CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

125526Orig1s000

PHARMACOLOGY REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date:	October 29, 2015
From:	Timothy J. McGovern, PhD, ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA:	125526
Agency receipt date:	November 4, 2014
Drug:	NUCALA (Mepolizumab)
Sponsor:	GlaxoSmithKline LLC
Indication:	Add-on maintenance treatment of patients with severe asthma aged 12 years and older and with an eosinophilic phenotype as reflected by blood eosinophil count
Reviewing Division:	Division of Pulmonary, Allergy, and Rheumatology Products

The primary pharmacology/toxicology reviewer and team leader concluded that the nonclinical data for NUCALA (mepolizumab) support approval for the indication listed above.

NUCALA is a subcutaneous (SC) injection product containing the active pharmaceutical ingredient mepolizumab containing 100 mg mepolizumab/vial. Mepolizumab is a humanized monoclonal antibody (IgG1 kappa), specific for interleukin-5 (IL-5). The recommended dose of NUCALA is 100 mg administered once every 4 weeks by SC injection into the upper arm, thigh, or abdomen. The Established Pharmacologic Class (EPC) for mepolizumab was discussed internally and is "interleukin-5 antagonist (IgG kappa)".

Pivotal nonclinical studies were conducted in cynomolgus monkeys; cynomolgus monkeys were identified as a pharmacologically relevant species in a series of pharmacology studies. In toxicology studies up to 6 months duration, no target organs of toxicity were identified at a SC dose of 10 mg/kg or intravenous (IV) doses of 10-100 mg/kg administered once every 4 weeks for a total of 7 doses. Eosinophil counts were reduced in all treatment groups; evaluation of bone marrow suggested a block of maturation and/or release of eosinophils from the bone marrow rather than a depletion of the eosinophil lineage cells. Systemic exposure to mepolizumab was similar at comparable IV and SC doses. The NOAEL was the high IV dose of 100 mg/kg q4 weeks or the SC dose of 10 mg/kg q4 weeks; the NOAEL for IV dosing provided a safety margin of approximately 70-fold compared to the maximum recommended clinical dose based on systemic exposure comparisons.

Genetic toxicity studies were not applicable for this therapeutic biologic protein. The need for a carcinogenicity study was discussed with the Executive Carcinogenicity Assessment Committee and it was concluded that a carcinogenicity study was not required given the lack of related findings in the 6-month toxicology study in monkeys and since conduct of a study in rodents did not appear to be technically feasible.

Information from the published literature regarding the potential roles of IL-5 and eosinophils in cancer is included in the product label.

Development and reproductive endpoints were evaluated as part of the 6-month chronic toxicology study of mepolizumab in monkeys (fertility based on histopathology of male and female reproductive organs), a pre- and postnatal development study of mepolizumab in pregnant female cynomolgus monkeys, and a study in mice that were treated with a surrogate monoclonal antibody (IgG2b). No effects on fertility or development parameters were identified in monkeys at doses up to 100 mg/kg IV; exposure margins of approximately 70-fold and 30-fold were available for fertility and developmental parameters, respectively. Similarly, no effects on fertility or embryofetal development parameters were identified in mice treated with the surrogate antibody.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this BLA can be approved from the pharmacology/toxicology perspective. I agree that the decision to waive the carcinogenicity study is reasonable and the EPC for mepolizumab is appropriate. I have discussed and am in agreement with labeling revisions proposed by the Division.

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TIMOTHY J MCGOVERN
10/29/2015

Pharmacology and Toxicology Review for BLA 125526

TO: BLA 125526 (NUCALA; Mepolizumab)

FROM: Timothy W. Robison, Ph.D., D.A.B.T.
Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products

Subject: Response to Sponsor's Submission dated September 10, 2015

The Clinical Team noted in controlled clinical trials, two serious adverse reactions of herpes zoster occurred in subjects treated with NUCALA compared to none in placebo. Five additional cases of herpes zoster were reported in the open label extension studies with NUCALA.

In a submission dated September 10, 2015, the Sponsor provided a response that "given the differences in murine and primate/human immune systems with regard to IL-5 biology and the absence of effects of mepolizumab on the host defense pathways involved with Herpes Zoster infections in primates and humans, there does not appear to be a risk for opportunistic infections in patients receiving mepolizumab."

The Sponsor provided clinical and nonclinical data demonstrating that mepolizumab can produce significant decreases of circulating eosinophils in Humans and Cynomolgus monkeys. Further, a surrogate anti-IL-5 antibody was shown to produce decreases of circulating eosinophils in CD-1 mice.

The published scientific literature provides evidence that eosinophils can play a role in the antiviral host response. Human eosinophils constitutively express Toll-like receptor (TLR)-1, TLR-4, TLR-7, TLR-9, and TLR-10, all of which coordinate innate and acquired immune responses. Recognition of viral nucleic acids, including double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and dsDNA, occurs by activation of TLR-3, TLR-7, and TLR-9, respectively, and can result in the production of type I interferons (IFNs) and the initiation of the antiviral host response. Further, eosinophils also express MHC-I and MHC-II, antiviral ribonucleases, cytokines, and chemokines, and can engage T cells, which supports the concept that this cell may contribute to the regulation of both innate and adaptive immunity. These cellular properties support a role for eosinophils in the antiviral host response.

Eosinophil recruitment has been reported in response to acute severe respiratory syncytial virus (RSV) infection in human infants (Journal of Pediatrics 1992; 120: 28-32; Archives of Disease in Children 1994; 71:428-432; American Journal of Respiratory and Critical Care Medicine 1999; 159:1918-1924). Further, eosinophil recruitment and degranulation in lung tissue has been observed in response to RSV infection in humans; however, the role of these cells was unclear (American Journal of Respiratory and Critical Care Medicine 1994; 150:1646-1652; American Journal of Respiratory and Critical Care Medicine 2001; 164:109-116).

Phibbs et al. (Blood 2007; 110: 1578-1586) conducted investigations to understand the role of eosinophils in host defense against RSV. Airway eosinophilia present constitutively in hypereosinophilic (IL-5 Tg) mice or introduced by transfer of eosinophils directly to the lungs resulted in accelerated clearance of RSV and reduction in associated airway dysfunction as measured by mucus hypersecretion and airway hyperreactivity. Eosinophil-deficient mice diplayed delayed clearance of RSV and increases of mucus hypersecretion and airway hyperreactivity. Single-stranded RNA, the ligand for TLR-7, induced functional activation of eosinophils leading to degranulation and increased expression of the phagocytic receptor, CD11b. These results appeared to be consistent with observations that eosinophils from infants with lower respiratory tract RSV disease demonstrated increased expression of CD11b (Clinical and Experimental Immunology 2006; 144: 409-417). Eosinophil-mediated accelerated RSV clearance and attenuated lung dysfunction were dependent on the toll IL-1 receptor resistance (TIR) domain adaptor protein, MyD88, and the production of nitric oxide (NO) by nitric oxide synthetase-2 (NOS-2). These findings support a role for eosinophils in innate immunity.

Adamko et al. (Journal of Experimental Medicine 1999; 190: 1465-1478) demonstrated that the clearance of parainfluenza virus in guinea pigs was enhanced by prior sensitization to ovalbumin and the generation of T helper 2 (Th2)-mediated pulmonary eosinophilia, which could be reversed by depletion of eosinophils with an anti-IL-5 monoclonal antibody.

Domachowske et al. (Journal of Infectious Diseases 1998; 177: 1458-1464) and Rosenberg (Journal of Leukocyte Biology 2001; 70: 691-698) found in vitro that eosinophils limit virus infectivity through the expression of eosinophil-associated ribonucleases, eosinophil-derived neurotoxin and eosinophilic cationic protein (ECP).

RSV infection of human epithelial cells of the human lower respiratory tract causes release of the chemokines such as RANTES, monocyte chemotactic protein 1, and macrophage inhibitory protein 1a (American Journal of Physiology 1997; 272: L512-L520 and Journal of Virology 1998; 72: 4756-4764) that can direct eosinophil migration. After infection with RSV, eosinophils have been shown to activate T cells by acting as antigen presenting cells (Journal of Immunology 1998; 160: 1279-1284). These investigations demonstrated that eosinophils can be recruited and activated by viral infections. Incubation of eosinophils with RSV-infected epithelial cells increases expression of the adhesion molecule CD18 on the eosinophils (Journal of Immunology 1998; 160: 4889-4895). Upregulation of Mac-1 (CD11b/CD18) is critical to eosinophil activation (American Journal of Respiratory and Critical Care Medicine 1998; 18: 675-686), allowing the eosinophils to interact with infected respiratory epithelium and to release ECP. The increased expression of CD18 also allows virus specific T cells to bind and activate eosinophils.

Varicella-zoster virus (VZV) causes varicella (chicken pox) and establishes latency in ganglia, from where it reactivates to cause herpes zoster (shingles), which is often followed by postherpetic neuralgia (PHN), causing severe neuropathic pain that can last

for years after the rash. The nature and kinetics of the virus-immune cell interactions that result in ganglion damage are not well known. Infected skin samples from both varicella and herpes zoster showed significant numbers of anti-T-cell intracellular antigen (TIA)- and granzyme B-expressing cytotoxic T cells (Journal of Virology 2002; 76: 11425–11433 and Journal of Experimental Medicine 2004; 200: 917–925). Gowrishankar et al. (Journal of Virology 2010; 84: 8861-8870) obtained material consisting of seven sensory ganglia from three donors who had suffered from herpes zoster between 1 and 4.5 months before death. Immunostaining of the site of VZV infection and to phenotype immune cells in these ganglia was conducted. VZV antigen was localized almost exclusively to neurons. The large immune infiltrate consisted of non-cytolytic CD8+ T cells with lesser numbers of CD4+ T cells, B cells, NK cells, and macrophages, and no dendritic cells. VZV antigen-positive neurons did not express detectable major histocompatibility complex (MHC) class I, nor did CD8+ T cells surround infected neurons, suggesting that mechanisms of immune control may not be dependent on direct contact. Further work is needed to define the nature of the immune response in ganglia following herpes zoster.

There are a large number of peer-reviewed scientific publications providing evidence that eosinophils can play a role in the antiviral host response. There are no specific publications describing eosinophils in the antiviral response to Herpes zoster, a dsDNA virus. However, there is clear scientific plausibility that eosinophils could potentially be involved in the host response to viruses such as Herpes zoster.

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TIMOTHY W ROBISON
09/20/2015

Secondary Pharmacology and Toxicology Review for BLA 125-526

TO: BLA 125-526 (GlaxoSmithKline LLC)

FROM: Marcie Wood, Ph.D.
Supervisory Pharmacologist
Division of Pulmonary, Allergy, and Rheumatology Products

DATE: July 17, 2015

Overview: I concur with the recommendation of Dr. Timothy Robison (detailed in a nonclinical review dated June 29, 2015) that the pharmacology and toxicology of NUCALA (mepolizumab) have been adequately studied and the drug product should be approved from a nonclinical perspective.

Background: Mepolizumab (Code name: SB240563) is a humanized monoclonal antibody (IgG1 kappa), specific for interleukin-5 (IL-5). It is indicated for add-on maintenance treatment in patients aged 12 years and older with severe eosinophilic asthma as identified by blood eosinophils greater than or equal to 150 cells/μL at initiation of treatment or blood eosinophils greater than or equal to 300 cells/μL in the past 12 months. The recommended dose of NUCALA is 100 mg administered once every 4 weeks by SC injection into the upper arm, thigh, or abdomen.

Pharmacology: Mepolizumab is a humanized monoclonal antibody (IgG1 kappa) that targets human IL-5. IL-5 is the major cytokine responsible for the growth and differentiation, recruitment, activation, and survival of eosinophils. The pharmacology of mepolizumab was evaluated both *in vitro* and *in vivo*. *In vitro*, mepolizumab inhibited binding of IL-5 to its receptor with an IC_{50} value <1.0 nM. The binding affinity of mepolizumab for human IL-5 ranged from 110 to 258 pM. Mepolizumab also inhibited exogenous IL-5-induced differentiation of eosinophils obtained from the bone marrow of healthy human volunteers and Cynomolgus monkeys. *In vivo*, mepolizumab decreased peripheral eosinophil counts in Cynomolgus monkeys and reduced pulmonary eosinophilia in response to *A. suum* challenge in an acute model of asthma.

Toxicology: The nonclinical program with mepolizumab was conducted in Cynomolgus monkeys, which were determined to be the only pharmacologically relevant nonclinical test species. In a chronic 6-month toxicology study with Cynomolgus monkeys that received mepolizumab by the IV route at doses up to 100 mg/kg q4 weeks or the SC route at a dose of 10 mg/kg q4 weeks, eosinophil counts were decreased by up to 95% at all doses from days 29 (first time point) to the end of the study. Evaluation of bone marrow suggested a block of maturation and/or release of eosinophils from the bone marrow and not depletion by mepolizumab of eosinophil lineage cells. There were no adverse histopathological findings. The NOAEL of 100 mg/kg q4 weeks provides adequate safety margin (greater than 90-fold on an AUC basis) for the proposed clinical dose of 100 mg SC q4 weeks.

Carcinogenicity: Given the absence of pre-neoplastic or neoplastic lesions in the 6-month toxicology study with monkeys and that the rodent (i.e., mouse or rat) was not a pharmacologically relevant species; a carcinogenicity study with mepolizumab was not required. Concurrence was obtained from the Executive Carcinogenicity Assessment Committee.

Reproductive and Developmental Toxicology: Male and female fertility was assessed in sexually mature monkeys as part of the chronic 6-month toxicology study with Cynomolgus monkeys discussed above. Male and female fertility was unaffected based upon no adverse findings from histopathological examinations of reproductive organs. In separate reproductive toxicity studies, there were no adverse findings in a pre- and post-natal development study with monkeys that were treated with mepolizumab at IV doses up to 100 mg/kg q4 weeks or a fertility and embryofetal development study with mice that received an analogous antibody, which inhibits the activity of murine IL-5.

Labeling: Dr. Robison provided labeling recommendations for the following sections: Indications and Usage (under Highlights of Prescribing Information), Section 8.1 (Pregnancy), (b) (4) Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). The Established Pharmacologic Class (EPC) was discussed with Dr. Paul Brown, Section 8.1 was modified in consultation with the Maternal Health Team to comply with the Pregnancy and Lactation Labeling Rule, and language regarding the carcinogenic potential of mepolizumab in Section 13.1 was modified in consultation with the Executive Carcinogenicity Assessment Committee. Complete product labeling details, including rationale for proposed product labeling, are found in Section 11 of Dr. Robison's review. I concur with the proposed labeling language for NUCALA.

There are no outstanding Pharmacology and Toxicology issues for this product.

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/s/

MARCIE L WOOD
07/17/2015

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:	125526
Supporting document/s:	001, 022, and 027
Applicant's letter date:	November 4, 2014 April 30, 2015 May 14, 2015
CDER stamp date:	November 4, 2015 April 30, 2015 May 14, 2015
Product:	NUCALA™ (Mepolizumab; Anti-IL-5 monoclonal antibody)
Indication:	Asthma
Applicant:	GlaxoSmithKline LLC
Review Division:	Pulmonary, Allergy, and Rheumatology Products
Reviewer/Team Leader:	Timothy W. Robison, Ph.D., D.A.B.T.
Supervisor:	Marcie Wood, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Nina Ton

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Mepolizumab (NUCALA™) is a humanized monoclonal antibody (IgG1 kappa), specific for interleukin-5. NUCALA™ is indicated for add-on maintenance treatment in patients aged 12 years and older with severe eosinophilic asthma as identified by blood eosinophils greater than or equal to 150 cells/μL at initiation of treatment or blood eosinophils greater than or equal to 300 cells/μL in the past 12 months.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical program with mepolizumab was conducted in Cynomolgus monkeys, which were determined to be the only pharmacologically relevant nonclinical test species. Mepolizumab was equipotent for inhibiting exogenous IL-5-induced differentiation of eosinophils, obtained from the bone marrow of human volunteers or Cynomolgus monkeys, with EC_{50} values of 13.3 pM. Further, the amino acid sequence of monkey IL-5 differs from human IL-5 by two conservative substitutions in a region not related to the presumed mepolizumab binding epitope on human IL-5.

In a chronic 6-month toxicology study with Cynomolgus monkeys that received mepolizumab by the IV route at doses up to 100 mg/kg q4 weeks or the SC route at a dose of 10 mg/kg q4 weeks, eosinophil counts were decreased by up to 95% at all doses from days 29 (first time point) to the end of the study. Evaluation of bone marrow suggested a block of maturation and/or release of eosinophils from the bone marrow and not depletion by mepolizumab of eosinophil lineage cells. There were no adverse histopathological findings. Male and female fertility was unaffected based upon no adverse findings from histopathological examinations of reproductive organs.

In reproductive toxicity studies, there were no adverse findings in a pre- and post-natal development study with monkeys that were treated with mepolizumab at IV doses up to 100 mg/kg q4 weeks or a fertility and embryofetal development study with mice that received an analogous antibody, which inhibits the activity of murine IL-5.

Given the absence of pre-neoplastic or neoplastic lesions in the 6-month toxicology study with monkeys and that the rodent (i.e., mouse or rat) was not a pharmacologically relevant species; a carcinogenicity study with mepolizumab was not required. Concurrence was obtained from the Executive Carcinogenicity Assessment Committee.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, the BLA is recommended for approval.

There are no outstanding nonclinical issues.

1.3.2 Additional Non Clinical Recommendations

None

1.3.3 Labeling

Labeling recommendations were provided for Indications and Usage (under Highlights of Prescribing Information), Section 8.1, Section (b) (4), Section 12.1, and Section 13. The Maternal Health Team was consulted for Sections 8.1 (b) (4) to achieve compliance with the Pregnancy and Lactation Labeling Rule.

Indications and Usage:

(b) (4)

8.1 Pregnancy

Pregnancy Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to NUCALA during pregnancy. Healthcare providers can enroll patients or encourage patients to enroll themselves by calling 1-877-xxx-xxxx or visiting www.nucalapregnancyregistry.com.

(b) (4)

(b) (4) Risk Summary

There are no data with NUCALA use in pregnant women to inform a drug associated risk. Monoclonal antibodies, such as mepolizumab, are transported across the placenta in a linear fashion as pregnancy progresses; therefore, potential effects on a fetus are likely to be greater during the second and third trimester of pregnancy. In an animal pre- and post-natal development study conducted in cynomolgus monkeys, there was no evidence of fetal harm with administration of intravenous mepolizumab throughout pregnancy at doses that produced exposures up to approximately 30 times the exposure at the maximum recommended human dose (MRHD) of 100 mg *[see Data]*. Consider the benefits and risks of NUCALA when prescribing NUCALA to a pregnant woman.

In the U.S. general population, the estimated background risk of major birth defects and

miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

(b) (4)

Clinical Considerations

Disease-associated maternal and/or embryo-fetal risk

In women with poorly or moderately controlled asthma, evidence demonstrates that there is an increased risk of preeclampsia in the mother and prematurity, low birth weight and small-for-gestational age for the neonate. The level of asthma control should be closely monitored in pregnant women and treatment adjusted as necessary to maintain optimal control.

(b) (4)

Data

Animal data

In a pre- and post-natal development study, pregnant cynomolgus monkeys received mepolizumab from gestation days 20 to 140 at doses that produced exposures up to approximately 30 times that achieved with the MRHD (on an AUC basis with maternal intravenous doses up to 100 mg/kg once every 4 weeks). Mepolizumab did not elicit adverse effects on fetal or neonatal growth (including immune function) up to 9 months after birth. Examinations for internal or skeletal malformations were not performed. Mepolizumab crossed the placenta in cynomolgus monkeys. Concentrations of mepolizumab were approximately 2.4 times higher in infants than in mothers for several months post-partum. Mepolizumab was quantifiable in infant plasma samples continuing through day 178 postpartum. Levels of mepolizumab in milk were 0.006 to 0.028% of maternal serum concentration.

In a fertility, early embryonic and embryofetal development study, pregnant CD-1 mice received an analogous antibody, which inhibits the activity of murine IL-5, at an intravenous dose of 50 mg/kg once per week throughout gestation. The analogous antibody was not teratogenic in mice. Embryofetal development of IL-5 deficient mice has been reported to be generally unaffected relative to wild-type mice.

(b) (4)

~~Mepolizumab crossed the placenta in cynomolgus monkeys. Concentrations of mepolizumab were approximately 2.4 times higher in infants than in mothers (b) (4) post partum (b) (4) .~~

(b) (4)

11 DESCRIPTION

(b) (4) mepolizumab (b) (4) ~~IL~~interleukin-5 antagonist (IgG1 kappa).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Mepolizumab is (b) (4) (IgG1 kappa) (b) (4) Mepolizumab binds to IL-5, with a dissociation constant of 100 pM, inhibiting the bioactivity of IL-5 (b) (4) by blocking (b) (4) ~~its~~ binding (b) (4) ~~to~~ to the alpha chain of the IL-5 receptor complex expressed on the eosinophil cell surface. (b) (4) Mepolizumab, by

inhibiting IL-5 signaling, (b) (4) reduces (b) (4) the production and survival of eosinophils. (b) (4) .

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of mepolizumab. (b) (4) . Published literature using mouse models suggests that IL-5 and eosinophils are part of an early inflammatory reaction at the site of tumorigenesis and can promote tumor rejection. However, other reports indicate that eosinophil infiltration into tumors can promote tumor growth. Therefore, the malignancy risk in humans from an antibody to IL-5 such as mepolizumab is unknown (b) (4)

Male and female fertility were unaffected based upon no adverse histopathological findings in the reproductive organs from Cynomolgus monkeys treated with mepolizumab for 6 months at intravenous doses up to 100 mg/kg once every 4 weeks (approximately 30 times the maximum recommended human dose on an AUC basis). Mating and reproductive performance were unaffected in male and female CD-1 mice treated with an analogous antibody, which inhibits the activity of murine IL-5, at an intravenous dose of 50 mg/kg once per week.

(b) (4)

2 Drug Information

2.1 Drug

Tradename: NUCALA®

Generic Name: Mepolizumab

Code Name: SB240563

Molecular Weight: (b) (4) kDa (b) (4))

Biochemical Description: Mepolizumab is a fully humanized IgG Ab (IgG_1, kappa) with (b) (4) . Mepolizumab is a humanized monoclonal antibody produced by a recombinant CHO mammalian cell (b) (4)

Pharmacologic Class: NUCALA is a humanized interleukin-5 antagonist (IgG1 kappa).

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 6971 (GlaxoSmithKline, Mepolizumab for Asthma)
IND (b) (4)
IND

2.3 Drug Formulation

Mepolizumab for Injection, 100 mg/vial, drug product (DP) is a white lyophilized cake, (b) (4) containing (b) (4) mg/mL mepolizumab, (b) (4) sodium phosphate dibasic heptahydrate, (b) (4) sucrose (b) (4) and (b) (4) polysorbate 80 (b) (4), at pH 7.0. Mepolizumab for Injection, 100 mg/vial, is filled and lyophilized in 10 mL Type 1 clear glass vials, sealed with gray (b) (4) rubber (b) (4) stoppers and aluminum overseals with red flip-off caps. After reconstitution with 1.2 mL of sterile Water for Injection (WFI), it forms a clear-to-opalescent, colorless to-pale–yellow or pale–brown solution that is essentially particle-free. The sterile WFI used for reconstituting the lyophilized DP is not supplied. The product is intended for administration by subcutaneous injection.

The components and quantitative composition per vial of Mepolizumab for Injection, 100 mg/vial, are shown in the table below.

Table 1 Composition of mepolizumab for injection, 100 mg/vial

Table 1 Composition of Mepolizumab for Injection, 100 mg/vial

Component	Quantity per vial[1] (mg)	Function	Quality Standards
Mepolizumab	(b) (4)	Drug Substance	GSK, Non-compendial[3]
Sucrose	(b) (4)		USP/NF[4], EP[5], and JP[6]
Sodium Phosphate Dibasic, Heptahydrate			USP
Polysorbate 80			USP/NF, EP, and JP
			USP/NF, EP, and JP
Water for Injection[9]			USP/NF, EP, and JP

Notes:

1. Minimum and maximum values per vial are provided for excipients.
2. The product contains (b) (4) mg/vial; the label claim of 100 mg/vial is based on a withdrawable volume of 1.0 mL following reconstitution of the lyophile. A (b) (4) overfill allows a withdrawal volume of 1.0 mL after reconstitution with 1.2 mL of SWFI. This volume accounts for the solids contribution to the final volume and results in a final concentration of 100mg/ml. No overages are included.
3. Refer to Section S.4.1.Specification for the drug substance specification.
4. USP/NF = United States Pharmacopeia/National Formulary
5. EP = European Pharmacopeia
6. JP = Japanese Pharmacopeia
7. N/A = Not applicable
8. (b) (4)
9. Water for injection is removed during lyophilization.
10. The (b) (4) per vial is approximately (b) (4) mL prior to lyophilization; it is determined by the (b) (4) which is adjusted according to mepolizumab (b) (4) may vary between (b) (4) (b) (4)

(Excer

2.4 Comments on Novel Excipients

None

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

NUCALA™ is indicated for add-on maintenance treatment in patients aged 12 years and older with severe eosinophilic asthma as identified by blood eosinophils greater than or equal to 150 cells/μL at initiation of treatment or blood eosinophils greater than or equal to 300 cells/μL in the past 12 months. NUCALA™ has been shown to reduce exacerbations of asthma in patients with an exacerbation history.

NUCALA is for subcutaneous (SC) use only.

The recommended dose of NUCALA is 100 mg administered once every 4 weeks by SC injection into the upper arm, thigh, or abdomen.

2.7 Regulatory Background

IND 6971 was opened in CBER on December 24, 1996. The IND was transferred to the Division of Pulmonary, Allergy, and Rheumatology Products (DPARP) in October of 2005.

DPARP nonclinical concerns during IND development were primarily focused on the adequacy of the 6-month toxicology study with monkeys and the need for a carcinogenicity study with mepolizumab as shown below.

A meeting under IND 6971 was held with the Sponsor on January 23, 2006, to discuss the pivotal clinical trials program to support their BLA program (see meeting minutes dated April 13, 2006). The Sponsor's nonclinical question, the Division's response, and the meeting discussion were reproduced below.

M. Do you agree that the toxicology program conducted with mepolizumab has adequately characterized the molecule and that no additional studies would be required for product registration?

Response:

We do not agree. At this time, the Division has concerns that the assessment of the toxicological potential is incomplete with particular regard to the effects of chronic exposure (e.g., cumulative, toxicity, delayed toxic effects, carcinogenicity).

Half-lives of mepolizumab in monkeys and humans are 12-14 days and 16-20 days, respectively. Steady-state plasma drug concentrations were not achieved in the 6-month monkey study until after approximately 3 months of treatment. Animals were not chronically exposed to steady-state plasma drug concentrations for at least 6 months.

