, discolored	0	0	0	0	0	0	0	1 mild
Cecum, mucosa, single nodule,	0	0	0	0	1	0	1	0
Ovary, enlarged, discoloration	NA	0	NA	0	NA	1	NA	1
Schedule Necropsy Week 26, n=3/sex								
Adrenal, nodule, tan	0	0	0	0	0	1 min	0	0
Lung foci, tan, 1-4 mm at different lobes	1 mild	1 mild	1 min	0	1	0	1 min	3 min
Spleen, nodule, tan 1mm	0	0	0	0	0	0	0	1 min
Stomach, foci, red, 1-2 mm	0	0	0	1 min	1	0	1 min	0
Recovery Necropsy, Week 60, n=2/sex								
Cecum: nodule, black	0	1	0	1	0	1	0	0
Colon, discoloration foci, nodules	0	2	2	2	0	0	0	0
Ileocecal valve red			0	1 mild	0	0	0	1 mod
Liver: accentuated lobular pattern	0	0	0	0	0	0	0	1 min
Lungs: foci, tan –gray, 1-3 mm at different lobes	0	0	0	2	0	2	0	0
Pancreas: nodules, red, 1 mm	1	0	0	1	0	1	0	0
Spleen accentuated follicular pattern	0	0	0	0	0	0	0	1 min
Tonsil (size increased)	1	0	1	0	0	0	1 mild	1 min
Thymus (size decreased)	0	0	1	0	0	1	1	1

Organ Weights

The organs listed in efore fixation, paired organs were weighed together.

Table 49 List of Organs Weighed

Adrenal	Liver	Thyroids w/ parathyroids	Epididymes
Brain	Lungs	Thymus	Testes
Kidney	Pituitary		ovaries
Heart	Spleen		

There was an increase in the absolute weights of some of the organs such as adrenals, heart, lungs at Week-13; however, no changes in the relative weights of these organs (compared to the brain) were noted.

The relative weights of the spleen (refer to table) increased more than 30% in all test article treated animals at week 13. However, there was a decrease in relative spleen weight at Week 26 with all doses (40%↓, 35%↓, and 40%↓ of control at low, mid and high dose, respectively). At recovery, Week-60, splenic weight at all doses was approximately 25% lower than those of the controls indicating recovery. This finding correlates with the histological findings of a decrease in number and size of lymphoid tissues in the splenic white pulp in all treatment groups.

There was an increase (not dose related) in the weight of the (relative to brain) thyroids at Week 13 in the belimumab treated animals. The weight of the thyroids, however, decreased in the belimumab treated groups at Weeks 26 and 60. Microscopic findings showed follicular degeneration of the thyroids in the belimumab treated animals at Weeks 13 and 26, but not at Week 60.

The weight of the thymus increased at Week 26 in the belimumab treated animals but decreased at Week 60, the finding was dose independent, the significance of the finding is not known. A non-dose related increase in the weight of the adrenal glands was observed at Week 60 with no histological correlation. There was a non dose related decrease in the weight of the kidneys from the animals sacrificed at Week 26; however, there was no histological correlation of this finding.

The relative (to brain) weights of the ovaries decreased at Weeks 13 and 26 from all of the belimumab treated animals compared to those of the controls. The finding is not dose related, however, increased hyperplasia of the interstitial cells, mineralization, and corpus lutea were noted in the ovaries from the belimumab treated animal. There were no changes in the weight of the ovaries at recovery. The histopathology of the ovaries and the observation that females had menses suggested that the animals were sexually matured; there were minimum individual variation of ovaries within the same treatment group. The age of the animals at the initiation of the study was 2.1-7.4 years. The females necropsied at 3, 6, and 8-months in the study period were sexually mature.

Table 50 Summary of Organ Weights (relative to brain, percent control)

Tissue/s	Low Dose	Mid Dose	High Dose
Terminal Necropsy Week 13, n=3/sex			
Spleen	41 ↑	32 ↑	31 ↑
Thyroid	17 ↑	30 ↑	NC
Ovaries	11 ↓	25 ↓	25 ↓
Terminal Necropsy, Week 26, n=3/sex			
Kidney	29 ↓	22 ↓	10 ↓
Spleen	47 ↓	38 ↓	38 ↓
Thymus	25 ↑	21 ↑	NC
Thyroid	50* ↓	29 ↓	25 ↓
Ovaries	50 ↓	33 ↑	33 ↑
Recovery Necropsy, Week 60, n=3/sex			
Adrenal	25 ↑	40 ↑	15 ↑
Spleen	38 ↓	24 ↓	41 ↓
Thymus	34 ↓	NC	37 ↓
Thyroid	34 ↓	12 ↓	34 ↓

*NC-no change

The weight of the male reproductive organs such as testes and epididymides at Weeks 13, 26, and 60 showed too much intra- and inter-treatment group variation. Most of the animals in the control, as well as test article treated groups, appear to be sexually immature as indicated by the testicular weights. Interestingly 3/8 animals from the recovery group appeared to be immature at Week 60 that is one and half years after the initiation of the study (age at study initiation 2.5-5.3 years). Also noted, testes weights were not always related to the body weight indicating that the body weight is not always a marker of sexual maturity in males. The results from this study indicate that male reproductive organ could not be appropriately evaluated from the current 6-month repeat dose toxicity study. The lack of complete fertility evaluations in males will be captured in belimumab labeling.

Histopathology

Adequate Battery: Yes

Peer Review: Yes

Histological Findings

The tissues (full battery), from all of the animals at terminal and recovery necropsy were collected, and stained in hematoxylin–eosin for microscopic observation subjected to histopathological evaluation. All of the tissues from all dose groups were evaluated in histopathology. The Applicant did not mention the fixatives used for processing the tissues. In addition, the tissues were also stained with Acid Fast Brown and Brenn's solution for identifying the bacteria.

Table 51 List of Tissues: 3 and 6-Month Study

Aorta	Rectum	Kidney	Pituitary
Heart	Pancreas	Urinary Bladder	Thyroid/parathyroids
Salivary Glands	Liver	Testes	Skin/Mammary Gland
Tongue	Gall Bladder	Epididymes	Skeletal Muscle (thigh)
Esophagus	Trachea	Prostrate	Bone (femoral)
Stomach	Lungs	Seminal Vesicles	Bones (7 th rib)
Duodenum	Bone Marrow	ovaries	Eyes
Jejunum	Thymus	Uterus	Sciatic Nerves
Ileum	Spleen	Cervix	Brain
Cecum	Mandibular Lymph Node	Vagina	Spinal cord (thoracic)
Colon	Mesenteric Lymph nodes	Adrenal	Injection Sites

The histopathological findings are grouped as the findings from the lymphoid organs (which are believed to be the primary target organ for the product), the tissues of the digestive system (which are observed to be affected with different bacterial infection), other tissues and the reproductive tissues.

The histological lesions in the lymphoid tissues consisted of the following findings (refer to table):

1. At Week 13, decreased lymphoid follicle size/number was observed in the spleen with a severity of minimal to marked. The spleen of one of the two affected mid dose males was completely devoid of the lymphoid follicles in the section examined. At Week 26, the reduction in the lymphoid follicles remained. The severity index of the findings ranged from minimal to moderate. One of the females from the mid dose group had complete loss of lymphoid follicles. The Applicant mentioned that in the affected lymphoid follicles (predominantly B-lymphocytes), the range of the decreased intrafollicular cell concentration was normal to mild. The decrease in the follicular size of the spleen was correlated with the decrease in the B-cells as observed by the flow cytometric analysis of the splenocytes. No such changes in the histology of spleen were noted at Week 60 indicating recovery. Hyperplasia was however, noted in spleen at high dose in 2/4 animals which may be a rebound effect due to recovery from the B-cell depletion.
2. Decreased thickness of the periartertolar lymphoid sheath (PALS) region of the spleen in the belimumab treated monkeys at Week 13 was observed. The alteration of the PALS region (predominantly T-lymphocytes) was characterized by a relative decrease in the number of small lymphocytes surrounding splenic arterioles. Decreased thickness of the PALS region of the spleen was only present in belimumab treated monkeys at Week 13 suggesting this finding to be an effect of test article administration; however,

this change was not identified in treated monkeys at Week 26 and at recovery. The lymphocyte subset analysis in the spleen at Week 13 (by flowcytometry) showed no difference in the relative percentage of T-lymphocytes comparing belimumab treated monkeys to control.

3. Hyperplasia of the mesenteric lymph node (minimal) was observed at Week 13. No such changes were noted at Week 26. However, at Week 60, animals respectively had hyperplasia of the mesenteric lymph node (severity index of minimal).
4. Extramedullary hematopoiesis (minimal to mild) of the mandibular lymph nodes was observed in animals at Week 13 and Week 26 with similar incidence (mild). At Week 60 (recovery), this finding was still observed.
5. Other findings which persisted at recovery are mononuclear cell infiltration in mandibular gland and mandibular lymph nodes, the biological significance of the findings are not known. Also, there was a decrease in IgG, IgM, and IgE levels in the belimumab treated animals until Week 26 (throughout the treatment period) compared to the vehicle treated animals. Paracortical expansion (minimal) of the mesenteric lymph node was apparent at recovery in 2/6 animals at low dose and 1/6 animals at high dose.

Table 52 Effects on Lymphoid Organs at Week 13, 26, and 60

Parameters	Dose (mg/kg)							
	0		5		15		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 13 n= 3/sex in treatment group, 2/sex in control								
MSLN/ hyperplasia	0	0	0	0	1	0	1	0
MSLN/ hemorrhage	0	0	0	0	0	0	0	1
MD LN/hemorrhage	0	0	0	0	0	0	1	0
MDLN, EMDH	0	1	2	2	2	3	1	1
Spleen, decrease lymphoid cell thickness	0	0	1	0	2	1	1	0
Spleen, decrease follicle size	0	0	1	0	2	1	2	0
Spleen, decreased lymphoid cell thickness (PALS)	0	0	1	0	2	1	1	0
Week 26 n= 3/sex in treatment group, 2/sex in control								
MSLN, lymphoid mass decrease	0	0	0	0	1	0	1	1
MSLN, sinus histiocytosis	0	0	0	0	2	0	1	1
MSLN, lymphoid depletion	0	0	0	0	0	1	1	0
MDLN, hyperplasia	1	0	1	3	1	3	2	1
MDLN, EMDH	1	1	2	2	0	3	1	2
MDLN/ paracortical expansion	1	1	1	0	1	0	2	1
MD, salivary gland fibrosis	0	0	0	0	0	0	1	0
MD, salivary gland mineralization	0	0	0	0	0	0	0	1
Spleen, protein deposition	1	0	1	0	0	0	2	0
Spleen, decrease follicle size, number	0	0	3	3	1	2	2	1
Spleen/fibrosis	1	1	2	1	2	1	1	2
Week 60 n= 2/sex in treatment group, 2/sex in control								
MSLN, hyperplasia	0	0	0	0	1	0	1	1
MSLN, eosinophilia	0	0	0	0	1	0	0	1
MSLN, paracortical expansion	0	0	1	1	0	0	1	0
MDLN, EMDH.	0	0	0	1	0	1	2	1
MDLN, erythrophagocytosis	0	0	0	0	0	0	0	1
MDLN, mononuclear cell infiltration	0	0	0	0	1	0	2	0
MD salivary gland mononuclear cell infiltration	0	1	0	0	1	1	2	1
Spleen, lymphoid hyperplasia	0	1	0	0	0	1	1	1

The histological lesions in the GI tract consisted of the following findings (refer to table):

1. At Week 13 and 26, lymphoid hyperplasia was observed in the cecum in males with no changes noted in females. No such changes were noted at Week 60,

suggesting recovery. At Week 60, 2/2 females, however showed vasculitis in cecum. Ciliate parasites were noted at Weeks 13 and 26 in all belimumab treated animal. The incidence of the parasite infection was higher in the belimumab treated animals compare to those of the controls. The incidence of parasite infection reduced at Week 60 in the test article treated animals indicating recovery.

- At Week 26, lymphoid hyperplasias were noted in a few belimumab treated animals in the colon and rectum, the incidences were not dose related, and were not observed at Week 60 indicating recovery. Ciliate parasites were noted in the colon and rectum of the belimumab treated animal. The number of incidences was higher than those of the controls. Therefore, the findings are believed to be treatment related, however, the parasite infection of colon and rectum were not observed at Week 60, indicating recovery.

Table 53 Effects on GI tract at Week 13, 26, and 60

Tissue/s	Dose (mg/kg)							
	0		5		15		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 13 n= 3/sex in treatment group, 2/sex in control								
Cecum, lymphoid hyperplasia	0	0	1	0	1	0	0	1
Cecum, ciliate parasite	0	2	2	1	2	2	2	2
Colon, ciliate parasite	0	1	2	0	1	1	2	2
Week 26 n= 3/sex in treatment group, 2/sex in control								
Stomach, lymphoid hyperplasia	0	0	0	0	0	0	1	1
Stomach, hemorrhage	0	0	0	1	0	0	1	0
Colon, lymphoid hyperplasia	0	1	0	0	1	1	0	1
Rectum, lymphoid hyperplasia	0	0	1	1	0	1	0	1
Cecum/lymphoid hyperplasia	0	0	0	0	1	0	2	0
Colon, ciliate parasite	0	0	2	1	1	0	2	0
Rectum, ciliate parasite	0	0	1	0	0	0	0	2
Cecum, ciliate parasite	0	0	2	1	2	1	2	1
Cecum, mononuclear cell infiltration	0	0	0	0	0	0	0	1
Week 60 n= 2/sex in treatment group, 2/sex in control								
Stomach, lymphoid hyperplasia	1	0	0	0	1	0	1	2
Cecum, mononuclear cell infiltration	0	1	1	0	1	0	2	1
Cecum, vasculitis	0	0	0	0	0	0	0	2
Cecum, ciliate parasite	1	0	0	1	2	0	1	1
Ileocecal valve, arterial & lymphoid hyperplasia, vasculitis,	0	0	0	0	0	0	0	1

The histological lesions in different tissues consisted of the following findings (refer to table):

1. At Week 13, the mononuclear cell infiltrations were noted in several tissues (heart, thyroid, and pancreas). At Week 26, similar findings were noted in heart and thyroid. This effect was considered treatment related as in most cases the effect was dose related and higher than those of the controls. However, no changes were noted at Week 60, indicating recovery. The heart findings at recovery consisted vasculitis in one female and hyperplasia of epicardium in another female. The thyroid findings at Weeks 13 and 26 consisted of follicular degeneration; however, no such findings were noted at recovery.
2. At Weeks 13 and 26, mineralizations were observed in the parenchyma and meninges in the brain from the test article treated animals, the incidences were absent in the control animals, therefore the findings are considered treatment related. These observations were not seen after recovery. The brain findings at recovery consisted of hemorrhage in the parenchyma from ½ males.
3. At Weeks 13 and 26, incidences of regeneration of tubules, glomerular thickening, mineralization, and hyperplasia were noted in the kidneys of the animals treated with belimumab. All of these incidences were higher than those of the controls, therefore the incidences were considered treatment related. All of these incidences were observed to be recovered at Week 60.
4. At Weeks, 13 and 26 pneumoconiosis were noted in lungs and similar findings were noted in the lung of the control animals. However, the numbers of such incidences were higher in the treated animals. At recovery, the finding was still observed, however, the number of incidences decreased, suggesting recovery.

Infections such as sarcocystosis and dermatitis were also noted at higher incidences in the belimumab treated animals at Weeks 13 and 26, the number of infections decreased at Week 60 suggesting recovery.

Table 54 Effect on Major Organ System at Week 13, 26 and Week 60

Tissue/s	Dose (mg/kg)							
	0		5		15		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 13 n=3/sex in treatment group, 2/sex in control								
Aorta, subintimal thickening	0	0	0	0	0	1	0	1
Brain, parenchyma, meningis & mineralization	0	0	0	0	1	0	1	1
Heart, mononuclear cell infiltration	0	1	0	2	0	2	0	2
Injection site/fibrosis	0	0	0	0	1	1	2	2
Liver, leukocyte foci	1	2	3	3	2	2	1	3
Liver, granuloma	0	0	0	0	0	1	1	0
Lung, pneumoconiosis	0	1	2	0	2	0	1	0
Kidney, regeneration of tubule	0	0	0	0	1	2	1	1
Kidney, glomerular thickening	0	0	0	0	0	1	0	2
Kidney, mineralization	1	0	1	1	0	0	1	1
Pancreas, mononuclear cell infiltration	0	0	0	0	0	0	1	1
Pancreas, fibrosis	0	0	0	0	0	0	0	1
Thyroid, mononuclear cell infiltration	0	0	0	0	0	1	1	1
Thyroid, follicular degeneration	0	0	3	2	1	0	0	1
Thyroid, ectopic thymus	0	0	0	2	0	1	1	0
Week 26 n=3/sex in treatment group, 2/sex in control								
Aorta/endothelium thickening	0	0	0	0	0	0	0	1
Brain, mineralization	0	0	0	0	0	0	1	1
Heart, fibrosis	0	0	0	0	0	0	0	1
Heart, mononuclear cell infiltration	0	0	0	1	3	0	2	1
Injection site, epidermal hyperplasia	0	0	1	1	0	1	1	2
Injection site, dermatitis	0	0	1	0	0	1	1	1
Liver, leukocyte foci	1	2	2	3	2	2	2	3
Lung, pneumoconiosis	2	2	3	0	3	2	3	3
Lung, alveolar macrophages	0	0	0	0	1	0	0	2
Lung, alveolitis	0	0	1	0	1	0	1	0
Lung, bronchiectasis	0	0	0	0	0	0	1	0
Kidney, hyperplasia (tubular epithelium)	0	0	0	0	0	0	1	0
Kidney, lymphoid follicle (pelvis)	0	0	0	0	0	0	1	0

Tissue/s	Dose (mg/kg)							
	0		5		15		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Kidney, mineralization (papilla & interstitium)	0	0	2	0	2	0	2	0
Thyroid, follicular degeneration	1	1	0	0	1	1	3	1
Thyroid, ectopic thymus	0	2	1	1	2	0	2	1
Thyroid, mononuclear cell infiltration	0	1	1	1	2	1	2	1
Sarcocystis, skeletal muscle	0	0	0	0	1	1	0	0
Sarcocystis, tongue	0	0	0	0	0	1	0	0
Sarcocystis, injection site	0	0	0	0	1	1	0	0
Dermatitis, neutrophilic, injection site	0	0	0	0	1	0	1	1
Week 60 n=2/sex in treatment group, 2/sex in control								
Adrenal gland, mineralization	0	0	0	0	0	0	0	1
Brain, hemorrhage, parenchyma	0	0	0	0	0	0	1	0
Sciatic nerve, vasculitis	0	0	0	0	0	0	0	1
Lung/pneumoconiosis	1	1	1	2	0	2	2	1
Kidney, vasculitis	0	0	0	0	0	0	0	1
Kidney, arterial hyperplasia	0	0	0	0	0	0	0	2
Cervix, vasculitis	0	0	0	0	0	0	0	1
Heart, vasculitis	0	0	0	0	0	0	0	1
Heart, hyperplasia, epicardial	0	0	0	0	0	0	0	1
Injection site, epidermal hyperplasia	0	0	0	0	0	0	0	2
Thymus/cyst	0	0	1	1	1	2	2	1
Sarcocystis, skeletal muscle, injection site, and tongue	0	0	0	0	0	0	1	0

The effect of belimumab on the male reproductive organs could not be appropriately evaluated because males were sexual immature. The histological changes in the ovary consisted of interstitial and germinal cell hyperplasia at Weeks 13 and 26, the incidence of findings was higher than those of the controls; the findings persisted at all doses in Week 60 suggesting no recovery. At Weeks 13 and 26, protein depositions were observed in the uterus, the incidences were higher than those of the controls. Therefore, the findings are considered treatment related, however, no such findings were noted at Week 60 suggesting recovery. The uterine findings at recovery consisted of vasculitis in ½ females from the high dose in the recovery group with no such findings were in control animals, therefore, the finding is considered treatment related. The sponsor did not conduct hormone analysis; menstrual cyclicity was assessed during clinical observation. Thus, the fertility assessments in males and females were incomplete.