1. With regard to the 6-month toxicology study with monkeys:

a. *Provide an explanation for the selection of doses used in the 6-month monkey study.*

b. *It is noted that histopathological examinations of the cecum, rectum, cervix, tongue, tonsils, larynx, sternebrae, and lumbar vertebra were not performed in the 6-month study, although our primary concerns are for the cecum, rectum, cervix, tongue, tonsils, and larynx. If these tissues are still available, microscopic examinations should be conducted and results submitted to the Division.*

2. *A long-term study to assess the effects of chronic exposure in terms of potential carcinogenicity, delayed toxicity, and cumulative toxicity is needed. It is recommended that this study be conducted with mice and be 18 to 24 months in duration. Possible approaches might involve the use of (1) the rat anti-human IL-5 monoclonal antibody, that cross reacts with murine IL-5, administered to an appropriate mouse strain or (2) the untreated IL-5 knockout mouse. This study should be available with a BLA. For option 1, a dose selection proposal and study protocol for the carcinogenicity study should be submitted to Division to obtain concurrence from the Executive Carcinogenicity Assessment Committee. It is recommended that the dose selection proposal be based upon the results of a dose range finding study. For option 2, a study protocol should be submitted to the Division.*

Discussion:

GSK asked the Agency to clarify the definition of "steady state" in the chronic monkey studies. The Agency noted in the 6-month toxicology study with monkeys that plasma concentrations appeared to increase with each successive dose of SB 240563, with trough concentrations reaching a plateau approximately after the fourth dose (see pages 111 and 315 of the submission).

The Agency assesses the safety of clinical doses on an AUC, mg/kg, or mg/m^2 basis as appropriate.

The Agency noted concerns that no target organs of toxicity and/or dose-limiting toxicity had been identified for mepolizumab.

GSK stated that selection of the high dose for 6-month toxicology study with monkeys at a 10-fold multiple of the human dose was based on a meeting with CBER representatives in the 1990s.

Further, GSK noted in a single dose study that monkeys had received 300 mg/kg, although no dose-limiting toxicity was observed.

The Agency noted that mepolizumab has a long half-life, and there are concerns that potential delayed and/or cumulative toxicity have not been adequately assessed. The 6-month toxicology study with monkeys did not include a recovery period. Further, it is generally standard practice now for monoclonal antibodies with a half-life of similar duration to mepolizumab to request a 9-month toxicology study or a 6-month toxicology study with an adequate recovery period.

Further, the Agency stated that carcinogenic potential of mepolizumab should be assessed. It is not always possible to assess the carcinogenic potential of a biological agent based upon results of the 6-month toxicology study. The potential carcinogenic signal noted in Phase 3 clinical trials with Xolair was noted as an example, which was unexpected based upon mechanistic considerations and results of chronic toxicology studies with monkeys.

GSK stated that antigenicity was a significant problem in the reproductive study with mice and would not be a feasible approach for a long-term carcinogenicity study.

GSK stated that the absence of a historical tumor database for the IL-5 knockout mouse would confound interpretation of potential tumor findings. Further, stability of the knockout could make such a long-term study problematic.

GSK suggested a chronic toxicology study using monkeys with duration of at least 1 year might be the most appropriate approach. The Agency has significant concerns that this monkey study will not address the issue of assessing carcinogenic potential. However, the Agency suggested GSK submit a proposal and justification for review.

The Agency also requested the remaining tissues from the 6-month monkey study be sectioned and the results submitted for review. It was noted that the tonsils were positive in the tissue cross reactivity study. However, the tonsils from monkeys in the 6-month toxicology study had not been submitted to histopathological examination.

Post-meeting Addendum:

The following information was obtained from an established supplier of IL5 knockout (IL5<tm1Kopf>) mice. The strain was developed using a C57BL/6-derived ES cell line, and no other strains have been introduced into the genetic background. C57BL/6J or wildtype mice from heterozygote X heterozygote crosses may be used as controls. Most targeted mutant alleles are stable, and there is no information which suggest that the Il5<tm1Kopf> mutation is unstable.

A meeting under IND (b) (4) was held with the Sponsor on (b) (4) to discuss the development program for mepolizumab (see meeting minutes dated (b) (4) The Sponsor's nonclinical question and the Division's response were reproduced below.

5. Does the Agency agree with GSK's assessment that carcinogenicity studies with mepolizumab are not warranted because carcinogenicity studies in currently available IL5-/- mice would not provide clearly interpretable safety data, and long term studies with mice with the surrogate antibody are not technically feasible?

FDA Response:

We concur. A carcinogenicity study is waived for mepolizumab due to the lack of an appropriate animal model.

In the BLA submission, address what has been learned relevant to prediction of carcinogenicity for mepolizumab (e.g., hyperplastic response or immunosuppression) based upon the nonclinical chronic study with monkeys that received mepolizumab and results from clinical trials with mepolizumab as well as the published scientific literature for IL-5, and other products with the same or a very similar target.

See the CARCINOGENICITY section for a discussion of the basis of the Division's decision, in consultation with the Executive Carcinogenicity Assessment Committee, to waive a carcinogenicity study.

A meeting under IND 6971 was held with the Sponsor on April 21, 2009, to discuss their Phase 2b/Phase 3 clinical development plans for mepolizumab (see meeting minutes dated May 20, 2009). The Sponsor's nonclinical questions, the Division's responses, and one additional Nonclinical Comment were reproduced below.

8. ***At the*** [redacted] (b) (4) ***the FDA agreed that the presentation of the additional histological analysis of monkey tissue samples satisfied the FDA's concerns regarding the toxicological potential of mepolizumab and the FDA also confirmed that the requirement for a carcinogenicity study with mepolizumab was waived due to the lack of an appropriate animal model.***

   a. ***Does the FDA agree that the presentation of the additional histological analysis of monkey tissue samples*** [redacted] (b) (4) [redacted] (b) (4) ***satisfies the FDA's concerns regarding the toxicological potential of mepolizumab for the treatment of asthma BB IND 6,971?***

FDA Response:

Yes, we agree.

   b. ***With respect to the requirement for a carcinogenicity study the FDA agreed that submission of results from clinical trials with mepolizumab, as well as published scientific literature for anti-IL-5 and other products with the same or a very similar target was acceptable*** [redacted] (b) (4) [redacted] (b) (4) ***Does the FDA also agree that this approach is acceptable for the BLA submission for mepolizumab treatment of asthma (BB IND 6,971)?***

FDA Response:

Yes, we agree.

3. *Clinical exposures (AUC0-4wk) of greater than approximately 2500 µg*day/mL (AUC0-4wk in monkeys that received SC doses of 10 mg/kg/4 weeks) following subcutaneous administration need to be adequately justified.*

Discussion:

GSK asked for clarification of this response. The FDA stated that GSK would need nonclinical support and the AUC data currently does not support doses greater than 250 mg. GSK asked if they decide to increase to doses higher than 250mg SC, would toxicology studies conducted 2 to 3 times higher than 250 mg be needed. The FDA stated that they will confer with the Pharmacology/Toxicology team and provide clarification in the meeting minutes.

POST-MEETING NOTE:

Systemic toxicity has been assessed in monkeys that received intravenous doses up to 100 mg/kg/4 week (AUC = 33707 µg·day/mL). However, there are concerns for local toxicity with subcutaneous doses >10 mg/kg/4 week. Conduct a study with duration of at least 4 weeks in monkeys with doses >10 mg/kg/4 weeks to assess local toxicity at injection sites and surrounding tissues (e.g., lymph nodes). A NOAEL with an appropriate safety margin should be identified. Alternatively, provide justification that such a study is not needed.

3 Studies Submitted

3.1 Studies Reviewed

Study Title:	Study Number:
Pharmacology Studies:	
Characterization of Anti-IL-5 Antibodies, SB240563 and 2B6	SB240563/RSD-100LD3/1
(b) (4)	2014N189114_00
In vitro binding properties of Mepolizumab (SB240563) to IL-5 determined by Biacore™	SH2009/0001 0/00
Mepolizumab (SB240563): In vitro Species Specificity	ch2005-00951
Intravenous Pharmacokinetic and Pharmacodynamic Study in Rabbits	SB240563/RSD-100XMR/1
Activity of Human and Monkey Recombinant IL-5 on Monkey and Human Cell Proliferation	CH2008/00044/00
Comparison of Different Recombinant Forms of Human IL-5 and their Neutralization by Mepolizumab	CR2009/00004/00
Preliminary Pharmacokinetics of a Humanized Monoclonal Antibody Directed to Interleukin-5, SB240563, in Female Cynomolgus Monkeys Following a Bolus Intravenous Dose of 1 mg/kg	SB240563/RSD-100LKP/2
Mepolizumab (SB240563): An Efficacy Study in a Model of Asthma in Cynomolgus Monkeys	SB240563/RSD1013L8/1
A Study to Evaluate the Efficacy of a Test Article in a Model of Asthma in Cynomolgus Monkeys	CD2005/00631/00
The Effect on Antigen-Induced Airway Eosinophilia in Guinea Pigs by 2B6 Monoclonal Antibody Directed at Interleukin-5	CH2005/00956/00
Safety Pharmacology Studies:	
General Pharmacological Study in Cynomolgus Monkeys	RSD-100SHZ/1
ADME Studies:	Summary
General Toxicology Studies:	
Single Intravenous Dose Toxicity Study in Monkeys	SB-240563/RSD-100FOG/1
Repeat Dose Intravenous Toxicity and Pharmacology Study in Cynomolgus Monkeys	SB-240563-RSD-100KN9/1
6-Month Toxicity Study in Cynomolgus Monkeys	SB240563/RSD-100X0L/1
Genetic Toxicity:	Not Applicable
Carcinogenicity:	Carcinogenicity Assessment
Reproductive and Developmental Toxicology:	
- **Fertility and Early Embryonic Development**	Summary
- **Embryonic Fetal Development**	
Intravenous Study of Male and Female Fertility, Early Embryonic and Embryo-Fetal Development in CD-1 Mice	SB240563/RSD-100P8V/1
SB-264091: Intravenous Dose Study to Assess Pharmacokinetics, Pharmacology, and Antigenicity in Female CD-1 Mice	SB240563/RSD-100MZP/2
- **Prenatal and Postnatal Development**	
Intravenous Study for Effects on pre- and Postnatal Development in Cynomolgus Monkeys	CD2003/01020/00
Other Toxicology Studies:	
Mepolizumab (SB240563): Immunocytochemical Analysis of Binding to	SB-240563/RSD-100KGT/1

Normal Human Tissues	
SB-264091: Effect on Host Defense against *Mesocestoides corti* Infection in Mice (CD2005/01090/00)	CD2005/01090/00

3.2 Studies Not Reviewed

Study Title:	**Study Number:**
Assay Validation:	
Quantitative ELISA Method Validation for the Determination of SB-240563 in Monkey Plasma	CD2006/00287/00
SB240563: Validation of a Second Generation Method to Quantitate Antibodies Against SB240563 in Serum and Plasma of Cynomolgus Monkeys	CD2006/01098/00
Determination of 58-240563 in Rabbit Plasma by Electrochemiluminescent Immunoassay	SB240563/RSD-100JMH/1
Determination of SB-240563 in Monkey Plasma by Electrochemiluminescent Immunoassay	SB-240563/RSD-100KFN/1
Determination of 58-240563 in Monkey Bronchoalveolar Lavage Fluid by Electrochemiluminescent Immunoassay	SB-240563/RSD-100M84/1
Validation of an Electrochemiluminescent (ECL) Immunoassay for Antibodies Against SB-240563	SB-240563/RSD-100N23/1
Purification and Characterization of Anti-SB-240563 and Anti-SB-240563 Idiotype Antibodies	SB-240563/RSD-100SLC/1
Development of an electrochemiluminescent (ECL) immunoassay for SB240563 in monkey plasma	SB-240563/RSD-1005M6/1
Determination of SB-240563 in Monkey Plasma by Time-Resolved Fluorescent Immunoassay	SB-240563/RSD-1017X9/1
[redacted]	
ADME Studies:	
Preliminary pharmacokinetics of an anti-interleukin-5 humanized monoclonal antibody, SB240563 (HzA), in male Sprague-Dawley rats following bolus intravenous administration of 1 mg/kg	SB-240563/RSD-1006F4/1
[redacted] (b) (4)	
Dosing and Sample Collection to Allow Comparison of the Pharmacokinetics of Two Commercial Supplies of SB-240563 Following Intravenous Administration to the Cynomolgus Monkey	CD2006/00846/00
[redacted] (b) (4)	
Dosing and Sample Collection to Allow Comparison of the Pharmacokinetics of the Phase III Supplies and Phase I/II Supplies of SB-240563 Following Intravenous Administration to the Cynomolgus Monkey	SB-240563/RSD-101FNT/1
Pharmacokinetics of a Humanized MAb (SB-240563, anti-IL5) in Cynomolgus Monkeys Following a Single Inhalation Exposure	CD2008/00299/00
[redacted]	
Metabolism:	
An In Vitro Evaluation of the Effect of Mepolizumab (SB-240563) and Cytokines IL-5 and IL-6 on the mRNA Levels of Cytochrome P450 3A4 in Cultured Human Hepatocytes	2011N121217_00
[redacted]	
Local Tolerance Studies:	
Subcutaneous Tolerability and Pharmacokinetic Study in Female Monkeys	SB-240563/RSD-100PLM/1

with the Medi-Jector Choice Needle-Free Injector	
Pharmacokinetic and Pharmacodynamic Subcutaneous Study in Male Monkeys with the Medi-Jector Choice Needle-Free Injector	SB-240563/RSD-100TV7/1
Subcutaneous Pharmacology, Irritancy and Antigenicity Study in Female Cynomolgus Monkeys	SB-240563/RSD-100ZHH/1
[redacted]	
Other Studies:	
Immunolocalization of Interleukin-5 in *Drosophila* cells	SB-240563/RSD-100DBX/2

3.3 Previous Reviews Referenced

1. Pharmacology and Toxicology Review dated February 8, 2006*
2. Pharmacology and Toxicology Review dated September 25, 2009*
3. Pharmacology and Toxicology Review dated November 3, 2010*

* Not attached; however, the contents of reviews were transferred to the present review.

4 Pharmacology

4.1 Primary Pharmacology

Characterization of Anti-IL-5 Antibodies, Mepolizumab (SB240563) and 2B6 (Study Report: SB240563/RSD-100LD3/1): The binding and biological properties of anti-IL-5 monoclonal antibodies, 2B6 and mepolizumab (SB240563), were characterized.

The murine monoclonal antibody 2B6 was prepared [redacted] (b) (4)

The humanized version of mAb 2B6, designated as mepolizumab, was prepared using standardized techniques. The complementarity determining regions were grafted from the murine antibody, 2B6, by molecular genetic techniques.

[redacted] (b) (4)

The binding kinetics of mepolizumab to IL-5 were analyzed at 25°C in a BIAcore biosensor. In these experiments, IL-5 (100 nM) was immobilized on a biosensor chip and mepolizumab was in solution. The k_{off} rate of mepolizumab was too slow to measure accurately, but was estimated at $1.7 \times 10^{-5} s^{-1}$. The k_{on} rate was 1.6×10^{5} $M^{-1}s^{-1}$. From the ratio of the k_{on} and k_{off} rates, the K_d of mepolizumab was calculated at approximately 100 pM.

Inhibition of binding of ^{125}I-labeled IL-5 to Drosophila cell membranes expressing the IL-5Rα chain by mepolizumab and 2B6 were measured. The IL-5R consists of both α and β subunits. The α subunit recognizes IL-5 while the β subunit recognizes the IL-5/α subunit complex and is required for signal transduction. Antibodies were pre-incubated with radiolabeled IL-5 and membranes were added. Radioactivity bound to membranes was measured. Mepolizumab inhibited binding of IL-5 to its receptor with an IC_{50} value <1.0 nM. Similar results were obtained with mAb 2B6.

Inhibition of IL-5-induced proliferation of murine LyH7.B13 cells by mepolizumab was measured. Mepolizumab inhibited the IL-5 dependent response of LyH7.B13 cells with an IC_{50} value <100 pM. Similar results were obtained with mAb 2B6.

Inhibition of IL-5-induced proliferation of human TF-1.28 cells by SB240563 was measured. SB240563 inhibited the response of TF-1.28 cells to IL-5 with an IC_{50} value <150pM. Addition of 300pM SB240563 produced greater than 90% inhibition of cell activity. Comparable results were obtained with mAb 2B6.

Table 2 Inhibition of IL-5 binding and IL-5-induced cell proliferation with SB240563, mAb 2B6, and IL-5CH

Table 2 Summary of binding and cell proliferative results obtained with SB 240563, mAb 2B6 and IL-5CH

Results are expressed as a mean [range]n, where n is the number of individual experiments.

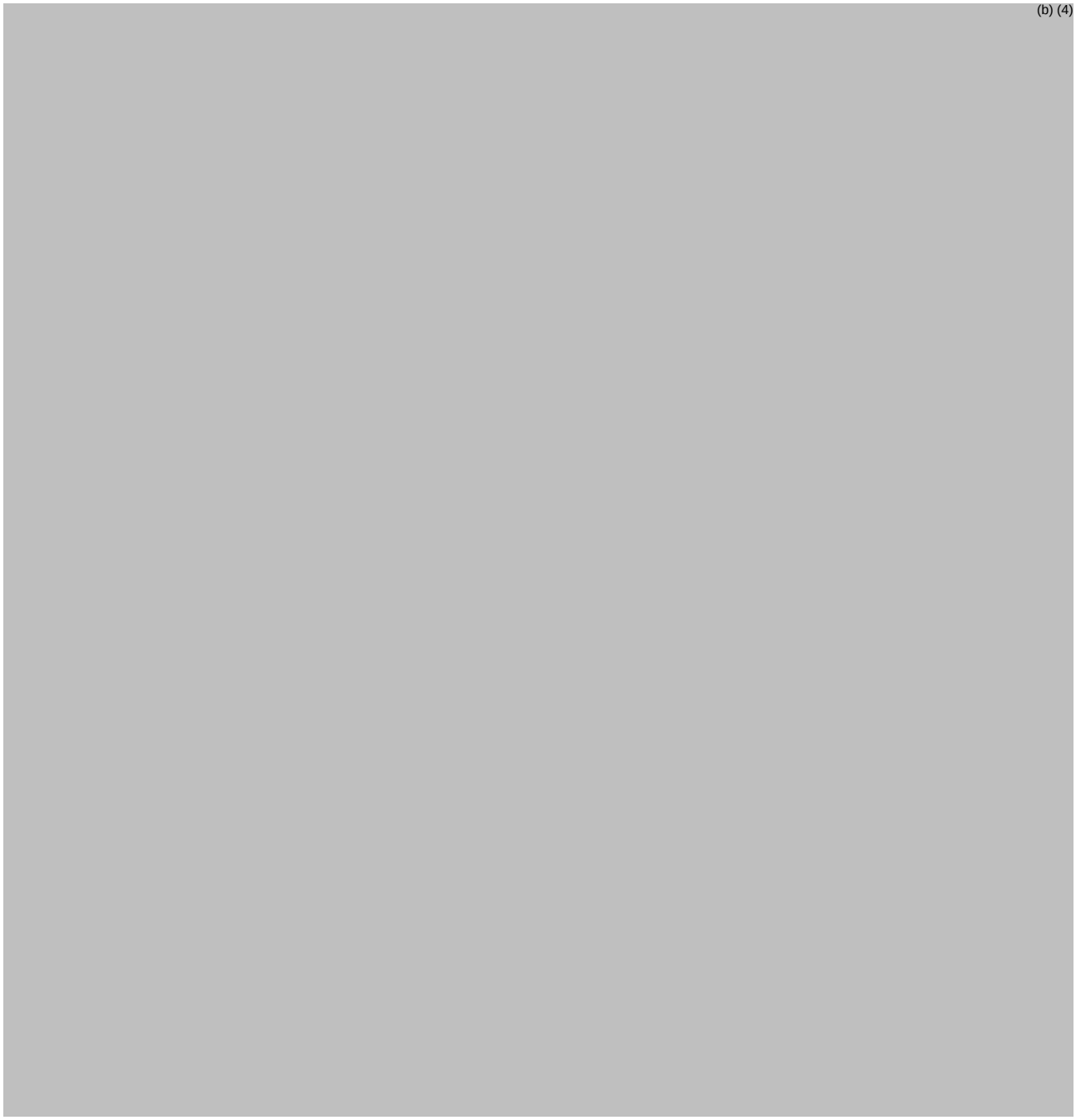
Antibody	IL-5/IL-5Ralpha IC50 nM	TF-1.28 assay IC50 pM	B13 assay IC50 pM
SB 240563	0.94 [0.70-1.2]3	73 [38-130]3	31 [15-55]5
2B6	0.70	30	74 [26-200]6
IL5CH	0.57 [0.39-0.75]2	38	34 [25-53]3
SB 240564	0.82	54	65 [51-79]2

(Excerpted from the Sponsor's submission)

Inhibition of IL-5-dependent eosinophil differentiation by mepolizumab was measured. Human bone marrow mononuclear cells were incubated with 10 ng/mL IL-5 in the presence of increasing concentrations of mepolizumab over a 14- or 21-day period. There was a dose-dependent increase in the inhibition of eosinophil differentiation in the presence of increasing concentrations of mepolizumab at both time points. At both time points ≥70% inhibition was achieved with 100 pM antibody.

Unpurified cynomolgus monkey lymph node supernatants stimulated with ionomycin and PMA were able to increase the proliferative response of LyH7.B13 cells. The addition of increasing concentrations of the parent mAb, 2B6, caused a dose-dependent decrease in activity. This might be considered preliminary evidence, using the parental antibody (2B6) that the monkey is a pharmacologically relevant species.

Mepolizumab failed to inhibit the activity of mouse IL-5 as measured by proliferation of LyH7.B13 cells. This result suggested that the mouse was not a pharmacologically relevant species with mepolizumab.




(b) (4)

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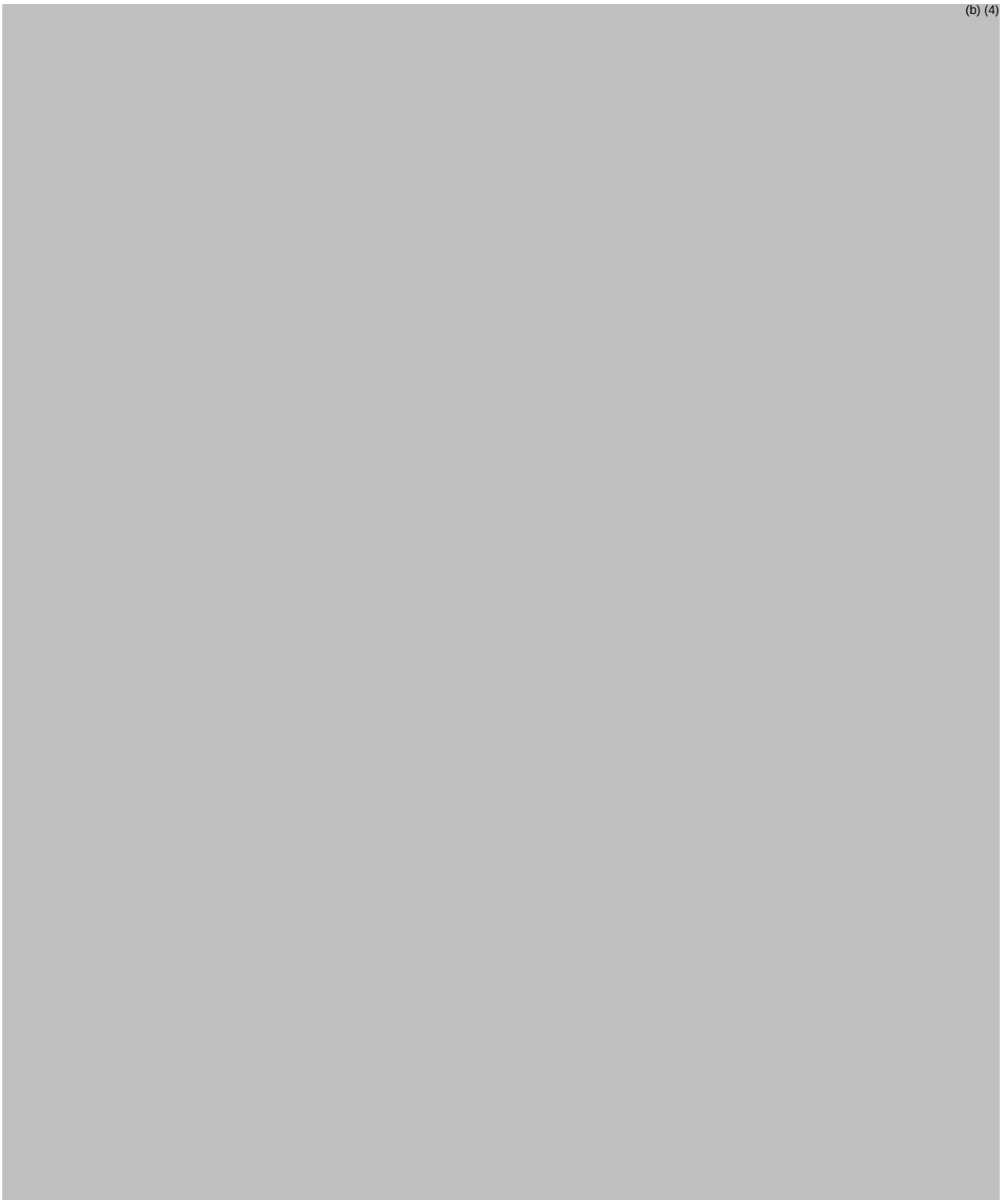
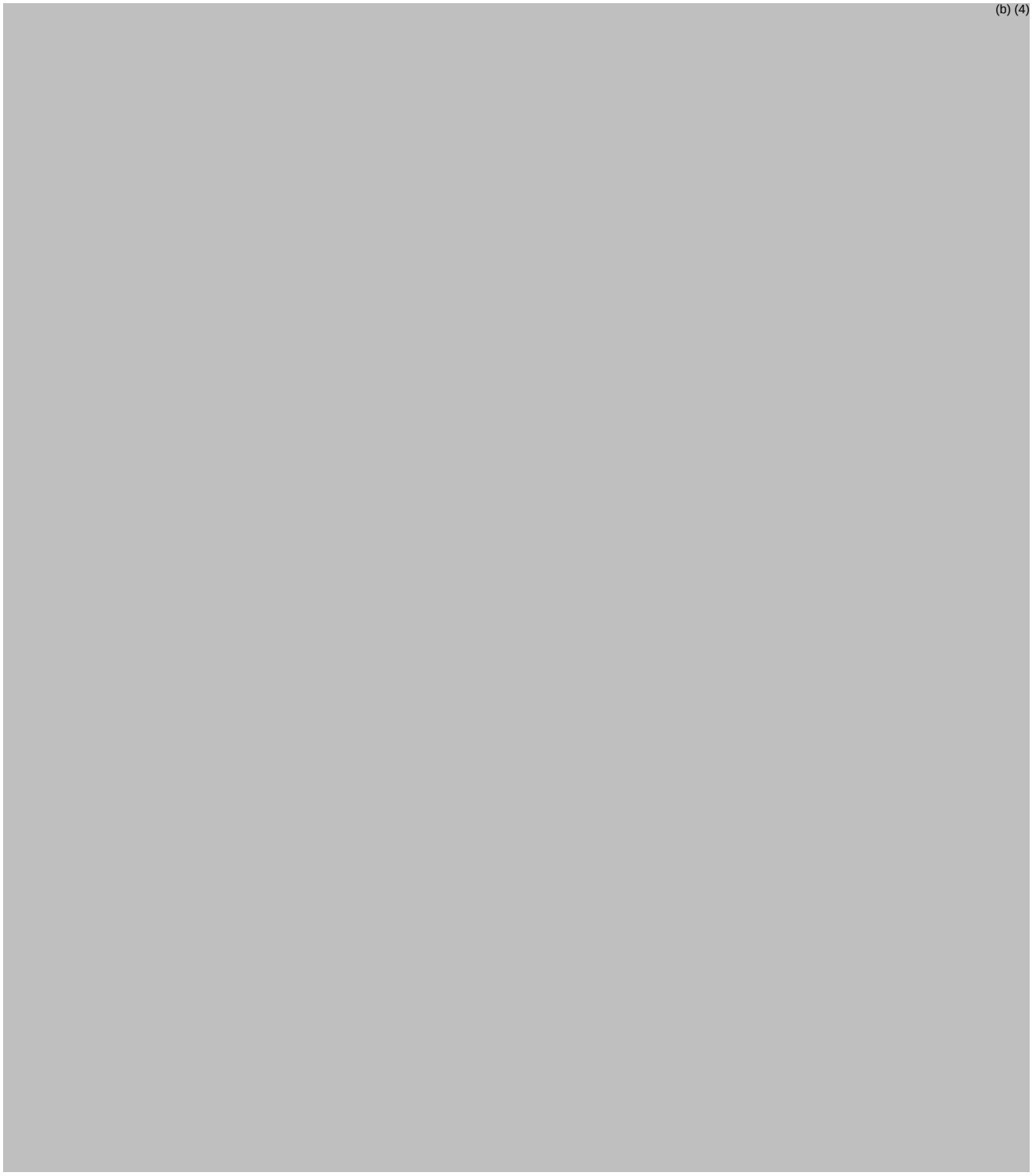




In vitro binding properties of Mepolizumab (SB240563) to IL-5 determined by Biacore™ (Study Report: SH2009/0001 0/00): Binding kinetics and affinity constants for binding of mepolizumab to human IL-5 were determined using plasmon surface resonance (Biacore™). The overall affinity for human IL-5 was high, ranging from 110 to 258 pM at room temperature in two experiments.

Mepolizumab (SB240563): In vitro Species Specificity (Study Report: ch2005-00951): The ability of mepolizumab to inhibit exogenous IL-5-induced proliferation of the murine IL-5/IL-3 dependent LyH7.B13 cell line and human IL-5 responsive TF-1.28 cell line was examined. Recombinant IL-5 was only available for mouse and human. Purified spleen cells were obtained from rats, beagle dogs, and rabbits and cultured with phytohemagglutinin, concanavalin A, or ionomycin/phorbol myristate acetate for 24 hr to stimulate cytokine release. The supernatants were collected and frozen for later use. Serum samples were collected from Cynomolgus monkeys. Lung tissue was collected from a guinea pig eosinophilia model.

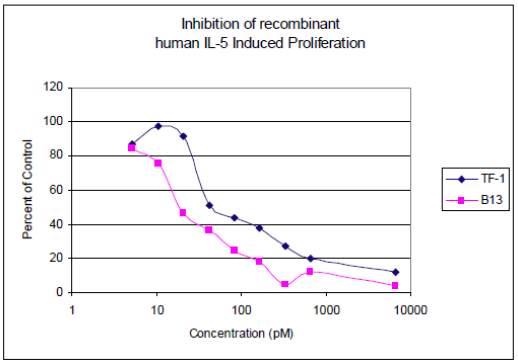
Recombinant murine and human IL-5 and supernatant from dog spleen cells stimulated the proliferation of both LyH7.B13 and human TF-1.28 cells. Supernatant from rat and rabbit spleen cells stimulated the proliferation of LyH7.B13 cells. Monkey serum samples and guinea pig lung tissue did not stimulate the proliferation of either LyH7.B13 or human TF-1.28 cells and were not tested any further.

Mepolizumab produced a concentration-dependent inhibition of recombinant human IL-5 induced proliferation (the IC_{50} was between 0.01 and 0.1 nM; see figure below). For samples from nonclinical test species (i.e., recombinant mouse IL-5 and supernatants from dog, rat, and rabbit spleen cells) that induced proliferation of either B-13 or TF-1.28 cells, addition of mepolizumab at concentrations of 1 or 10 nM failed to inhibit cell proliferation.

The mouse was judged to not be a pharmacologically relevant species for mepolizumab. Available data with spleen cell supernatants appeared to suggest that dog, rat, and rabbit were not pharmacologically relevant species, although this data might not be considered definitive. No conclusions regarding the pharmacological relevancy of guinea pig and Cynomolgus monkey could be drawn from this study.

Figure 5 Mepolizumab (SB240563) inhibition of human IL-5 induced cell proliferation

Figure 1 SB-240563 Inhibition of human IL-5 Induced Cell Proliferation



(Excerpted from the Sponsor's submission)

Table 3 Ability of Mepolizumab (SB240563) to inhibit exogenous IL-5-induced proliferation of LyH7.B13 and TF-1.28 cells (Recombinant IL-5 was only available for mouse and human. Supernatants from purified spleen cells, obtained from rats, beagle dogs, and rabbits, cultured in the presence of PHA, ConA, or ionomycin/PMA to stimulate cytokine release were used in other studies.)

Table 1 Species Specificity

Species	Tissue or cytokine	Stimulation conditions	Proliferation[1] (IL-5 Responsive Cell Line)	Antibody treatment[2] (SB-240563)	Notebook/ Pages
Human	Recombinant IL-5	NA	TF-1.28 – positive B13 – positive	Positive Positive	28042 pages 140-143, 154-157, 160-162
Guinea Pig	Lung	none	TF-1.28 – negative B13 – negative	Not tested Not tested	28242 pages 1-4, 16-17
Rat	Spleen	PHA Con A Ionomycin /PMA PHA Ionomycin/PMA	TF-1.28 – Negative Negative Negative B13 – positive positive [3]	Not tested Not tested Not tested Negative Not tested	28242 pages 20-24, 37-40, 44-46
Dog	Spleen	PHA Con A Ionomycin /PMA Ionomycin/PMA	TF-1.28 – positive positive positive B13 – positive[3]	Negative Negative Negative Not tested	28242 pages 56-61, 71-74, 132-134
Mouse	Recombinant IL-5	NA	TF-1.28 – Positive B-13 – Positive	Negative Negative	28042 pages 140-144, 154-157
Monkey	Serum	None	TF-1.28 – negative B13 – negative	Not tested Not tested	28242 pages 89-92
Rabbit	Spleen	PHA Con A Ionomycin /PMA PHA Con A	TF-1.28 – negative negative negative B13 – positive positive	Not tested Not tested Not tested Negative Negative	28242 pages 97, 99-100, 109-110, 132-134, 155-156, 180-182

1. negative = no proliferation; positive = proliferation
2. Negative = did not block proliferation; Positive = blocked proliferation
3. Positive in n=1 assay, not confirmed in subsequent assays

(Excerpted from the Sponsor's submission)

<u>Intravenous Pharmacokinetic and Pharmacodynamic Study in Rabbits</u> (Study Report: SB240563/RSD-100XMR/1): This study evaluated the pharmacokinetics, pharmacologic activity, and antigenicity of mepolizumab (Lot No. VJC-17622-286) in female New Zealand White rabbits (n=3) following an intravenous dose of 125 mg/kg. Animals were monitored for 1 month after receiving a single IV dose. Mepolizumab at an IV dose of 125 mg/kg had no effects on basal eosinophil levels or other hematologic parameters. A single dose of 125 mg/kg SB240563 was not antigenic in rabbits.

Two additional groups of 3 rabbits were used to evaluate the effects of mepolizumab on recombinant human (rh) IL-2-induced eosinophilia. These animals received IV doses of 0 (vehicle) or 250 mg/kg mepolizumab as well as 3 subcutaneous doses of 22 µg/kg rhIL-2 every-other day and were monitored for 1 week. Administration of rhIL-2 produced treatment-related changes of red cell mass and total and differential white cell counts that included a 7- to 10-fold increase of peripheral blood eosinophil counts. Treatment with mepolizumab at an IV dose of 250 mg/kg did not significantly alter hematologic parameters or antagonize the IL-2 induced eosinophilia.

These experiments demonstrated that the rabbit was not a pharmacologically relevant species with mepolizumab.

Activity of Human and Monkey Recombinant IL-5 on Monkey and Human Cell Proliferation (Study Report: CH2008/00044/00): The ability of mepolizumab (SB240563) to inhibit exogenous IL-5-induced proliferation of the murine IL-5/IL-3 dependent LyH7.B13 cell line and human IL-5 responsive TF-1.28 cell line was examined. In addition, the ability of mepolizumab to inhibit exogenous IL-5-induced differentiation of eosinophils, obtained from the bone marrow of healthy human volunteers or Cynomolgus monkeys, was examined.

Cells were treated with recombinant monkey or human IL-5. The EC_{50} values for both human and monkey IL-5, as measured by the proliferative responses of mouse LyH7.B13 and human TF-1.28 cells, were 0.7-6 pM. Both recombinant human and monkey IL-5 were equipotent in causing the differentiation of human and monkey eosinophils from cultures of bone marrow mononuclear cells with EC_{50} values of 13.3 pM. Mepolizumab inhibited the biological activity of recombinant human and monkey IL-5 in all assays and was equally effective against both cytokines with IC_{50} values of 70-116 pM.

It is noted that the amino acid sequence of monkey IL-5 differs from human IL-5 by two conservative substitutions in a region not related to the presumed mepolizumab binding epitope on hIL-5. Thus, it might be expected that mepolizumab would cross react between the two species and inhibit the biological activity of both human and Cynomolgus IL-5.

The Cynomolgus monkey was judged to be a pharmacologically relevant species for mepolizumab.

Table 4 Effect of rhIL-5 and mkIL-5 on the proliferation of IL-5 receptor-positive cells and eosinophil differentiation

Recombinant IL-5	Response of IL-5R Positive Cell Lines (EC_{50} pM)		Eosinophil Differentiation (EC_{50} pM)	
	TF-1 Cell Proliferation	B13 Cell Proliferation	Human Bone Marrow	Monkey Bone Marrow
Human	0.7	2.7	13.3	13.3
Monkey	0.7	6.0	NA*	13.3

*Raw data for this experiment could not be located at the time that the report was prepared by the Sponsor.
(Excerpted from the Sponsor's submission)

Table 5 Neutralization activity of Mepolizumab on proliferation of IL-5 receptor-positive cells and eosinophil differentiation

Recombinant IL-5	(IC_{50} pM)		(IC_{50} pM)	
	TF-1 Cell Proliferation	B13 Cell Proliferation	Human Bone Marrow	Monkey Bone Marrow
Human	75	83	70	104
Monkey	84	79	NA*	116

*Raw data for this experiment could not be located at the time that the report was prepared by the Sponsor.
(Excerpted from the Sponsor's submission)

Comparison of Different Recombinant Forms of Human IL-5 and their Neutralization by Mepolizumab (Study Report: CR2009/00004/00): The ability of mepolizumab to inhibit the biological activity of rhIL-5 generated from Drosophila, the Human Embryonic Kidney 293 (HEK293) cell line, and Chinese Hamster Ovary (CHO) cells was examined. The activity of rhIL-5 was assessed by examining IL-5-induced proliferation of the murine LyH7.B13 and human TF-1.28 cell lines.

The EC_{50} values for Drosophila (sF9), HEK 293 cells, and CHO cells expressed rhIL-5 for inducing proliferative responses were determined over a concentration range of 0.0001 to 100 ng/mL rhIL-5. The ED_{50} values for proliferation of the human (TF-1.28) receptor positive cell line were 0.07-0.3 ng/mL. The ED_{50} values for proliferation of the mouse (B13) IL-5 receptor positive cell line were 0.3-7 ng/mL. Mepolizumab inhibited the biologic activity of the three differently expressed rhIL-5 proteins (1 ng/mL rhIL-5) in both cellular assays with IC_{50} values ranging from 20-60 ng/mL. The glycosylation pattern of rhIL-5 did not affect the biological activity of the cytokine or the ability of mepolizumab to neutralize that activity.

Table 6 Neutralization activity of SB240563 on recombinant human IL-5-induced proliferation of IL-5 receptor-positive cells

rhIL-5 expression system	IC50 values (ng/mL)	
	TF-1.28	B13
Drosophila (sF9)	60	20
HEK293	20	30
CHO	20	40

(Excerpted from the Sponsor's submission)

Preliminary Pharmacokinetics of a Humanized Monoclonal Antibody Directed to Interleukin-5, SB240563, in Female Cynomolgus Monkeys Following a Bolus Intravenous Dose of 1 mg/kg (Study Report: SB240563/RSD-100LKP/2): Four female Cynomolgus monkeys received a 1 mg/kg intravenous dose of mepolizumab (SB240563) and approximately three months later a 1 mg/kg subcutaneous dose. Plasma concentrations of mepolizumab were determined from blood samples obtained up to 8 weeks postdose using an electrochemiluminescent (ECL) immunoassay with a lower limit of quantitation of 10.0 ng/mL. Eosinophil counts were also determined at baseline (prior to IV and SC dosing) and during the first 14 weeks following subcutaneous administration of SB240563.

Mepolizumab was completely bioavailable following subcutaneous administration. The observed mean terminal half-life (14.5 days) following SC administration was similar to that (13.1 days) obtained following IV administration.

Table 7 Mean (SD) pharmacokinetic parameters following IV and SC administration

Parameter	Intravenous	Subcutaneous
AUC (0-inf) (ug.h/mL)	6385.3 (1204.6)	7519.9 (1604.0)
Cmax (ug/mL)	27.7 (1.3)	11.4 (1.9)
Tmax (h)*	0.30 (0.1-1.00)	84.0 (48.0- 96.0)
T1/2 (days)	13.1 (1.5)	14.5 (3.8)
CL (mL/h/kg)	0.157 (0.018)	
Vss (mL/kg)	65.6 (5.0)	
F		1.18 (0.16)

* Median and range are reported.

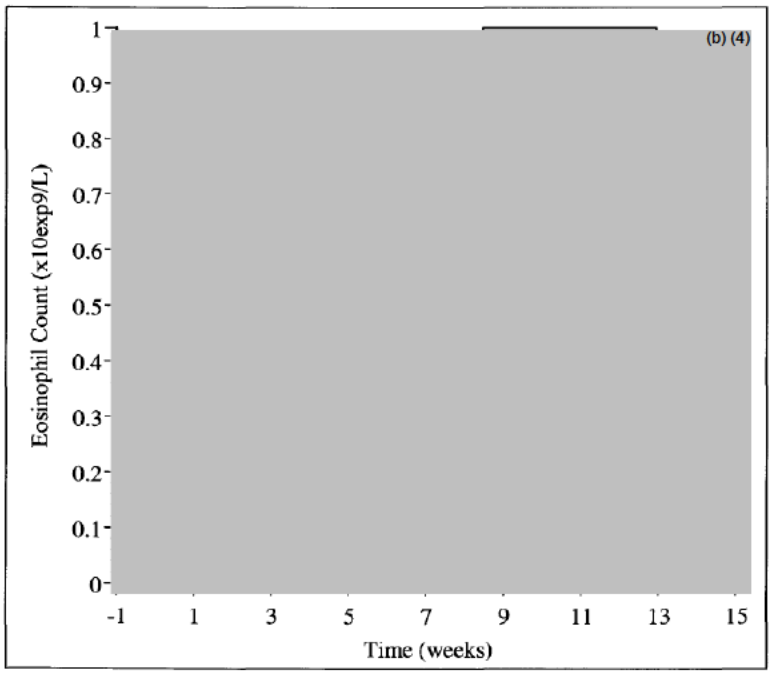
(Excerpted from the Sponsor's submission)

Mepolizumab reduced circulating eosinophil counts at an IV dose of 1 mg/kg. A time lag was noted between the plasma mepolizumab concentration-time profile and reduction of eosinophil counts. This observation was consistent with an indirect pharmacologic response. Following subcutaneous administration of mepolizumab, peripheral eosinophil counts decreased in a time dependent manner. The maximum percent decrease (81 to 96%) relative to baseline (just prior to administration of SC dose) was observed at three weeks postdose, while maximal concentrations were observed at 2 to 4 days postdose.

Figure 6 Eosinophil Counts versus Time following administration of Mepolizumab (SB240563)

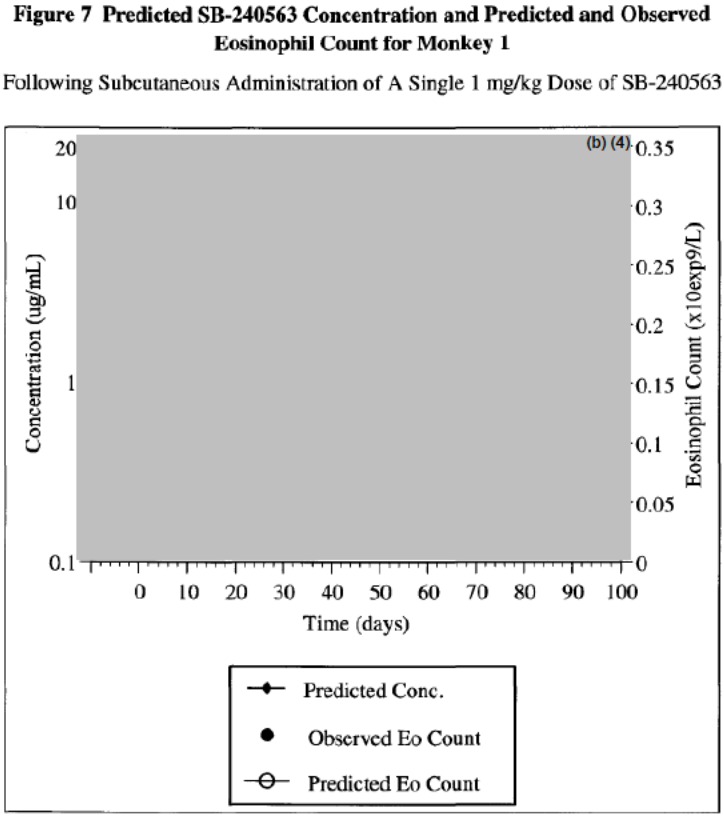
Figure 6 Eosinophil Count versus Time

Following Subcutaneous Administration of A Single 1 mg/kg Dose of SB-240563



(Excerpted from the Sponsor's submission)

Figure 7 Predicted Mepolizumab (SB240563) Concentration vs. Predicted and Observed Eosinophil Counts (Monkey 1)



(Excerpted from the Sponsor's submission)

Table 8 Predicted IC_{50} values of Mepolizumab (µg/mL)

Parameter	Mean (SD) (n=4)
Kin (count.10^9/L/day)	0.127 (0.088)
Kout (day^{-1})	0.326 (0.226)
IC50 (ug/mL)	1.43 (0.21)

(Excerpted from the Sponsor's submission)

Mepolizumab (SB240563): An Efficacy Study in a Model of Asthma in Cynomolgus Monkeys (Study Report: SB240563/RSD1013L8/1): The efficacy of mepolizumab was evaluated in an acute model of asthma in Cynomolgus monkeys. Eight male Cynomolgus monkeys were selected for this study on the basis of a positive bronchoconstrictor response to a specific dose of inhaled *Ascaris suum* antigen. The monkeys were randomly assigned to two groups and received either vehicle (placebo: 20 mM sodium phosphate, 6.2% sucrose, 0.2% Tween-80, pH 7.0) or 10 mg/kg mepolizumab (Batch No. U96257) by intravenous injection on Study Day 1. At 24 hr postdose and 3 and 6 weeks postdose, the monkeys received an aerosol challenge to

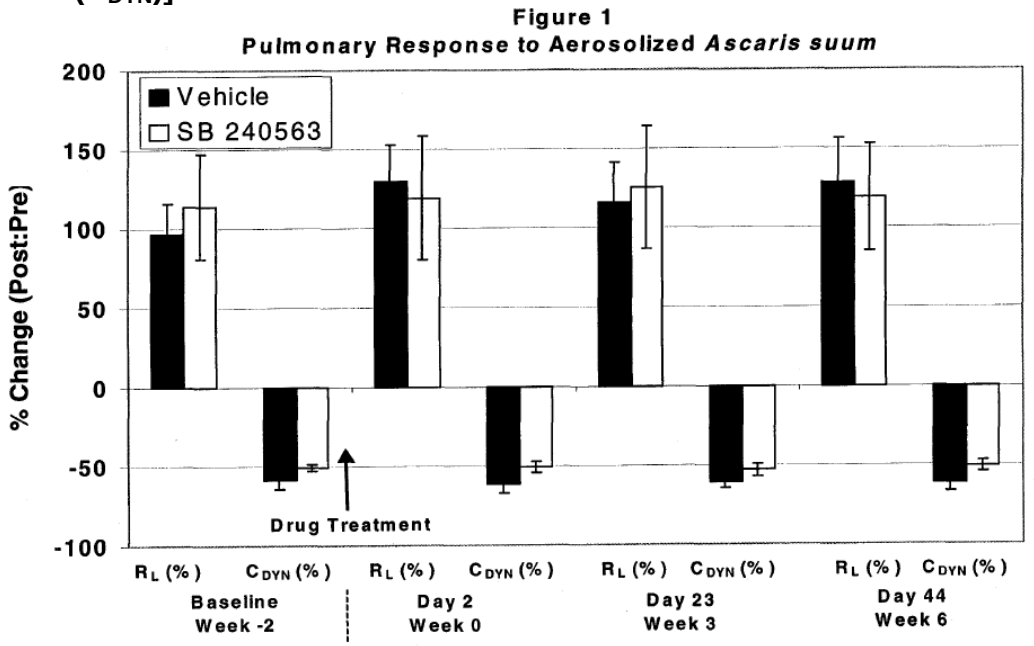
A. suum. Bronchoalveolar lavage (BAL) was performed and blood samples were collected prior to and at approximately 24 hr after each antigen challenge.

All animals exhibited a severe bronchoconstrictor response to *A. suum* challenge, which was associated with increases in lung resistance (R_L) and decreases in dynamic compliance (C_{DYN}). There was evidence of pulmonary eosinophilia by 24-hr postdose.

The vehicle had no effects on the bronchoconstrictor response or the pulmonary eosinophilia in response to *A. suum* challenge.

Mepolizumab at 10 mg/kg had no effect on the bronchoconstrictor response to *A. suum* challenge as measured by R_L and C_{DYN}.

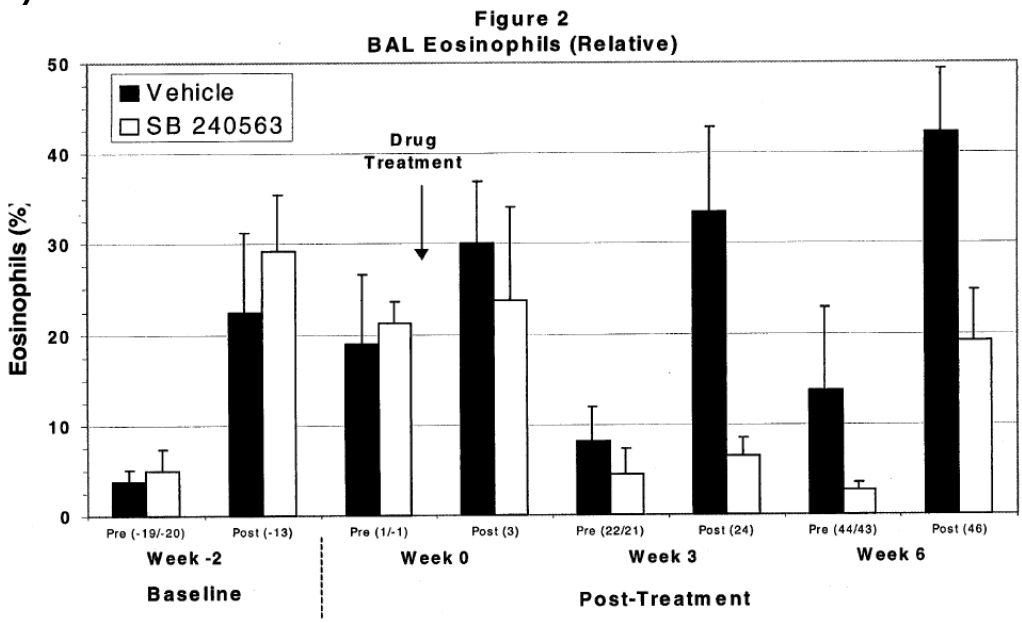
Figure 8 Pulmonary response [lung resistance (R_L) and decreases in dynamic compliance (C_{DYN})] to Aerosolized *Ascaris suum*



(Excerpted from the Sponsor's submission)

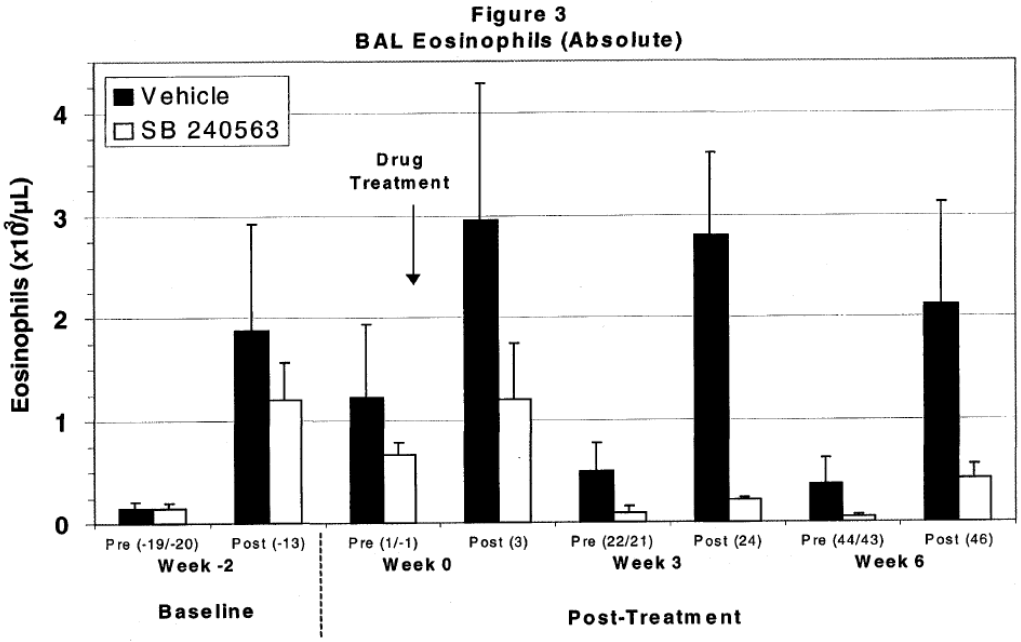
Mepolizumab at 10 mg/kg had no effects on pulmonary eosinophilia at 24-hr postdose; however, marked inhibition of pulmonary eosinophilia in response to *A. suum* challenge was observed at 3- and 6-weeks postdose.

Figure 9 Effect of Mepolizumab (SB240563) on levels of BAL eosinophils (Relative)



(Excerpted from the Sponsor's submission)

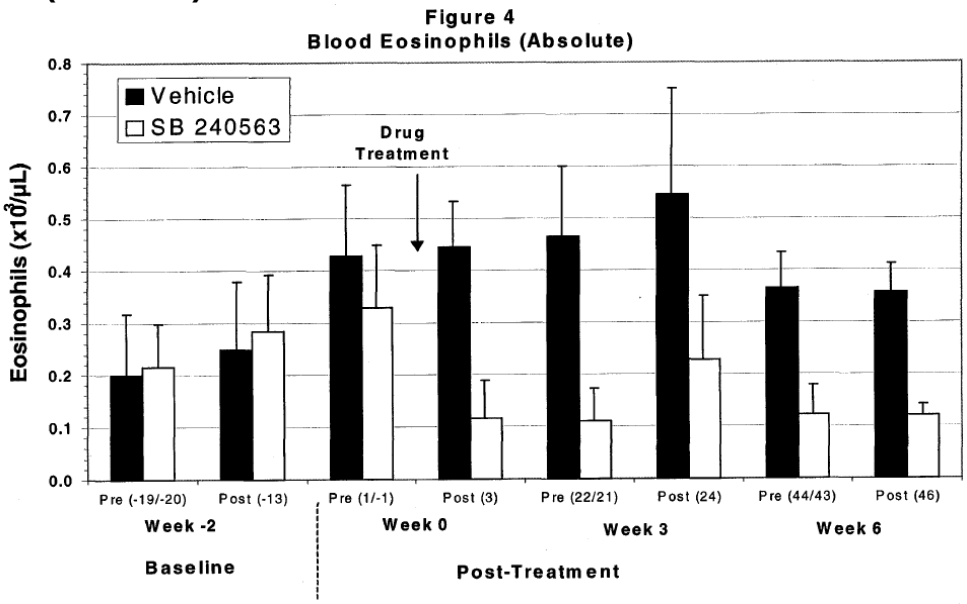
Figure 10 Effect of Mepolizumab (SB240563) on levels of BAL eosinophils (Absolute)



(Excerpted from the Sponsor's submission)

Mepolizumab at 10 mg/kg produced marked decreases in peripheral eosinophil counts beginning 24-hr postdose and continuing at 3 and 6 weeks postdose.