Table 55 Effects on Reproductive System Weeks 13, 26, and 60

T issue/s	Dose (mg/kg)							
	0		5		15		50	
	♂	♀	♂	♀	♂	♀	♂	♀
Week 13 n=3/sex in treatment group, 2/sex in control								
Testes, incomplete spermatogenesis	2	NA	0	NA	1	NA	3	NA
Testes, fibrosis	1	NA	0	NA	0	NA	2	NA
Prostrate, mononuclear cell infiltration	1	NA	2	NA	2	NA	1	NA
Uterus, protein deposition	NA	0	NA	2	NA	2	NA	0
Ovary, hyperplasia of interstitial cell	NA	0	NA	3	NA	2	NA	1
Ovary, follicular cyst	NA	0	NA	1	NA	0	NA	2
Ovary, corpus luteum	NA	0	0	1	0	2	0	2
Week 26 n=3/sex in treatment group, 2/sex in control								
Testes, incomplete spermatogenesis	0	NA	2	NA	1	NA	2	NA
Prostrate, mononuclear cell infiltration	0	NA	1	NA	1	NA	1	NA
Uterus, protein deposition	0	1	0	0	0	2	0	1
Ovary, hyperplasia of interstitial cell	NA	1	NA	1	NA	3	NA	2
Ovary, corpus luteum	NA	1	0	1	0	3	0	2
Ovary, mineralization	NA	0	NA	0	NA	0	NA	2
Week 60 n=2/sex in treatment group, 2/sex in control								
Testes, incomplete spermatogenesis	1	NA	1	NA	1	NA	0	NA
Uterus, vasculitis	NA	0	NA	0	NA	0	NA	1
Ovary, hyperplasia of interstitial & germinal cell	NA	0	NA	2	NA	2	NA	2

Special Evaluation

Peripheral Blood Mononuclear Cell Populations:

The blood samples were collected from all animals on predose period, Weeks 13 and 26 of the treatment period and Week 60 of the recovery period. At each time point, the samples were quantitatively analyzed for the (refer to table) absolute lymphocyte populations, T lymphocytes (CD3⁺, CD3⁺/CD4⁺, CD3⁺/CD8⁺), monocytes (CD14⁺), and B lymphocytes (CD20⁺, CD20⁺CD21⁺).

Table 56 Effect of Belimumab on WBC Markers from PBMCs

Time of Investigation/ Dosages (mg/kg)	CD20 ⁺			CD20 ⁺ /CD21 ⁺			CD14 ⁺		
	5	15	50	5	15	50	5	15	50
Week 13	38* ↓	42* ↓	49* ↓	25 ↓	42* ↓	55* ↓	37 ↓	45 ↓	33 ↓
Week 26	59* ↓	58* ↓	65* ↓	60* ↓	69* ↓	73* ↓	1 ↑	33* ↑	42 ↓
Week 60	26 ↓	17 ↓	14 ↑	23 ↓	4 ↑	37 ↑	4 ↓	42 ↓	38 ↑

The results show the following (refer to table)

1. There were no differences in CD3⁺, CD3⁺/CD4⁺, and CD4⁺/CD8⁺ positive lymphocytes quantitatively. The magnitude of change from the baseline at each time point was similar between the belimumab treated and the control animals. The results indicate that belimumab is not affecting the number of total lymphocytes, T-helper/cytotoxic cells, and monocytes.
2. There was a statistically significant decrease in CD20⁺ and CD20⁺/CD21⁺ positive B lymphocytes in the peripheral blood from the belimumab (5, 15, and 50 mg/kg) treated animals compared to the control animals at Weeks 13 and 26. The decrease in the B lymphocyte populations were not dose related. There were no differences between the observed decrease in the CD20⁺ and CD20⁺/21⁺ cells indicating that the effect of belimumab within the B-cell population (such as immature and mature B-cells) does not vary significantly. The reduction in the B-cells was partially recovered at Week 60. The effect of belimumab observed is considered as its pharmacologic activity.
3. There were no meaningful changes in the monocyte population at Weeks 13 and 26 of the treatment period, except an increase (33% of baseline) at mid dose in Week 26. The biological significance of the finding is not known.

Determination of Mononuclear Cell Populations from Lymphoid Tissues

Flow cytometric analyses were conducted to characterize different subpopulations of the mononuclear cells from spleen and mesenteric lymph node (LN) at Weeks 13, 26, and 60.

The following changes in the lymphocyte population were noted.

1. There was a substantial decrease in the total B-cell (CD20⁺) and the mature B-cell population (CD 20⁺/CD21) in spleen and mesenteric lymph nodes at Weeks 13 and 26 in the belimumab treated animals. The decrease in the CD20⁺ cells and CD 20⁺/CD21⁺ positive cells in spleen and mesenteric lymph nodes were greater than 50 and 70% of the controls, respectively, at all doses. The changes were statistically significant (p=0.001); however, the changes were not dose dependent. The decrease in the B-cell population is considered the

pharmacologic effect of the drug product. The decrease in the B-cell population appeared to recover at Week 60.

- The monocyte population (CD3-/CD14+) decreased (> 60%) at Weeks 13 and 26 in the mesenteric lymph nodes in all belimumab treated animals. This effect was observed to be recovered at Week 60. There were no treatment related changes in the monocytes population in the spleens.
- There were no changes in the immature T lymphocytes (CD3+) and the subset of T-helper cells (CD3+/CD4+) in the spleen and mesenteric lymph nodes at Week 13 in the belimumab treated animals. At Week 26, however, there was an increase in the immature T lymphocytes (CD3+) and the subset of T-helper cells (CD3+/CD4+) in the spleen but not in the mesenteric lymph nodes. The changes were statistically significant (p=0.01), however the changes were not dose dependent. The changes in the T lymphocyte population might be a reflection of decreased B-cell population.

Table 57 Effect of Belimumab on WBC Markers from Spleen & Mesenteric LN

Immune Cell Markers/dosages(mg/kg)	Spleen			Mesenteric Lymph Node		
	5	15	50	5	15	50
CD20⁺						
Week 13	50 ↓*	54 ↓*	46 ↓*	71 ↓*	72 ↓*	77 ↓
Week 26	60 ↓*	53 ↓*	63 ↓*	72 ↓*	69 ↓*	75 ↓*
Week 60	37 ↓	34 ↓	5 ↓	26 ↓	NC	NC
CD20⁺/CD21⁺						
Week 13	64 ↓*	70 ↓*	62 ↓*	75 ↓	88 ↓	70 ↓
Week 26	74 ↓*	65 ↓*	72 ↓*	88 ↓*	91 ↓*	91 ↓*
Week 60	34 ↓	27 ↓	9 ↓	20 ↓	NC	NC
CD3/CD14⁺						
Week 13	NC	NC	NC	66 ↓	73 ↓	73 ↓
Week 26	NC	42 ↓	35 ↓	72 ↓*	82 ↓*	62 ↓*
Week 60	NC	NC	NC	17 ↑	46 ↑	30 ↑
CD3+						
Week 26	20 ↑*	32 ↑*	32 ↑*	NC	NC	NC
CD3+/CD8+						
Week 26	33 ↑*	13 ↑	33 ↑*	NC	NC	NC

NC- No change

Determination of Immunoglobulins (Igs) as a Measure of Immunomodulation:

The blood samples were collected at predose (Week 1) and Weeks 6, 13, 22, 34, 39, 52, and 60 for evaluating total immunoglobulins as well as IgA, IgM, IgE, and IgG from the monkeys treated intravenously (2x/week) with 0, 5, 15, and 50 mg/kg of belimumab. The immunoglobulin levels were analyzed by (b) (4) in a solid-phase ELISA in which affinity purified goat anti-human Ig capture antisera was adsorbed to individual wells of a 96 well micro titer plate and probed with various dilution of the serum from the belimumab treated monkeys.

The results show that the administration of belimumab (5, 15, and 50 mg/kg) produced different effects on the serum levels of different immunoglobulins in the monkeys. There was a decrease in IgG, IgM, and IgE levels in the belimumab treated animals until Week 26 (throughout the treatment period) compared to the vehicle treated animals. Among the degree of the reductions in between these three classes of immunoglobulins, the reduction was highest for the IgE levels followed by the IgM and IgG levels. The reduction in the IgE levels continued post dosing up to Week 39. At Week 60, all of these three types of immunoglobulin showed an increase. The IgA level, however, showed an increasing trend from Week 26 and this trend continues in the post dosing period. Note that none of these findings were dose related and not statistically significant. The biological significance of these changes is not known. Total Ig levels were measured (IgG +IgM+ IgA but not IgE, page 583 of the study report #1175-95), the levels of the total Igs at Week 1, 13, 26, 39, and 60 were 11, 12, 21, 5, and 7% of the control respectively at high dose (50 mg/kg). At week 26, the decrease in total immunoglobulin level was 21% which was observed to be slightly statistically significant (p=0.02). At Week 26, the reduction of the total immunoglobulin levels with 5 and 15 mg/kg doses were 23 (p=0.02) and 14 (p=0.4), respectively. The results show that at Week 26 of the treatment, the total immunoglobulin levels decreased after belimumab treatment. The decrease was not dose dependent and only slightly statistically significant. However, the finding is related to the pharmacological effect of the drug product and an immunosuppressive property of the product at this time point may be predicted. At Week 26, IgA level showed an increase while IgG and IgM levels were still decreasing indicating a compensatory mechanism for the immunoglobulin changes.

Table 58 Percent Changes in Different Ig Levels from 6-Month Monkey Study

Time/ Dose	IgG (mg/dL)			IgA (mg/dL)			IgM (mg/dL)			IgE (ng/mL)		
	Dosages (mg/kg)											
	5	15	50	5	15	50	5	15	50	5	15	50
Week 1	4↓	4↓	11↓	7↓	5↓	6↓	5↓	16↓	16↓	17↓	30↓	67↓
Week 13	4↓	7↑	11↓	5↓	12↓	16↓	21↓	31↓	31↓	46↓	58↓	23↓
Week 26	25↓	12↓	18↓	10↑	8↑	6↑	42↓	35↓	42↓	90↓	93↓	38↓
Week 39	NC	NC	20↓	30↑	25↑	35↑	27↑	4↓	15↑	73↓	28↓	52↓
Week 60	10↑	30↑	11↓	30↑	21↑	35↑	26↑	10↑	8↓	53↑	20↑	17↑

Toxicokinetics

The TK parameters of belimumab in the monkeys from the 6-month repeat dose toxicity study were measured by sandwich type ELISA in HGS INC. The blood sample were collected for ELISA analyses of the belimumab prior to dosing and every two weeks throughout the 26 weeks of dosing period and 8-months of the post dosing period. The last blood sample was collected at Day 406 (considering Day 1 is the initiation of the treatment). The serum concentration of the compound increased with the increase in dose. The increase in the serum concentration of the compound at the low and mid dose, however, was slightly more than dose proportional at Day 14 and Day 84. For example an approximate 4-6 fold increase in serum concentration was noted for a 3-fold

increase in dose (5-15 mg/kg) for both males and females (23 vs 95 mcg/mL at Days 14 and 45 vs 188 µg/mL at Day 84 in females and 33 vs 113 mcg/mL at Day 14 and 42 vs 255 mcg/mL at Day 84 in males). There serum concentrations of the compound in the males and the females were comparable. Similarly, the increase in the serum concentration from 5-50 mg/kg (10-fold) was approximately 11-14-fold.

No appreciable level of belimumab was noted with low and mid dose at Days 309 and 351 respectively. In high dose animals, ¼ animals showed belimumab level at Day 407. One male (FN19058M) from low dose group (at Week 10) and one female (FN19086F) from high dose group (at Week 16) showed altered PK for the belimumab, which confirmed the development of the antibody against the drug product in these two animals.

The serum concentration was observed to reach the steady state between Day 71. The accumulation of the compound was approximately 2-fold. During the recovery period, the serum concentrations of belimumab declined approximately monoexponentially with half-lives ranging from 9.05 to 15.8 days. In the low-dose group, there was no detectable belimumab in 3 out of 4 recovery monkeys by Day 253 and 4 out of 4 by Day 323. In the mid-dose group, there was no detectable belimumab in 3 out of 4 recovery monkeys by Day 309 and 4 out of 4 by Day 351. In the high-dose group, there was no detectable belimumab in 3 out of 4 recovery monkeys by Day 394, but one monkey still had low levels (0.1 mcg/mL) of belimumab at Day 407.

Mean dose-normalized serum concentrations of belimumab in Cynomolgus monkeys following IV injection of 5, 15, or 50 mg/kg. [Points represent the mean of 16 monkeys through Day 84, 10 monkeys from Day 98 through 182, and 4 monkeys after Day 182. Concentrations below the limit of quantification of the ELISA were averaged in as zero for graphing purposes only. Error bars represent standard deviation.]

Figure 34 Belimumab Serum Concentration: 3, 6 Month Repeat Dose Study

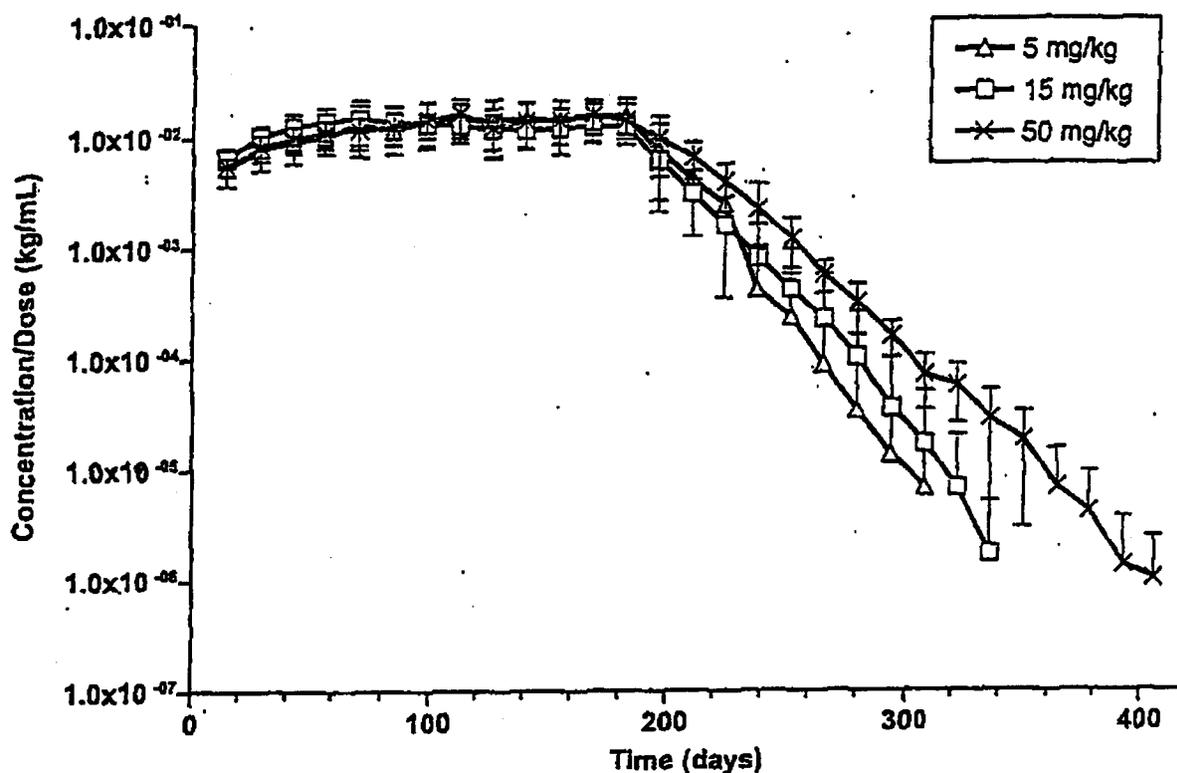


Table 59 Serum Concentration of Belimumab: 3 & 6 Month monkey Study (mcg/mL)

Dose/Time	Male	Female
5 mg/kg		
Day 14	33±4.1	23.3±2.7
Day 84	42.3±10	45.6±20
Day 182	93±31.4	65±10.8
Day 266	LOQ	LOQ 1/2
Day 406	LOQ	LOQ
15 mg/kg		
Day 14	113.8±8.5	95.5±7.5
Day 84	255.2±39	188.8±22.5
Day 182	199.33±20.3	181.6±15.4
Day 266	2.0±1.2	2.5±1.1
Day 406	LOQ	LOQ
50 mg/kg		
Day 14	270±14	279±26
Day 84	540.5±41	658±103
Day 182	608±6	872±83
Day 266	25±5.1	35 (1/2)
Day 406	0.1 (1/2)	LOQ

Table 60 Individual Animal Serum Belimumab Concentrations (mcg/mL)

TREATMENT (5 mg/kg)						RECOVERY					
Male		Female		Male		Female		Male		Female	
Day 14	42	26	Day 84	26	26	Day 182	61	59	Day 14	29	24
	27	34		52	78		144	81	Day 84	20	44
	21	20		49	33		74	52	Day 182	LOQ	58
	42	25	Mean	42.3	45.6		93	65	Day 266	LOQ	LOQ
	39	16	SE	10	20		31.4	10.8	Day 406	LOQ	LOQ
	27	19							Day 14	16	34
Mean	33	23.3							Day 84	34	75
SE	4.1	2.7							Day 182	41	121
									Day 266	LOQ	2
									Day 406	LOQ	LOQ
TREATMENT (15 mg/kg)						RECOVERY					
Male		Female		Male		Female		Male		Female	
Day 14	121	61	Day 84	348	90	Day 182	219	211	Day 14	90	108
	108	93		326	153		220	160	Day 84	190	227
	91	101		129	257		159	174	Day 182	196	268
	112	108		240	227		199.333	181.67	Day 266	4	5
	150	113		329	198		20.3	15.35	Day 406	LOQ	LOQ
	101	99		159	208				Day 14	80	90
Mean	113.8	95.5	Mean	255.1667	188.83				Day 84	147	116
SE	8.5	7.6	SE	39.2	22.5				Day 182	126	132
									Day 266	0.2	0.3
									Day 406	LOQ	LOQ
TREATMENT (50 mg/kg)						RECOVERY					
Male		Female		Male		Female		Male		Female	
Day 14	322	366	Day 84	707	1070	Day 182	447	720	Day 14	357	300
	304	329		602	784		853	891	Day 84	921	862
	262	174		491	354		526	1007	Day 182	1114	317
	271	256		549	439		608.667	872.67	Day 266	31	35
	237	316		390	509		123	83.5	Day 406	0.1	LOQ
	224	235		504	795				Day 14	273	231
Mean	270	279.33	Mean	540.5	658.5				Day 84	531	347
SE	14.3	26.6	SE	40.9	102.9				Day 182	430	39
									Day 266	18	LOQ
									Day 406	LOQ	LOQ

Determination of Immunogenicity:

The blood samples for the assessment of serum immunoglobulins specific for belimumab were collected during Week 0 (pre-dose) and Week 13 for all monkeys, and for all remaining monkeys in each dose group at Weeks 26, 52 and 60. The presence of belimumab-specific antibodies was determined by standard ELISA techniques with detection using anti-IgA, anti-IgM, anti-IgG-Fc, and anti-kappa-light-chain.