Figure 11 Effect of Mepolizumab on levels of peripheral blood levels of eosinophils (Absolute)



(Excerpted from the Sponsor's submission)

A Study to Evaluate the Efficacy of a Test Article in a Model of Asthma in Cynomolgus Monkeys (Study Report: CD2005/00631/00): The objective of this study was to determine the effects of the anti-IL-4 antibody (SB240683) alone or in combination with the anti-IL-5 antibody (SB240563) on pulmonary function and bronchoalveolar lavage cell counts in Cynomolgus monkeys sensitive to *Ascaris suum*-induced bronchoconstriction. A total of 12 animals (9 males and 3 females) were randomized into 3 groups of 4 animals each.

Sensitivity of animals to an aerosol challenge with *A. suum* was verified in independent sessions (#1 and #3) before and after the experimental session (#2). There was a separation of 3 weeks between the experimental session (#2) and session #3. The dose of A. suum was intended to elicit a >40% increase in lung resistance (RL) and a >35% decrease in dynamic compliance (C_{DYN}). Animals were anesthetized with a combination of ketamine (IM, 10 mg/kg) and propofol (IV, 3 mg/kg followed by 0.2 mg/kg/min as necessary) for challenges with *A. suum*.

On Day 1 of Session #2, Group 1 animals received the vehicle by IV administration, Group 2 animals received an IV dose of 10 mg/kg SB240683, and Group 3 animals received IV doses of 10 mg/kg SB240683 and 10 mg/kg mepolizumab. On Day 2 a single dose of *A. suum* antigen was administered by aerosol inhalation. Pulmonary function values (resistance [R_L] and compliance [C_{DYN}]) were monitored and recorded throughout the challenge periods. Bronchoalveolar lavage (BAL) samples for determination of BAL cell morphology and differential and blood samples for hematology and plasma for the Sponsor were collected on Days 1 and 3 (24 hr after challenge).

Treatment with either SB240683 alone (Group 2) or in combination with mepolizumab (Group 3) did not inhibit the acute bronchoconstrictor response to *A. suum* antigen based on the significant increases in lung resistance and decreases in dynamic compliance seen in all animals in response to challenge.

Challenge with *A. suum* led to a significant influx of eosinophils into the lungs (based upon the comparison of values at baseline to 24-hr after challenge). During Session #2, treatment with either SB240683 alone (Group 2) or in combination with mepolizumab (Group 3) had no effect on the influx of eosinophils.

During Session 3, the mean baseline eosinophil percentage for Group 1 at 36% was slightly higher than in previous sessions (7-18%). Following challenge, the eosinophil percentage increased to 61%, which was similar to results obtained in Session 2. Three weeks after treatment with SB 240683 alone (Group 2), no effects were seen on the eosinophil percentage. The mean baseline eosinophil percentage was at 15% and increased to 43% following challenge. Three weeks after treatment with the combination of SB240683 and SB240563 (Group 3), the baseline eosinophil percentage at 16% was lower than compared to Sessions 1 or 2. After challenge with *A. suum* antigen, a significant inhibition of the expected eosinophilia was observed as the eosinophil percentage was only at 25% compared to values at 46 to 47% in Sessions 1 and 2.

Table 9 Bronchoalveolar lavage: Total cell count and percent eosinophils

Group	Session #1				Session #2				Session #3			
	Baseline		24-hr		Baseline		24-hr		Baseline		24-hr	
	10^3/µL	%	10^3/µL	%	10^3/µL	%	10^3/µL	%	10^3/µL	%	10^3/µL	%
1	5.8	7	9.6	48	6.4	18	15.3	65	12.9	36	17.0	61
2	5.4	4	12.6	48	5.7	8	16.7	50	7.2	15	16.3	43
3	4.6	10	10.5	46	5.2	20	10.2	47	6.3	16	7.9	25

Circulating eosinophils were reduced following treatment with the combination of SB240683 and mepolizumab (Group 3) in Session 2. Reduced eosinophil counts were still evident 3 weeks later at baseline for Session 3 as well as 24-hr after challenge with *A. suum*.

Table 10 Circulating eosinophils: Absolute counts and percentages

Group	Session #1				Session #2				Session #3			
	Baseline		24-hr		Baseline		24-hr		Baseline		24-hr	
	10^3/L	%	10^3/L	%	10^3/L	%	10^3/L	%	10^3/L	%	10^3/L	%
1	0.270	3.8	0.430	5.5	0.345	5.8	0.553	8.5	0.660	7.8	0.530	7.0
2	0.455	5.3	0.803	10.3	0.315	5.3	0.648	9.3	0.335	5.3	0.583	6.8
3	0.205	2.5	0.485	6.5	0.363	5.0	0.148	1.8	0.168	2.3	0.098	1.3

In Session 1, challenge with *A. suum* increased levels of IL-6, IL-8, and RANTES. In Session #2, treatment with SB240683 in combination with mepolizumab (Group 3) produced a slight reduction of BAL IL-6 levels. Treatment with SB240683 (Group 2) or SB240683 in combination with mepolizumab (Group 3) reduced RANTES levels compared with the Group 1 vehicle-treated animals. There was no effect of treatment on IL-8 levels.

Table 11 Effects of SB240683 (Group 2) or SB240683 in combination with mepolizumab (Group 3) on IL-6, IL-8, and RANTES levels

Gr	IL-6, pg/mL						IL-8, pg/mL						RANTES, pg/mL					
	S #1		S #2		S #3		S #1		S #2		S #3		S #1		S #2		S #3	
	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3	D1	D3
1	1.97	2.83	2.98	4.44	13.48	5.41	10.01	16.7	10.4	17.8	18.7	43.1	2.6	15.0	5.3	29.5	12.8	21.9
2	0.41	1.83	0.44	2.72	0.99	2.42	2.9	12.4	3.5	9.7	7.1	9.7	0.6	13.5	1.0	13.5	2.1	8.9
3	2.73	15.16	1.20	3.48	3.97	17.63	20.6	48.1	11.3	21.3	18.0	54.1	4.7	13.6	10.1	15.0	4.5	13.7

The Effect on Antigen-Induced Airway Eosinophilia in Guinea Pigs by 2B6 Monoclonal Antibody Directed at Interleukin-5 (Study Report: CH2005/00956/00): The present study was conducted to determine if 2B6, an anti-IL5 monoclonal antibody, reduces bronchoconstriction or airway eosinophilia in guinea pigs following ovalbumin challenge. Guinea pigs were sensitized to chicken ovalbumin. Two hr prior to antigen challenge, animals were treated by the IP route with vehicle (PBS), 2B6 mAb (0.3 mg/kg), or H3 mAb control (0.3 mg/kg). Twenty min prior to antigen challenge, animals were treated with chlorpheniramine (0.1 mg/kg SC). Conscious guinea pigs were placed into a double flow body plethysmograph, ovalbumin aerosol (1% for 10 sec) was generated by an ultrasonic nebulizer and delivered at a rate of 250 mL/min, and

bronchoconstriction was measured. Animals were euthanized 24 hr later and lungs were lavaged to obtain cells for total and differential cell counts. The anti-IL5 mAb, 2B6, had no effect on the bronchoconstriction, measured as sGaw, induced by antigen challenge in conscious guinea pigs. 2B6 produced a slight but insignificant decrease in the lung eosinophilia seen 24 hr post-challenge, which was comparable to that obtained with the murine IgG1 control antibody H3. This data suggests that guinea pigs are not a pharmacologically relevant species with mepolizumab.

4.2 Secondary Pharmacology

No studies to assess the secondary pharmacology of mepolizumab were conducted.

4.3 Safety Pharmacology

Study title: General Pharmacological Study in Cynomolgus Monkeys.

Study no.:	RSD-100SHZ/1
Study report location:	EDR
Conducting laboratory and location:	Toxicology Department SmithKline Beecham Pharmaceuticals King of Prussia, PA
Date of study initiation:	October 6, 1997 (First day of treatment)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Mepolizumab (SB240563), batch number BAG-26765-18 SB240563 was supplied as a lyophile with a nominal concentration of (b) (4) mg/mL in a vehicle containing (b) (4) sodium phosphate, (b) (4) sucrose, and (b) (4) Tween 80 at pH 7.0.

Key Study Findings

● Potential cardiovascular, respiratory, and renal effects of mepolizumab (SB240563) were assessed in three male Cynomolgus monkeys that received intravenous doses of the vehicle (twice) and mepolizumab at 10 and 100 mg/kg. There was one week between each treatment.

● Mepolizumab at single intravenous doses up to 100 mg/kg had no effects on mean arterial pressure, heart rate, respiratory rate, urinary pH and water, osmolality, sodium, potassium, chloride, and creatinine excretion rates. There were no treatment-related effects on total urinary water, osmolality, sodium, potassium, chloride, and creatinine excretion.

Methods

Doses:	Each monkey was given vehicle (2 times), 10 and 100 mg/kg mepolizumab by the intravenous route at weekly intervals using a dose escalation design
Frequency of dosing:	Vehicle or drug was administered at weekly intervals using a dose escalation design
Route of administration:	Intravenous Monkeys were previously outfitted with a Vascular Access Port® into the femoral artery. Ports were maintained by bi-weekly flushing with heparinized saline (5 IU/mL) and locked with a 50% aqueous glucose/500 IU Heparin solution.
Dose volume:	Vehicle and mepolizumab were administered by the intravenous route using a dose volume of 4 mL/kg
Formulation/Vehicle:	(b) (4) sodium phosphate, (b) (4) sucrose, and (b) (4) Tween 80 at pH 7.0
Species/Strain:	Male Cynomolgus monkeys were obtained from (b) (4)
Number/Sex/Group:	3 male monkeys were used in this study
Age:	Monkeys ranged from 3 to 10 years old at the start of dosing
Weight:	Monkeys had a body weight range of 3 to 5 kg at the start of dosing
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Deviations were minor and did not impact the integrity of the study.

Methods: Monkeys were treated with vehicle or mepolizumab (SB240563) on successive weeks as shown in the table below. There were approximately 7 days between each treatment. Arterial pressure (systolic, diastolic, and mean) and heart rate were monitored continuously from 1 hr prior to dosing to 3 hr after dosing. Rectal temperatures were recorded at 1-hr interval from 1 hr prior to dosing up to 3 hr after dosing. Respiratory rate was observed from 1 hr prior to dosing to 3 hr after dosing. Urine was collected in two 30-min collection periods during the 60 min prior to dosing. Following dosing, urine was collected in 30-min urine collection periods for a total of 3 hr after dosing. Urine volume and pH were recorded at the time of collection. Urine samples were analyzed for electrolytes (sodium, potassium, and chloride), osmolality, and creatinine. Serum electrolytes and creatinine were measured in blood samples once prior to initiation of the baseline period and once after final urine collection.

Table 12 Design of safety pharmacology study in male Cynomolgus monkeys

Study Week	Treatment
1	Vehicle

2	Vehicle
3	10 mg/kg SB240563
4	100 mg/kg SB240563

Results: Mepolizumab at single intravenous doses up to 100 mg/kg had no effects on mean arterial pressure, heart rate, respiratory rate, urinary pH, and urinary water, osmolality, sodium, potassium, chloride, and creatinine excretion rates. There were no treatment-related effects on total urinary water, osmolality, sodium, potassium, chloride, and creatinine excretion.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

See review of 6-month toxicology study with monkeys (SB240563/RSD-100X0L/1) listed below

5.2 Toxicokinetics

See review of 6-month toxicology study with monkeys (SB240563/RSD-100X0L/1) listed below

6 General Toxicology

6.1 Single-Dose Toxicity

Single Intravenous Dose Toxicity Study in Monkeys (SB-240563/RSD-100FOG/1)

Methods: Mepolizumab (Lot VJC-17622-263; (b) (4) mg/mL) was administered as a single intravenous dose of 0 (vehicle), 3.0, or 304 mg/kg to Cynomolgus monkeys (1 monkey/sex/group). The vehicle control group and the 304 mg/kg group received an injection volume of 20 mL/kg as a short-term infusion (10 mL/min). The 3.0 mg/kg group received a bolus injection at a dosing volume of 0.2 mL/kg. Monkeys were monitored for one month at which time the animals were killed and a complete necropsy was performed. The following were evaluated: clinical observations, body weight, hematology, hemostasis, chemistry, urinalysis, leukocyte phenotyping, mitogen responses, anti-SB240563 antibodies, toxicokinetics, organ weights, and histology.

Results: The low number of animals per group rendered interpretation of the study difficult. The review was confined to an examination of eosinophil counts and toxicokinetics based upon a review of the 6-month toxicology study with Cynomolgus monkeys.

Eosinophil counts for the female in the 300 mg/kg group were decreased on days 8 and 28 to 13.6% of the control baseline (0.22×10^9/L). The eosinophil counts for the male in the 300 mg/kg group were low prior to the IV dose of mepolizumab and no further reduction was evident on days 8 or 28.

There were no marked differences in systemic exposure (C_{max} and AUC) between males and females based on the limited data available. AUC values increased in an approximate dose-proportional manner. The half-life had a mean value of 11.4 days (range, 9.9 to 12.4 days). The mean clearance was 0.213 mL/hr/kg and the mean V_{ss} was estimated to be 78.3 mL/kg, which is similar to blood volume in the monkey. No anti-SB 240563 antibodies were detected.

Table 13 Toxicokinetic parameters in monkeys that received a single IV dose of mepolizumab

	3.0 mg/kg		304 mg/kg	
	P96M-1305	P96F-1306	P96M-1307	P96F-1308
AUC(0-28 days) (mg·h/mL)	12.6	11.3	1101	1177
AUC(0-inf)* (mg·h/mL)	15.9 (21)	13.2 (14)	1359 (19)	1450 (19)
Cmax (mg/mL)	0.0935	0.0846	6.39	7.01
T½† (days)	12.4 [3]	9.94 [3]	11.6 [3]	11.8 [3]
CL (mL/h/kg)	0.189	0.227	0.224	0.210
Vss (mL/kg)	76.0	73.2	85.2	78.9

* Numbers in parentheses indicate the percent of the area extrapolated (t-inf).
† Numbers in brackets indicate the number of points used to determine T½.

(Excerpted from the Sponsor's submission)

6.2 Repeat-Dose Toxicity

Repeat Dose Intravenous Toxicity and Pharmacology Study in Cynomolgus Monkeys (SB-240563-RSD-100KN9/1)

Methods: Cynomolgus monkeys (2/sex/group) received mepolizumab (Lot VJC-I7622-274; (b) (4) mg/mL) by the intravenous route at doses of 0 (vehicle), 0.05, 0.5, or 50 mg/kg on days 1 and 29. Two control groups received the vehicle on days 1 and 29. The vehicle consisted of (b) (4) sodium phosphate, (b) (4) sucrose, and (b) (4) polysorbate 80 at pH 7.0. The dose volume was 2 mL/kg. Animals weighed approximately 2.2-3.9 kg and were approximately 2-4 years of age at the initiation of dosing.

To evaluate the pharmacology of mepolizumab, beginning on study Day 30, all mepolizumab-dosed monkeys and one control group received six subcutaneous doses of PROLEUKIN™ (rhIL-2; 22ug/kg/dose) given as one dose every other day (days 30, 32, 34, 36, 38, and 40). The second control group received 6 subcutaneous doses of the vehicle given as 1 dose every other day. Administration of recombinant human IL-2

(rhIL-2, PROLEUKIN®) to humans has been found to induce a significant eosinophilia that was attributed to increased IL-5 secretion by stimulated T lymphocytes.

All monkeys were monitored for 13 weeks after the second dose of mepolizumab. The following were evaluated: clinical observations, body weight, electrocardiography, ophthalmology, hematology, hemostasis, chemistry, urinalysis, leukocyte phenotyping, anti-SB240563 antibodies, and toxicokinetics. Animals were not killed.

Results: The low number of animals per group rendered interpretation of the study difficult. The review was confined to an examination of eosinophil counts and toxicokinetics based upon a review of the 6-month toxicology study with Cynomolgus monkeys.

Pharmacologic activity of mepolizumab in monkeys consisted of decreased circulating eosinophils (relative to controls or baseline values) and blockade of rhIL-2 (PROLEUKIN®)-induced eosinophilia (relative to monkeys that only received rhIL-2).

Administration of mepolizumab at doses of 5 and 50 mg/kg produced significant suppression (89-94%) of eosinophil counts by day 13 or 27. Following the second dose of mepolizumab on day 29, eosinophil counts in monkeys that received 5 or 50 mg/kg remained suppressed (82-88%) through day 77. Eosinophil counts by day 99 had returned to pre-drug values in most monkeys that received mepolizumab at 5 or 50 mg/kg. However, mean eosinophil counts for some monkeys that received 50 mg/kg remained decreased compared to baseline and concurrent control values.

On day 35, following administration of 3 doses of rhIL-2, the mean eosinophil counts for monkeys that received rhIL-2 only (Group 2) were increased in comparison to the concurrent control (Group1). The maximal response to rhIL-2 was observed on Day 41 after the 6th dose of rhIL-2; see Group 1 vs. Group 2.

A dose-dependent inhibition of rhIL-2 induced eosinophilia was observed in monkeys that received mepolizumab. There was a significant blockade of eosinophilia in monkeys dosed with mepolizumab at 0.5 mg/kg (85-87%) and 5 or 50 mg/kg (>95%). The mepolizumab dose of 0.05 mg/kg had no effects on rhIL-2 induced eosinophilia. No changes in the eosinophil cell surface activation marker, CD11b, were detected as a result of rhIL-2 or mepolizumab treatment.

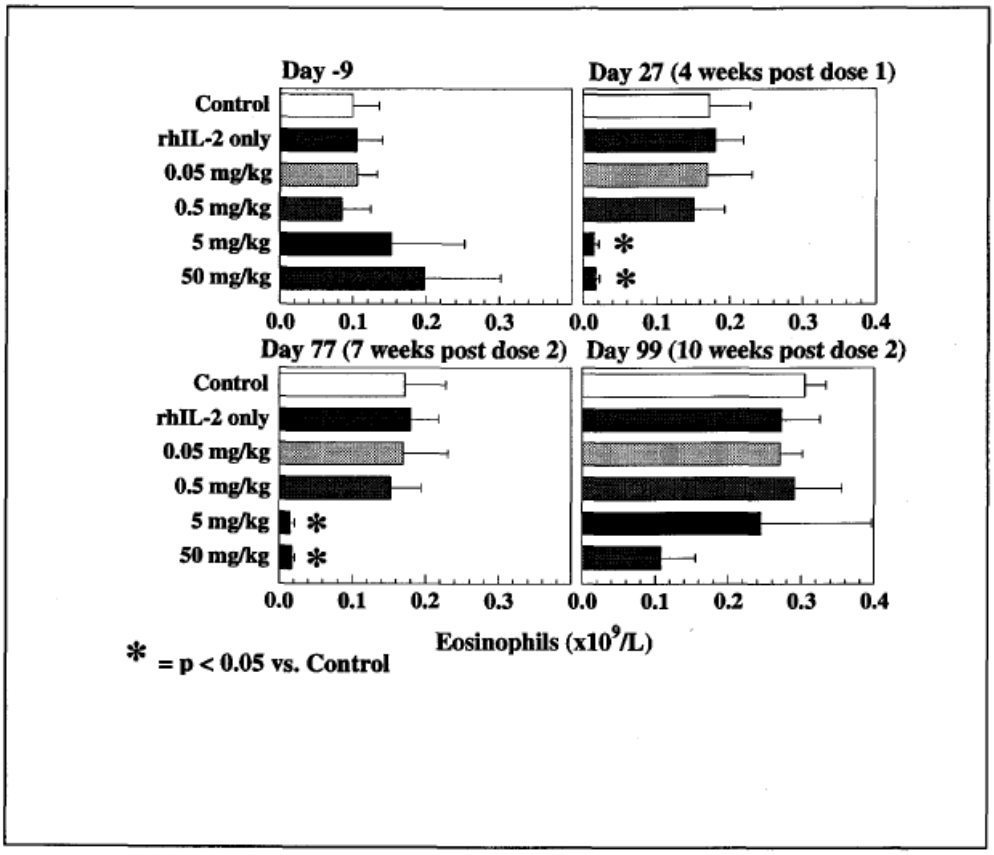
Table 14 Mean Absolute eosinophil counts (male and female combined), x 10^9/L

Group	Day -9	Day 13	Day 27	Day 35	Day 41	Day 56	Day 77	Day 99	Day 120
0 mg/kg-1	0.10	0.11	0.18	0.24	0.31	0.20	0.17	0.31	0.22
0 mg/kg-2	0.11	0.07	0.14	1.25	5.54	0.3	0.18	0.27	0.22
0.05 mg/kg	0.11	0.32	0.15	0.75	3.81	0.45	0.17	0.27	0.14
0.5 mg/kg	0.09	0.07	0.09	0.19	0.71	0.71	0.15	0.29	0.15
5 mg/kg	0.15	0.04	0.02	0.03	0.07	0.09	0.02	0.25	0.27

50 mg/kg	0.20	0.03	0.01	0.06	0.10	0.04	0.03	0.11	0.10

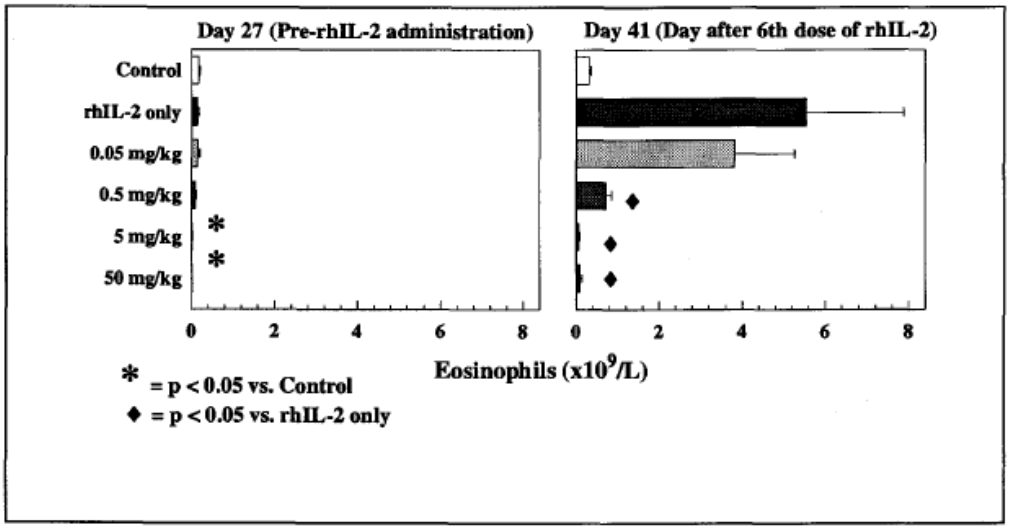
Note: Beginning on study Day 30, all mepolizumab-dosed monkeys and one control group (#2) received six subcutaneous doses of PROLEUKIN™ (rhIL-2; 22ug/kg/dose) given as one dose every other day (days 30, 32, 34, 36, 38, and 40). The second control group (#1) received 6 subcutaneous doses of the vehicle given as 1 dose every other day.

Figure 12 Reduction of circulating eosinophil counts in monkeys in monkeys treated with mepolizumab at IV doses of 0.05 to 50 mg/kg



(Excerpted from the Sponsor's submission)

Figure 13 Inhibition of recombinant human IL-2 (rhIL-2) - induced eosinophilia by mepolizumab



(Excerpted from the Sponsor's submission)

Mepolizumab was detectable in the plasma of drug-treated monkeys at all sampling time points. C_{max} and AUC values for mepolizumab in males and females increased in an approximate dose proportional manner. Exposures were slightly higher after the second dose suggesting some drug accumulation. Mean half-lives ranged from 8.97 to 13.9 days. Mean clearance values ranged from 0.156 to 0.219 mL/hr/kg and slightly decreased with increasing dose of mepolizumab. Volume of distribution values ranged from 65.7 to 74.9 mL/kg, which were slightly greater than the plasma volume in monkeys (45 mL/kg). There were no differences of toxicokinetic parameters between male and female monkeys.

An analysis of mepolizumab plasma concentration versus eosinophil counts found that concentrations ≥5 µg/mL were associated with decreases in eosinophil counts relative to controls and blocking of rhIL-2-induced eosinophilia. There was some evidence of a time delay to response as evidenced on day 13 where many but not all monkeys with plasma concentrations ≥5 µg/mL had decreases in eosinophil counts. However, by day 27, the plasma concentration/response was clearly evident. After the second dose of mepolizumab on day 29, eosinophil counts in most monkeys had returned to their respective pre-study levels by day 99. The effects of mepolizumab were reversible; however, there was a time lag that did not necessarily correlate to the declining plasma concentration of mepolizumab.

Figure 14 Toxicokinetic parameters in monkeys that received two IV doses of mepolizumab

		Cmax[1] (ug/mL)		AUC(0-inf)[2] (ug.h/mL)		T½[3] (days)	CL[4] (mL/h/kg)	Vss[5] (mL/kg)
Dose (mg/kg)	Mean (n=2)	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 1	Dose 1
0.05	males	1.21	1.01	233	234a	8.97	0.219	66.7
	females	1.22	1.26	269	292	10.4	0.187	65.7
0.5	males	13.0	14.8	3002	3421	12.6	0.171	69.5
	females	12.9	13.9	2980	3099	13.0	0.168	72.3
5	males	151	155	25173	32301	9.80	0.199	64.3
	females	123	137	30750	37251	13.9	0.163	75.2
50	males	1309	1518	321029	413618	13.4	0.156	70.0
	females	1131	1228	299902	363551	13.3	0.168	74.9

a indicates that n=1.
1 Cmax = maximum observed plasma concentration following each dose of SB 240563.
2 AUC(0-inf) = areas under the plasma concentration-time curve following each dose of SB 240563.
3 T½ = terminal phase half-lives of SB 240563 estimated using data obtained from Days 1 to 27.
4 CL = plasma clearance of SB 240563 estimated using data obtained from Days 1 to 27.
5 Vss = volume of distribution at steady-state estimated using data obtained from Days 1 to 27.
Note: T½, CL and Vss values were reported based on data obtained following the first dose of SB 240563 (Days 1-27) only, as the subsequent dose of drug had the potential to affect these pharmacokinetic parameters.

(Excerpted from the Sponsor's submission)

Study Title: 6-Month Toxicity Study in Cynomolgus Monkeys

Study no.:	SB240563/RSD-100X0L/1
Study report location:	EDR
Conducting laboratory and location:	Safety Assessment and Drug Metabolism and Pharmacokinetics (DMPK) SmithKline Beecham Pharmaceuticals King of Prussia, PA
Date of study initiation:	May 14, 1997 (First day of treatment)
GLP compliance:	Yes, except for the assay of concentrated bronchoalveolar lavage fluid
QA statement:	Yes
Drug, lot #, and % purity:	Mepolizumab (SB240563), batch number BAG-26765-18 was supplied as a lyophile containing (b) (4) mg SB240563/vial. Upon reconstitution with 2.0 mL Sterile Water for Injection (WFI), each vial contained (b) (4) SB240563/mL, (b) (4) sodium phosphate, (b) (4) sucrose, and (b) (4) Tween-80 at pH 7.0. Upon recertification of SB240563, batch number BAG-26765-18, each vial reconstituted with 2.0 mL of WFI was determined to contain (b) (4) mg SB240563/mL

Key Study Findings

● In a 6-month toxicology study, Cynomolgus monkeys received mepolizumab (SB240563) at a subcutaneous dose of 10 mg/kg or an intravenous dose of 10 or 100 mg/kg once every 4 weeks for a total of 7 doses (i.e., administration of mepolizumab on days 1, 29, 59, 85, 114, 141, and 169). Control animals received both an intravenous dose and a subcutaneous dose of the vehicle at each time point.

● Eosinophil counts were decreased for all male and female treatment groups from days 29 through 169. Neutrophil counts were increased for male treatment groups from days 29 through 169; however, dose-response relationships were not present or flat at all time points. Neutrophil counts were increased for females in the 100 mg/kg IV group on days 114, 141, and 169.

● Evaluation of bone marrow suggested a block of maturation and/or release of eosinophils from the bone marrow and not depletion by mepolizumab of eosinophil lineage cells.

● Histopathological examinations of organs and tissues did not identify any target organs of toxicity.

● Mepolizumab at IV doses up to 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks had no effects on male or female fertility based upon no adverse findings from histopathological examinations of male and female reproductive organs.

● C_{max} and AUC values for the 10 and 100 mg/kg IV groups were dose proportional. C_{max} and AUC values for the 10 mg/kg SC and 10 mg/kg IV groups were generally comparable suggesting high bioavailability following subcutaneous administration. SB240563 was detectable in the bronchoalveolar fluid from all treatment groups. Three control monkeys (M1044, M1046, and F1048) were detected with anti-SB240563 antibodies on day 169.

● The NOAEL was identified as the IV dose of 100 mg/kg q4 weeks or SC dose of 10 mg/kg q4 weeks based upon no treatment-related histopathological findings in any organs or tissues. Systemic exposure at 100 mg/kg q4 weeks was 808975 μg·hr/mL.

Methods

Doses:	Mepolizumab (SB240563) was administered at intravenous doses of 0 (Vehicle), 10, and 100 mg/kg or subcutaneous doses of 0 and 10 mg/kg every 4 weeks for a total of 7 doses (i.e., SB240563 was administered on days 1, 29, 59, 85, 114, 141, and 169). Control animals received both an intravenous dose and a subcutaneous dose of the vehicle at each time point.
Frequency of dosing:	Once every 4 weeks
Route of administration:	Intravenous or subcutaneous
Dose volume:	Reconstituted SB240563 ((b) (4) mg/mL stock solution) was administered to the 100 mg/kg intravenous and 10 mg/kg subcutaneous dose groups at dose volumes of 4.4 mL/kg or 0.44 mL/kg, respectively. The stock solution was diluted 10-fold with vehicle, to yield a concentration of (b) (4) mg/mL, for the 10 mg/kg intravenous dose group for the corresponding 4.4 mL/kg injection. Control monkeys received the vehicle (b) (4) sodium phosphate, (b) (4) Sucrose, (b) (4) Tween-80 in WFI, pH 7.0) by the intravenous and subcutaneous routes at dose volumes of 4.4 and 0.44 mL/kg, respectively.
Formulation/Vehicle:	(b) (4) sodium phosphate, (b) (4) Sucrose, (b) (4) Tween-80 in WFI, pH 7.0
Species/Strain:	Twelve male and twelve female Cynomolgus monkeys (*Macaca fascicularis*; (b) (4) were used in this study. Twenty-three of 24 monkeys in the present study were used in the previously reported repeat dose intravenous study (SB240563/RSD100KN9/1; SB240563 was administered on days 1 and 29). Monkeys were allowed a 6-month recovery period before use in the present study. These 23 monkeys along with one naïve monkey were assigned to the present study as indicated in the table above.
Number/Sex/Group:	3 monkeys/sex/group
Age:	4 to 8.5 years old on day 1 of treatment In response to Information request dated April 21, 2015, the Sponsor stated that the ages of monkeys, at the time of necropsy, were 3.9 to

	5.5 years old for females and 3.9 to 8.8 years old for males.
Weight:	Body weight ranges on day 1 of treatment were 2.56-4.96 kg for males and 2.36-3.18 kg for females
Satellite groups:	None
Unique study design:	Twenty-three of 24 monkeys in the present study were used in the previously reported repeat dose intravenous study (SB240563/ RSD100KN9/1; SB240563 was administered on days 1 and 29). Monkeys were allowed a 6-month recovery period before use in the present study. These 23 monkeys along with one naïve monkey were assigned to the present study.
Deviation from study protocol:	Deviations were minor and did not impact the integrity of the study.

Table 15 Design of the 6-month IV/SC toxicology study with monkeys

Group	SB 240563 IV Dose (mg/kg)	SB 240563 SC Dose (mg/kg)	Number of Animals	Animal Identification Numbers[2]	Previous Group Assignment[3]
1	0	0	3M/3F	P97M-1044 to 1046 P97F-1047 to 1049	Group 1- 2M/2F Group 2- 1M*/1F*
2	--	10	3M/3F	P97M-1050 to 1052 P97F-1053 to 1055	Group 2- 1M*/1F* Group 3- 2M/2F
3	10	--	3M/3F	P97M-1056 to 1058 P97F-1059 to 1061	Group 4- 2M/2F Group 5- 1M*/1F**
4	100	--	3M/3F	P97M-1062 to 1064 P97F-1065 to 1067	Group 5- 1M*/1F* Group 6- 2M/2F

* Selected randomly from animals previously assigned to Groups 2 or 5 (G96107).

** One female from study G96107 (Group 5) was considered unacceptable for assignment to this study because of intestinal entamoebiasis and a probable arterio-venous fistula, therefore an additional naïve monkey was selected to replace this animal.

(Excerpted from the Sponsor's submission)

Note: 23 of 24 monkeys in the present study were used in the previously reported repeat dose intravenous study (SB240563/RSD100KN9/1; SB240563 was administered on days 1 and 29). Monkeys were allowed a 6-month recovery period before use in the present study. These 23 monkeys along with one naïve monkey were assigned to the present study as indicated in the table above.

Observations and Results

Mortality: Monkeys were observed daily for viability.

There were no unscheduled/premature deaths in the study.

Clinical Signs: All monkeys were observed daily for clinical signs beginning approximately 42 days prior to the initiation of dosing and continuing until study termination. Detailed clinical examinations were performed prior to the start of treatment and on days 1, 29, 59, 85, 114, 141, and 169.

There were no treatment-related clinical signs.

Body Weights: Body weights were measured prior to the start of treatment and on days 1, 29, 59, 85, 114, 141, and 169.

There were no treatment-related effects on body weight gain.

Feed Consumption: Approximately 6 (female) or 8 (male) biscuits twice a day of Monkey Diet #5038 (b) (4)) and a daily allotment of fresh fruit was offered to each monkey.

Food consumption was not reported.

Ophthalmoscopy: Prior to the start of treatment and on day 167, ophthalmic examinations were conducted.

No treatment-related ophthalmic findings were observed.

ECG: Prior to the start of treatment and on day 164, leads I, II, III, aVR, aVL, aVF, mV1, mV2, and mV3 were monitored using a three-channel electrocardiograph.

There were no treatment-related effects on heart rate or ECG parameters.

Hematology: Blood samples for measurement of hematology parameters were collected from all monkeys prior to the start of treatment and on days 29, 59, 85, 114, 141, and 169. Blood samples for flow cytometric analysis of lymphocyte phenotype markers were collected prior to start of treatment and on days 85 and 169. Samples were analyzed for lymphocyte cell surface CD markers (CD2 - total T cells; CD4 - T helper cells; CD8 - T cytotoxic cells; CD-16 - NK cells; and CD20 - total B cells).

Eosinophil counts were decreased for all male and female treatment groups from days 29 through 169 as shown in the table and figure below.

Neutrophil counts were increased for male treatment groups from days 29 through 169; however, dose-response relationships were not present or flat at all time points. Neutrophil counts were increased for females in the 100 mg/kg IV group on days 114 and 169.

Lymphocyte counts were decreased for male and female treatment groups from days 29 through 169, although dose-response relationships were not present and any relation of observed differences to treatment was questionable.

Analysis of lymphocyte subsets (i.e., CD8+ T lymphocytes, CD4+ T lymphocytes, CD2+ T lymphocytes, CD20+ B lymphocytes, and CD16+ NK cells) on days 85 and 169 found no treatment-related changes of percentages or absolute counts.

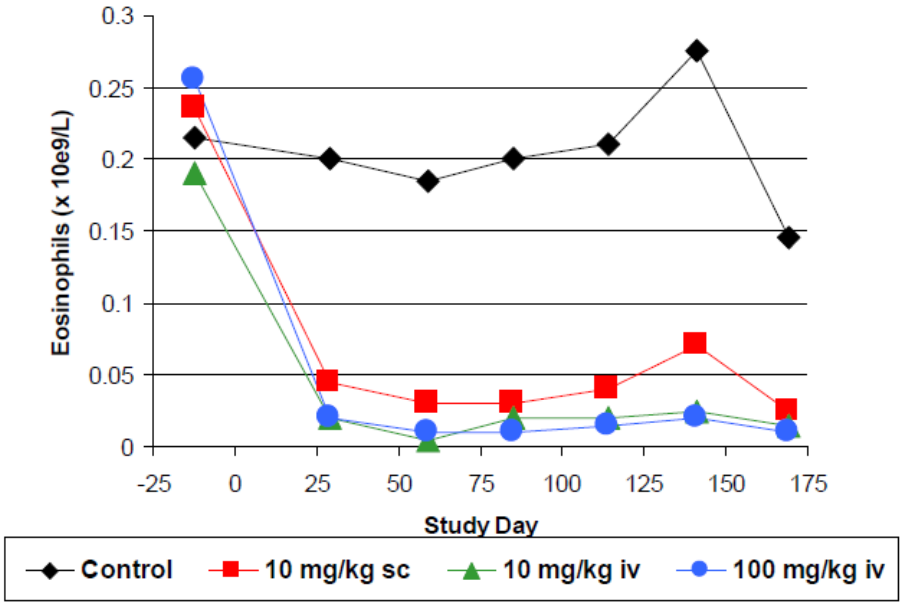
Decreased red blood cell counts, hemoglobin levels, and hematocrit were observed for 1 female in the high dose group; however, these differences from the control were small and evident prior to the start of treatment.

Table 16 Hematology parameters on days -12, 29, 59, 85, 114 141, and 169 (values in parentheses represent percent of control)

Parameter	Day	Males				Females			
		0	10-SC	10-IV	100-IV	0	10-SC	10-IV	100-IV
Eosinophils x 10^9/L	-12	0.26	0.16	0.12	0.38	0.17	0.31	0.26	0.13
	29	0.19	0.03 (16%)	0.01 (5%)	0.02 (11%)	0.21	0.06 (29%)	0.03 (14%)	0.02 (10%)
	59	0.21	0.02 (10%)	0.00 (0%)	0.01 (5%)	0.16	0.04 (25%)	0.01 (6%)	0.01 (6%)
	85	0.19	0.03 (16%)	0.01 (5%)	0.01 (5%)	0.21	0.03 (14%)	0.03 (14%)	0.01 (5%)
	114	0.24	0.05 (21%)	0.01 (4%)	0.01 (4%)	0.18	0.03 (17%)	0.03 (17%)	0.02 (11%)
	141	0.26	0.07 (27%)	0.03 (12%)	0.03 (12%)	0.29	0.07 (24%)	0.02 (7%)	0.02 (7%)
	169	0.17	0.03 (18%)	0.01 (6%)	0.01 (6%)	0.12	0.02 (17%)	0.02 (17%)	0.01 (8%)
Neutrophil counts x 10^9/L	-12	3.72	3.64	3.55	5.03 (135%)	3.90	2.23	2.47	4.20
	29	3.74	8.73 (233%)	8.11 (217%)	5.54 (148%)	7.61	3.98	6.68	8.60
	59	2.71	6.45 (238%)	6.20 (229%)	6.80 (251%)	7.12	4.40	4.80	7.93
	85	3.64	7.86 (216%)	8.53 (234%)	8.60 (236%)	6.31	5.31	4.37	6.46
	114	3.45	9.99 (290%)	9.16 (266%)	10.28 (298%)	3.91	4.82 (123%)	5.18 (133%)	10.09 (258%)
	141	3.59	7.12 (198%)	7.27 (203%)	9.15 (255%)	4.84	3.58	4.05	7.21
	169	3.72	6.98 (188%)	5.94 (160%)	5.38 (145%)	6.50	2.73	2.82	10.59 (163%)
Lymphocyte counts x 10^9/L	-12	10.77	8.50	7.50	10.01	6.30	5.85	7.74	5.93
	29	7.62	7.00 (92%)	4.29 (56%)	5.68 (75%)	5.94	4.67 (79%)	5.21 (88%)	4.64 (78%)
	59	7.29	6.28 (86%)	3.40 (47%)	5.24 (72%)	4.54	3.47 (76%)	4.15 (91%)	3.63 (80%)
	85	7.86	5.92 (75%)	3.87 (49%)	5.76 (73%)	5.54	3.82 (69%)	5.59 (101%)	4.91 (89%)
	114	9.28	7.02 (76%)	4.16 (45%)	4.99 (54%)	5.98	3.79 (63%)	5.85 (98%)	5.28 (88%)
	141	9.28	7.03 (76%)	4.97 (54%)	5.97 (64%)	6.43	3.98 (62%)	5.75 (89%)	4.32 (67%)

	169	7.74	6.13 (79%)	3.93 (51%)	5.94 (77%)	3.81	2.80 (74%)	4.81 (126%)	3.59 (94%)

Table 17 Effects of mepolizumab (SB240563) on circulating eosinophil counts



(Excerpted from the Sponsor's submission)

Bone marrow eosinophil evaluation: Bone marrow was collected at the time of necropsy. Eosinophilopoeisis was assessed in histologic sections of rib bone marrow stained with Luna's method. For each slide, ten high-power (500x) fields (HPF) were evaluated using an eyepiece reticle (1 cm x 1 cm). Fields were chosen based on cellularity and uniformity of staining quality. The total number of eosinophils in ten high-power fields were counted and classified as mature (segmented) or immature (blast-to-band forms).

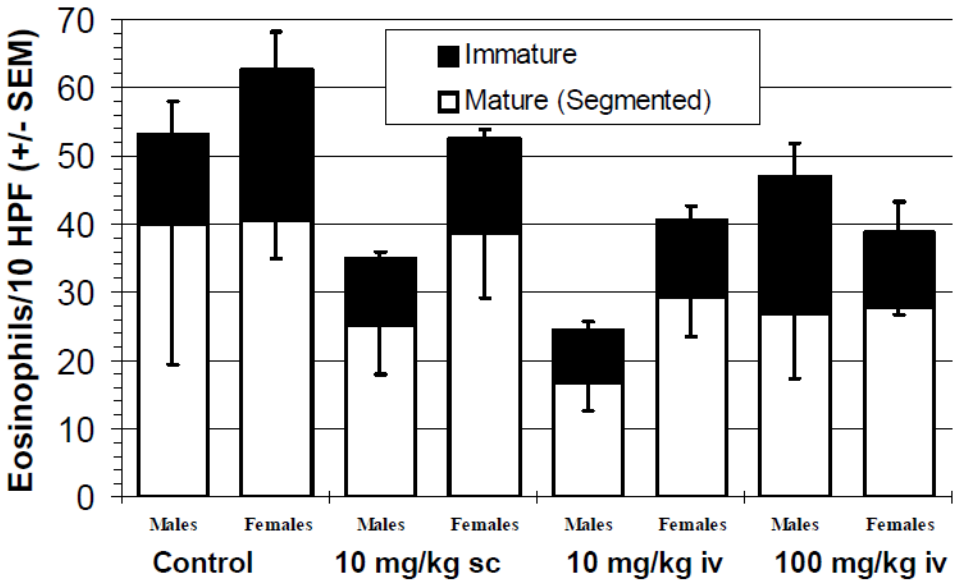
Considerable variability in the number of mature and immature eosinophils was observed in rib sections with the ranges in drug-treated monkeys overlapping that observed in control monkeys. However, there was evidence of a trend toward lower numbers of mature eosinophils. The data suggests a block of maturation and/or release of eosinophils from the bone marrow and not depletion of eosinophil lineage cells by mepolizumab.

Table 18 Effects of SB240563 on one marrow eosinophils (values in parentheses represent percent of control)

Parameter	Males				Females			
	0	10-SC	10-IV	100-IV	0	10-SC	10-IV	100-IV
Total #eosinophils/10 HPF	53.0	35.0 (66%)	24.3 (46%)	47.0 (89%)	62.7	52.3 (83%)	40.7 (65%)	38.7 (62%)
#Mature eosinophils/10 HPF	40.0	25.0 (63%)	16.7 (42%)	27.0 (68%)	40.7	38.7 (95%)	29.3 (72%)	27.7 (68%)
#Immature eosinophils/10 HPF	13.0	10.0 (77%)	7.7 (59%)	20.0 (154%)	22.0	13.7 (62%)	11.3 (51%)	11.0 (50%)

Figure 15 Effects of SB240563 on bone marrow eosinophils

Figure C. Bone Marrow Eosinophil Evaluation



(Excerpted from the Sponsor's submission)

Clinical Chemistry: Blood samples for measurements of clinical chemistry parameters were collected from all monkeys prior to the start of treatment and on days 29, 85, and 169.

Potassium concentrations were increased for males in the 10 and 100 mg/kg-IV groups on days 85 and 169. Glucose levels were increased for all male treatment groups and females in the high dose group on day 85. Aspartate aminotransferase (AST) activity was increased for females in the high dose group on day 169, although there were no corresponding histopathological findings in the liver. Serum IgG were slightly increased for males in the 10 and 100 mg/kg-IV groups, which might be attributed to administration of SB240563.

Table 19 Clinical chemistry parameters on days -12, 29, 85, and 169 (values in parentheses represent percent of control)

Parameter	Day	Males				Females			
		0	10-SC	10-IV	100-IV	0	10-SC	10-IV	100-IV
Potassium mmol/L	85	3.6	3.8	4.0 (111%)	4.5 (125%)	3.7	3.8	4.5	4.1
	169	3.9	4.2 (108%)	4.9 (126%)	4.9 (126%)	4.5	4.2	4.3	4.6
Glucose mmol/L	85	2.31	2.80 (121%)	2.60 (113%)	3.51 (152%)	2.49	2.39	2.78	3.19 (128%)
AST U/L	169	45	30	47	42	38	31	28	65 (171%)
IgG g/L	114[1]	9.84	9.84	11.25 (114%)	11.75 (119%)	9.51	8.48	9.52	9.07

1. Separate analysis on day 114.

Urinalysis: Urine samples for measurement of urinalysis parameters were collected from all monkeys prior to the start of treatment and on days 29, 85, and 169.

Urinary pH for males in the 100 mg/kg IV group on day 169 was 6.2 as compared to 9.0 for the control. There were no histopathological findings in the kidneys.

Gross Pathology: Monkeys were killed and necropsied on days 175 and 176 (i.e., 6 or 7 days after the last dose). Tissues were collected and preserved.

Not reported.

Organ Weights: Absolute and relative organ weights were measured for the adrenals, brain, heart, kidneys, liver, ovaries, testes with epididymides, and thymus.

Absolute and relative thymus weights were decreased for males in the 100 mg/kg IV group. These differences were primarily attributed to one male (M1062) in the 100 mg/kg-IV group.

Table 20 Absolute and relative organ weights (values in parentheses are % of control)

Organ	Males				Females			
	0	10-SC	10-IV	100-IV	0	10-SC	10-IV	100-IV
Thymus g	2.59	2.43	3.17	1.74 (67%)	2.76	2.72	3.32	3.33
Thymus %BW	0.076	0.070	0.091	0.041 (54%)	0.086	0.091	0.109	0.114

Histopathology:

Adequate Battery: Yes. Organ and tissue samples from all monkeys were examined by light microscopy. Eosinophilopoeisis was assessed in histologic sections of rib bone marrow stained with Luna's method.

Histopathological examinations of the cecum, rectum, tongue, tonsils, larynx, sternebrae, and lumbar vertebra were provided in a submission dated September 3, 2009.

Table 21 Histopathology inventory of tissues collected, weighed, and examined by light microscopy:

Study	6-month IV and SC study
Species	Cynomolgus monkey
Adrenals	X*
Aorta	X
Bone Marrow smear	
Bone (femur)	(not processed or examined)
Brain	X*
Cecum	XX
Cervix	(not processed or examined)
Colon	X
Duodenum	X
Epididymis	X* (w/testes)
Esophagus	
Eye	X (w/optic nerve-right)
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X*
Lachrymal gland	
Larynx	XX
Liver	X* (left and right lateral lobe)
Lungs	(right caudal lobe)
Lymph nodes, axillary	X
Lymph nodes, cervical	
Lymph nodes, inguinal	X
Lymph nodes mandibular	
Lymph nodes, mesenteric	X
Mammary Gland	X (females only)
Nasal cavity	
Optic nerves	X (w/eye-right)
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve	

Pharynx	
Pituitary	X
Prostate	X
Rectum	XX
Rib	X (CCJ and bone marrow)
Salivary gland	X (mandibular and parotid)
Sciatic nerve	X
Seminal vesicles	X
Skeletal muscle	X
Skin	X (Abdominal)
Spinal cord	X
Spleen	X
Sternebra	XX
Sternum	
Stomach	X
Testes	X* (w/epididymides)
Thymus	X*
Thyroid	X
Tongue	XX
Tonsils	XX
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Vertebra, lumbar	XX
Zymbal gland	

X, histopathology performed
XX, Results provided in a submission dated September 3, 2009
*, organ weight obtained

Peer Review: Yes. The Peer Review Pathologist reviewed all tissue sections from the following monkeys.

Group 1: 1044, 1048
Group 2: 1052, 1054
Group 3: 1056, 1059
Group 4: 1062, 1066

Histological Findings:
Histopathological examinations of organs and tissues did not identify any target organs of toxicity. Several animals from control and treatment groups had evidence of parasitic infection, which may have been responsible for the findings listed in the table below.

Table 22 Histopathological findings from monkeys treated with the vehicle, 10 mg/kg SC, 10 mg/kg IV, or 100 mg/kg IV q4 weeks for a total of 7 doses

Organ/Tissue	Sex	0 IV	10 SC	10 IV	100 IV
Liver					
-granuloma	M	0/3	0/3	0/3	1/3
	F	0/3	0/3	0/3	1/3
Colon					

-granuloma, parasitic	M F	0/3 0/3	0/3 0/3	0/3 0/3	1/3 0/3
Stomach -fibrosis, serosal, focal	M F	0/3 0/3	0/3 0/3	0/3 0/3	1/3 0/3
Spleen -infiltrate, foamy macrophages, focal	M F	0/3 0/3	0/3 0/3	0/3 0/3	0/3 1/3
Injection site, IV -fibrin, subendothelial, focal	M F	0/3 0/3	- -	0/3 0/3	0/3 1/3
Thyroid -cyst	M F	0/3 0/3	1/3 0/3	1/3 1/3	1/3 1/3
Brain -mineralization	M F	0/3 0/3	0/3 0/3	0/3 0/3	0/3 1/3

Assessment for Potential Effects on Male and Female Fertility: In accordance with Section 5.2 Fertility in ICH S6 (R1), a review of the age, terminal body weight, weight of selected reproductive organs (paired testes with epididymides, paired ovaries), and histopathologic assessment of the male and female reproductive tracts determined that all the monkeys, with the exception of 1 male in the control group, would be considered sexually mature during the majority of the time they were exposed to mepolizumab on the 6 month study [Reference G97054 in Table 1]. The one male in the control group had the lowest body weight, lowest testes plus epididymides weight, and a histologic diagnosis of immature testes. For the male monkeys, the endpoints assessed were consistent with the animals being sexually mature (Smedley et al., Contemporary Topics 41:18-20, 2002). While menstruation was not assessed in female monkeys, the age and body weight were consistent with the animals being sexually mature (Luetjens and Weinbauer, Toxicologist 132:448, 2013).

Mepolizumab at IV doses up to 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks had no effects on male or female fertility based upon no adverse findings from histopathological examinations of male and female reproductive organs.

Table 23 Comparison of monkey age, body weight, reproductive organ weight, and histopathology from toxicology studies with Mepolizumab

Animal Tatoo	Sex	Date of Birth	G96107 Dose (mg/kg)	Animal ID	G97054 Dose (mg/kg)	Animal ID	Age at Necropsy	Term BW	Thymus	Testis + Epi (paired) Weight	Testis	Prostate	Ovary (paired) Weight	Ovary	Other
(b) (4)															

Animal Tatoo	Sex	Date of Birth	G96107 Dose (mg/kg)	Animal ID	G97054 Dose (mg/kg)	Animal ID	Age at Necropsy	Term BW	Thymus	Testis + Epi (paired) Weight	Testis	Prostate	Ovary (paired) Weight	Ovary	Other
(b) (4)															

Key:
NAD = no abnormality detected
*Female 2359 was considered unacceptable for assignment to Study G97054 because of intestinal entamoebiasis and probable arterio-venous fistula. Findings were not considered related to prior last dose of mepolizumab approximately 10 months prior to start of 6 month study.

(The Sponsor provided the table in a submission dated April 30, 2015, as a response to an Information Request dated April 21, 2015)

Special Evaluation: Bronchoalveolar lavage samples from all monkeys were collected and evaluated prior to the start of treatment and on day 104. Lavage samples were centrifuged and the supernatant was collected and stored at -80°C. Cell pellets were

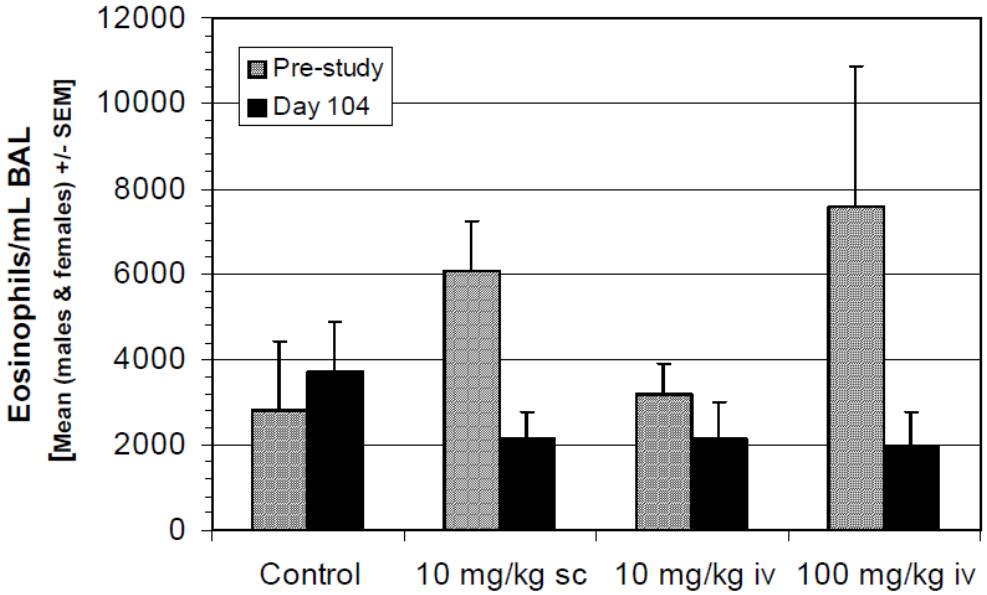
resuspended and aliquots were processed for total and differential cell counts. Samples of lavage fluid were analyzed for total protein, total IgG, and SB240563 concentrations.