Immunogenicity was analyzed for the anti drug antibodies (ADA) directed to the drug product (belimumab). In brief, ADAs were evaluated on two microplates: one coated with the whole drug product and the other with the Fab fragment of the drug product. Diluted sera (1:100) from the belimumab treated animals were incubated on both of these plates. Detection of the immunoglobulins in the monkey sera that adhere to the belimumab coated plate was accomplished by using an HRP-conjugated anti-human kappa light chain specific antibody. This conjugated immunoglobulin recognizes all the immunoglobulins that contain kappa light chains. The conjugate used on the microplates coated with the belimumab Fab fragments recognizes all IgG, IgA, and IgM immunoglobulins that have bound to the microplates regardless of the light chain. This assay is not a highly sensitive, quantitative determination of ADA especially in sera with high concentrations of belimumab.

The presence of monkey antibodies to belimumab was defined by an increase in absorption of at least two fold from sera samples obtained post treatment compared to the absorption obtained from the predose sera from the same monkey. Based on this criterion, 2 of 60 monkeys (FN19058hf and Fbl19084F) were positive during the treatment phase.

Table 61 Immunogenicity Results: 3 & 6-Month Monkey Study

<u>Monkey ID</u>	<u>LymphoStat-B</u>	<u>Assay</u>	<u>Week 0 (pre-dose)</u>	<u>Week 13</u>	<u>Week 26</u>	<u>Week 52</u>	<u>Week 60</u>
FN19097F	0 mg/kg	Fab	0.080	0.093	0.090	0.094	0.088
		IgG	0.196	0.141	0.145	0.133	0.139
FN19058M	05 mg/kg	Fab	0.092	0.162	0.967	0.122	0.152
		IgG	0.112	0.874	1.943	0.183	0.190
FN19019M	15 mg/kg	Fab	0.092	0.083	0.083	0.076	0.080
		IgG	0.088	0.090	0.108	0.094	0.092
FN19086F	50 mg/kg	Fab	0.078	0.184	0.295	0.094	0.110
		IgG	0.266	0.296	0.435	0.286	0.260

Monkey No. FN19058M had decreased serum belimumab as compared to other low dose group monkeys (5 mg/kg/dose) that was first apparent on study Day 43 (during Week 6), and no belimumab was detected on or after Day 84. The data demonstrated a positive response for IgG at both Weeks 13 and 26 and for Fab only at Week 26.

Although generally consistent, the tabular data for this monkey from assays run during the study indicated a positive response to both Fab and IgG at both Weeks 13 and 26. Monkey No. FN19086F had decreased serum belimumab as compared to other monkeys from the high dose group monkeys (50 mg/kg/dose) that was apparent by study Day 168 (during Week 24) and no belimumab was detected on or after Day 196; This monkey was positive for anti-Fab antibodies as early as Week 13. The tabular data for this monkey from assays run during the study indicated a positive response to Fab at Week 26 only.

In summary, the data indicated that of the 60 monkeys in this study, two monkeys developed an antibody response to belimumab. Positive monkeys included one male from the low-dose group (5 mg/kg) and one female from the high-dose group (50 mg/kg). The anti-LymnphoStat-3 antibody produced was variably directed at the whole molecule or Fab portion of the belimumab-B antibody molecule.

Dosing solution analyses

Analysis of the dosing solutions indicated that all solutions ranged between 90% and 100% of the target concentration.

Study title: 22-Week Subcutaneous Injection Immunogenicity and Toxicokinetic Study with LymnphoStat-B in Cynomolgus Monkeys, (b) (4) Study # 6962-161

Study no.: (b) (4) Study # 6962-161
 Study report location: EDR
 Conducting laboratory and location: (b) (4)
 Date of study initiation: May 09, 2005
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: LymnphoStat-B (belimumab), Lot # 00A06019, 99% pure; placebo Lot #: 710001

Key Study Findings

- The Cynomolgus monkey (5/sex/group) was administered the lyophilized formulation of belimumab SC for 13 weeks (1 mg/kg/every 2x/ week and 4x/week), 3/sex/group were sacrificed at Week 13 and 2/sex/group were allowed to recover for an additional 9 weeks and sacrificed at Week 22.
- Study endpoints included mortality, clinical signs, clinical pathology, toxicokinetics evaluations, immunophenotyping, and immunogenicity results. No histology was conducted within this experimental procedure. The study was conducted mainly to evaluate toxicokinetics, immunogenicity, and pharmacodynamics of the product after subcutaneous administration.
- The systemic exposures of belimumab as described by AUC, increased with the increase in the doses (administered 2 times weekly or 4 times weekly). However, the increase was less than dose proportional at Week 1 (AUC_{0-7d} mcg.day/mL

for 2x weekly and 4x weekly were approximately 96 and 126 respectively) as well as at Week 13 (AUC_{0-7d} mcg.day/mL for 2x weekly and 4x weekly were approximately 444 and 780 respectively). Similar observation was made with C_{max}. The systemic exposures declined steadily after the treatment period was over and during the 9 weeks of recovery. The detectable serum concentrations of belimumab were, however, still present in 18 of 20 belimumab-treated animals on Study Day 154.

- The serum concentrations prior to weekly administration of the compound (as indicated from the predose values, see the table below) increased with the duration of the treatment up to Week 8. The pre dose serum concentrations with the low dose were approximately 18 and 60 at Week 1 and Week 8 respectively. The pre dose serum concentrations with the high dose were approximately 35 and 109 at Week 1 and Week 8 respectively. There were no increases in the serum concentrations with the increases in the treatment period indicating that the steady state was reached at Week 8 for both of the doses studied. The t_{1/2} at Week 13 was determined to be 11-12 days.
- For both dosing regimens, the C_{max}, AUC_{0-7d}, and C_{7d} (serum concentration) values following the Week 13, after the SC dosing were significantly higher than the corresponding values following Week 1 dosing. There were an average of 3.5-, 4.7-, and 3.7-fold accumulation at Week 13 for C_{max}, AUC_{0-7d}, and C_{7d}, respectively, at the 1mg/kg twice-weekly doses. The average accumulation at Week 13 was 3.5-fold for C_{max}, 6.2-fold for AUC_{0-7d}, and 3.0-fold for C_{7d}, with the 1mg/kg four times weekly doses. This accumulation might be due to the long half life of the compound.
- The compound showed minimal immunogenicity.
- Belimumab was administered through Day 85, and immune cell phenotyping continued for an additional 9 weeks, through Day 154. A reduction CD20⁺ B cells in the belimumab-treated monkeys was observed at Day 85. This reduction was statistically significant (p < 0.05, two-sample t-test with unequal variance) and did not reverse by the end of the study at Day 154.
- There were apparent increases in T-helper, cytotoxic T cells, and all T cells between Days 75-100. At this time period statistically significant B-cell depletion was observed. The results indicate that a compensatory mechanism, T lymphoid cell proliferation, occurred at this time. However, a decrease in the T cell populations was noted in the serum after this period, although B- Cell depletion continues.

Methods

Doses: 0, 1 mg/kg
Frequency of dosing: 2x/week or 4x /week for 22 weeks
Route of administration: Subcutaneous
Dose volume: 0.25 mL/ injection
Formulation/Vehicle: LSB (belimumab) was formulated in 10 mM sodium citrate, 8% sucrose, 0.04% (w/v) polysorbate 80, pH 6.5, the placebo contains all the components except the protein.
Species/Strain: Cynomolgus monkeys
Number/Sex/Group: 5/sex/group
Age: 2.8-4 years
Weight: Males 2.8 -4.0 kg; Females: 2.3-3.4 kg
Satellite groups: 2 animals/sex/group were maintained for recovery for 9 weeks, post treatment
Unique study design: Refer to study design table submitted by Applicant, the study design is not unique
Deviation from study protocol: The protocol deviations were documented in page 59 of the study report, according to the reviewer, the study results are not affected by the protocol deviations.

Table 62 Study Design (Applicant's table): 22-Week Monkey (SC) Study

Group	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
	Male	Female		
1 (Placebo)	5	5	0	0
2 (Low - 2 times/week dosing)	5	5	1	4
3 (High - 4 times/week dosing)	5	5	1	4

Mortality

Animals were checked twice daily for mortality and morbidity. There was no mortality or morbidity in this study.

Clinical Signs

Animals were checked twice daily for mortality and abnormalities, and signs of pain or distress. Detailed observations were made once weekly and on the day of scheduled sacrifice. Daily cage side observations were made for each animal approximately 1 to 2 hours post dose, except on days when detailed observations were conducted. There was no test article related clinical signs in this study.

Body Weights

The body weights were taken four times during the predose phase, on the first day of dosing, weekly thereafter through Week 14, and during Weeks 18 and 22 (day of scheduled sacrifice). No test article related changes in the body weight were observed.

Feed Consumption

Qualitative food consumption was assessed once daily. The feed consumption appeared to be within the normal variation throughout the study.

Special Evaluation

Immunophenotyping:

Blood samples were drawn from the femoral vein at the following time points: Days -7 (Day 22 of pre dose phase), 1 (pre dose), 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, and 154 for quantization the following parameters. The following cell surface receptors were stained with the respective antibodies and counted by flow cytometry.

Lymphocyte Subsets Phenotype

Total T cells CD3⁺

Helper T cells CD4⁺

Cytotoxic T cells CD8⁺

B cells CD20⁺

Natural killer cells CD3⁻/CD16⁺

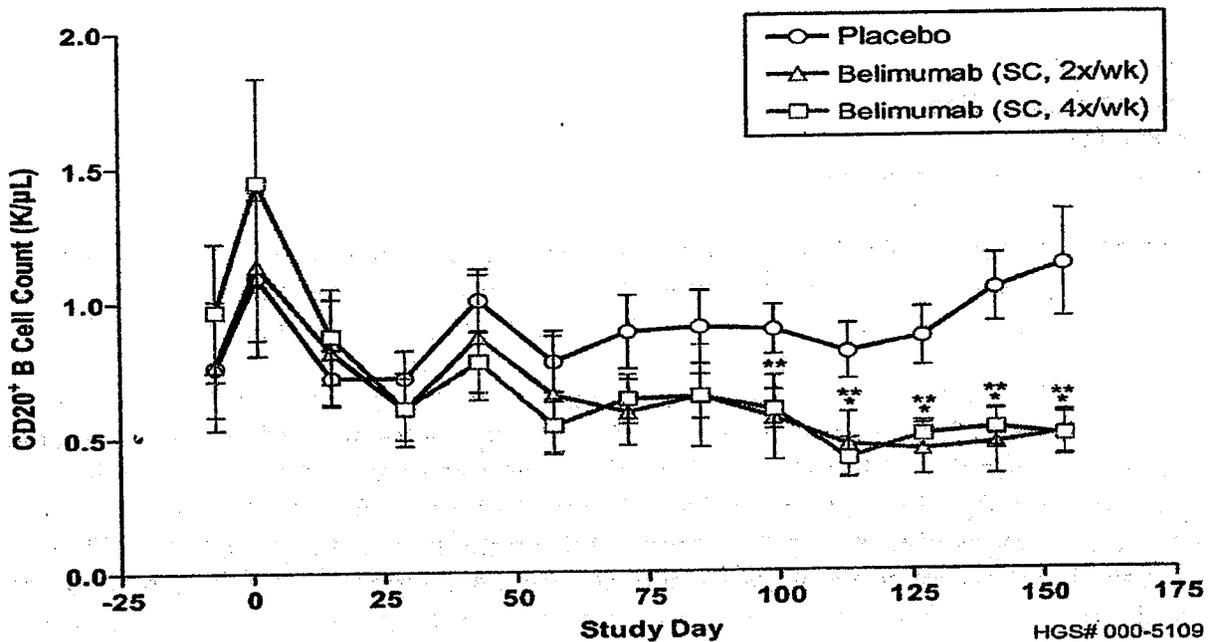
The number of the B-cells, T-helper, cytotoxic, and natural killer cells were calculated from different treatment days and compared to their predose level (refer to table). The number of the T helper and cytotoxic T-cell did not change compare to their pre-dose values. However, there was a decreasing trend in the number of the CD 20⁺ B lymphocytes from Day 29 onwards. This statistically significant (p<0.0001) reduction of B-cells in belimumab treated animals persisted during the treatment-free period and did not reverse by the end of the study at Day 154. The decrease in the number of B-cells compared to those of the controls at Days 85 and 154 were 25 and 50% respectively. Both of the dosages showed similar degree of the reduction in B-cell numbers. There were detectable serum concentrations of belimumab still present in 18 of 20 belimumab-treated monkeys on Day 154 which might explain the reduction of B-cell number at 9-weeks post dosing. Interestingly, the numbers of natural killer cells were found to be increased from Day 57 of the treatment through the recovery period up to Day154 in the low dose treated animals. The absolute cell numbers of the natural killer cell from the high dose group animals did not show similar findings. Therefore, the significance of such findings is not known.

Table 63 Summary of Findings: Immunophenotyping

Days of Administration	Absolute Cell Count (Percent Control)									
	CD3+		CD4+		CD8+		CD20+		CD3-/CD16+	
	LD	HD	LD	HD	LD	HD	LD	HD	LD	HD
Treatment Period										
Day 29	4 ↓	4 ↓	13 ↓	7 ↓	9 ↑	1 ↓	4 ↑	32 ↑	12 ↓	8 ↓
Day 57	17 ↑	1 ↑	4 ↑	6 ↓	39 ↑	9 ↑	16 ↓	16 ↓	3 ↑	15 ↓
Day 85	6 ↑	4 ↑	4 ↓	1 ↓	24 ↑	17 ↑	28 ↓	28 ↓	9 ↑	2 ↓
Recovery Period										
Day 127	6 ↓	3 ↓	15 ↓	2 ↓	7 ↑	2 ↑	49 ↓	42 ↓	22 ↑	6 ↓
Day 154	2 ↓	15 ↓	7 ↓	18 ↓	9 ↑	11 ↓	55 ↓	56 ↓	48 ↑	10 ↓

CD20+ B cell counts: the mean and the SEM are shown using a repeated measures model, there was a significant difference between the placebo group and the 2x and 4x per week group (Study Day 113 through end of study **p < 0.05) insert figure.

Figure 37 CD 20+Positive B-Cell Counts



Toxicokinetics

The blood samples were collected from the low dose group at pre dose, 24 hrs, and 48 hrs following test article administration from Day 1 and weekly thereafter. Similarly, the blood samples were taken from the high dose group at pre dose, 24 hrs, 48 hrs, and 96 hrs following test article administration from Day 1 and weekly thereafter. The blood samples were collected as indicated above during the treatment as well as the recovery period. The serum concentration of belimumab was quantified by ELISA. The serum

concentration of belimumab exposure is shown in the table below. There was no apparent gender difference.

The systemic exposures of the belimumab as described by AUC, increased with the increase in the doses (administered 2 times weekly or 4 times weekly). However, the increase was less than dose proportional at Week 1 (AUC_{0-7d} mcg.day/mL for 2x weekly and 4x weekly were approximately 96 and 126 respectively) as well as at Week 13 (AUC_{0-7d} mcg.day/mL for 2x weekly and 4x weekly were approximately 444 and 780 respectively). Similar observation was made with C_{max}. The systemic exposure declined steadily after the treatment period was over and during the 9 weeks of recovery. The detectable serum concentrations of belimumab were, however, still present in 18 of 20 belimumab-treated animals on Study Day 154.

The serum concentration prior to weekly administration of the compound (as indicated from the predose values, see the table below) increased with the duration of the treatment up to Week 8. The pre-dose serum concentrations with the low dose were approximately 18 and 60 at Week 1 and Week 8, respectively. The pre-dose serum concentrations with the high dose were approximately 35 and 109 at Week 1 and Week 8 respectively. There were no increases in the serum concentrations with the increase in the treatment period indicating that the steady state was reached at Week 8 for both of the doses studied. The t_{1/2} at Week 13 was determined to be 11-14 days. For dosing regimens the C_{max}, AUC_{0-7d}, and C_{7d} (serum concentration) values following the Week 13, after the SC dosing were significantly higher than the corresponding values following Week 1 dosing. There were an average of 3.5, 4.7, and 3.7fold accumulations at Week 13 for C_{max}, AUC_{0-7d}, and C_{7d} respectively, at the 1mg/kg twice-weekly doses. The average accumulations at Week 13 were 3.5-fold for C_{max}, 6.2-fold for AUC_{0-7d}, and 3.0-fold for C_{7d}, with the 1 mg/kg four times weekly doses. These accumulations might be due to the long half life of the compound.