Total BAL fluid cell counts were decreased for female treatment groups in a dose-related manner. BAL fluid eosinophil counts were decreased for male and female treatment groups, although dose-response relationships were not present. BAL fluid neutrophil cell counts were increased for males in the high dose group and all female treatment groups. BAL fluid macrophage counts were increased for all female treatment groups.

Table 24 Effects of SB240563 on bronchoalveolar lavage fluid cell counts (values in parentheses represent percent of control)

Parameter	Males				Females			
	0	10-SC	10-IV	100-IV	0	10-SC	10-IV	100-IV
Total cells x 10^3/mL	123.6	107.6	170.1	134.0	165.6	152.7 (92%)	133.3 (81%)	124.9 (75%)
Eosinophils x 10^3/mL	4.3	2.9 (67%)	3.2 (74%)	2.3 (54%)	3.1	1.3 (42%)	1.1 (36%)	1.6 (52%)
Neutrophils x 10^3/mL	1.4	1.5	0.9	2.6 (186%)	1.3	2.6 (200%)	1.9 (146%)	4.0 (308%)
Macrophages x 10^3/mL	101.9	80.7	134.3	100.3	43.90	133.4 (304%)	110.9 (253%)	106.4 (242%)

Figure 16 Effects of SB240563 on BAL eosinophils



(Excerpted from the Sponsor's submission)

Analysis of BAL fluid found that IgG concentrations on day 104 for males in the 10 and 100 mg/kg-IV groups were increased to 128.4 and 168.8% of the control (10.9 µg/mL;

BAL fluid was concentrated 5-fold). The significance of this difference from the control is not known.

Toxicokinetics: Plasma and bronchoalveolar lavage fluid samples were analyzed for SB240563. Serum samples were analyzed for anti-SB240563 antibodies.

Blood samples for measurement of SB240563 were collected from drug-treated animals prior to dosing on day 1 and then monthly prior to each of the 7 dose administrations of SB240563. Additional blood samples were collected at 24 and 168 hr following the first and fifth doses of SB240563. Samples of bronchoalveolar lavage (BAL) fluid were also analyzed for SB240563. Preliminary analysis indicated that SB240563 concentrations in BAL fluid for the 10 mg/kg SC and 10 mg/kg IV groups were near the limit of quantification. Aliquots of 5-fold concentrated BAL supernatant were prepared and analyzed. Both plasma and BAL fluid samples were analyzed for SB240563 using an electrochemiluminescent immunoassay. The lower limit of quantification was 50 ng/mL in both plasma and BAL fluid using 50 µL of a 10-fold diluted sample.

Blood samples for measurement of anti-SB240563 antibodies were collected from all monkeys prior to start of treatment and on days 29, 59, 85, 114, 141, and 169. Serum samples were processed and then treated with both TAG (ruthenium)-SB 240563 and biotin-SB 240563. Streptavidin-coated paramagnetic microbeads were added to samples. ORIGEN® assay buffer was added to stop the reaction and the electrochemiluminescent (ECL) response was recorded by the ORIGEN® Analyzer.

Two monkeys (P97F-1065 and P97F-1066) in the 100 mg/kg IV group had quantifiable baseline plasma concentrations of SB240563 before receiving the first dose of study drug. These residual concentrations were from the previous treatment with SB240563 that these monkeys received as part of study SB240563/RSD100KN9/1 approximately 9 months prior to the start of the present study. The residual concentrations detected were generally less than 4-fold greater than the assay lower limit of quantitation and were estimated to contribute less than 0.01% to total AUC values calculated, so no correction to the baseline was deemed necessary during subsequent pharmacokinetic evaluations.

Plasma concentrations of SB240563 increased with each successive dose. C_{max} and AUC values for all three treatment groups were greater after the 5th dose as compared to the 1st dose suggesting accumulation of SB240563. C_{max} and AUC values for the 10 and 100 mg/kg IV groups were dose proportional. C_{max} and AUC values for the 10 mg/kg SC and 10 mg/kg IV groups were generally comparable suggesting high bioavailability following subcutaneous administration. C_{max} and AUC values were generally slightly greater for male monkeys as compared to female monkeys. In general, trough concentrations reached a plateau after the 4th dose for most animals.

Table 25 Toxicokinetic parameters in monkeys that received IV doses of 10 or 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks

Group Assignment	Sex (n=3)	Observed Mean Cmax* [ug/mL] (S.D.)		Mean AUC(0-4wk) [ug·h/mL] (S.D.)	
		Dose 1	Dose 5	Dose 1	Dose 5
2 (10 mg/kg, sc)	M	112 (27)	139 (36)	44908 (8162)	63130 (13275)
3 (10 mg/kg, iv)	M	179 (12)	264 (21)	50156 (1894)	75812 (3614)
4 (100 mg/kg, iv)	M	2008 (521)	2670 (660)	605949 (94062)	883071 (175089)
2 (10 mg/kg, sc)	F	105 (9)	145 (18)	43522 (2967)	57995 (2844)
3 (10 mg/kg, iv)	F	157 (1)	221 (12)	43683 (3170)	59327 (8217)
4 (100 mg/kg, iv)	F	1731 (57)	2194 (160)	539203 (34725)	734879 (67633)

* Cmax values were observed at the first sampling time (approximately 24 hours post-dosing) and may not represent the true maximum concentrations achieved.

(Excerpted from the Sponsor's submission)

SB240563 was detectable in the bronchoalveolar fluid from all treatment groups after concentrating it 5-fold. Concentrations in the 10 mg/kg SC and 10 mg/kg IV groups were generally comparable and concentrations in the 100 mg/kg IV group were approximately 10-fold higher. It appeared that SB240563 was transferred from the blood to alveoli along with other IgG (i.e., there was no specialized transport).

Table 26 Concentrations of SB240563 in the BAL fluid collected on day 104

Appendix 50 SB 240563 in BAL Fluid

Group	Animal	Predose	Day 104 (ug/mL)	Day 104 5x concentrated (ug/mL)
Group 1	1044M	NQ	NQ	NQ
	1045M	NQ	NQ	NQ
	1046M	NQ	NQ	NQ
	1047F	NQ	NQ	NQ
	1048F	NQ	NQ	NQ
	1049F	NQ	NQ	NQ
Group 2	1050M	NQ	0.0880	0.1831
	1051M	NQ	0.0624	0.0675
	1052M	NQ	NQ	0.0795
	1053F	NQ	NQ	0.1195
	1054F	NQ	0.0508	0.0697
	1055F	NQ	0.0706	0.2196
Group 3	1056M	NQ	NQ	0.0760
	1057M	NQ	NQ	0.1029
	1058M	NQ	NQ	0.0583
	1059F	NQ	NQ	0.0747
	1060F	NQ	NQ	0.0959
	1061F	NQ	NQ	0.0885
Group 4	1062M	NQ	0.2047	0.7387
	1063M	NQ	0.3096	1.2071
	1064M	NQ	0.2568	1.0621
	1065F	NQ	0.4322	1.4798
	1066F	NQ	0.3500	1.4869
	1067F	NQ	0.2386	1.0578

NQ = Not Quantifiable.

(Excerpted from the Sponsor's submission)

No anti-SB240563 antibodies were detected in treated monkeys throughout the course of the treatment period. However, three control monkeys (M1044, M1046, and F1048) had detectable anti-SB240563 antibodies on day 169, with concentrations ranging from 47 to 209 ng/mL.

Dosing Formulation Analysis: SB240563 dosing solutions were prepared on the 7 respective days of dosing (Days 1, 29, 59, 85, 114, 141 and 169). Samples of the SB240563 dosing solutions used on Days 1 and 169 were assayed for SB240563 concentrations by the Department of Pharmaceutical Technologies, SmithKline Beecham Pharmaceuticals.

Dosing solutions were 96-106% of nominal concentrations.

7 Genetic Toxicology

Not applicable for a therapeutic biological protein produced by recombinant DNA technology.

8 Carcinogenicity

The carcinogenic potential of mepolizumab and the need for a carcinogenicity study were discussed with Drs. David Jacobson-Kram, Abby Jacobs, and Paul Brown of the Executive Carcinogenicity Assessment Committee in emails dated May 22 and 23, 2008.

Principles issues of the discussion were as follows:

1. In a 6-month toxicology study with Cynomolgus monkeys that received mepolizumab by the intravenous or subcutaneous route, there was no evidence of pre-neoplastic or neoplastic lesions.

2. The species specificity of mepolizumab was restricted to human and nonhuman primate IL-5 only. Mepolizumab did not bind to mouse or rat IL-5. Thus, a rodent carcinogenicity study with mepolizumab did not appear to be technically feasible.

3. SB264091 (5D3), a rat anti-human IL-5 monoclonal antibody (lgG2b), was available for study. SB264091 was determined to bind to murine and human IL-5. SB264091 was administered to CD-1 mice at IV doses up to 50 mg/kg/week to assess potential effects on male and female fertility and early embryonic and embryofetal development (See review of Study number SB240563/RSD-100P8V1 below). Male and female CD-1 mice received a total of 5 or 6 weekly doses. A few deaths were observed in the study that the Sponsor attributed to anaphylactoid reactions, although no specific investigations were conducted to confirm causes of death. The Sponsor contended that conducting a 2-year carcinogenicity study with SB264091 was not feasible due to formation of anti-SB264091 antibodies.

Per the ICH S6 (R1) Guidance (finalized in 2011), a study with a murine surrogate anti-IL-5 antibody was not required. Results of the 6-month toxicology study with monkeys do not suggest the need for such a study.

4. IL-5 knockout mice are reported to be viable and generally healthy. The IL-5 knockout mouse has not been systemically studied for the development of tumors (animals have not been followed on a lifetime basis to assess development of tumors versus wild-type mice). Further, the relevance of this model was questioned given that these animals still possess high levels of eosinophils although their functionally is altered compared to the wild-type mice. In contrast, monkeys and humans treated with mepolizumab shown significant reductions of eosinophil counts.

5. Evaluation of the published scientific literature regarding the roles for IL-5 and eosinophils in cancer.

Although recent publications have proposed roles for IL-5 and eosinophils in immune tumor surveillance; conversely, other references have reported a tumor-supporting role for eosinophils. Further, eosinophils themselves can express transforming growth factor-α (TGF-α) and potentially contribute to tumor-associated pathological responses.

- Innate non-T IL-5–producing cells localized most abundantly in the lung and proliferated and upregulated IL-5 production in response to IL-25 and IL-33. IL-33 was more effective than IL-25. These cells contribute to maintaining sufficient numbers of lung eosinophils and are important for eosinophil recruitment mediated by IL-25 and IL-33. Given that eosinophils are shown to possess antitumor activity, lung tumor metastasis was studied in IL-5/Venus KI mice and it was found that innate IL-5-producing cells were increased in response to tumor invasion, and their regulation of eosinophils was critical to suppress tumor metastasis. Genetic blockade or neutralization of IL-5 impaired eosinophil recruitment into the lung and resulted in increased tumor metastasis. Conversely, exogenous IL-5 treatment resulted in suppressed tumor metastasis and augmented eosinophil infiltration. These results suggested that innate IL-5–producing cells play a role in tumor surveillance through lung eosinophils and may contribute to development of novel immunotherapies for cancer (The Journal of Immunology 188: 703–713, 2012).

- Two recently-published papers have proposed roles for IL-5 and eosinophils in immune tumor surveillance (Journal of Immunology 160: 345-350, 1998; Journal of Leukocyte Biology 79: 1131-1139, 2006). These publications suggest that eosinophils are part of an early inflammatory reaction at the site of tumorigenesis and, when recruited into tumors, can very effectively eradicate transplantable tumors. It was also found that methylcholanthrene (MCA)-induced tumor incidence and growth were significantly attenuated in IL-5 transgenic mice (over-expression of IL-5) of both the BALB/c strain and C57BL/6 background. Histological examination revealed that the protective effect of IL-5 was associated with massively enhanced numbers of eosinophils within and surrounding tumors.

- Conversely, it was reported that the plasmacytoma, J558L, and the mammary adenocarcinoma, TS/A, were transfected with an expression vector encoding the mouse gene for IL-5. Injection of parietal cells, mock transfectants, and IL-5 producing cells into syngeneic mice showed that local IL-5 secretion indeed induced rapid tumor infiltration by eosinophils as evidenced by immunohistochemical staining, but did not alter the tumor growth kinetics of IL-5 transfectants. It was concluded that the presence of both IL-5 and eosinophils did not suppress tumor growth (European Journal of Immunology 23: 992-995; 1993).

- In another study, MCA205 cells were transfected with the IL-5 gene, resulting in MCA205-IL5. IL-$5^{-/-}$ and wild-type mice were injected with 2.5 x 10^5 cells/mouse of the bulk culture. Analysis of the tumor growth showed no change in the

tumorigenicity between parental and IL-5 transduced tumor cells in either wild-type or IL-$5^{-/-}$ mice (Journal of Immunology 160: 345-350, 1998).

- There are no known reports of IL-5 deficient mice developing an increased rate or risk of neoplasia as compared to wild-type control mice (b) (4)); however, there is no publication reporting that IL-5 knockout mice have been systemically studied for the development of tumors (i.e., knockout mice have not been followed on a lifetime basis to assess development of tumors versus wild-type mice). Mice deficient in IL-5, although lacking in eosinophilia, exhibit normal antibody and cytotoxic T-cell responses, indicative that if anti-tumor CTL responses are needed, they would likely be functional even in the absence of systemic IL-5 (Immunity 4: 15-24, 1996).

- It has been reported that host-derived IL-5 promoted malignant pleural effusions (MPE) in C57BL/6 mice following intraperitoneal injections of Lewis lung cancer (LLC) or colon (MC38) adenocarcinoma cells using wild-type (IL-$5^{+/+}$) and IL-5-deficient (IL-$5^{-/-}$) mice. Exogenous IL-5 promoted MPE formation in both IL-$5^{+/+}$ and IL-$5^{-/-}$ mice while anti-IL-5 antibody treatment limited experimental MPE in IL-$5^{+/+}$ mice (Stathopoulos et al., American Journal of Respiratory and Critical Care Medicine 2010).

- Studies have reported both favorable and unfavorable progress for patients with tumors exhibiting tumor-associated tissue eosinophilia (TATE). In attempting to elucidate the potential role of eosinophils in squamous cell carcinoma development, a carcinogen (7,12-dimethylbenz(a)anthracene [DMBA])-induced hamster oral cancer model was utilized. Eosinophils were determined to progressively infiltrate into this model, and when ablated by the use of an anti-interleukin-5 monoclonal antibody, resulted in a smaller tumor burden and delayed onset of tumor development as compared with control animals. It was proposed that eosinophils may have a tumor-promoting role in that eosinophils have been demonstrated to express TGFα. Transgenic mice overexpressing TGFα develop mammary and lacrimal tumors as well as demonstrating accelerated development of DMBA-induced mammary tumors (Oral Oncology 35: 496-501, 1999; American Journal of Pathology 137: 1425-1434, 1990; Cancer Research 52: 389-393, 1992). It has also been reported that human eosinophils also express TGF-α and may contribute to physiological, immunological, and pathological responses (Journal of Experimental Medicine 172: 673-681, 1990).

- IL-5 has been reported to show activity on B-cells in mice, but not in humans (McKenzie et al 1991). Murine and human IL-5 polypeptides exhibit 70% sequence homology and display species-specific activity to some extent. While the cytokine (IL-5) induces eosinophilic production and activation in both species, murine IL-5 has additional activity on B-cells in mice. To this end, even though the interactions of IL-5 with its receptor may be similar between mouse and

human, differences in downstream activity may make the mouse of more limited relevance for the assessment of human safety.

• Literature references indicate, based principally on the mechanism of action and/or pharmacologic/toxicologic properties of IL-5, that certain differences in activities of IL-5 exist between mice and humans, thus suggesting that the mouse model may have limited relevance regarding the assessment of projected safety in humans.

Conclusions: It was concluded that a carcinogenicity study with mepolizumab was not required. Key factors were the lack of pre-neoplastic or neoplastic findings in the 6-month toxicology study with monkeys and given that the species specificity of mepolizumab was limited to humans and Cynomolgus monkeys, a rodent carcinogenicity study did not appear to be technically feasible.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Potential effects of mepolizumab on fertility were assessed on adult male and female monkeys treated for 6 months (Study no. SB240563/RSD-100X0L/1)
. Mepolizumab at IV doses up to 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks had no effects on male or female fertility based upon no adverse findings from histopathological examinations of male and female reproductive organs.

Potential effects on fertility were also evaluated with male and female CD-1 mice treated with SB264091, a rat anti-human IL-5 surrogate monoclonal antibody (lgG2b), which cross reacts with human and murine IL-5. Male and female mice were treated with IV doses of SB-264091 at 0, 0.5, and 50 mg/kg/week. Male mice (F_0) were dosed on day 1 of the study and continued on a once-weekly basis until termination (for a total of 5 to 6 weekly doses). Female mice (F_0) were dosed 1 time per week for 2 weeks preceding cohabitation, 1 time per week during cohabitation (2 weeks maximum) until mated, and on gestation days 0, 7, and 14 (for a total of 5 to 6 weekly doses). Circulating concentrations of SB264091 as well as formation of anti-SB264091 antibodies in male and female mice that received up to 5 or 6 weekly doses were not examined. Mating and pregnancy incidences were unaffected with doses up to 50 mg/kg/week (see below for more details).

9.2 Embryonic Fetal Development

Study title: Intravenous Study of Male and Female Fertility, Early Embryonic and Embryo-Fetal Development in CD-1 Mice

Study no.:	SB240563/RSD-100P8V/1 and SB240563/RSD-100MZP/2 (Dose range finding and toxicokinetic study)
Study report location:	EDR
Conducting laboratory and location:	Toxicology Department SmithKline Beecham Pharmaceuticals King of Prussia, PA
Date of study initiation:	April 23, 1997
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SB-264091 [(5D3), a rat anti-human IL-5 monoclonal antibody (IgG2b)], Lot number 28908-76, was supplied as a solution containing (b) (4) mg/mL SB-264091 in vehicle ((b) (4) sodium phosphate, (b) (4) pH (b) (4)) by the Department of Protein Biochemistry, SmithKline Beecham Pharmaceuticals. The (b) (4) mg/mL dosing solution was made by serial dilution of the (b) (4) mg/mL stock solution with vehicle. The dosing solutions were stored at approximately 4°C.

Key Study Findings

● Fertility and embryofetal development were evaluated with male and female CD-1 mice treated with SB264091, a rat anti-human IL-5 surrogate monoclonal antibody (IgG2b), which cross reacts with human and murine IL-5. Male and female mice were treated with IV doses of SB-264091 at 0, 0.5, and 50 mg/kg/week.

● Male mice (F_0) were dosed on day 1 of the study and continued on a once-weekly basis until termination (for a total of 5 to 6 weekly doses). Female mice (F_0) were dosed 1 time per week for 2 weeks preceding cohabitation, 1 time per week during cohabitation (2 weeks maximum) until mated, and on gestation days 0, 7, and 14 (for a total of 5 to 6 weekly doses).

● Circulating concentrations of SB264091 as well as formation of anti-SB264091 antibodies in male and female mice that received up to 5 or 6 weekly doses were not

examined. The only available data was to extrapolate from measurements of ADA titer and eosinophil counts collected from female mice that received 1, 2, or 3 weekly doses of 5 or 50 mg/kg/week in a dose range finding study (SB240563/RSD-100MZP/2). No data was available for the dose of 0.5 mg/kg/week. For female mice in the 5 or 50 mg/kg/week groups that had received 1 to 3 doses of drug (on days 1, 8, and 15), eosinophil counts were decreased on days 8, 15, and 29. The pharmacodynamic (PD) effect (i.e., decreased eosinophils) persisted through day 29, although the last dose of SB264091 was on day 15. It can generally be inferred that the PD effect for the 50 mg/kg/week group persisted through at least day 29, which was extrapolated to the present study to suggest that drug exposure/pharmacodynamic activity was more than likely present and considered acceptable. These circumstances were not considered ideal.

● In the 50 mg/kg/week group, 1 female (#7823) was found dead on day 28, one day after the 3rd dose, and another female (#7820) was found dead on GD 7, one day after the 5th dose. The Sponsor attributed these deaths to anaphylactoid reactions, although no investigations were conducted to definitively determine causes of death.

● Body weights were unaffected in male and female mice that received doses up to 50 mg/kg/week.

● Mating and pregnancy incidences were unaffected with doses up to 50 mg/kg/week.

● Estrous cycle duration was unaffected with doses up to 50 mg/kg/week.

● Weights of male reproductive organs and sperm concentration and motility were unaffected in male mice that received doses up to 50 mg/kg/week.

● SB-264091 was not teratogenic in CD-1 mice that received a dose of 50 mg/kg/week. It must be noted that SB-264091 is not the clinical candidate (mepolizumab). This surrogate anti-IL-5 monoclonal antibody provides a qualitative hazard assessment; however, it would not be appropriate to extrapolate exposure multiples relative to the clinical exposure.

Methods

Doses:	0, 0.5, or 50 mg/kg/week
Frequency of dosing:	Once per week
Dose volume:	5.6 mL/kg
Route of administration:	Intravenous
Formulation/Vehicle:	(b) (4) sodium phosphate, (b) (4) pH (b) (4)
Species/Strain:	CD-1 mice obtained from (b) (4)
	Mated females were housed individually in clear polycarbonate boxes.

Number/Sex/Group:	25 F_0 mice/sex/group The (F_0) animals were approximately 11 weeks of age (body weight range of approximately 24 to 40 grams) at the initiation of dosing.
Satellite groups:	None
Study design:	Dose selection was based upon a range finding study with mice that received IV doses of SB-264091 at 5 or 50 mg/kg (SB240563/RSD-100MZP/2). Maximal pharmacologic activity (i.e., reduction in circulating eosinophil counts) was observed at doses ≥5 mg/kg. Single and weekly doses of ≥5 mg/kg were antigenic in some mice, but repeated weekly doses did not produce adverse clinical signs. Following a single dose, SB-264091 was found to have a half-life of approximately 5 days in mice. For the present study, a high dose of 50 mg/kg/week was selected based upon an approximate 10-fold dose multiple relative to the maximal pharmacological dose in mice. A low dose of 0.5 mg/kg was selected to evaluate potential dose-response relationships and to represent a dose in the range of that proposed for the clinical setting. Male mice (F_0) were dosed on day 1 of the study and continued on a once-weekly basis until termination (for a total of 5 to 6 weekly doses). Female mice (F_0) were dosed 1 time per week for 2 weeks preceding cohabitation, 1 time per week during cohabitation (2 weeks maximum) until mated, and on gestation days 0, 7, and 14 (for a total of 5 to 6 weekly doses). Mated females were killed on gestation day 18. Surviving males were killed the week following successful mating or the week following cohabitation.
Deviation from study protocol:	Female #7768 in the 50 mg/kg/week group naturally delivered 12 live pups

Table 27 Study Design

Group	Dose (mg/kg/day)	Number of Males	Number of Females
1	0 (vehicle)	25	25
2	0.5	25	25
3	50	25	25

(Excerpted from the Sponsor's submission)

Observations and Results

Mortality: Animals were checked for viability on a daily basis.

In the 50 mg/kg/week group, 1 female (#7823) was found dead on day 28, one day after the 3rd dose, and another female (#7820) was found dead on GD 7, one day after the 5th dose. The Sponsor attributed these deaths to anaphylactoid reactions, although no investigations were conducted to definitively determine causes of death. Necropsy of female #7823 found that the left kidney was enlarged and pale. There was a soft, yellowish material around the papilla. In addition, the mucosal surface of the stomach was reddened. For female #7820, there were no observable abnormalities at necropsy.

One male (#7710) in the 50 mg/kg/day group was found dead on day 5, 4 days after receiving the first dose. The necropsy examination did not identify any observable abnormalities. Based upon the timing of the death, a relationship to treatment appeared unlikely.

One female (#7836) in the 0.5 mg/kg/week group on day 23 began bleeding from the nose and convulsing following removal from the cage. The animal subsequently died on day 23. The necropsy examination revealed a break in the cervical vertebrae. The death was attributed to an accident.

Clinical Signs: Animals were observed daily for clinical signs. Detailed clinical observations were performed for males on study day 1 and the day of termination and for females on the first day of treatment, on the first day of cohabitation in the mating trial, and on gestation days 0 and 18.

There were no treatment-related clinical signs for males and females surviving to scheduled terminations.

Body Weight: Males were weighed weekly during the treatment period. Females were weighed weekly during the pre-treatment and treatment periods, and on gestation days 0, 3, 7, 10, 14, and 18.

Body weight gains for males in the 0.5 and 50 mg/kg/week groups from days 0 to 35 were unaffected relative to the control.

Body weight gain was increased for females in the 50 mg/kg/week group during the 2-week dosing period prior to mating, although the significance of this finding was unclear.

Body weight gains for females in the 0.5 and 50 mg/kg/week groups from gestation days 0 to 18 were unaffected relative to the control.

Table 28 Body weight gains for female control and SB-264091-treated groups during the 2-week dosing period prior to mating and from gestation days 0 to 18

Parameter	Control 0 mg/kg/week	0.5 mg/kg/week	50 mg/kg/week
BW, Day 15	29.4	28.9	28.6
BW, Day 28	30.1	29.6	29.8
▲, g	0.7	0.7	0.8
BW gain % of day 1 BW	2.38	2.42	4.20
BW gain % of control	100.0	101.7	176.2
BW, GD 0	29.8	29.4	29.5
BW, GD 18	57.6	55.9	55.5
▲, g	27.8	26.5	26.0
BW gain % of GD 1 BW	93.3	90.1	88.1
BW gain % of control	100.0	96.6	95.5

Feed Consumption: Feed bins were weighed weekly, except during cohabitation, starting on Day 1 for males and starting on the first day of estrous cycle evaluation (pretreatment) for females; then also for mated females on corresponding days to measure food consumption for the following intervals: gestation days 0-7, 7-15, and 15-18.

Food consumption was unaffected for males in the 0.5 and 50 mg/kg/week groups from days 1 to 28.

Food consumption was unaffected for females in the 0.5 and 50 mg/kg/week groups for the 2-week dosing period prior to mating and gestation days 0 to 18.

Toxicokinetics: Serum concentrations of SB264061 and anti-SB264061 antibodies were not measured in the present study. However, serum concentrations of SB264061 and anti-SB264061 antibodies as well as pharmacodynamic activity (i.e., decreased eosinophil counts) were measured in a dose range finding study (SB240563/RSD-100MZP/2) initiated on March 4, 1997. Female CD-1 mice received IV doses of 5 or 50 mg/kg/week for a total of 1 to 3 doses. There is no toxicokinetic data for the dose of 0.5 mg/kg/day that was used in the present study.