Table 64 Summary of Toxicokinetics Parameters

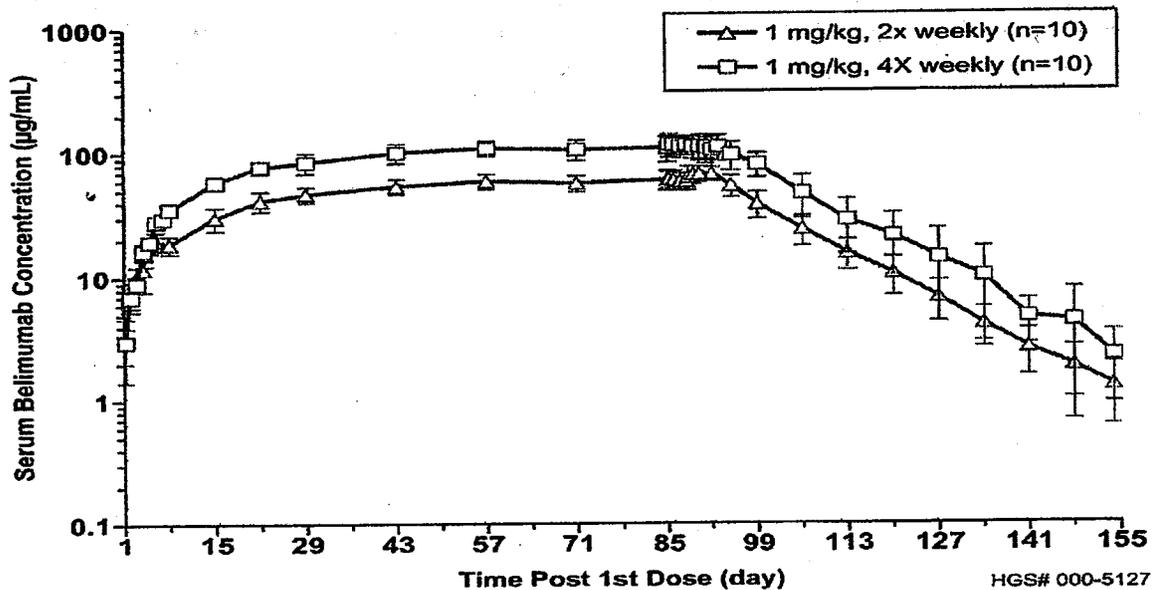
Parameters	Low Dose	High Dose
Cmax, week 1(mcg/mL)	21.0 ± 2.9	35.8 ± 3.9
Cmax, week 13 (mcg/mL)	72.6 ± 10.4	124.1 ± 22.4
AUC _{0-7d} , week 1 (mcg.day/mL)	96.7 ± 13.3	126.7 ± 12.8
AUC _{0-7d} , week 13 (mcg.day/mL)	444.1 ± 60.9	780.2 ± 156.5
Serum concentration at 7 days, post Week 1 dosing	18.7 ± 3.0	35.8 ± 3.9
C7 days, post Week 13 dosing	67.3 ± 8.5	104.8 ± 28.8
C Week 4	41.9 ± 7.8	77.5 ± 7.8
C Week 9	59.5 ± 8.2	109.1 ± 15.5
C Week 11	57.8 ± 8.4	106. ± 19.7
C at Week 13	60.8 ± 10.9	109.2 ± 25.7
Cmax.n (mcg/mL)*	3.52 ^a	3.5 ^a
AUC _{0-7d.n} (mcg.day/mL)*	4.65 ^a	6.22 ^a
C _{7d.n} mcg/mL*	3.66 ^a	2.95 ^a
Css mcg/mL**	0.97	0.97
T1/2	11.58 ± 3.01	10.92 ± 2.81

*ratio of values Week13 vs Week 1

** Ratio of values Week 11 vs Week 9

^a p<000.1

Figure 38 Mean Serum Concentration of belimumab in Cynomolgus Monkeys



Immunogenicity

For immunogenicity evaluation, blood samples were drawn at the following time points: Days 1 (predose), 8, 15, 22, 29, 43, 57, 71, 85, 92, 99, 106, 113, 120, 127, 134, 141, 148, and 154. The immunogenicity was measured using ELISA first by capturing the belimumab specific antibodies from the monkey serum diluted at 1:35; the specificity was then confirmed by the serial dilution of the serum. One male at high dose was confirmed to have the anti product antibody in 13/19 blood samples. However, 5/10 monkeys (3 males and 2 females) showed positive reaction for the anti product antibody with high dose at the same time which could not be confirmed by the titrating of the serum at the same time point. 3/5 females (no males) showed positive reaction for the anti belimumab antibody with low dose, which can not be confirmed by titrating. It is not clear from the report at what time point these antibodies were detected in each animal.

Dosing solution analyses

The concentration verification results from Days 1, 31, 32, 60, 61, 88, and 91 showed that the belimumab formulations were within 10% of the expected concentration for all preparation dates. Therefore, the dose formulations were considered acceptable for use on the study. Analysis of the dosing solutions indicated that all solutions ranged between 90% and 100% of the target concentration.

7 Genetic Toxicology

No genotoxicity study was submitted in this application. Per ICH S6 and S6 addendum, no genotoxicity studies are required for a monoclonal antibody.

8 Carcinogenicity

No carcinogenicity studies were submitted to this application.

Carcinogenicity studies were not done for belimumab due to the lack of ability to complete standard 2-year bioassays in mice. High level of anti-drug-antibody formation and death were noted in mice after repeated administration of belimumab and mice specific anti BLyS antibody production. Also, increased neoplasia was not observed in the A/WySNJ mouse that has a non-functional BR3/BAFF-R. In addition, no neoplasia was observed in the repeat dose toxicity studies in monkey. The reviewer believes that the carcinogenicity study is not feasible in rodents due to immunogenicity. The lack of carcinogenic assessment will be captured in the belimumab label.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

No male or female fertility studies were conducted. Conducting a female fertility study in monkey is not deemed feasible. The Applicant evaluated male and female reproductive organs in the chronic intravenous toxicity study [HG19300-T05]. The

reproductive organs from the female monkeys were assessed. However, no hormone analysis or menstrual cyclicity was conducted. The male reproductive toxicity could not be determined because of the inclusion of sexually immature males in the repeat dose toxicity study. The evaluations of the reproductive organs are discussed in the review of the chronic toxicity study. Per agreements with the Agency dated March 26, 2003, no fertility study is required; the belimumab product labeling will capture the lack of fertility assessment data.

9.2 Embryonic Fetal Development

The Applicant conducted a combined embryo fetal development and peri/post-natal development in the Cynomolgus Monkey. This approach is considered acceptable per ICH Guidance S6 and S6 Addendum and ICH M3 (R2).

9.3 Prenatal and Postnatal Development

Study title: A Dose-Tolerance Study of Lymphostat-B™ Administered by Intravenous Injection to Non-Pregnant Female Cynomolgus Monkeys
Study number: 1736-95

This is a non-GLP dose range finding study conducted to achieve the maximum tolerated dose for the combined embryo-fetal/peri-post natal reproductive toxicity study. Three female monkeys were administered IV doses at approximately 121 mg/kg of belimumab on Day 1, 97 mg/kg on Days 4, and 125 mg/kg on Day 15 by slow bolus IV injection (refer to study design table). The Applicant targeted a Cmax of approximately 3700 mcg/mL. The target was predicted for the administration of 150 mg/kg biweekly administration for 22 weeks in monkeys which will give an approximate safety margin of 10-fold over belimumab administration in human. The animals were necropsied at days 17-18. There were no test article related changes in the clinical observations, feed consumption, and body weights and no test article related gross lesions were observed.

The bioanalytical analyses showed that the mean serum belimumab levels from the 3 monkeys after the 1st, 2nd and 3rd dosing were approximately 2531, 2805, and 3785 mcg/mL, respectively, indicating that the target level was achieved. Based on this study the Applicant initiated the pivotal reproductive toxicity study with the maximum dosing of 150 mg/kg.

Table 65 Study Design: Dose Tolerance Study (Applicant's table)

Phase	Animal No.	Body Weight	Study Day	Actual Dose Volume (mL)	Dose Volume per Body Weight (mL/kg)	Actual Dose Solution Concentration (mg/mL)	Dose Level (mg/kg/dose)
1	F9504F	2.1 ^a	1	13.0	6.2 ^b	20	123.8 ^b
		2.1 ^a	4	13.0	6.2 ^b	16	99.0 ^b
		2.3	15	17.5	7.5	16.67	125.0
	F18176F	2.2 ^a	1	13.5	6.1 ^b	20	122.7 ^b
		2.2 ^a	4	13.5	6.1 ^b	16	98.2 ^b
		2.3	15	17.5	7.5	16.67	125.0
	F23460F	2.55 ^a	1	15.0	5.9 ^b	20	117.6 ^b
		2.55 ^a	4	15.0	5.9 ^b	16	94.1 ^b
		2.7	15	20.5	7.5	16.67	125.0
2	F21388F	2.95 ^a	1	18.0	6.1 ^b	0	0
		2.95 ^a	4	18.0	6.1 ^b	0	0
		3.1	15	23.5	7.5	0	0
	F23885F	2.65 ^a	1	16.5	6.2 ^b	0	0
		2.65 ^a	4	16.5	6.2 ^b	0	0
		2.9	15	22.0	7.5	0	0
	F23287F	3.4 ^a	1	22.0	6.5 ^b	0	0
		3.4 ^a	4	22.0	6.5 ^b	0	0
		3.5	15	26.5	7.5	0	0

- a The body weight used to determine Day 1 and 4 dose levels was based on the Day -1 body weights which were collected improperly. The body weight for each animal for Days 1 and 4 was estimated based on the average of the body weights collected on Day -7 and Days 6 (Phase 2) or 8 (Phase 1). The Day 15 body weight was collected properly prior to dosing on Day 15.
- b The dose volume by body weight and dose level used was estimated using the average of the body weights collected on Day -7 and Days 6 (Phase 2) or 8 (Phase 1); therefore, dose levels listed for Days 1 and 4 are estimated. Dose levels for Day 15 are actual, based on Day 15 body weight.

Study title: Maternal, Fetal and Neonatal Toxicity Study of LymphoStat-B™ Administered Bi-Weekly by Intravenous (Bolus) Injection to Pregnant Cynomolgus Monkeys, Including a One Year Postnatal Evaluation

Study no: 1721-95
 Study report location: EDR
 Conducting laboratory and location: [REDACTED] (b) (4)
 Date of study initiation: July 19, 2004
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity: LymphoStat-B (belimumab), Lot No. 03A06117

Key Study Findings

- The combined embryo-fetal and peri/postnatal toxicity study was conducted by biweekly, intravenous administration of 0, 5, and 150 mg/kg in pregnant Cynomolgus monkeys.
- There were 3 (fetus), 8 (6 fetus+ 2 infants), and 4 (3 fetus+ 1 infants) deaths in this study in 5, and 150 mg/kg dose group respectively. The percent of fetal losses were 14 and 20%, respectively, from the control and test article treated animal (historical control for fetal death is 17.6%). The percent of infant deaths were 0 and 7% in control and test article treated animals, respectively (historical control for infant death is 10-12%). The percent of fetal and infant deaths in the belimumab treated animals were higher than the control group under this experimental condition. The cause of deaths for the fetus and the infants were unknown. However, most of the deaths were associated with atelectasis of the lungs.
- There were two infants from the high dose group which showed sleepiness, no muscle tone, and one infant from the low dose group also showed non responsiveness (this one died at BD7); two infants from the control also had similar findings. The degree of the severity of these findings from the high dose group might be higher than that of the control group; the data is not clear from the Applicant's description.
- The serum immunoglobulin level (IgM) was decreased one year post partum in the infants. No changes in IgG, IgA, and IgE were noted.
- Serum belimumab concentration was observed to be linear in adult females. Belimumab was transported through placenta and the serum belimumab levels in infants were 5-fold less than the mothers. Belimumab was observed in milk.
- The immunogenicity assay was not sensitive due to the interference of belimumab in serum. However, one adult female had ADA formation and the fetus from this animal was normal. The female however, showed clinical signs of muscular dystrophy and the histopathology observation from this animal showed

atrophy of axial muscle in thigh and sciatic nerved proximal to it showed axonal degeneration.

Methods

Doses: 0, 5, 150 mg/kg
 Frequency of dosing: Once every 2-weeks
 Dose volume: 7.5 mL/kg
 Route of administration: Intravenous (slow bolus)
 Formulation/Vehicle: LymphoStat-B™ (belimumab) was formulated as a liquid solution of 20 mg/mL of protein in 1.9% glycine, 0.5% sucrose, 10 mL sodium citrate, and 0.01% polysorbate 80 at a pH of 6.5 ± 0.4; the formulation without the protein was used as vehicle
 Species/Strain: Cynomolgus monkeys
 Number/Sex/Group: Refer to study design table
 Satellite groups: None
 Study design: Refer to Applicant's table

Table 66 Study Design: Embryofetal/Peri-Postnatal Toxicity Study

Group No	No ♀	Body wt (kg)	Belimumab (mg/kg)	Dose Conc. mg/mL	Number of pregnant ♀			Mother Released from Study
					C-section	Natural Delivery	Fetal** Losses	
1	11	3.00 0.4	0	0	9	--	2; 1 ea at GD 49, 97	After C-section
2	10	3.30 0.4	0	0	--	9	1, GD 133	1 year PPD
3	13	3.60 0.7	5	0.67	9	--	4; 2, 1, 1 at GDs 50; 52, 87, respectively	After C-section
4	12	3.50 0.7	5	0.67	--	10	2, 1 ea GDs 38, and 50 respectively	1 year PPD
5	9	3.10 0.4	150	20	9	--	0	After C-section
6	11	3.30 0.4	150	20	--	8	3, 1 ea at GDs 125, 161, and 151	1 year PPD

*Volume administered 7.5 mL

** Note that there were three infant deaths in this study, two from low dose group at BDs 7 and 11, and one from high dose group at 2 hrs after birth at GD 146

Unique Methodology: Cynomolgus monkeys, 3.8 to 15 years of age, and weighing 2.4 kg to 5.2 kg prior to pregnancy, were assigned to dose groups as shown in the study

design table. Different concentrations of belimumab or vehicle control were administered to pregnant Cynomolgus monkeys via intravenous injection (slow bolus) within two days of confirmed (by ultrasound) pregnancy (GD20 to GD22), GD34 and every 14 days thereafter until GD150. Ultrasounds were performed (\pm 3 days) on GD50 (approximate end of major organogenesis), GD90, GD115, and GD140 (near end of dosing) to evaluate the general condition, heart rate, and developmental landmarks of the embryo/fetus. The study consisted of two phases. Phase 1 consisted of the study data for Groups 1, 3, and 5 prior to mating through GD150 Cesarean section or day of early parturition and for Groups 2, 4 and 6 prior to mating through the day of parturition. Phase 2 includes the study data for Group 2, 4 and 6 adult females and infants from the day of parturition through approximately 1 year after birth.

Observations and Results

All of the pregnant animals survived; however, the animal #1392 had muscular atrophy in the right leg and preferred using the left leg. The animal was euthanized 15 days after C-section (the fetus # 9114 was observed to be normal). Prior to euthanizing blood samples were collected to assess clinical pathology, flowcytometric analyses of B-cell markers, immunogenicity and toxicokinetics. High ADA was observed in the screening assay and the specificity of the ADA was determined in the confirmatory assay. The data showed that at GD 150, the time of necropsy, there were significant amounts of ADA formation (belimumab concentration is still 207 mcg/mL). The neutralization assay failed to produce a clear result due to high titer of the drug product.

Table 67 Immunogenicity Finding from Female No. F1392F

<u>Assay</u>	<u>Predose</u>	<u>Timepoint Description</u>			
		<u>GD104</u>	<u>GD132</u>	<u>Cordblood</u>	<u>Necropsy^a</u>
TK value ($\mu\text{g/mL}$)	BLOQ	150.733	213.862	142.963	207.821
Assay A titer	< 50	8262	1314	507	1058
Assay B titer w/o inhibitor	< 50	7180	1748	406	773
Assay B titer with inhibitor	< 50	5100	1018	219	436
Assay B % drop	N/A	29%	42%	46%	44%
Outcome ^b	Negative	Negative	Positive	Positive	Positive

^aNecropsy performed 15 days after C-section and 19 days after the last dose on GD146.

^bTo be considered positive, a sample must have a titer above 50 in Assay A and greater than a 30% drop in titer with the addition of inhibitor in Assay B.

The clinical pathology findings include increases in WBCs which are related to inflammation associated with C-section. A 58% decrease in the B-cell markers were noted which is similar to the findings from the same dose group. There was a minimal increase in body weight from GD 81 onwards. This female appeared to generate antiproduct antibody formation. Serum belimumab concentrations were 2 to 5-fold lower than the corresponding mean concentrations for the remaining females in the group. The serum concentration at the time of necropsy was 208 mcg/mL, which was about 3.5-fold lower than the expected mean serum concentration for Group 5 females

at this time point. The histology findings consisted of the following changes which were associated with the gross pathology findings:

1. Diffuse moderate atrophy of the (assumed) distal portion of the axial muscles, dorsal to the wing of the ileum. The changes were more severe away from the normal portion of the muscle and only a few damaged fibers remained in the most affected portions. Skeletal muscle from the thigh showed bilateral moderate atrophy and mild adipose infiltration.
2. Vascular damage was noted in aortic sections at the L1-L2 level, and at the bifurcation. Mild to moderate intimal thickening was noted in both locations. Additionally, disruption of the internal elastic lamella was present on left side (at or just distal to the bifurcation). This change was observed to be significant and may have contributed to the narrowing of the aortic or femoral artery narrowing. This might be the cause of progressive loss of use of the lower limbs. The Applicant could not determine whether the intimal lesions were pre-existing, or were caused by the pressure of the developing pregnancy. The proximal contributory branch of the left sciatic nerve showed mild axonal degeneration.
3. Lymphoid depletion was noted in thymus and spleen. Hyperplasia of the adrenal zona fasciculata and secretory depletion of the same area were noted. These lesions are typical of chronic stress. The pituitary contained multifocal cysts, in both pars intermedia and pars distalis, graded mild. The Applicant mentioned that the cysts were similar to spontaneously occurring changes commonly seen in Cynomolgus monkeys at the facility. The eosinophil cells of the pars distalis appeared to be mildly hyperplastic which may be due to the hormone fluxes related to pregnancy.
4. A moderate myeloid hypercellularity was noted in the bone marrow which may be associated with the pharmacological activity of the drug product. The enlarged iliac lymph node noted grossly contained mild paracortical expansion and a neutrophilic infiltrate.

All of these changes are considered to be associated with the immunogenicity findings by the reviewer.

Infant No. 7 was rejected by its birth mother (Adult Female No. 1014) on PP7. The infant was cared for manually until it was successfully cross-fostered on PP8 to Adult Female No. 1072 (Group 1). The infant for Adult Female No. 1072 (Infant No. 978) was born on GD146 and euthanized per study protocol in Phase 1, which was one day prior to the birth of Infant No 7. Both the birth mother (Female No. 1014, Group 4) and the foster mother (Female No. 1072, Group 1) remained on study until Infant No 7 was euthanized approximately 1 year after birth. The reviewer noted that there were no test article related changes in the female #1014.

Fetal/Infant Losses (Refer to table):

The total numbers of fetal losses in this study were 3/21 (14%), 6/25 (24%), and 3/20 (15%) from control, low and high dose group females, respectively. The total fetal losses from the belimumab treated animals were 9/45 (20%) compared to controls 3/21 (14%). Historical control fetal losses are 17.6% in monkeys. The total number of infant losses in this study was 0/21 (0%), 2/25 (8%), and 1/20 (5%) from control, low, and high dose, respectively. The sum of the belimumab treated infant losses were 3/45 (7 %) compared to controls (0%). The historical control infant losses are 10-12% in monkeys. When summing the total number of deaths (fetal plus infant deaths) for controls, LD and MD, the respective values were 14%, 32%, and 20 %. The details for fetal and/or infant deaths are discussed below.

In the low dose group one fetus (965) was observed to be dead by ultrasound at GD 87; no abnormalities were noted from the fetus and the mother. Five other fetuses were aborted between GDs 38, 50, and 52. In addition there were two more infant deaths from the low dose group.

The 3 fetuses from the high dose group, fetus Nos. 9112, 989, and 999 died at GDs, 151, 161, and 125 respectively. One of these deaths (fetus #9112) was an intra uterine death. The fetus # 9112 of the animal # 23249 had heavy bleeding at Day 151, the fetus was in a breech position, the delivery was conducted by C-section but the baby was dead when delivered. Interestingly, this animal was observed to be ADA positive in the primary assay (level 0.2 mcg/mL), however in the confirmatory assay, no titer of the antibody was noted. An abnormality (thick, opaque non disc membrane with tan or mottled infarct) on the maternal side of the placenta was noted. The histological findings from the fetus # 9112 consisted of a single red focus that correlated microscopically with ectopic spleen. The lungs had minimal atelectasis and minimal amniotic debris in the alveolar spaces. Several organs were congested and there was multifocal hemorrhage on the epicardium. The fetus No. 999 (female #, 1081) was a still born fetus observed by ultrasound at GD125; the histology findings from this fetus showed brain and skull were red, correlating microscopically with congestion and hemorrhage respectively. There was amniotic debris in the pulmonary alveolar spaces indicating distress. Several organs were congested. The fetus No. 989 of the female # 130101 aborted at GD161, the lungs were diffusely dark red, correlating microscopically with congestion. The lungs had diffuse atelectasis, consistent with an animal that had never breathed. There was a slight amount of amniotic debris in the alveolar spaces. A few other organs were congested. All of the fetal losses from the high dose group are considered test article related by the reviewer, the cause of the fetal losses were not mentioned by the Applicant, however, it appeared from the study description that the deaths were caused by breathing difficulties caused by distressed lungs. The percent fetal death from the high dose group (12.5%) was, however, within the historical control.

One infant was lost from the high dose group animals after natural delivery. Infant no.9128 from the female No. 23179 gave birth naturally on GD 142; no abnormalities were noted in the female. The infant was weak and died 2 hrs after birth; the fetal measurements were recorded and were observed to be within the normal range. The histological findings from this infant consisted of hemorrhage in the meninges and

parenchyma of the brain which may suggest hypoxia. Also, the lungs contained amniotic debris and had areas of atelectasis indicating that the inflation of lung was not complete after birth. The reviewer believes that the fetus might have died from the respiratory disturbances resulted from lung/heart malfunction.

There were two infant deaths (nos. 27 and 55 from the female No. 23179 and 23242 respectively) from the low dose group. The infants were born on GDs 154/155; no abnormalities were noted in the female. The infant no. 27 was observed to be weak, tired, and not very responsive, it died at birth day (BD) 7. Infant no. 55 was observed to have neurological somnolence. The histological findings from Infant No. 27 consisted of moderate involution of thymus, minimal hyaline droplet formation in some renal tubular epithelium, diffuse vacuolation of renal tubular epithelium, amniotic debris and a minimal increase in alveolar macrophages in the alveolar spaces of the lungs suggesting fetal distress prior to birth. This infant died at BD11 The histological findings from infant No.55 consisted of moderate enlargement of adrenal, increased thickness of the skin of the right side of the thorax and abdomen (correlated histologically with subcutaneous edema and was likely due to subcutaneous fluid administration) multifocal discoloration of one lobe of the lung due to (mild multifocal intra-alveolar hemorrhage, which appeared to affect only the right apical lobe) and diffuse atelectasis of the lung. Although the cause of death is not confirmed by the Applicant, the reviewer believes that breathing trouble associated with lung/heart malfunction might be the cause of the deaths for these infants.

Table 68 Summary of Fetal /Infant losses

Adult Female#	Fetal #	No Doses	GDs	Comments
1342 (C)	1482	2	49	Aborted (detected via ultrasound)
23227 (C)	9117	6	97	Aborted (tissue obtained, M)
23237 (C)	9123	9	133	Aborted (tissue obtained, F)
Low Dose-5 mg/kg				
1328	NA	3	50	Aborted (detected via ultrasound)
1332	965	5	87	Aborted (tissue obtained)
1352	NA	3	50	Aborted (detected via ultrasound)
23255	NA	3	52	Aborted (detected via ultrasound)
1014	7	11	165	Infant cross fostered w/adult female
1321	NA	2	38	Aborted (detected via ultrasound)
10106	27	10	155	Infant died BD 7(F)
23173	NA	3	50	Aborted (detected via ultrasound)
23242	55	10	154	Infant died BD 11(F)
High Dose-150 mg/kg				
1081	989	8	161	Aborted, tissue obtained (M)
13101	999	11	125	Still born (M)
23179	9128	11	142	Infant died BD1(F)
23249 (ADA+)	9112	10	151	Fetus dead (removed by C-section, F)

Phase 1- F₀ Dams

Pregnant females from Groups 1, 3 and 5 (refer to study design table) were scheduled for C-section delivery. The mothers were released from the study once their fetuses were euthanized for teratologic evaluation.

Clinical signs: The clinical signs were recorded twice daily, no treatment related changes were noted [except female # 1392 described in text].

Body weight: The body weights were recorded once on the day of mating, and GDs 7, 14, 18, and weekly thereafter; there were no treatment related changes.

Feed consumption: The feed consumption was recorded once daily; there were no treatment related changes.

Hematology: The blood samples were collected via venipunctures at pre-mating, GDs, 90, 142. The standard battery (RBC, WBC, reticulocyte, platelet count, blood morphology, Hgb, hematocrit, MCH, MCV, MCHC) of hematological parameters were assessed; there were no test article related changes.

Peripheral blood flow cytometry analyses: The blood samples were collected via venipunctures at pre-mating, GDs 90, 142 for flowcytometric analyses using CD45+

(leukocyte differentiation), CD3+ (T cell), CD3+/CD4+ (T helper cells), CD3+/CD8+, CD3-/CD16+ (NK cells), CD3-/CD14+ (monocytes), CD 20+ (B-cells), CD20+/CD21+ (mature-B cells). No changes in the T cells and monocytes were noted. There was a decrease in B-cell population. This decrease is not dose related, however, considered as the pharmacologic effect of the drug product.

Table 69 Phase 1-F₀ Dams: Summary of Changes in B-cell markers

Parameters, % predose)	Dosing (mg/kg)			
	5		150	
	CD20+	CD20+/CD21+	CD20+	CD20+/CD21+
GD 90	51* ↓	46* ↓	58* ↓	58* ↓
GD 142	41* ↓	35* ↓	40* ↓	36* ↓

*statistically significant (p=0.001)

Serum immunoglobulin: The blood samples were collected via venipunctures at pre-mating, GDs 90 and 142 for Immunoglobulin (IgG, IgA, IgM, and IgE) analyses. There were no changes in the different immunoglobulin level at GD 90, however, at GD 140 slight increase in IgA and IgM were noted at low and high dose. The biological significance of these changes is not known. There were no changes in the IgG at GDs 90 and 140.

Table 70 Phase 1-F₀ Dams: Summary of Changes in Immunoglobulin Levels

Time/ Immunoglobulins (% predose)	Dosing (mg/kg)					
	5			150		
	IgA	IgM	IgE	IgA	IgM	IgE
GD 90	NC	NC	NC	NC	NC	NC
GD 142	26 ↑	24* ↑ p=0.01	9 ↓	25 ↑	19 ↑	10 ↓

NC---no change, there were no treatment related changes in IgG

Placenta: The placentae were collected, weighed and grossly examined. The placenta and the chorionamnionic membrane and umbilical cord were fixed (10% formalin) embedded in paraffin, sectioned, stained with hematoxylin and eosin and examined by a Testing Facility ACVP-certified pathologist.

Table 71 Phase 1-F₀ Dams: Summary of Changes in Placenta

Placental Changes	Dosing (mg/kg)		
	0	5	150
	Fetus No/Female No		
Color	1; 993/1360 discoloration	-	-
Thickness	-	1; 991/1079 Primary disc was thicker & secondary disc is thinner	1; 9108/1055 Maternal side is thick & amniotic sac did not separate
Single disc	2; 993/1360 9102/1368	992/1363	
Diffuse, neutrophils infiltration (moderate)	-	-	994/1331
Secondary disc length (mm)	86	55* (37%↓, p=0.01)	71 (17%↓)

Umbilical cord: The blood samples were collected during C-section and natural deliveries for the analyses of belimumab and ADA. The concentration of belimumab in the umbilical cord was observed to be 4-fold lower than that observed in the maternal blood (refer to Applicant's table). The data show that belimumab crosses the placental barrier and the fetuses were exposed to belimumab.

Table 72 Phase 1-F₀ Dams: Summary of Toxicokinetics from Umbilical Cord

Parameters	5 mg/kg (n = 7)	150 mg/kg (n = 9) ^b
C _{maternal} (µg/mL)	57.0 ± 31.1	1854.8 ± 683.1
C _{umbilical cord} (µg/mL)	35.7 ± 36.7	582.4 ± 381.7
C _{maternal} /C _{umbilical cord} Ratio ^a	4.10 ± 3.35	4.15 ± 1.74

Source: Appendix 30.

^aThe mean ratio was calculated using ratios from individual animals.

^bFemale No. 1392 was positive for anti-belimumab antibodies starting on GD132 and had an altered TK. Therefore, the serum concentrations for this animal were excluded from the descriptive statistics.

Amniotic fluid: The amniotic fluids were collected during C-section and natural deliveries for the analysis of belimumab; the data showed that belimumab exposure in the blood samples were collected via venipunctures at pre-mating, GDs 90 and 142 amniotic fluid was 38-39-fold lower than that in the maternal blood. The data indicate that belimumab crosses the placenta as expected for an IgG1 antibody.

Table 73 Phase 1-F₀ Dams: Summary of Toxicokinetics from Amniotic Fluids

<u>Parameters</u>	<u>5 mg/kg</u> <u>(n = 9)</u>	<u>150 mg/kg</u> <u>(n = 8)^b</u>
C _{serum} (µg/mL)	57.0 ± 27.0	1874.7 ± 727.5
C _{amniotic fluid} (µg/mL)	2.93 ± 3.32	52.2 ± 14.6
C _{serum} /C _{amniotic fluid} Ratio ^a	31.5 ± 22.6	36.0 ± 11.6

Source: Appendix 33.

Abbreviations: C_{maternal}, maternal serum belimumab concentration on GD150; C_{amniotic fluid}, amniotic fluid drug concentration.

^aThe mean ratio was calculated using ratios from individual animals.

^bFemale No. 1392 was positive for anti-belimumab antibodies starting on GD132 and had an altered TK. Therefore, the serum concentrations for this animal were excluded from the descriptive statistics.

Immunogenicity: The blood samples were collected by venipunctures, prior to dosing and 5 mins and 6, 24, 72, 168 and 336 hours after the first dose (approximately GD20) and GD132 for immunogenicity analyses. The method is not very sensitive and the level of detection is low when plasma concentration of Belimumab is high (refer to table.)

Table 74 Anti-Belimumab Antibody Level (in the presence of belimumab)

<u>LymphoStat-B</u> <u>Concentration (µg/mL)</u>	<u>LOD</u> <u>(µg/mL)</u>
0	NA
25	2.0
50	2.0
75	2.0
100	4.0
200	4.0

At least two pregnant females developed immunogenicity #1392 and #23249h (ADA formation from these two females has already been described).

Toxicokinetics: The blood samples were collected by venipunctures, prior to dosing and 5 mins and 6, 24, 72, 168 and 336 hours after the first dose (approximately GD20) and GD132 for bioanalytical analyses of belimumab. As observed from the attached table, the AUC(mcg.mL/day) increased proportionally to dose, while the mean C_{max} increased slightly more than dose proportionally.

Table 75 Phase 1-F₀ Dams: Summary of Toxicokinetics from Serum

Parameters	5 mg/kg		150 mg/kg	
	Week 1	Week 17	Week 1	Week 17
AUC (mcg.day/mL)	700 ± 91	19,994 ± 3416	1,023 ± 98	26,944 ± 3789
Cmax mcg/mL	145 ± 98	5025 ± 1115	193 ± 25	5077 ± 911
Accumulation (fold)Week17/Week1	1.39 (P<0.0001)		1.1 (P<0.0001)	

Phase 1- F₁ Generation Fetus

Fetuses obtained after C-section are called F1 fetuses. These fetuses were scheduled for necropsy for visceral and skeletal evaluation. The body weights and fetal measurements were also recorded.

Body weight: The body weights were taken within 24 hrs of birth. The mean fetal body weights of the high dose group animals were observed to be 6% lower than that of the controls.

Fetal Measurement: The following fetal measurements were assessed from all of the fetuses at C-section on GD150 and by natural birth before GD 150 from the Phase 1 group were evaluated: head circumference, occipital frontal diameter, foot length, femur length, chest circumference, crown-hip length, crown-rump length, biparietal diameter, and angiogenital distance. There were no test article related changes. The ultrasound measurements of the head circumferences and occipital frontal diameter at different GDs, however, showed a dose related statistically significant (p=.0001) decrease at GDs 140-142, the biological significance of the findings are not known.

Table 76 Phase 1-F₁ Generation Fetus: Summary of External Findings

GDs 140-142	Dosages (mg/kg)		
	0	5	150
Head circumferences (mm)	156	152	147*
Occipital diameter (mm)	54	51	48*

Visceral observation: All of the visceral organs from all of the fetuses were evaluated. Following variations were noted in the heart of the fetuses. The variations are within the normal historical control range

Table 77 Phase 1-F₁ Generation Fetus: Summary of Visceral Changes

Fetal Heart, Variations	Dosages (mg/kg)		
	0	5	150
Number	2 (993, 9124)	2 (991, 9131)	3 (990, 974, 9110)
Type of changes	Thickened mitral valve, slightly dilated right ventricle	Fibrotic tricuspid & mitral valve, Mitral valve w/one thick and one thin leaflet	Left atrium enlarged, Right atrium enlarged, Small septum secundum

Skeletal observation: Skull (cranial base, cranial vault, face), forelimb (humerus, radius/ulna, carpals, metacarpals, digits), hind limb (femur, tibia/fibula, tarsal, digits), sternum, vertebrae, (cervical, thoracic, sacral, coccygeal), pectoral and pelvic girdle from all of the fetuses born by C-section and by natural birth before GD 150 from the Phase 1 group were evaluated. There were no treatment related changes in the number and morphology of the bones.

Organ weights: The following organs were weighed: adrenals, brain, kidneys, liver, spleen, thymus, thyroids and parathyroids, ovaries or testes and epididymides. There were approximately 20 and 25% decrease in the weight of the spleens from the low and high dose group respectively; no gross/histological correlation was noted. No other test article related changes were observed.

Gross/Histopathology: All of the tissues mentioned in the tissue list in the review of study # 1175 were collected. The following tissues were evaluated histologically: adrenals, abdominal cavity, cervix, epididymides, live, lymph nodes (inguinal, mandibular, and mesenteric), ovary, oviduct, pancreas, prostate, seminal vesicles, skin, spleen, testes, thymus, urinary bladder, uterus, vagina, placenta, umbilical cord, and chorioamniotic membrane. The test article related changes are tabulated below. Note that the tissue for histology was not available from all of the fetuses. However, no major histological changes were noted.

Table 78 Phase 1-F₁ Generation Fetus: Summary of histological changes

Parameters	Dosages (mg/kg)					
	0		5		150	
	♂	♀	♂	♀	♂	♀
Liver, vacuolation, hepatocyte	0/2	1/1	1/1	0	0/1	0
Inguinal lymph node, erythrocytosis	0/8	0/7	0/3	0/1	0/2	1/5
Mesenteric lymph node, congestion	0/8	0/7	0/3	0/1	0/2	1/5
Thymus, congestion	0/8	0/7	0/3	0/1	0/2	2/6
Urinary bladder, congestion, edema	0	0	0	0	0	1/1
Uterus, dilation, congestion	NA	NA	NA	0/1	0/2	1/5
Chorioamniotic membrane, acute inflammation(diffused)	0/7	0/6	0/3	0	0/2	1/5

Phase 2- F₀ Dams

Pregnant females from Groups 2, 4 and 6 (refer to study design table) were allowed to deliver their infants by natural birth. The mothers were observed with their infants for one year; the infants were euthanized one year after they were born (post birth day 366-368). The mothers were released from the study once their infants were euthanized.

Survival: All dams survived.

Clinical signs: The clinical signs were recorded twice daily; there were no treatment related changes.