The design of the dose range finding study (SB240563/RSD-100MZP/2) with female mice that received IV doses of 5 or 50 mg/kg/week is as follows:

Table 29 Design of dose range finding study (SB240563/RSD-100MZP/2)

Group	Dose (mg/kg/dose)	Number of Animals	Day(s) of Dosing	Animal Identification Numbers[2]
1	0	15	1, 8, 15	M97F-0650 to M97F-0664
2	5	15	1, 8, 15	M97F-0665 to M97F-0679
3	50	15	1, 8, 15	M97F-0680 to M97F-0694
4*	5	24	1	M97F-0695 to M97F-0718
5*	50	24	1	M97F-0719 to M97F-0742

* Animals used for toxicokinetic evaluation.

(Excerpted from the Sponsor's submission)

Pharmacokinetic parameters for SB264091 were assessed in female mice following a single dose of 5 or 50 mg/kg (Groups 4 and 5; n=3 females/sampling time). Blood samples were collected immediately postdose, at 24 hr postdose, and on days 5, 8, 12, 15, and 29 postdose. SB264091 was quantifiable immediately following dose administration and during the 29 day sampling period in all animals evaluated. However, there was considerable inter-animal variability of plasma concentrations obtained from mice in the 5 and 50 mg/kg groups particularly from Days 8 to 29. Eleven animals had plasma concentrations that were much lower (differences of up to approximately 200-fold) than other animals sampled at the same nominal times. The majority of these samples (7 of 11; 5 animals at 5 mg/kg and 2 animals at 50 mg/kg) were found to be positive for anti-SB264091 antibodies, which appeared to affect calculated pharmacokinetic parameters. For mice given 5 mg/kg with detectable ADA, 2 were detected on Day 8, 2 on Day 15, and 1 on Day 29. For mice given 50 mg/kg with detectable ADA, 1 was detected on Day 12 and 1 on Day 29. There were three additional mice (2 given 5 mg/kg and 1 given 50 mg/kg) with lower SB-264091 plasma levels compared to group mean or predicted values, but did not have detectable ADA.

Pharmacokinetic parameters were calculated with and without plasma concentrations obtained from the antibody-positive animals. Plasma concentrations of SB264091 declined slowly with a terminal elimination half-life of approximately 5 days. Half-lives for mice in the 5 and 50 mg/kg groups, negative for anti-SB264091 antibodies, were 5.7 and 7.5 days, respectively. C_{max} and AUC values from 5 to 50 mg/kg increased in a greater than dose proportional manner. The T½ was approximately 4.9 days for ADA positive and negative animals. Clearance and the volume of distribution at steady state were relatively comparable with IV doses of 5 and 50 mg/kg. The volume of distribution was approximately 2- to 3-times the plasma volume (50 mL/kg).

Table 30 Pharmacokinetic parameters of SB264061 in female mice following a single dose of 5 or 50 mg/kg

Parameter	5 mg/kg	50 mg/kg
Cmax* (ug/mL)	50.6	965
AUC(0-inf) (ug.h/mL)	4048 (1.2)**	54414 (1.3)**
T½ (days)	4.96	4.80
CL (mL/h/kg)	1.24	0.919
Vss (mL/kg)	149	121

* Observed Cmax at 4-6 minutes following dose administration.

** Numbers in parentheses indicate the percent of the area extrapolated (t-inf).

(Excerpted from the Sponsor's submission)

Female mice in Groups 1, 2, or 3 received IV doses of SB264091 at 5 or 50 mg/kg/week, respectively, for a total of 1, 2, or 3 weekly doses. Terminal blood samples were collected from the first 5 mice per group from Groups 1-3 on day 8 (7 days after the first dose, prior to the second dose), day 15 (7 days after the second dose, prior to the third dose), and day 29 (14 days after the third dose). Analysis of serum samples from these mice revealed positive anti-SB264091 antibody responses only in the 5 mg/kg/week group following 2 weekly doses (Day 15, 1 of 5 mice) or 3 weekly doses (Day 29, 2 of 5 mice) of SB264091. The absence of a detectable anti-SB264091 antibody response in the 50 mg/kg group might be attributed to the presence of high plasma concentrations of SB264091 that were competitory in the assay.

For female mice in the 5 or 50 mg/kg/week that had received 1 to 3 doses of drug (on days 1, 8, and 15), eosinophil counts were decreased on days 8, 15, and 29. The pharmacodynamic effect (i.e., decreased eosinophils) persisted through day 29, although the last dose of SB264091 was on day 15. For mouse #672 in the 5 mg/kg group that had received 2 doses of drug, there was evidence of ADA on day 15. However, eosinophil counts (0.04 x 10^9/L) were still decreased. For mouse #678 in the 5 mg/kg group that had had 3 doses of drug, there was evidence of ADA on day 29. Eosinophil counts in mouse #678 (0.12 x 10^9/L vs. 0.04, 0.08, and 0.04 x 10^9/L for other mice in the 5 mg/kg group) appeared to have reversed toward the control (0.06, 0.13, 0.20, 0.26, and 0.25 x10^9/L).

Table 31 Eosinophil counts (x10^9/L) in female mice on days 8, 15, and 29 following 2 or 3 weekly intravenous dose of SB264091 at 0, 5, or 50 mg/kg/week

Dose, mg/kg	Day 8	Day 15	Day 29
0	0.14 ± 0.04	0.36 ± 0.19	0.18 ± 0.04
5	0.04 ± 0.01	0.03 ± 0.01	0.08 ± 0.03
50	0.04 ± 0.01	0.03 ± 0.01	0.06 ± 0.02

Table 32 Anti-SB264091 antibody concentrations in plasma samples from female mice in Groups 2 or 3 that received IV doses of SB264091 at 5 or 50 mg/kg/week, respectively, for a total of 1, 2, or 3 weekly doses

Anti-SB 264091 Antibody Concentrations in Mouse Serum Samples from Groups 1-3*

Dose Group	Day 8		Day 15		Day 29	
(mg/kg/day)	I.D. No.	ug/mL	I.D. No.	ug/mL	I.D. No.	ug/mL
0	M97F-650	0	M97F-655	0	M97F-660	0
	M97F-651	0	M97F-656	0	M97F-661	0
	M97F-652	0	M97F-657	0	M97F-662	0
	M97F-653	0	M97F-658	0	M97F-663	0
	M97F-654	0	M97F-659	0	M97F-664	0
5	M97F-665	0	M97F-670	0	M97F-675	0
	M97F-666	0	M97F-671	0	M97F-676	0
	M97F-667	0	M97F-672	11.75	M97F-677	0
	M97F-668	0	M97F-673	0	M97F-678	11.17
	M97F-669	0	M97F-674	0	M97F-679	3.75
50	M97F-680	0	M97F-685	0	M97F-690	0
	M97F-681	0	M97F-686	0	M97F-691	0
	M97F-682	0	M97F-687	0	M97F-692	0
	M97F-683	0	M97F-688	0	M97F-693	0
	M97F-684	0	M97F-689	0	M97F-694	0

(Excerpted from the Sponsor's submission)

Circulating concentrations of SB264091 as well as formation of anti-SB264091 antibodies in male and female mice that received up to 5 or 6 weekly doses were not examined. The only available data was to extrapolate from female mice that received 1, 2, or 3 weekly doses.

In response to and Information Request dated April 21, 2015, the Sponsor noted that "the pharmacodynamic response, decreased eosinophils (both group mean and individual values), was consistently observed at all 3 time points in both dose groups suggesting that anti-IL5 pharmacologic activity was maintained. The eosinophil counts in 2 of 3 mice at 5 mg/kg with detectable ADA were equal to or less than the group means while the remaining mouse had an eosinophil count that was comparable to control suggesting a neutralizing effect of the ADA. Thus, while a repeat dose toxicokinetic assessment was not conducted; weekly dosing appeared to reduce the ADA response and maintained the pharmacologic activity of SB-264091 in mice. While male mice were not included on this study, prior experience with monoclonal antibodies in laboratory animals has not detected a sex-related difference for TK or generating an ADA response."

"While the incidence of ADA in mice used for the reproductive assessments was unknown, the impact on PD was assumed to be low based on once weekly dosing in the TK study. In females dosed with 50 mg/kg, 2 of 25 mice died following the third and fifth dose (1 each, respectively) following presumed anaphylactoid reactions; similar responses have occurred with other biologic agents in rodents. Given the large number of mice per group (25/sex/group) and a conservative estimate of 20% ADA, a sufficient

number of mice would have received exposure to active SB-264091 over the dosing periods to allow for an adequate evaluation of potential reproductive toxic effects to be conducted. Based on this, it is estimated that greater than 17 to 18 litters would have been exposed to pharmacologically relevant levels of SB-264091, which is considered to be an adequate number of litters for detecting a reproductive hazard per the ICH S5 (R2) Guidance."

Reviewer's Evaluation: Pharmacodynamic activity was demonstrated to persist through at least 4 weeks in the dose range finding study with 1 to 3 weekly doses. This period appears adequate to cover the 4-week dosing period prior to mating as well as the mating period (mean = 2 days) for male mice. This period also appears adequate to cover the 2-week dosing period prior to mating, the mating period (mean = 2 days), and through gestation day 14 for female mice. Thus, the study to assess effects of SB-264091 on male and female fertility, early embryonic and embryo-fetal development in CD-1 mice was considered to be valid.

Dosing Formulation Analysis: Analyses of dosing solutions were performed 2 times during the study. Measured concentrations of drug were 102 to 124% of nominal concentrations.

Necropsy: Surviving males were killed by CO_2 asphyxiation and necropsied between days 35 and 42 (the week following successful mating or the week following cohabitation). Reproductive organs were weighed and sperm analysis was conducted as discussed below.

Surviving females were sacrificed on gestation day 18 for examination as discussed below.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.):

1. Duration of Estrous Cycle

The vaginal cytology of each female was determined daily for 14 days preceding treatment and during 14 days of treatment preceding cohabitation in order to evaluate the estrous cycle.

The duration of estrous cycle was unaffected for females in the 0.5 and 5 mg/kg/week groups (see the table below).

2. Mating and Pregnancy Incidences

After the pre-mating treatment period (males received a total of 5 doses and females received a total of 3 doses), males and females from the same dose group were cohabited (1:1) for up to 14 days. The females were checked twice daily for the presence of an intravaginal copulatory plug. Females with an intravaginal copulatory plug were weighed and separated from the male and this was designated as gestation day 0.

All males and females in the 0, 0.5, and 5 mg/kg/week groups mated (mating incidence was 100% for all groups). There was no treatment-related effect on the period required for mating. Incidences of pregnancy were unaffected for females in the 0.5 and 50 mg/kg/week groups relative to the control. Mating and pregnancy (or fertility) indexes were unaffected by treatment with doses up 50 mg/kg/week.

Table 33 Estrous cycle duration, day needed for mating, and the mating and pregnancy incidences

Female (F0) Reproductive Performance - Summary

		-No. of Estrous Cycles During-				
		Pre-Treatment	Treatment	Days needed For Mating	Mating Incidence (a) (%)	Pregnancy Incidence (b) (%)
Test Used:		KW-	KW-	KW-	CA-	CA-
Dose (mg/kg/day)						
0 Control	Mean	2.9	2.8	2.1	25/25 (100%)	22/25 (88%)
	SEM	0.1	0.1	0.3		
	N	24	24	24		
0.5	Mean	2.8	2.7	1.8	24/24 (100%)	21/24 (87%)
	SEM	0.1	0.2	0.2		
	N	23	23	24		
50	Mean	2.7	2.8	2.1	24/24 (100%)	20/24 (83%)
	SEM	0.1	0.1	0.3		
	N	24	24	23		

Note: (a) Mating Incidence = Number of Females Inseminated/Number of Females Evaluated
(b) Pregnancy Incidence = Number of Females Pregnant/Number of Females Inseminated

(Excerpted from the Sponsor's submission)

3. Weights of male reproductive organs and sperm evaluation:
Surviving males were killed the week following successful mating or the week following cohabitation. Each testis, each epididymis (left intact; right caput and corpus together, right cauda separate), ventral prostate, and one seminal vesicle with coagulating gland from each male were weighed. The testes and epididymides (except for right cauda) were fixed. The right cauda epididymides were frozen at approximately -20°C. The frozen cauda epididymis was minced and homogenized. Homogenization-resistant sperm were counted in a hemocytometer by light microscopy. Spermatozoa were collected at necropsy from the vas deferens and examined for numbers of motile and non-motile spermatozoa.

Absolute and relative weights of the left testis, right testis, left epididymis, right caput and corpus epididymis, right cauda epididymis, ventral prostate, and seminal vesicle were unaffected by treatment with SB264091 at doses up to 50 mg/kg/week.

Sperm counts per cauda epididymis and per gram cauda epididymis as well as percent motile sperm were unaffected by treatment with SB264091 at doses up to 50 mg/kg/week.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.):
Mated females were killed on gestation day 18 and necropsied. The ovaries were removed and the corpora lutea were counted. The uterus was weighed and examined

for implantation sites, resorptions, and live and dead fetuses with their positions recorded. A gross examination of each placenta was performed.

All fetuses were resorbed for dam #7797 in the 0.5 mg/kg/week group and dam #7805 in the 50 mg/kg/week group. Only 1 live fetus was delivered from dam #7832 in the 0.5 mg/kg/week group.

The number of implants/dam was slightly reduced for females at 50 mg/kg/day as reflected by a larger pre-implantation loss. Total resorptions were increased for females at 0.5 and 50 mg/kg/day, which might be attributed to higher incidences of late resorptions. Numbers of live fetuses were slightly reduced for females at 0.5 and 50 mg/kg/week. There were no dose-response relationships for increased late and total resorptions or decreased live fetuses. Further, these changes were relatively small. Relationships of these observations to treatment with SB-264091 were questionable. Uterus weights were reduced for females at 50 mg/kg/week.

Table 34 Female (F_0) Cesarean Section data

Parameter	Control	0.5 mg/kg/week	50 mg/kg/week
Total number of female mice	25	25	25
Deaths (before cohabitation)	0	1	1
Female mice mated	25	24	24
Not pregnant	3	3	4
Number of pregnant mice	22	21	20
Deaths (after cohabitation)	0	0	1
Number of pregnant females evaluated	22	21	19
Total litter resorption	0	1	1
Number of litters examined	22	20 (or 19*)	18
Number of corpora lutea/dam	12.9	13.3	12.6
Number of implants/dam	12.1	12.4	11.3
% Pre-Implantation Loss	6.0	7.6	10.8
Early Resorptions per dam	1.0	2.0	1.2
Late Resorptions per dam	0.1	**0.5**	**0.6**
Total Resorptions per dam	1.1	**2.5**	**1.8**
% Implants Resorbed	9.3	22.0	16.5
Number of live fetuses (M/F) per dam	10.9 (5.1/5.8)	**9.9 (5.3/4.6)**	**9.4 (5.1/4.3)**
% Live male and female fetuses	45.8/54.2	57.4/42.6	56.0/44.0
Dead fetuses	0.1	0.0	0.1
Live Birth Index, %	98.6	100.0	99.6
Uterus weight, mg	20.0	19.2	17.6
Male/Female Fetal BW, g	1.39/1.33	1.41/1.32	1.47/1.42

*Only 1 live fetus was delivered from dam #7832 in the 0.5 mg/kg/week group.

In a submission dated May 14, 2015, the Sponsor responded to an Information Request dated May 6, 2015, and provided historical control data regarding the incidences of early, late, and total resorptions from 1995 to 1999 and 2007 to 2012 as shown in the table below. Incidences of late and total resorptions from the present study conducted in 1997 were higher than the historical control data provided by the Sponsor. The Sponsor attributed these findings to total or near total litter loss for dams #7797 and 7832 in the

0.5 mg/kg/week group and dam #7805 in the 50 mg/kg/week group as noted above. Further, there was lack of a dose-response relationship for these observations.

There were no control females with totally resorbed litters in the Historical Control Compendium. The Sponsor noted that “immune reactions such as anti-drug antibody responses in some mice may explain these observations. Takayama (Congenital Anomalies 21: 175-186, 1981) noted a statistically significant decrease in the number of live fetuses at cesarean section from dams that had anaphylaxis induced on Day 8.5 of gestation. While the incidence of ADA in mice on this reproductive toxicity study was not known, it was anticipated to affect about 20% of mice based on the dose range finding study (SB240563/RSD-100MZP/2).”

Reviewer evaluation: Increased incidences of late and total resorptions in SB-264091-treated groups were considered unlikely to be related to drug treatment based upon lack of dose-response relationships. These findings were attributed to total or near total litter loss for dams #7797 and 7832 in the 0.5 mg/kg/week group and dam #7805 in the 50 mg/kg/week group.

Table 35 Sponsor's historical control data - range of incidence of fetal resorption (early, late, and total)

	Early Resorptions	Late Resorptions	Total Resorptions	Live Fetuses
Years: 1995 to 1999 (N = Litters)				
Study 1 (N=22)	0.6	0.2	0.8	11.3
Study 2 (N=17)	0.9	0.1	1.0	11.2
Study 3 (N=31)	0.8	0.0	0.8	10.7
Study 4 (N=20)	0.5	0.0	0.5	12.2
Study 5 (N=22)	1.0	0.0	1.0	11.6
Study 6 (N=22)	1.0	0.1	1.1	10.9
Mean (N=134)	0.8	0.1	0.9	11.3
Years: 2007 to 2012				
Study 1 (N=25)	0.7	0.0	0.7	11.7
Study 2 (N=23)	0.6	0.0	0.7	13.0
Study 3 (N=25)	0.7	0.0	0.8	12.0
Study 4 (N=7)	0.6	0.1	0.7	12.0
Study 5 (N=6)	0.3	0.0	0.3	14.5
Mean (N=86)	0.6	0.0	0.6	12.6

(Excerpted from the Sponsor's submission)

Offspring (Malformations, Variations, etc.): Fetuses were killed by an overdose of sodium pentobarbital given orally. Each fetus and late resorption was examined externally. Live fetuses were individually weighed. Abdominal and thoracic viscera in approximately one-half of the fetuses were examined by a modified Staples' technique. These fetuses were decapitated and the heads preserved in Bouin's solution and subsequently sectioned and examined by the Wilson's technique. The remaining fetuses were eviscerated with sex identified by internal examination, processed for skeletal staining with Alizarin Red S, and then the skeletons were examined.

The incidence of cleft palate was increased for fetuses in the 50 mg/kg/week group (4 of 179 fetuses (2.23%) or 2 of 18 litters (11.1%)). The observed incidence appears to exceed the published background incidence of 0.17% (4 of 2352 fetuses; Laboratory Animal Science 26: 293-300, 1976).

In a submission dated May 14, 2015, the Sponsor responded to an Information Request dated May 6, 2015, and provided historical control data regarding the incidence of cleft palate. The Sponsor's historical control compendium for the period when the study was conducted (1997) consisted of 6 studies (between 1995 and 1999) in which a total of 1532 fetuses in 136 litters were examined. Cleft palate was detected in 7 fetuses from 3 of the 6 studies. The mean fetuses affected and range were 0.46% (7/1532) and 0 to 1.17%, respectively. The mean litters affected and range were 4.4% (6/136) and 0 to

14.3%, respectively. The Sponsor also provided historical control data from 5 studies conducted between 2007 and 2012 in which a total of 1029 fetuses in 83 litters were examined. Cleft palate was detected in 8 fetuses from 2 of the 5 studies. The mean fetuses affected and range were 0.78% (8/1029) and 0 to 2.17%, respectively. The mean litters affected and range were 7.2% (6/83) and 0 to 17.4%, respectively. The historical control data provided by the Sponsor suggests that the observations of cleft palate in the present study were more than likely background in nature and unrelated to treatment with SB264091.

Table 36 Fetal external observations (#fetuses/#litters)

Observation	Control	0.5 mg/kg/week	50 mg/kg/week
Number of fetuses/litters examined	243/22	208/22	179/18
External mouth and jaw – cleft palate	1/1 (0.41%)	0/0	4/2 (2.23%)

One fetus in the 50 mg/kg/week group was observed with dilation of the subcutaneous space in the brain. One fetus in the 50 mg/kg/week group (7787-6L) was observed with multiple findings in the brain as well as a finding in the lung. Another fetus in the 50 mg/kg/week group had a dilated renal pelvis. The relationships of these findings in single fetuses from the 50 mg/kg/week group to treatment with SB264091 were unclear.

Table 37 Fetal visceral observations (#fetuses/#litters)

Parameter	Control	0.5 mg/kg/week	50 mg/kg/week
Number of fetuses/litters examined	119/22	103/20	93/18
Brain – dilation of subcutaneous space	0/0	0/0	1/1 (0.93 ± 0.93%)
Brain – dilated third ventricle	0/0	0/0	1/1 (0.93 ± 0.93%)[1]
Brain – encephalocele	0/0	0/0	1/1 (0.93 ± 0.93%)[1]
Brain – diencephalon abnormally shaped	0/0	1/1 (0.83±0.83%)	1/1 (0.93 ± 0.93%)[1]
Kidney(s) Renal Pelvis – moderately dilated	0/0	0/0	1/1 (0.93 ± 0.93%)
Lung – one or more small lobe(s) small	0/0	0/0	1/1 (0.93 ± 0.93%)[1]

[1.] Findings in the brain and lung were observed in a dead fetus (7787-6L)

There were no fetal skeletal observations attributed to treatment with SB264091.

Table 38 Fetal skeletal observations (#fetuses/#litters)

Parameter	Control	0.5 mg/kg/week	50 mg/kg/week
Number of fetuses/litters examined	124/22	105/19	86/18
Cervical vertebrae – one or more arch variation in shape	0/0	2/1 (1.75±1.75%)	1/1 (0.79±0.79%)
Cervical Rib	12/7 (11.40±4.49%)	5/4 (4.17±2.09%)	2/2 (5.56±3.81%)
Hindpaw – calcaneous not evident	25/11 (22.80±6.85%)	21/6 (19.35±8.48%)	11/5 (10.63±4.51%)

9.3 Prenatal and Postnatal Development

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

Study no.:	CD2003/01020/00
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 6, 1999 (Start of treatment)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SB240563, Batch/Lot Numbers U96257 and U97281 The vial (55 mg/vial) was reconstituted with 2.0 mL of sterile water for injection. After reconstitution, the concentration was approximately (b) (4) mg/mL mepolizumab (SB240563). For the 10 mg/kg group, the (b) (4) mg/mL dosing solution was diluted 1 to 10 (i.e., (b) (4) mg/mL). Analysis of dosing formulations on two occasions determined that the actual drug concentration for the nominal (b) (4) mg/mL solution was (b) (4) mg/mL and for the nominal (b) (4) mg/mL solution was (b) (4) mg/mL.

Key Study Findings

● In a pre- and postnatal development study, 12 pregnant female Cynomolgus monkeys/group received mepolizumab (SB240563) at intravenous doses of 0, 10, or 100 mg/kg once per month on gestation days 20, 50, 80, 110, and 140. These animals were allowed to naturally deliver their offspring. The development of offspring was followed for 9 months after birth. Surviving animals and their offspring were returned to the primate colony at the end of the study.

● There was no evidence of treatment-related maternal toxicity or effects on pregnancy outcome or natural delivery. Eight or nine monkeys from each group gave birth to live offspring (1 infant per mother).

● Offspring were passively exposed to mepolizumab *in utero* through transplacental transfer or consumption of breast milk after birth, and drug was detectable in infant plasma through 6 months post-partum.

● There was no evidence of treatment-related effects on physical, hematologic, or immunologic development of offspring. Following immunization with Tripedia®, there

were no treatment-related effects on development of specific titers to the vaccine components.

● Anti-mepolizumab antibodies were detected in one mother and its infant from the 10 mg/kg group.

● Mepolizumab (SB240563) at intravenous doses ≤100 mg/kg administered to mothers had no effects on the pre- and postnatal development of their offspring up to 9 months after birth. The present study is considered adequate; however, it should be noted that this study was small with only 8 or 9 infant monkeys per group for assessment. Visual and physical examinations of infants did not identify any malformations. Infants were not examined for skeletal or visceral malformations and variations. The results of this study should be judged with caution given the limitations in study design and resources.

Methods

Doses:	Pregnant Cynomolgus monkeys were treated with the vehicle (sterile aqueous solution of (b) (4) polysorbate 80, (b) (4) sucrose, (b) (4) sodium phosphate dibasic, pH 7) or SB240563 at doses of 10 or 100 mg/kg administered once per month by the intravenous route into the saphenous vein on gestation days 20, 50, 80, 110, and 140.
Frequency of dosing:	Starting on gestation day 20, SB240563 was administered once every 30 days. The last dose was administered on day 140.
Dose volume:	Vehicle and drug solutions were administered by the intravenous route using a dose volume of 4 mL/kg.
Route of administration:	Intravenous route
Formulation/Vehicle:	Sterile aqueous solution of (b) (4) polysorbate 80, (b) (4) sucrose, (b) (4) sodium phosphate dibasic, pH 7
Species/Strain:	Female Cynomolgus monkeys (*Macaca fascicularis*) (b) (4) (b) (4) (b) (4). These female monkeys were purpose-bred, sexually mature, and not previously used in any study. Female animals were paired with untreated fertile male animals 1 or 2 days before the theoretical middle of the menstrual cycle. The duration of mating was maximally 18 hr. The day on which mating terminated was designated Day 0 of gestation (or post-coitum). On days 18, 19, or 20 post-coitum, an ultrasonic examination was performed to identify pregnancy. If the lumen of the uterus was detectable in the ultrasonogram as a dark gap, the female was considered to be pregnant. Females had a body weight range of 2.6 to 5.8 kg and were at least 3 years old at the start of the study (gestation day 20).
Number/Sex/Group:	12 female monkeys/group
Satellite groups:	None
Study design:	This study evaluated the potential effects of SB240563 when administered by the intravenous route to female Cynomolgus monkeys on pregnancy, parturition, and lactation, and on survival, growth, and postnatal

development of offspring. Pregnant female Cynomolgus monkeys received SB240563 by the intravenous route at doses of 0, 10, or 100 mg/kg on gestation days 20, 50, 80, 110, and 140. Mothers were allowed to naturally deliver infants. Growth and development of infants was assessed for up to 9 months after delivery. At the end of the study, all remaining monkeys and their infants were returned to the primate colony.

Deviation from study protocol: Deviations were generally minor and did not affect the integrity of the study.

Table 39 Design of the pre- and post-natal development study with monkeys

Group Number	Group Description	Color Code	Number of Pregnant Females	Dose Level mg/kg/day	Application Volume mL/kg/day
1	Control	white	12	0	4
2	Low	blue	12	10	4
3	High	red	12	100	4

(Excerpted from the Sponsor's submission)

Table 40 Maternal and infant blood samples were collected for measurements of SB240563, anti-SB240563 antibodies, hematology parameters, lymphocyte subset analysis, and immunoglobulins (IgA, IgG, and IgM)

Study Day	SB-240563 (Plasma and Milk)	Anti-SB-240563 Antibody (Serum)	Hematology (Blood)	Lymphocyte Subsets (Blood)	Immunoglobulin (Serum)	Note
pre-dose	M	M	M		M	1
20 p.c.	M					2
21 p.c.	M					2
27 p.c.	M					2
50 p.c.	M					2
80 p.c.	M	M	M		M	1
110 p.c.	M					2
140 p.c.	M	M	M		M	1
141 p.c.	M					2
147 p.c.	M					2
14 p.p.	M O				M O	3,4,5
28 p.p.	M O		O		M O	3,4,6
91 p.p.	M O	M O	M O	+O	M O	1,4,7
178 p.p.	M O	M O	M O	++O	M O	1,4,7

M = mother animal
O = offspring
\+ = on Day 91 ± 1 *post-partum*
++ = on Day 178 ± 1 *post-partum*

(Excerpted from the Sponsor's submission)

Observations and Results

F_0 Dams

Survival: Animals were observed daily for morbidity/mortality from gestation day 20 to the end of the study.