Body weight: The body weights were recorded once at postpartum day 1 (PPD1), PPD14, and during postpartum Weeks 4, 8, 13, 26, 39 and 53; there were no treatment related changes.

Feed consumption: The feed consumption was measured once daily; there were no treatment related changes.

Hematology: The blood samples were collected via venipunctures at preparting, GDs 90, 142, and PPDs 7, 28, 91, 182, 273, and 365. The standard battery (RBC, WBC, reticulocyte, platelet count, blood morphology, Hgb, hematocrit, MCH, MCV, MCHC) of hematological parameters were assessed. There were no test article related changes.

Peripheral blood Flow Cytometry Analyses: The blood samples were collected via venipunctures at preparting, GDs 90, 142, and PPDs 7, 28, 91, 182, 273, and 365. for flow cytometric analyses using CD45+ (leukocyte differentiation), CD3+ (T cell), CD3+/CD4+ (T helper cells), CD3+/CD8+, CD3-/CD16+ (NK cells), CD3-/CD14+ (monocytes), CD 20+ (B-cells), CD20+/CD21+ (mature-B cells). There were no changes in the T cells and monocytes. There was a decrease (similar to phase 1 females) in B-cell population at GDs 90 and 142. The decrease was not dose related, however, considered as the pharmacologic effect of the drug product. There were no differences in the B-cell.

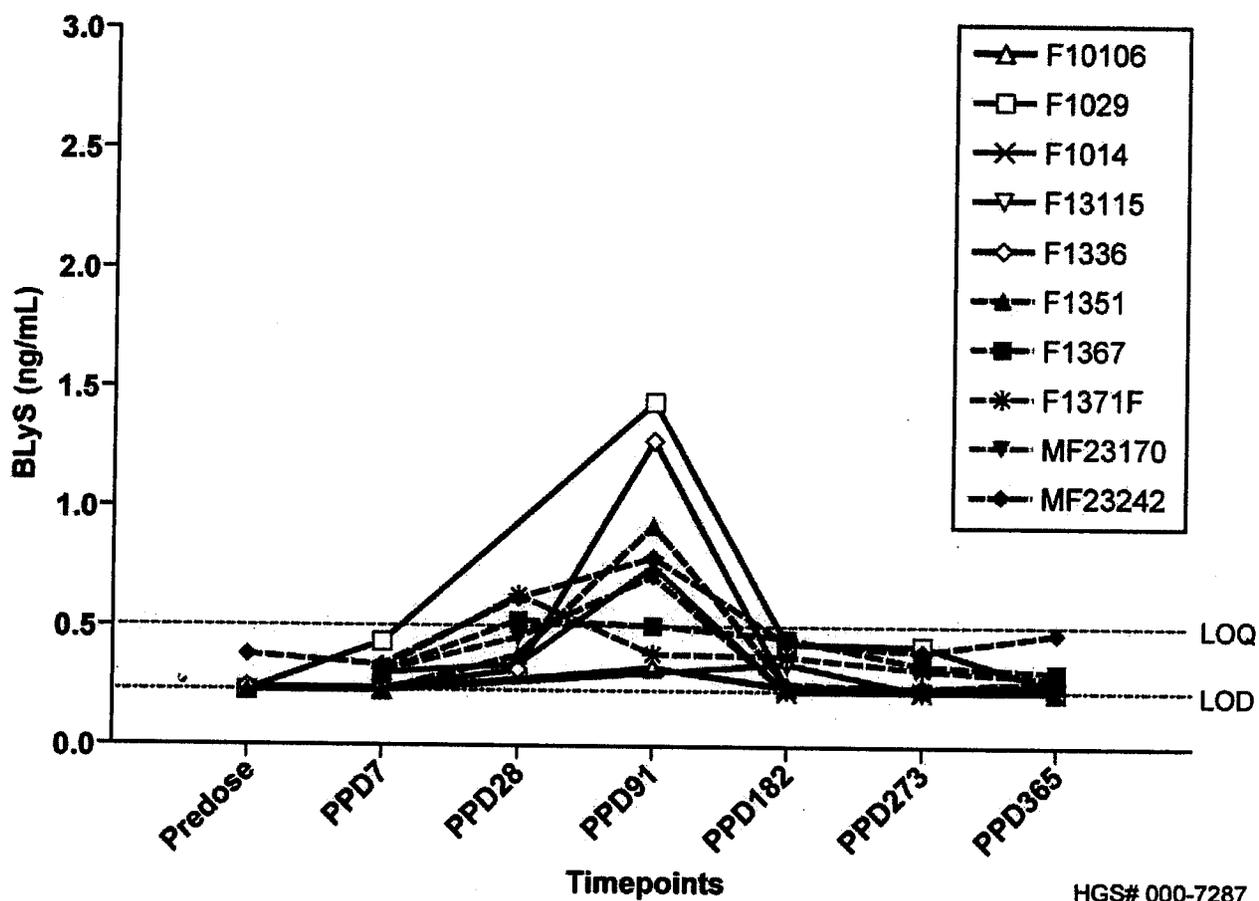
Table 79 Phase 2-F₀ Dams: Summary of Changes in B-cells Markers

Time/ Parameters (B- cell markers, % predose)	Dosing (mg/kg)			
	5		150	
	CD20+	CD20+/CD21+	CD20+	CD20+/CD21+
PPD 7	25 ↓	18 ↓	35 ↓	29 ↓
PPD 28	33 ↓	29 ↓	32 ↓	28 ↓
PPD 91	30 ↓	22 ↓	35 ↓	33 ↓
PPD 182	28 ↓	9 ↓	29 ↓	22 ↓
PPD 273	7 ↓	5 ↑	20 ↓	6 ↓

Serum immunoglobulin: The blood samples were collected via venipunctures at pre mating, GDs 90, 142, and PPDs 7, 28, 91, 182, 273, and 365 for Immunoglobulin (IgG, IgA, IgM, and IgE) analyses. There were no test article related changes.

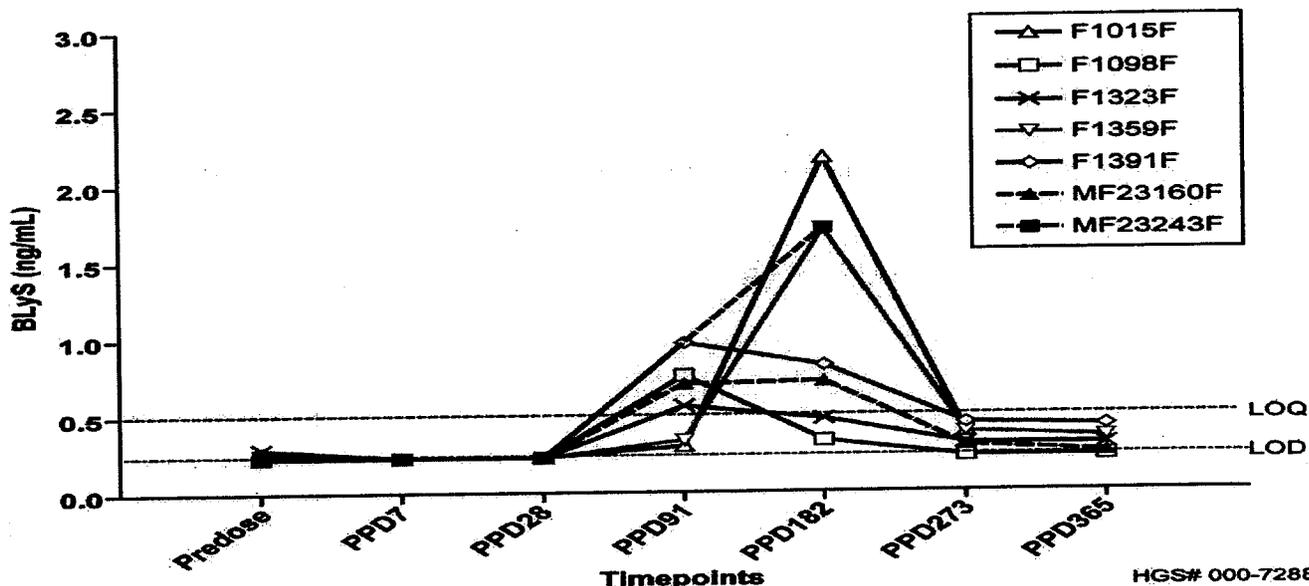
Toxicokinetics adult females: The samples were collected by venipunctures, prior to dosing (GD 20) and prior to dosing on GDs 62, 104, 132, and PPDs 7, 28, 91, 182, 273, and 365 for bioanalytical analyses of BLYS; the toxicokinetics of the females from Phase 1 and 2 are very similar as described in the attached figures from the Applicant.

Figure 39 Phase 2-F₀ Dams: BLYS Serum Concentration, 5 mg/kg group



HGS# 000-7287

Figure 40 Phase 2-F₀ Dams: BLyS Serum Concentration, 150 mg/kg group



Most of the adult females from the 5 mg/kg belimumab dose group had no measurable serum belimumab levels by PPD91. In the 150 mg/kg belimumab dose group most adult females had no measurable serum belimumab levels by PPD182. Following the last dose administration of belimumab prior to delivery, the mean t_{1/2} terminal for the adult females was 11 to 12 days.

Immunogenicity: The blood samples were collected from all of the females at different postpartum days for analyzing ADAs. The sensitivity of the ADA assay was low due to the interference of the drug product as described before. None of the animals showed a positive response.

Milk: Milk samples were collected from PPDs 7, 28, 91, 182 and 273 for the bioanalytical analyses of belimumab, ADA, and immunoglobulin. Only four milk samples could be collected from 2 females, both of these samples had measurable concentrations of belimumab indicating that the drug product could be secreted in milk.

Phase 2-F₁ Fetus

Infants were maintained with their mothers for the entire postnatal period. The infants were euthanized between BD366 and BD368. A full necropsy was conducted on all infants, and tissues were collected, preserved, processed and microscopically examined.

Clinical signs: Twice daily. Red/clear nasal discharge was noted 1, 1, and 4 females from the control, 5, and 150 mg/kg dose, the Applicant claimed that this is normal for.

housed animals. However, due to the higher findings at high dose, treatment related increased incidence of getting cold can not be eliminated.

Body weight: Once at birth day (BD)1, BD3, BD7 and BD14, and during postnatal Weeks 4, 8, 13, 26, 39 and 53); no treatment related changes.

Feed consumption: Once daily; No treatment related changes.

Physical development: Include measurements of head circumference, inspection of hard palate, spinal cord closure, digits, limbs, joint angles, and structural morphology;

One infant # 13 (mid dose) was observed to have small preputial opening and surgical intervention was needed. No other changes were reported.

Behavioral assessment: The muscle tones were assessed from the neonates on the morning after birth or approximately 4 to 6 hours after birth occurred during the day. For neonatal neurobehavioral tests, the infants were separated from the mother and placed in an incubator or isolated evaluation area for a short period (up to 20 minutes, adaptation period) at BDs 1, 3, 7, and 14. The behaviors evaluated were as follows: righting reflex, palmer grasp, clasp-grasp (monkey infant reflex for clinging to ventrum of the dam), visual following, prone progression, lipsmack orient (test for hearing), oral reflexes (rooting, sucking, snout reflex), eye reflexes (pupil constriction, nystagmus, glabellar tap [blink reflex]), Moro reflex, negative geotaxis (test for vestibular function); Each reflex/behavior was scored. Infants No. 34 (Group 6), 23 (control) and 40 (control) in the resting position displayed little or no bending in the knee, elbow or finger joints and limited head extension at BD1. The Applicant mentioned that with the exception of the head, attempts at movement of these joints (elicited response) showed little or no resistance neither in these locations nor in the hip, toes and shoulder. The physical exam and other neurobehavioral parameters for Infant No. 34 were within normal limits of variation, toxicokinetic assessment from the mother and the infant did not show any alteration compare to the animals from the same age group. Similar changes were noted in 2 fetuses from the control animals. It is not, however, clear from the submission whether the severity index of the finding is similar in the fetuses from the control and the high dose group. Neurological somnolence was observed from one infant (No. 27) that died. The biological significance of the finding is not yet known.

Table 80 Phase 2- F1 Generation: Summary of Behavioral Changes

Female #	Infant #	BD*	Parameters
1337 (control)	23	BD1	Muscle tonus less
1027 (control)	40	BD1	Muscle tonus less
1351 (low dose)	13	3,7	Dazzle response
1323 (high dose)	34	BD1	Muscle tonus (no resistance)

* BD=birth date

The Infant No. 13 (Group 4) displayed a dazzle response to light (i.e., closed eyelids right away) at BD3 and BD7, but not BD14. The infant also displayed strong glabellar tap on both days and variable and strong nystagmus on BD3 and BD7, respectively. Because the dazzle response was not dose-related and occurred only in one infant, and no abnormal toxicokinetic findings were noted in this infant and its mother, the reviewer agreed concluded that this is not test article related.

Hematology: The blood samples were collected via venipunctures at BD 365. The standard battery (RBC, WBC, reticulocyte, platelet count, blood morphology, Hgb, hematocrit, MCH, MCV, MCHC) of hematological parameters were assessed. The hematological parameter including WBC counts, neutrophil counts, and unclassified cells were observed to increase in the test article treated animals compared to those of the controls. The changes in the hematological parameters did not show any dose response. Most of the changes persist for one year. The biological significance of the changes is not known.

Table 81 Phase 2- F1 Generation: Hematology Changes

Time / (% control)	5 mg/kg				150 mg/kg			
	BD*7	BD28	BD 182	BD365	BD*7	BD28	BD 182	BD365
MCH	NC	NC	NC	NC	4** ↑	6 ↑	6 ↑	NC
WBCs	33 ↑	NC	15 ↑	21 ↑	33 ↑	38 ↑	10 ↑	21 ↑
Polysegmented neutrophils	68 ↑	15 ↑	41 ↑	22 ↑	60 ↑	36 ↑	17 ↑	46** ↑
Basophils	12 ↑	15 ↑	28 ↑	25 ↑	39 ↑	69 ↑	44 ↑	54** ↑
Unclassified cells	44 ↑	NC	43 ↑	30 ↑	44**	54 ↑	27 ↑	51 ↑

BD*=Birth day, ** statistically significant $p > 0.001$; NC=no change

Peripheral blood flow cytometry analyses: The blood samples were collected via venipunctures at BD 365 for flow cytometric analyses using CD45+ (leukocyte differentiation), CD3+ (T cell), CD3+/CD4+ (T helper cells), CD3+/CD8+, CD3-/CD16+ (NK cells), CD3-/CD14+ (monocytes), CD 20+ (B-cells), CD20+/CD21+ (mature-B cells). No changes in the T cells and monocytes were observed.

As noted in the following table, there was a decrease in B-cell population. This decrease is not dose related, however, it was considered as the pharmacologic effect of the drug product. There was a decrease in the CD20+ and CD20+/CD21+ B cells at BDs 7, 28, and 182 in the test article treated animals compared to those of the controls. The finding is considered the test article related pharmacological effect of the drug product. The findings, however, were observed to recover at one year.

Table 82 Phase 2- F1 Generation: Summary of Changes in B-cell Counts

Time/ B-cell markers (% control)	Dosing (mg/kg)			
	5		150	
	CD20+	CD20+/CD21+	CD20+	CD20+/CD21+
BD7	60 ↓	84 ↓	60 ↓	73 ↓
BD28	61 ↓	90 ↓	NC	52 ↓
BD182	27 ↓	73 ↓	26 ↓	34 ↓
BD 365	36 ↑	10 ↓	36 ↑	14 ↑

Serum immunoglobulin: The blood samples were collected via venipunctures at BD 365 for Immunoglobulin (IgG, IgA, IgM, and IgE) analyses. The IgG level from the high dose treated animals increased compared to those of the controls, this change might be related to the increase in the WBCs observed from these animals. The level of the IgMs however, decrease at all test articles treated animals at least up to Days 182 after birth compared to those of the controls animals. Although not dose related, the changes are considered test article related pharmacological effect of the drug product.

Table 83 Phase 2- F1 Generation: Summary of Immunoglobulin Levels

Time/ Immunoglobulins (% predose)	Dosing (mg/kg)							
	5				150			
	IgG	IgA	IgM	IgE	IgG	IgA	IgM	IgE
BD 7	NC	NC	75* ↓	NC	33 ↑	NC	75* ↓	NC
BD 28	NC	NC	57 ↓	NC	23 ↑	NC	22 ↓	NC
BD 182	NC	NC	28 ↓	NC	NC	NC	33 ↓	NC
BD 365	NC	NC	26 ↓	NC	13 ↑	NC	NC	NC

NC =no change; * statistically significant p=0.001

Coagulation: The blood samples were collected via venipunctures at BD 365 for the analyses of APTT, PT, and fibrinogen. There were no test article related changes.

Serum chemistry: The blood samples were collected via venipunctures at BD 365 for the analyses of the standard battery for serum chemistry (Na, K, Ca, P, CO₂, LDH, AST, ALT, GGT, ALP, BUN, creatinine, total protein, albumin, globulin, A: G ratio, glucose, cholesterol, triglycerides). There were no test article related changes.

Urinalyses: The urine samples were collected between BD 366-368 via cystocentesis at necropsy for analyzing color/character, specific gravity, pH, nitrite, urobilinogen, leukocyte esterase, protein, glucose, ketones, bilirubin, occult blood, and macroscopic debris in urine. There were no test article related changes.

Toxicokinetics infants: The blood samples were collected from BDs 7, 28, 91, 182, and 273 for the bioanalytical analyses of BLYS. During the postnatal period, most infants in the 5 mg/kg belimumab dose group had no measurable serum levels by BD91, while in the 150 mg/kg belimumab dose group most infants had no measurable serum belimumab levels by BD182. The serum concentrations of BLYS for infants in the 5 mg/kg dose group were similar to those for their mothers on BD7 and 2 and 13-fold lower than those for their mothers on BD28 and BD91, respectively. Mean belimumab exposure for infants in the 150 mg/kg dose group were 10, 9, 1.4, and 1.6-fold lower than those for their mothers on BD7, BD28, BD91 and BD182, respectively

Figure 41 BLYS Serum Concentration: Infants in 5 mg/kg group

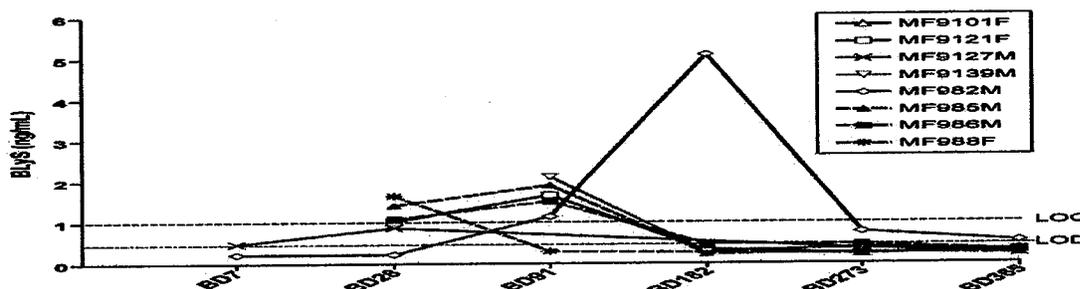
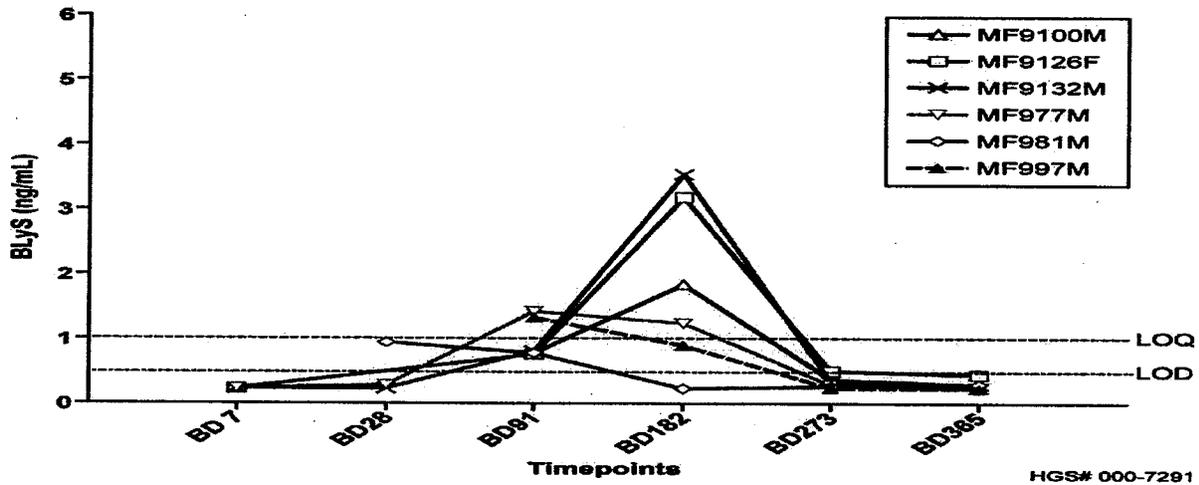


Figure 42 BLyS Serum Concentration: Infants in 150 mg/kg group



Immunogenicity: The blood samples were collected from BDs 7, 28, 91, 182 and 273 for the bioanalytical analyses of ADA; there were 4 infants whose serum were observed to be ADA positive in the screening assay, but the titer was observed not to be clearly positive. The sensitivity of the ADA assay was low due to the interference of the drug product. However, none of these infants had any abnormalities, therefore even if these animals are positive for ADA, it does not appear to interfere with the parameters tested.

Table 84 Phase 2- F1 Generation: Summary of ADA Levels in Infants

Female #	Infant #	Assay 1 (mcg/mL)	Time	Assay 2 Mean titer
1336(L)	9121	0.09	BD 365	<25
1015 (H)	977	0.09	BD 365	<25
1351 (L)	985	0.13	BD 365	<25
1367 (L)	986	0.09	BD182	< 25

Immunohistochemical evaluation: The spleen, lymph nodes (inguinal, mesenteric, and mandibular), and thymus were frozen and examined for CD 2+ (T cells), CD20+ (B cell), CD60+ (Macrophages), and CD68+ (NK cell). There were no test article related changes.

Gross/histopathology: The gross and histopathology observations for infants euthanized approximately one year after their birth were reported. There were no gross findings in infants exposed to belimumab in utero and euthanized one year after their birth.

Table 85 Phase 2- F1 generation: Summary of Histology Findings

Tissues/Number examined	Dosages mg/kg					
	0		5		150	
	♂	♀	♂	♀	♂	♀
	3	6	5	3	5	2
Artherosclerosis	0	0	0	0	1	0
Brain, mononuclear cell (meninges)	0	0	2	1	2	0
Brain, mononuclear cell (choroid plexus, multifocal)	0	0	0	0	0	1
Brain, mononuclear cell (parenchyma, focal)	0	0	0	0	0	1
Cervix, ciliate parasites	0	0	0	0	0	1
Heart, mononuclear cell (myocardial, multifocal)	0	0	0	1	1	2
Ileocecal valve (hemorrhage)	0	0	0	0	1	0
Kidney, protein cast (tubular, multifocal)	0	0	1	0	1	0
Liver, mononuclear cell (parenchyma, multifocal)	0	1	1	1	2	1
Liver, necrosis (hepatocyte, focal)	0	0	0	0	1	0
Liver, lipidosis (hepatocyte, multifocal)	0	0	1	0	1	0
Inguinal lymph node, EMDH	0	0	2	0	1	0
Inguinal lymph node, hyperplasia, paracortex	0	0	0	0	1	0
Mandibular lymph node, ENMDH	0	0	0	0	1	0
Mandibular lymph node, hyperplasia, paracortex	0	0	0	0	1	0
Mesenteric lymph node, hyperplasia, paracortex	0	0	0	0	1	0
Pancreas, ectopic spleen	0	0	1	1	0	2
Thymus, cyst	0	0	0	0	2	0
Thyroid, ectopic thymus	0	1	2	0	3	2
Thyroid, follicular atrophy	0	0	0	0	0	1
Prostrate, mononuclear cell (multifocal)	0	NA	0	NA	2	NA
Prostrate, inflammation (granulomatous)	0	NA	0	NA	1	NA

Dosing Solution Analysis: Formulation accuracy was verified by analysis of belimumab concentration in aliquots of dose solutions prepared for the first (approximately GD20), fifth (GD76) and ninth (GD132) doses, unless parturition or abortion occurred prior to the scheduled collection. Analysis of the dosing solutions indicated that all solutions ranged between 90% and 100% of the target concentration.

Protocol Deviations: There were several instances in which the serum samples for the TK analyses yielded results that were not consistent with the results for the remaining animals in the same dose group. One sample for a Group 1 [control] adult female had a high level of belimumab and one sample for a Group 5 [150 mg/kg] adult female had no measurable belimumab after dosing on GD20; both samples were

collected on the same calendar day. For one Group 5 [150 mg/kg] adult female on GD132, the predose sample had higher levels of belimumab than a 5 minute post dose sample. For two Group 3 [5 mg/kg] adult females and one Group 6 [150 mg/kg] adult female, the maternal serum sample concentrations were lower and umbilical cord serum sample concentrations were higher than the remaining animals in the same dose group. Some or all of these inconsistencies may be explained by mislabeling indicating serious protocol deviation; however, the impact on the data interpretation was minimized by exclusion of these inconsistent serum concentration data from the toxicokinetic evaluation and statistical analysis.

There were four cases in which the serum samples for toxicokinetic analyses yielded results that were not consistent with the results for the remaining animals in the same dose group. A BD28 and a BD91 sample for a Group 2 [control] infant had low to high levels of belimumab and a BD28 and a BD91 sample for a Group 6 [150 mg/kg] infant had no measurable belimumab the respective samples were collected on the same calendar day. These inconsistencies may be explained by mislabeling indicating serious protocol deviation; however, there is minimal impact on these inconsistencies on data interpretation as these data were excluded from the toxicokinetic evaluation and statistical analysis.

The reviewer believes that the toxicokinetic data could have been better interpreted if all the time points from different animals were correctly evaluated and included in the data analyses. The reviewer does not believe that the repetition of the study is necessary. The method for immunogenicity analyses could have been improved. Due to limited sensitivity of the immunogenicity assay, immunogenicity has been observed in a few animals with serious consequences.

10 Special Toxicology Studies

Study title: Subcutaneous Local Tolerance Study with LymphoStat-B in Cynomolgus Monkeys; ^{(b) (4)} Study # 6962-157

Key study findings:

- Cynomolgus monkeys (3/sex/group) were assigned to two groups. The Group 1 received the lyophilized preparation of the belimumab (and the respective placebo) and Group 2 received the liquid preparation (and the respective placebo). The test articles (or placebos) were administered by subcutaneous injection (25 mg/mL) one of six injection sites on either Day 7 or on Days 1, 3, 5, and 7 at a dose volume of 0.3125 mL/kg.
- The serum concentrations of belimumab after subcutaneous administration of the two different formulations were comparable indicating that the change in formulation from the lyophilized form to the liquid form did not result in any changes in the systemic absorption. No gender differences were noted.
- Increased incidence of erythema was noted with the lyophilized powder formulation of belimumab after multiple applications compared to those of the placebo in males. Desquamation was noted in 1/3 males (but not in females) with

the liquid formulation after multiple applications of belimumab (see dose site 2, group 1).

- There were higher incidences of mononuclear cell infiltration with the liquid formulation (0/3 with placebo, 3/3 males with liquid formulation, and 2/3 females with the liquid formulation) compared to that of the placebo. Higher incidences of the pleocellular infiltrates were also observed with the liquid formulation in females (1/3 in placebo vs 2/3 with the test article). In addition, 1/3 males showed erosion with liquid formulation (0/3 males showed erosion with placebo). The incidence of mononuclear cell infiltration with powder formulation was similar to the placebo. The above mentioned histopathological observations suggest that the subcutaneous administration of the liquid formulation might result in an inflammatory reaction at the local injection site. However, the incidence of the increased inflammatory response was minimal (1/3 in test article vs 0/3 in placebo) in most cases and the severity of the increase was considered minimal to slight by the peer reviewed pathologist.

Study (b) (4) Study # 6962-157

Volume # and page #: 1; Page 1-228

Conducting laboratory and location: (b) (4)

Date of study initiation: February, 09, 2005

GLP compliance: Yes

QA report: Yes

Drug, lot #, and % purity: LymphoStat-B (belimumab)

Lot # 04TP06004 (lyophilized powder) *placebo* 10 mM citrate, 8% sucrose, 0.04 % (w/v) Polysorbate 80, pH 6.5

Lot # 04TP 06007 (liquid) *placebo* 10 mM Histidine, 150 mM NaCl, 0.01 % (w/v) Polysorbate 80, pH 6.5

Lot # 04TA0601 ((lyophilized powder) 83 mg/mL Belimumab, 10 mM citrate, 8% sucrose, 0.04% (w/v) Polysorbate 80, pH 6.5

Lot # 04TA0602 (liquid) 79 mg/mL Belimumab, 10 mM Histidine, 150 mM NaCl, 0.01% (w/v) Polysorbate 80, pH 6.0

Methods:

The animals (3/sex/group) were assigned to two groups. Group 1 received the lyophilized preparation of the belimumab (and the respective placebo) and Group 2 received the liquid preparation (and the respective placebo). The test articles (or placebos) were administered by subcutaneous injection (25 mg/mL) into one of six injection sites on either Day 7 or on Days 1, 3, 5, and 7 at a dose volume of 0.3125 mL/kg as follows:

Figure 43 Map of the Injection site

Group 1		Group 2	
Site 1 Placebo 1 Days 1, 3, 5, and 7	Site 2 Test Article 1 Days 1, 3, 5, and 7	Site 1 Placebo 2 Days 1, 3, 5, and 7	Site 2 Test Article 2 Days 1, 3, 5, and 7
Site 3 Placebo 1 Day 7	Site 4 Test Article 1 Day 7	Site 3 Placebo 2 Day 7	Site 4 Test Article 2 Day 7
Site 5 Saline Days 1, 3, 5, and 7	Site 6 Saline Day 7	Site 5 Saline Days 1, 3, 5, and 7	Site 6 Saline Day 7

Table 86 Study Design: Local Tolerance in Monkey

Group	No. of Animals		Dose Level (mg/kg/dose)	Dose Concentration (mg/mL)
	Male	Female		
1 (lyophilized powder)	3	3	25	80
2 (liquid)	3	3	25	80

Assessment of local tolerance was based on mortality, clinical observations, dermal irritation, body weight change, and anatomic pathology evaluations. Blood samples for toxicokinetics analysis were taken prior to dosing on Days 1 and 3, and prior to necropsy on Day 8. Approximately 2 mL of blood was collected. Animals were sacrificed and necropsied on Day 8. Tissues (injection sites) were preserved in 10% neutral-buffered formalin.

Results:

There were no mortalities in this study. The clinical observation consisted of liquid stool. Skin scabs were noted with both placebo and test article administration. Desquamation was noted in 1/3 males with the liquid formulation after multiple applications of belimumab (see dose site 2, group 1). Increased incidence of erythema was also noted with powder formulation after multiple applications of belimumab (see dose site 2, group 1) compared to placebo (dose site 1 group 2). No such changes were noted with the application of the liquid formulation (see dose site 2, group 2) in females.

Table 87 Summary of Dermal Irritation

Table 2
Summary of Dermal Irritation Data

DAYS 1-8	CATEGORY	KEYWORD	QUALIFIER	NUMBER OF ANIMALS AFFECTED ¹								
				SEX:	--MALE--		--FEMALE--					
				GROUP:	1	2	1	2				
				DOSE:	25	25	25	25				
				NUMBER:	3	3	3	3				
*** TOP OF LIST ***												
	DOSE SITE 1											
	ERYTHEMA											
		NO ERYTHEMA							3	3	3	3
		VERY SLIGHT ERYTHEMA							1	0	1	1
	EDEMA											
		NO EDEMA							3	3	3	3
	ATONIA											
		NO							3	3	3	3
	DESQUAMATION											
		NO							3	3	3	3
	FISSURING											
		NO							3	3	3	3
	ECCHYM											
		NO							3	3	3	3
	DOSE SITE 2											
	ERYTHEMA											
		NO ERYTHEMA							3	3	3	3
		VERY SLIGHT ERYTHEMA							1	1	0	0
	EDEMA											
		NO EDEMA							3	3	3	3
	ATONIA											
		NO							3	3	3	3
	DESQUAMATION											
		NO							3	3	3	3
		YES							0	1	0	0
	FISSURING											
		NO							3	3	3	3
	ECCHYM											
		NO							3	3	3	3

Macroscopic observation of the injection sites showed dark area in the subcutaneous tissues associated with hemorrhage which varied from minimal to moderate in 1/3 males with liquid formulation. No such changes were observed in placebo or powder formulation in males. Dark area was, however, observed in 1/3 females with powder formulation.

Table 88 Incidence of Macroscopic Findings

**Table 5
Incidence of Macroscopic Observations**

TABLE INCLUDES: SEX=ALL;GROUP=ALL;WEEKS=1-2 DEATH=T;SUBSET=ALL		-- N U M B E R - O F				
		SEX: --MALE--		--FEMALE--		
GROUP:		-1-	-2-	-1-	-2-	
ORGAN AND KEYWORD(S) OR PHRASE		NUMBER:	3	3	3	3

*** TOP OF LIST ***						
INJECTION SITE 1 (IS0)		NUMBER EXAMINED:	3	3	3	3
		NOT REMARKABLE:	1	3	3	2
DARK AREA			2	0	0	0
H-DARK AREA			0	0	0	1
INJECTION SITE 2 (IS1)		NUMBER EXAMINED:	3	3	3	3
		NOT REMARKABLE:	3	2	2	3
DARK AREA			0	1	1	0
H-SORE			0	1	0	0

The following table compares the histology of the injection sites in males and females after the multiple administrations of liquid (Group 2) and lyophilized powder administration (Group 1) in the injection sites (placebo was injected at site 1, and test article was injected at site 2). There were higher incidences of mononuclear cell infiltration with liquid formulation compared to that of the placebo. Higher incidence of the pleocellular infiltrates were also observed with the liquid formulation in females. In addition, 1/3 males showed erosion with liquid formulation (0/3 males showed erosion with placebo). The incidence of mononuclear cell infiltration with powder formulation was similar to the placebo. The above mentioned histopathological observations suggest that the subcutaneous administration of the liquid formulation might result in an inflammatory reaction at the local injection site. However, the incidence of the increased inflammatory response was minimal in most cases and the severity of the increase was considered minimal to slight by the peer reviewed pathologist.

Table 89 Summary of Histology Findings

TABLE INCLUDES:		SEX: --MALE-- --FEMALE--			
SEX-ALL; GROUP-ALL; WEEKS-1-2		GROUP: -1- -2- -1- -2-			
DEATH-T; FIND-ALL; SUBSET-ALL		NUMBER:		3 3 3 3	
ORGAN AND FINDING DESCRIPTION					
*** TOP OF LIST ***					
INJECTION SITE 1 (ISO)	NUMBER EXAMINED:	3	3	3	3
	NOT REMARKABLE:	0	1	1	0
--HEMORRHAGE		3	1	1	2
--NEUTROPHIL INFILTRATE, PERIVASCULAR		2	0	0	0
--PLEOCYLLULAR INFILTRATE		2	1	2	1
--INFLAMMATION, CHRONIC		0	0	0	1
--COLLAGEN NECROSIS		2	0	0	0
--MYOFIBER DEGENERATION/NECROSIS, FOCAL		0	1	0	0
--EROSION		0	0	1	1
INJECTION SITE 2 (IS1)	NUMBER EXAMINED:	3	3	3	3
	NOT REMARKABLE:	1	0	1	0
--HEMORRHAGE		1	1	1	0
--MONONUCLEAR INFILTRATE, PERIVASCULAR		1	3	0	2
--PLEOCYLLULAR INFILTRATE		0	0	2	2
--COLLAGEN NECROSIS		0	0	1	0
--MYOFIBER DEGENERATION/NECROSIS, FOCAL		1	0	0	0
--ACANTHOSIS		0	1	0	0
--EROSION		0	1	0	0

Toxicokinetics

The serum concentrations of belimumab after subcutaneous of the two different formulations of the belimumab administration were comparable indicating that the change in formulation from the lyophilized form to the liquid form did not result any changes in the systemic absorption. No gender differences were noted.

Table 90 Summary Belimumab Serum Concentration: Local Tolerance Study

Appendix 2 Summary of LymphoStat-B serum concentrations (µg/mL) for male and female animals

<u>Group</u>	<u>Gender</u>	<u>Animal ID</u>	<u>Formulation</u>	<u>Day 1 Predose</u>	<u>Day 3 Predose</u>	<u>Day 8 Necropsy</u>
1	F	157789	lyophilized	0.000	230.720	1026.458
		157790	lyophilized	0.000	227.686	930.543
		157791	lyophilized	0.000	233.280	940.800
			N	3	3	3
			Mean	0.000	230.562	965.934
			SD	0.000	2.800	52.666
			SE	0.000	1.617	30.407
			Min	0.000	227.686	930.543
			Median	0.000	230.720	940.800
			Max	0.000	233.280	1026.458
			CV%	NC	1.2	5.5
			Geometric Mean	NC	230.551	964.995
			Lower 95% CI	0.000	223.606	835.104
			Upper 95% CI	0.000	237.518	1096.763
1	M	157783	lyophilized	0.000	194.394	711.142
		157784	lyophilized	0.000	242.432	974.848
		157785	lyophilized	0.000	255.987	935.178
			N	3	3	3
			Mean	0.000	230.938	873.723
			SD	0.000	32.365	142.189
			SE	0.000	18.686	82.093
			Min	0.000	194.394	711.142
			Median	0.000	242.432	935.178
			Max	0.000	255.987	974.848
			CV%	NC	14.0	16.3
			Geometric Mean	NC	229.349	865.491
			Lower 95% CI	0.000	150.538	520.505
			Upper 95% CI	0.000	311.338	1226.940
2	F	157792	liquid	0.000	268.902	876.544
		157793	liquid	0.000	251.571	830.549
		157794	liquid	0.000	189.043	659.136
			N	3	3	3
			Mean	0.000	236.505	788.743
			SD	0.000	42.007	114.575
			SE	0.000	24.253	66.150
			Min	0.000	189.043	659.136
			Median	0.000	251.571	830.549
			Max	0.000	268.902	876.544
			CV%	NC	17.8	14.5
			Geometric Mean	NC	233.851	782.897
			Lower 95% CI	0.000	132.154	504.124
			Upper 95% CI	0.000	340.857	1073.362

(continued)

Group	Gender	Animal ID	Formulation	Day 1 Predose	Day 3 Predose	Day 8 Necropsy
2	M	I57786	liquid	0.000	204.045	716.621
		I57787	liquid	0.000	197.606	687.654
		I57788	liquid	0.000	231.718	995.703
			N	3	3	3
			Mean	0.000	211.123	799.993
			SD	0.000	18.124	170.108
			SE	0.000	10.464	98.212
			Min	0.000	197.606	687.654
			Median	0.000	204.045	716.621
			Max	0.000	231.718	995.703
			CV%	NC	8.6	21.3
			Geometric Mean	NC	210.618	788.733
			Lower 95% CI	0.000	166.100	377.421
		Upper 95% CI	0.000	256.146	1222.564	

(concluded)

NC Not calculated.

Table 2 Mean (± SD) serum concentrations of LymphoStat-B in cynomolgus monkeys following repeated SC administration at 25 mg/kg doses

Study Day	Collection Time	Gender	Observed Concentration (µg/mL)		Predicted Concentration (µg/mL)
			Lyophilized Formulation	Liquid Formulation	
1	predose	M	0	0	0
		F	0	0	
		M & F	0	0	
3	predose	M	230.9 ± 32.4	211.1 ± 18.1	233
		F	230.6 ± 2.8	236.5 ± 42.0	
		M & F	230.8 ± 20.5	223.8 ± 32.1	
8	necropsy	M	873.7 ± 142.2	800.0 ± 170.1	984
		F	965.9 ± 52.7	788.7 ± 114.6	
		M & F	919.8 ± 108.4	794.4 ± 129.9	

M Male.
F Female.

11 Integrated Summary and Safety Evaluation

The BLA #125,370 for belimumab was submitted on June 09, 2010 for (b) (4) adult patients with active, autoantibody positive, systemic lupus erythematosus who are receiving standard therapy. The proposed treatment regimen is 10 mg/kg belimumab administered by intravenous (IV) infusion over 1 hour every 2 weeks for the first 3 infusions, then every 4 weeks thereafter. Phase 3 clinical studies have shown AUC values of 1849 mcg.h/mL in 563 SLE patients dosed with 10 mg of belimumab. The proposed commercial final drug product (FDP) is a lyophilized

formulation to be reconstituted with sterile Water for Injection (WFI) and further diluted to a final volume of 250 mL in normal saline for IV infusion.

Belimumab is a recombinant, fully humanized, monoclonal antibody, intended to inhibit BLYS, a B cell survival and differentiation factor. The belimumab target, BLYS protein, is derived from the BLYS gene that encodes for the generation of a (b) (4)

BLYS is a membrane bound protein expressed on cells of myeloid origin including monocytes, T-cells, dendritic cells, granulocytes, and activated neutrophils. Cleavage of the membrane bound BLYS results in a soluble (b) (4)

The amino acid sequence of human soluble BLYS is 98% identical to Cynomolgus monkey BLYS and the region of BLYS involved in receptor binding is 100% conserved. In contrast, human and mouse or rat BLYS share 84% identity, and some of the differences in amino acid residues fall within the receptor binding region. The Applicant showed that human and Cynomolgus monkey BLYS bind to human BR-3, TACI and BCMA with very similar kinetics. In addition to binding activity, the Applicant also demonstrated that belimumab was active on both human and monkey receptors using in vitro assays where belimumab inhibited BLYS induced proliferation of human PBMC and human and monkey BLYS induced proliferation of murine splenocytes. These data supported the selection of the Cynomolgus monkey as a relevant species in which to evaluate belimumab's potential toxicity.

The Applicant showed that the mean V_i in these studies ranged from 40-45 mL/kg, which is close to the plasma volume (45 mL/kg, Davies and Morris, 1993). The value for V_{ss} , ranging from 67-108 mL/kg, compared to the extracellular fluid volume (~170-210 mL/kg including plasma; Levine, 1990; Davies and Morris, 1993) may suggest that belimumab distribution is restricted to a space smaller than the extracellular fluid volume. Mean clearance values (ranging from 5.6-6.8 mL/day/kg of belimumab) are substantially less than the glomerular filtration rate for Cynomolgus monkeys (~3000 mL/day/kg; Davies and Morris, 1993), as expected for a large molecule such as an antibody (Gøbburu et al, 1998). Based on these studies, the mean terminal half-life of belimumab is approximately 7-14 days. No distribution, metabolism or excretion studies have been conducted with belimumab. The elimination of monoclonal antibody drugs is generally through cellular catabolism following nonspecific uptake by pinocytosis (Lobo et al, 2004).

Three toxicology studies were conducted in the Cynomolgus monkey in which they completed toxicokinetic evaluation. Two of these studies administered belimumab intravenously (1 for 4 weeks and 1 for 6-months) and one study administered belimumab subcutaneously for 13-weeks. After 4-week IV administration, belimumab serum concentrations increased with increased doses. In the 6-month IV study, serum concentrations also increased with increased doses for each the twice weekly dosing regimen. Interestingly, the twice weekly dosing regimen appeared to reach steady state at week 8. There were no significant gender differences in toxicokinetic parameters after IV administration. Belimumab is considered to be minimally immunogenic in

Cynomolgus monkeys. Because animals receiving belimumab showed expected pharmacological effects (reduction in B-lymphocytes), and there were no animals with belimumab exposures lower than expected, little anti-drug antibody formation occurred.

Repeat dose toxicity studies (1 and 6-month) were conducted in the Cynomolgus monkeys to support the chronic use of the product. The 1-month study dosed Cynomolgus monkeys with 0, 5, 15 and 50 mg/kg IV doses once weekly. The 6-month study dosed Cynomolgus monkeys with 0, 5, 15 and 50 mg/kg IV doses once every two weeks. The potential target organs of belimumab in the 1-month study were spleen (abscess), thyroid (follicular epithelial degeneration) and lymph node (granuloma). Similar findings were not observed in the 6-month toxicity study. The potential target organs of toxicity in the 6-month study were spleen (lymphoid depletion; hyperplasia), lymph nodes (extramedullary hematopoiesis, hyperplasia). These findings appear to be related to the exaggerated pharmacological effect of the drug product.

The levels of different immunoglobulins such as IgG, IgE, and IgM decreased during the course of treatment. The degree of the reduction in IgG, IgE, and IgG level was 18-25%, IgE levels were 18-25%, 38-90%, and 35-42% respectively. The finding is not dose related and recovered post dosing. The result showed that throughout the treatment of belimumab immunosuppressive effect might be observed.

Based on these findings a NOAEL of 15 mg/kg was identified. The Applicant did not include AUC data from the repeat dose toxicity studies, therefore, dose/body weight (mg/kg) have been used for the evaluation of the safety margins.

Table 91 Safety Margin

Species	NOAEL (mg/kg) M/F	Safety Margin Based on human dose 10 mg	Safety Margin Based on AUC ₍₀₋₂₄₎
Cynomolgus Monkey 28-Day study	5 mg/kg(LOAEL) (HED=300 mg), AUC ₀₋₂₄ @NOAEL=2868 mcg.h/mL	30 x	1.5 x
Cynomolgus Monkey 6-Month study**	15 mg/kg (HED=900 mg), (AUC _{0-inf} =2730 mcg.h/mL AUC)	90 x	Not available

*AUC in human: 1849 mcg.hr/mL at 10 mg/day

** Applicant did not provide AUC, blood samples were only collected from the trough, plasma serum concentration but not AUC was calculated by the Applicant from the toxicokinetic analyses

The Applicant did not complete an adequate assessment of male or female fertility. Based on discussions between the Agency and the Applicant at regulatory meetings on March, 08, 2010 and July 21, 2010 no formal fertility study is recommended. In the 6-month chronic IV Cynomolgus monkey study, no specific fertility endpoints were incorporated other than microscopic examination of male and female reproductive tissues. Immature testes were observed in the remaining male monkeys. The sperm analyses were not conducted. However, monoclonal antibodies are unable to cross a functional blood-testis barrier and would not be able to access Sertoli cells and germ cells in adult males. In female monkeys, periodic vaginal discharges consistent with actively cycling ovaries in all dose groups were observed. However, no systematic evaluation was completed.

The sponsor completed a combined embryo-fetal and peri- and post-natal development study in monkeys (0, 5, 150 mg/kg administered IV from GDs 20-150). Belimumab was shown to cross the placenta and was excreted in milk. There were 3 fetal, 8 (6 fetus+ 2 infants), and 4 (3 fetus+ 1 infant) deaths in 0, 5, and 150 mg/kg/bi-weekly dosing in the monkeys. The percent of fetal losses were 14 and 20% from the control and test article treated animal respectively (historical control for fetal death is 17.6%). The percent of infant deaths were 0 and 7 % in control and test article treated animals respectively (historical control for infant death is 10-12%). The cause of deaths of the fetus and the infants are unknown, however, most of the deaths were associated with atelectasis of the lungs. The drug is recommended as Pregnancy category C for labeling. A significant maternal and fetal morbidity, including spontaneous abortion, pre-eclampsia, intrauterine growth restriction, fetal death, and pre-term delivery has been reported in SLE patients (Molad et al, 2005). During the clinical trial, 47 pregnancies across the entire Phase 2 and 3 SLE experience were reported in the BLA. These subjects discontinued belimumab when the pregnancy was detected, and the Applicant followed the pregnancy to ascertain its outcome. The total fetal loss in patients treated with belimumab was 30% (11/37 patients), which is lower than in the placebo group (50%, 3/6 patients), but higher than the background estimated rate in patients with SLE [(15-25%) Andrade et al, 2008]. The nonclinical findings are reported in label.

Exposure of belimumab during development may alter the offspring's immune system. Therefore, infants from the embryofetal/postnatal reproductive toxicity study were evaluated for any changes in the immune system. There were 24 infant Cynomolgus monkeys were evaluated through 1 year post-partum. There were no belimumab-related changes in clinical signs, body weight, physical examinations, and or hematologic, serum chemistry, coagulation, or urinalysis parameters. The growth and development of the infants were within normal limits for infant Cynomolgus monkeys. Importantly, no infections were noted during the 1-year follow-up of the infants. Alterations in PBMCs of infant Cynomolgus monkeys born to females dosed with belimumab during pregnancy were limited to dose-independent reductions in the mean total B lymphocyte and mature B lymphocyte counts at BD7 and BD28. By BD91, the infant PBMCs were similar in all groups, including controls. IgG levels were not reduced. IgM levels were reduced from BD7 to BD91 (dose-independent; statistically significant at BD7 and BD91), but values were similar between belimumab-exposed and control infants from BD182 through the

end of the 1 year period following birth. T-lymphocytes (CD3+), T-helper lymphocytes (CD3+/CD4+), T-cytotoxic/suppressor lymphocytes (CD3+/CD8+), NK cells (CD3-/CD16+) and monocytes (CD3-/CD14+) were not reduced. The reduction in B lymphocytes identified by immunohistochemistry in the lymph nodes and spleen of fetuses sacrificed on GD150 (Phase 1) was reversed by 1 year after birth. After cessation of dosing and clearance of belimumab and just prior to (or concomitant with) B cell recovery, BLyS levels, which pre-dose were very low in adult females, transiently increased in both mothers and infants prior to returning to baseline levels. Based on the data generated in this study, exposure to belimumab in utero does not appear to confer a immunosuppression in the infant monkeys. Research in mice suggests that BLyS antagonism with a monoclonal anti-BLyS antibody diminishes primary B cell responses but leaves memory B cell pools intact (ie, capable of generating a secondary immune responses. However, monkeys dosed weekly with another BLyS antagonist, BR3-Fc, were able to mount relatively normal humoral immune responses to antigens as measured by primary response to KLH and Pneumovax 23 and recall [memory] response to tetanus and KLH. Based on all of this information, no further non clinical study is necessary to address the concern for immunosuppression.

No carcinogenicity study was conducted with belimumab. A literature review on the potential involvement of BLyS in tumor biology has been provided by the Applicant. There is no evidence to suggest ablation of BLyS would lead to the transformation of normal cells. There are data available from mice (AWySNJ) that have a non-functional BR3/BAFF-R receptor. The phenotype of the AWySNJ mice strain is very similar to the BLyS-knockout mice and is caused by an insertion in the BR3/BAFF-R gene that results in a non-functional receptor and mature B cell deficiency. Aged (22 months) AWySNJ mice do not have a higher rate of infection or neoplasia than their co-isogenic A/J counterparts, or other mouse strains routinely used for aging studies, supporting the conclusion that BLyS inhibition does not increase the risk of infection or neoplasia in animal models (Cancro et al 2005). These data lead the reviewer to believe that the carcinogenicity assessment of belimumab is not doable and might be addressed via labeling. In SLE patients an increased risk has been noted for lymphoma, lung cancer, and hepatobiliary cancer (Bernatsky et al, 2005), cervical and breast cancers (Kiss et al, 2010) compared to the general population. In the clinical trial with belimumab, the rates of malignancies, in patients with SLE were similar between placebo and belimumab groups. The malignancy rate was 0.29 with placebo and 0.20 with belimumab (Applicant's data). The risk for malignancy, however, can only be fully assessed in the post-marketing setting. The Applicant has proposed a post-marketing observational study to be conducted in 2,500 belimumab-exposed patients who will be followed for a period of 5 years. The malignancy findings from clinical trials have been relayed in labeling.

In summary, the BLA application for belimumab is approvable from non clinical perspective. There is no post marketing requirement from the Applicant from the non clinical perspective. The changes in the labeling were incorporated to

emphasize the mortality findings (fetus and infants) from the combined/peri-post natal toxicity studies.

12 Appendix

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BLA # 125,370

Reviewer Mamata De

Signature Page for BLA

[Redacted] (b) (6)

Primary Reviewer: Mamata De

Date November 24, 2010

Supervisory Pharmacologist: Molly Topper

[Redacted] (b) (6)

Nov 24, 2010

Date November 24, 2010

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA/BLA Number: 125370 Applicant: HGS Inc

Stamp Date: June 09, 2010

Product: Belimumab

BLA Type: NME

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	x		<p>* Carcinogenicity studies might not be doable. The Sponsor need to provide wordings for labeling to address the carcinogenicity issues</p> <p>* Male and female fertility studies were not conducted; Sponsor's rationale for not conducting these studies is inadequate. The Sponsor need to address potential male and female fertility toxicity associated with the product adequately.</p> <p>* Absorption and metabolism study not done, not required.</p> <p>* Safety pharmacology partially completed w/repeat dose toxicity study, further study might not be required (review issue).</p>
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	x		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	x		

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

	Content Parameter	Yes	No	Comment
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		
8	Has the applicant submitted all special toxicity studies/data requested by the Division during pre-submission discussions?		X	Division did ask to address the potential developmental toxicity associated with the offspring. The Sponsor need to adequately address the potential toxicity associated with the immune suppression in newborns.
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	x		<ul style="list-style-type: none"> • Need wordings for carcinogenicity part. • Reproductive toxicity battery not completed, however, the portion which is (Seg II, Seg III combined study) completed has adequate information for labeling.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	x		
11	Has the applicant addressed any abuse potential issues in the submission?		x	Not required
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?		x	Not relevant.

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

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- The potential for belimumab to alter male and female fertility parameters were not evaluated as per ICH S6 (R1) draft dated December 2009. At the Pre-BLA meeting the Sponsor was told ‘Although a dedicated fertility study in the primate is not an ideal means to address fertility aspects, specific assessments such as hormone measurements, sperm counts, morphology and motility were not included in the completed 6-month toxicology study. Your BLA submission should specifically discuss the reproductive parameters that were not assessed as per this draft guidance and the potential impact of your product on male and female fertility in light of the existing data and published literature regarding these endpoints.’

- Provide clear and adequate justification for not conducting male and female fertility parameter/studies with your product. Provide all of the existing data and published literature regarding these endpoints. Your justification should

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

specifically address potential toxicity (male and female fertility) associated with your product.

- The potential for belimumab to alter tumor development does not appear to have been evaluated as per ICHS6 (R1) draft dated December 2009.
 - You have not yet provided a clear rationale for not conducting carcinogenicity study with your product. You should specifically state how you intend to address the carcinogenicity section of your product labeling.
- Your embryo-fetal development/postnatal development study does not appear to have functional characterization of the impact of your product on the developing immune system. Exposure to this product during development can alter the offspring's immune system.

Your BLA submission does not provide a rationale to address the above mentioned safety concern. You must provide information as regards to what is known and expected and how you intend to address this concern in the product labeling.

<u>Mawala</u> 	<u>7/21/2010</u>
Reviewing Pharmacologist	Date
<u>Molly E. Topper, PhD</u>	<u>7/21/2010</u>
Team Leader/Supervisor	Date