There were no apparent treatment-related effects of SB240683 on survival of F_0 dams.

One female (F9454) that received the mid dose of 10 mg/kg was sacrificed in a moribund state on gestation day 62. Body weight declined from 3.4 kg on gestation day 27 to 2.2 kg on gestation day 62. Reduced food consumption was evident during this period. Clinical signs from gestation days 59 to 62 included apathy and sluggish. Necropsy examination of this animal found a gall bladder filled with dark-green fluid and two small (2 to 5 mm) red foci on the luminal surface. The relationship of these findings to the moribund sacrifice was unclear. The fetus was found to be small. This moribund sacrifice in the low dose group did not appear to be treatment-related given that no deaths occurred in the high dose group.

Clinical signs: Animals were observed daily for clinical signs from gestation day 20 to the end of the study. Vaginal smears were examined daily from gestation day 20 until delivery. Pregnancy status was monitored by ultrasonography on gestation days 30, 44, 58, 72, 86, 100, 114, 128, 142, and 156. Additional monitoring was conducted if animals showed signs of abortion.

There were no treatment-related clinical signs.

Abortion occurred for 2 of 12 monkeys in the control group (F9257 on unknown day and F9455 on day 76) and 2 of 12 monkeys in the high dose group (F9441 on day 43 and F9249 on day 117).

See below under "F_1 Infants" for survival status of F_1 infants.

Body weight: Body weights were measured on gestation days 20, 27, 34, 41, 48, 50, 55, 62, 69, 76, 80, 83, 90, 97, 104, 110, 111, 118, 125, 132, 139, 140, 146, 153, 160, 167, 174, and 181. After delivery, body weights of the mother were measured on postpartum days 1, 7, 14, and 28 and then at monthly intervals until termination of the study.

Body weight gains of F_0 female Cynomolgus monkeys during the gestation and postpartum periods were unaffected by treatment with SB240563.

Food consumption: Not measured.

Clinical pathology: Maternal blood samples were collected for measurements of hematology parameters, lymphocyte subset analysis, and immunoglobulins (IgA, IgG, and IgM) as shown in the table below.

There were no treatment-related effects on maternal hematology parameters or immunoglobulin levels during the gestation and postpartum periods. Eosinophil percentages were low for control and treatment groups during the gestation and postpartum periods.

Uterine content: F_0 dams were allowed to naturally deliver infants. The uterine contents were not examined.

Necropsy observation: One female (F9454) in the 10 mg/kg was sacrificed in a moribund state on gestation day 62 as descried above. At the end of the study, all remaining F_0 dams were returned to the primate colony.

Toxicokinetics: Maternal blood samples were collected for measurements of SB240563 and anti-SB240563 antibodies as shown in the table below. Maternal milk samples for measurement of SB240563 were collected on postpartum days 14, 28, 91, and 178. Monkey plasma and milk samples were assayed for SB240563 using a fluorescent immunoassay method. The lower limit of quantitation was 50 ng/mL using 100 µL of 10% plasma or milk. Anti-SB240563 antibodies were measured by two electrochemiluminescent (ECL) immunoassays. The first assay detected anti-SB240563 antibodies, consisting of anti-framework (against human IgG portion of SB240563) and anti-idiotype (against murine complementarity determining region, CDRs of SB240563) antibodies. The second assay is specific for anti-SB-240562 idiotype antibodies.

Two monkeys (#9447, 10 mg/kg and #9445, 100 mg/kg) had quantifiable plasma concentrations of SB240563 prior to the first dose of drug. The Sponsor had no explanation for these findings.

Serum concentrations: C_{24hr} (1st and 5th doses) and AUC values for pregnant female Cynomolgus monkeys in the 10 and 100 mg/kg groups were approximately dose proportional. The half-life was 12.16 to 14.34 days. Trough plasma concentrations for the 10 and 100 mg/kg groups measured prior to 2nd, 3rd, 4th, and 5th doses were comparable suggesting that steady-state drug concentrations were achieved.

Table 41 Mean maternal AUC(0-t) and C_{24h} values following the 1st dose of SB240563 and C_{24h} values following the 5th dose of SB240563

	†AUC(0-t) (µg·h/mL)	C_{24h} Dose 1* (µg/mL)	C_{24h} Dose 5* (µg/mL)
Dose (mg/kg)	(n=12)	(n=12)	(n=9)
10	38857	144	165‡
100	254111	1059	1159

† For AUC calculated after Dose 1, t=30 days (nominally). *The concentration at 24 hours post-dose has been reported, since a 5 min post-dose sample (expected observed Cmax) was not collected following Dose 5. ‡ n=8, as one animal was positive for anti-SB-240563 antibodies.

(Excerpted from the Sponsor's submission)

Table 42 Mean (SD) SB240563 maternal trough concentrations (µg/mL; n = 11 unless otherwise indicated)

Sample	Dose	
	10 mg/kg	100 mg/kg
Pre-Dose 2	15.1 (4.9)	106 (42)
Pre-Dose 3	13.2 (4.6)*	93.3 (24.7)
Pre-Dose 4	15.7 (8.2)*	95.5 (28.6)
Pre-Dose 5	15.8 (2.6)**	104 (35)***

* n = 10; ** n = 8; and *** n = 9

Breast milk concentrations: For the 10 mg/kg group, SB240563 was not detected in any maternal milk samples collected during the postpartum period. For the 100 mg/kg group, SB240563 was measured in 6 of 7 animals on postpartum day 14 and concentrations ranged from 67 to 328 ng/mL (these concentrations were 0.006 and 0.028% of the plasma concentration (C_{24hr} = 1159 µg/mL) after the 5th dose). On postpartum day 28, plasma concentrations were less than 100 ng/mL (two times the lower limit of quantitation).

Anti-SB240536 Antibodies: One monkey (F9243) in the 10 mg/kg group tested positive for anti-SB240563 antibodies on gestation days 80 and 140 (prior to the 3rd and 5th doses). Detectable anti-SB240563 antibodies of increasing concentration (0.222 to 2.369 µg/mL) were measured between gestation day 80 and postpartum day 178. Beginning on gestation day 50 (prior to dose 2), this animal had lower or non-quantifiable drug concentrations compared to other animals in the 10 mg/kg group, indicating a faster plasma clearance of SB240563. The $C_{24\ hr}$ value from this animal following the 5th dose was excluded. The infant of F9243 had no quantifiable plasma concentrations of SB240563 and tested positive for anti-SB240563 antibodies. On postpartum day 91, the anti-SB240563 antibody concentration was 1.531 µg/mL; however, on postpartum day 178, the concentration had dropped to 0.047 µg/mL. These data indicate that the infant passively acquired anti-SB240563 antibodies (by transplacental transport) and did not represent an immune response in the infant to SB240563. Both maternal and infant anti-SB240563 antibody samples had an anti-idiotype component in the antibody response.

Dosing Formulation: Concentrations of SB240563 (Batch numbers U96257 and U97281) in dosing formulations were determined twice during the study. Duplicate samples (2 mL) each were taken from selected dosing formulations on the day of preparation. One sample of each duplicate set was dispatched (packed at approximately 0 to 4°C and protected from light) to the Sponsor on the day of preparation. The remaining samples were retained at the Testing Facility as backup samples.

Analysis of dosing formulations on two occasions determined that the actual drug concentration for the nominally (b) (4) mg/mL solution was (b) (4) mg/mL and for the nominally (b) (4) mg/mL solution was (b) (4) mg/mL. Measured concentrations were sufficiently close to the nominal values.

F_1 Generation

Survival: F_1 infants were observed for survival from the time of delivery up to 9 months after birth.

Stillbirths occurred for one control animal (F9452) on gestation day 162, two animals of the 10 mg/kg group (F8743 and F1906/1) on gestation days 164 and 168, respectively, and two animals of the 100 mg/kg group (F9270 and F8592) on gestation days 160 and 167, respectively. Necropsy examinations were unable to determine causes of death. The fetus of F1906/1 in the 10 mg/kg group was covered with blood. The fetus of F9270 in the 100 mg/kg group was reported with a dark-red face, marked swelling of the face and eyelid, and the anus was smeared over with excrement.

A dead fetus was removed from one female (F9443) in the 10 mg/kg group on gestation day 117 following an ultrasonography examination.

Nine control animals gave birth to live infants between gestation days 150 and 172 with 3 males and 5 females surviving to 9 months. Eight animals in the 10 mg/kg group gave birth to live infants between gestation days 157 and 171 with 3 males and 4 females surviving to 9 months. Eight animals in the 100 mg/kg group gave birth to live infants between gestation days 138 and 169 with 4 males and 4 females surviving to 9 months.

Table 43 PPND Study: Pregnancy outcomes

Table 1 Pregnancy Outcome Summary CLE Study No. 802-485

Parameter	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 100 mg/kg/day
Number of pregnant females	12	12+	12
Number of females killed moribund	0	1	0
Number of females with a dead fetus and cesarean section	0	1	0
Number of females which aborted	2	0	2
Number of females which delivered at term	10	10	10
Mean duration (days) of gestation for all animals which delivered at term	160	162	162
Number of females with stillbirth	1	2	2

+ not included one female (No. 9458) replaced due to false positive pregnancy test

(Excerpted from the Sponsor's submission)

One infant of a female in the control group (F9467) died within a few hours after delivery on gestation day 150. One infant of a female in the 10 mg/kg group (F9254) was found dead on day 1 after delivery on gestation day 138. Another infant of a female in the 10 mg/kg group (F8590) was found dead on day 5 after delivery on gestation day 158. Necropsy examinations of these three infants were unable to determine causes of death. The fetus of F9254 in the 10 mg/kg group was reported to have a dark-red face.

Table 44 PPND Study: Infant observation data

Table 48 Infant Observation Data CLE Study No. 802-485

Parameter	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 100 mg/kg/day
Number of live infants on day of birth	9	8	8
Number of infants which died after a few hours	1	0	0
Number of infants which died on day 1 after birth	0	1	0
Number of infants which died on day 5 after birth	0	1	0
Number of surviving offspring	8	6	8

(Excerpted from the Sponsor's submission)

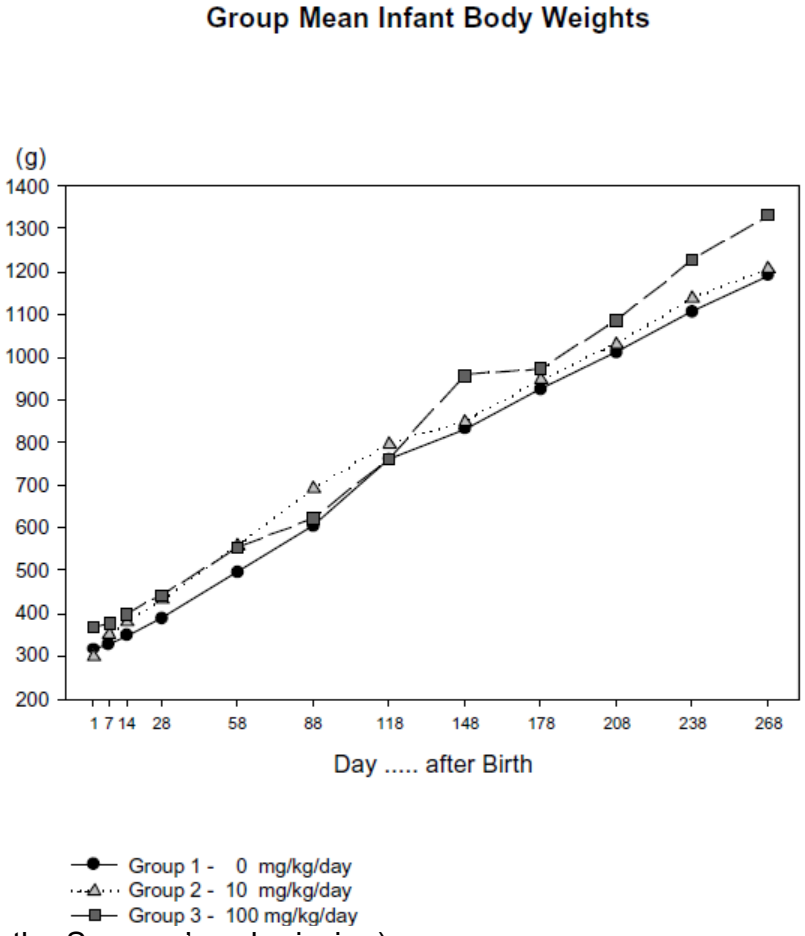
Clinical signs: All infants were examined twice daily for general health appearance and behavior.

There were no treatment-related clinical signs.

Body weight: After delivery, body weights of the infants were measured on postpartum days 1, 7, 14, and 28 and then at monthly intervals until termination of the study.

There were no treatment-related effects on body weight gains.

Table 45 Group mean infant body weights



(Excerpted from the Sponsor's submission)

Feed consumption: Not measured.

Clinical pathology: Infant blood samples were collected for measurements of hematology parameters, lymphocyte subset analysis, and immunoglobulins (IgA, IgG, and IgM).

There were no treatment-related effects on hematology parameters or lymphocyte subsets (CD2, CD2/CD4, CD2/CD8, CD2+CD16, CD20, and CD4/CD8) for F_1 infants. IgG levels for infants in the 10 and 100 mg/kg groups on day 14 were increased to 142 and 188.4% of the control (0.43 g/L), respectively, although there were no findings at later time points. IgA levels for infants in the 100 mg/kg group on day 178 were decreased to 64.5% of the control (1.07 g/L).

Physical development: The sex of each infant was determined on day 1 postpartum.

There were no treatment-related effects on physical development; however, it should be noted that this study was small with only 8 or 9 infant monkeys per group for

assessment. The Sponsor stated that no offspring malformations were evident. The results of this study should be judged with caution given the limitations in study design.

Neurological assessment: All infants were examined twice daily for general behavior.

There were no treatment-related behavioral effects in F_1 offspring. It does not appear that the study included any specific behavioral tests (e.g., learning and memory).

Reproduction: Reproduction of F_1 offspring was not assessed. F_1 offspring were observed for 9 months after birth and surviving animals were returned to the primate colony.

Necropsy: There were no necropsy examinations of F1 infants that survived to 9 months after delivery. At the end of the study, all remaining monkeys and their infants were returned to the primate colony.

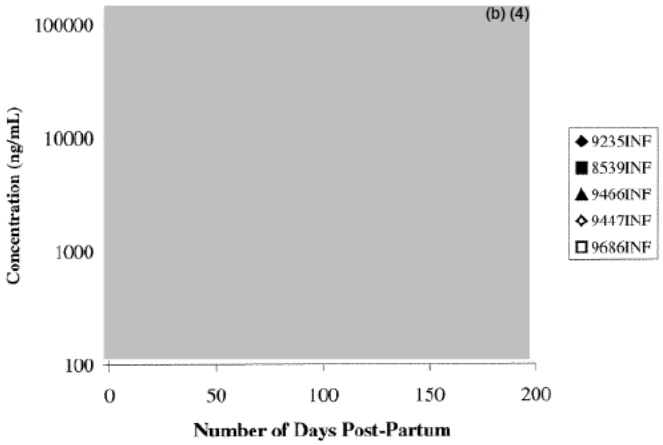
Toxicokinetics: Infant blood samples were collected for measurements of SB240563 and anti-SB240563 antibodies.

Serum concentrations of SB240563: SB240563 crossed the placenta and was generally quantifiable in infant plasma samples between the first *post-partum* sampling time on Day 14 and the Day 91 *post-partum* sample. SB240563 was generally quantifiable in infant plasma samples up to the Day 91 post-partum sample. Quantifiable SB240563 concentrations were measured on postpartum day 178 in 3 of 8 infants in the 100 mg/kg group. Infant plasma concentrations on average were 2.4-fold higher than maternal plasma concentrations. Active transport of the IgG1 subclass across the placenta by Fc receptors has been demonstrated. AUC values in infants, which were passively exposed to SB240563 in utero or by consumption of breast milk, were approximately one-half of those observed in mothers. The half-life in infants was 14.93 to 15.56 days.

Table 46 Plasma concentrations of SB240563 in infants from the 10 mg/kg group

Individual Infant Plasma SB-240563 Concentrations (ng/mL)

10 mg/kg

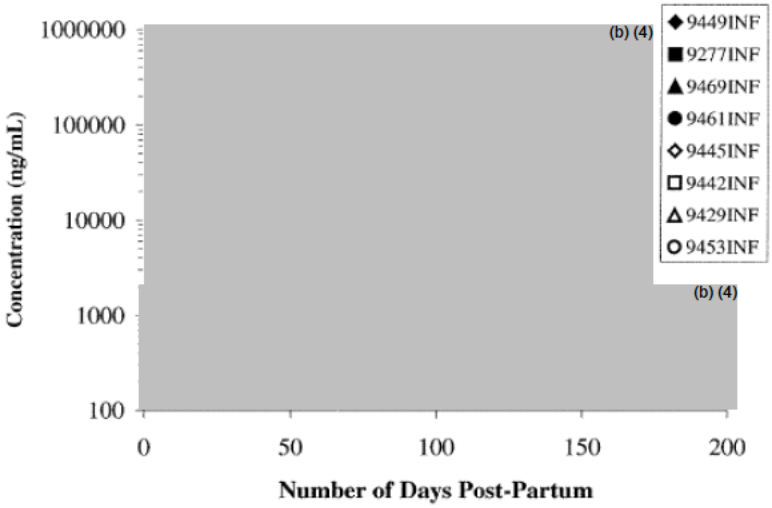


(Excerpted from the Sponsor's submission)

Table 47 Plasma concentrations of SB240563 in infants from the 100 mg/kg group

Individual Infant Plasma SB-240563 Concentrations (ng/mL)

100 mg/kg



(Excerpted from the Sponsor's submission)

The Sponsor stated that the higher concentration of SB240563 in the infants was consistent with higher concentrations of anti-tetanus antigen antibodies reported in human infants compared to their mothers. Active transport of SB240563 across the placenta would be expected based upon the IgG 1 subclass, which has been shown to

be transported more efficiently across the placenta by Fc receptors than IgG2 and IgG3 subclasses.

Anti-SB-240536 Antibodies: The infant of Dam F9243 (see above) had no quantifiable plasma concentrations of SB240563 and tested positive for anti-SB240563 antibodies. On postpartum day 91, the anti-SB240563 antibody concentration was 1.531 µg/mL; however, on postpartum day 178, the concentration had dropped to 0.047 µg/mL. These data indicate that the infant passively acquired anti-SB240563 antibodies (by transplacental transport) and did not represent an immune response in the infant to SB240563. Both maternal and infant anti-SB240563 antibody samples had an anti-idiotype component in the antibody response.

Other: To evaluate the immune system of offspring, infants were immunized with two treatments of Tripedia® vaccine at approximately 7 and 8 months of age. Four weeks after the second injection, blood samples were collected from infants to analyze antibody response to vaccine components (diphtheria and tetanus toxoids, pertussis toxin, filamentous hemagglutinin) and Pertactin using ELISA methods.

Treatment of pregnant monkeys with SB240563 had no effect on the ability of their offspring to develop a primary immune response to components of the pediatric vaccine, Tripedia®. A weak response to pertactin was seen in all infants; however, pertactin was not a specific component of the Tripedia® vaccine.

10 Special Toxicology Studies

Study title: Mepolizumab (SB240563): Immunocytochemical Analysis of Binding to Normal Human Tissues

Study no.:	SB-240563/RSD-100KGT/1
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 19, 1996
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	SB240563 (Lot No. MB-28061-192) was supplied biotinylated at a concentration of (b) (4) mg/mL in a buffer containing (b) (4) sodium phosphate and (b) (4), pH (b) (4). There were 5 biotin molecules per SB240563 molecule, and biotinylated-SB240563 had comparable binding to rhIL-5 as unmodified SB240563. Purity was greater than 90% by size exclusion chromatography. The biotinylated-SB240563 was used diluted in phosphate buffered saline (PBS) containing 2% bovine serum albumin (BSA).

Key Study Findings

● The distribution of mepolizumab (SB240563) binding to normal human tissues was assessed by immunocytochemical analysis.

● Staining was observed with bone marrow; weak to moderate staining was observed on cells of granulocyte lineages (eosinophils and/or neutrophils) present in the samples. These findings are generally consistent with the results of the 6-month toxicology study with monkeys.

● Staining was observed throughout the sections of lymph nodes tested.

● In the spleen, staining was observed in reticular cells or macrophages in the red pulp, and intense staining was seen in granulocytes (eosinophils and/or neutrophils). Equivocal to weak staining was observed in the white pulp.

● Staining in the tonsils was predominantly seen in the lymphoid cells in perifollicullar regions. Moderate staining was observed on dendritic cells in submucosa, germinal centers, and intraepithelial locations of the tonsil.

Methods: The objective of this study was to determine the distribution of mepolizumab (SB240563) binding to normal human tissues by immunocytochemical analysis.

Histologically normal human tissues were obtained fresh from surgical and autopsy specimens. Fresh tissues were embedded in OCT compound in cryomolds and frozen in liquid-nitrogen cooled isopentane. The tissues were cut at 5 µm, placed on slides, air-dried, and stored at -70°C until needed. White blood cells of the peripheral blood and bone marrow were prepared.

The humanized monoclonal antibody, CMHZOO (Lot No. MB-28061-63; IgG1, kappa), was biotinylated and used as the negative control antibody. Biotinylated-CMHZOO was supplied at a concentration of 5.1 mg/mL in a buffer containing (b) (4) mM sodium phosphate and (b) (4) mM sodium chloride, pH (b) (4). There were 4 biotin molecules per CMHZOO molecule. Purity was greater than 95% by size exclusion chromatography. The negative control antibody was diluted to the same working concentration as SB240563.

Peroxidase-conjugated streptavidin was used as the labeling reagent at a 1:500 dilution of the manufacturer's stock in PBS.

Immunocytochemical studies were performed using an avidin-biotin immunoperoxidase technique. The cryostat cut sections were processed to reduce endogenous peroxidase and biotin activities. Tissue sections were incubated with the primary antibody for 2 hours and then washed. A concentration of 25 µg/mL of SB240563 gave optimal results, which was defined as maximum staining without significant background staining of the negative control. A complete list of tissues was evaluated (see below). The labeling reagent, peroxidase-conjugated streptavidin, was applied for 20 minutes. Slides were then washed. The peroxidase reaction was visualized by incubating tissue sections for 3-5 minutes with 3-3'diaminobenzidine-tetrahydrochloride (DAB; 0.06%), 0.01% hydrogen peroxide, and 0.1% Triton X-100. Tissue sections were washed, counterstained with a modified Harris hematoxylin, dehydrated through graded alcohols and xylene, and cover slipped.

Frozen, unfixed smears of human IL-5 transfected *Drosophila* cells and respiratory syncytial virus F-protein transfected *Drosophila* cells were used as the positive and negative control samples, respectively, for immunostaining with SB240563. The negative staining control consisted of substitution of the primary antibody with an isotype-matched biotinylated antibody, CMHZOO. Fixation was conducted with methyl/acetone (1:1, v/v; 2-8°C) that provided histological preservation and optimal immunoreactivity with the antibody.

Table 48 Tissue specimen list for immunocytochemical analysis

(b) (4) ID	Tissue Type	Patient Sex
F88-033	Adrenal	NK
F94-001B	Adrenal	F
F94-007	Adrenal	M
F95-080	Bladder	M
F95-064	Bladder	M
F95-063	Bladder	M
F96-045	Bone Marrow	F
95-15247	Bone Marrow	F
95-14653	Bone Marrow	M
F95-053	Brain	F
F95-054	Brain	M
F95-087	Brain	M
F90-563	Breast	F
F90-273A	Breast	F
F94-025B	Breast	F
F96-014E	Eye	M
F96-013A	Eye	F
F90-405C	Eye	NK
F96-002	Fallopian Tube	F
F96-003	Fallopian Tube	F
F96-004	Fallopian Tube	F
F89-166Y1	Heart	M
F92-322	Heart	M
F89-294G6	Heart	NK
F94-004	Kidney	F
F94-010	Kidney	M
F92-432	Kidney	F
F92-410	Large Intestine	NK
F94-017A	Large Intestine	F
F92-407	Large Intestine	M

(b) (4) ID	Tissue Type	Patient Sex
F92-389	Liver	NK
F94-15B	Liver	F
F92-397	Liver	F
F95-070	Lung	F
F95-071	Lung	M
F89-134	Lung	NK
F94-031	Lymph Node	F
F94-027	Lymph Node	F
F94-028	Lymph Node	F
F92-380	Muscle, skeletal	F
F92-354	Muscle, skeletal	F
F88-034	Muscle, skeletal	NK
F90-285	Ovary	F
F92-327	Ovary	F
F95-085	Ovary	F
F95-067	Pancreas	M
F95-042	Pancreas	F
F95-069	Pancreas	M
F95-088	Pituitary	M
F89-152A	Placenta	F
F89-172C	Placenta	F
F96-005	Placenta	F
F92-350	Prostate	M
F92-020	Prostate	M
F96-038	Prostate	M
F89-294A1	Skin	NK
F89-239B2	Skin	F
F89-166BB3	Skin	M
F95-008	Small Intestine	M
F95-009	Small Intestine	F
F95-014	Small Intestine	M

(b) (4) ID	Tissue Type	Patient Sex
F92-215A1	Spinal Cord	M
F95-077	Spinal Cord	NK
F89-166B1	Spinal Cord	M
F94-040	Spleen	M
F94-042	Spleen	F
F94-043	Spleen	M
F88-039	Stomach	NK
F94-024B	Stomach	M
F95-075B	Stomach	F
F94-012	Testis	M
F95-072	Testis	M
F95-074	Testis	M
F95-058	Thymus	M
F95-026B	Thymus	F
F96-015	Thymus	NK
F94-006B	Thyroid	F
F94-022B	Thyroid	F
F94-023B	Thyroid	M
F97-001	Tonsil	NK
F96-002	Tonsil	NK
F96-003	Tonsil	NK
F96-001	Ureter	M
F95-017	Uterus	F
F95-019	Uterus	F
F88-041	Uterus	F
F94-038	White Blood Cells	M
F95-059	White Blood Cells	M
F94-036	White Blood Cells	M

(Excerpted from the Sponsor's submission)

Observations and Results: The optimal concentration of 25.0 µg/mL of SB240563 was selected as it gave maximum staining intensity without significant background staining of the negative control. Positive reactivity was observed in 80 to 100% of the IL-5-transfected *Drosophila* cells when tested with SB240563. The staining intensity of these cells was 3+ (range 0-3+).

Immunocytochemical analysis of SB240563 on human tissues demonstrated that the antigen recognized by the antibody exhibited a highly restricted pattern of distribution. Staining (1+ to 2+) was observed in all three specimens of bone marrow tested. Weak to moderate staining was observed on cells of granulocyte lineages (eosinophils and/or neutrophils) present in the samples.

Staining (1 to 2+) was observed throughout the sections of lymph nodes tested; however, reactivity of defined compartments could not be established due to suboptimal preservation of cellular architecture in frozen lymph node sections.

In the spleen, staining (1 to 3+) was observed in reticular cells or macrophages in the red pulp, and intense staining was seen in granulocytes (eosinophils and/or neutrophils). Equivocal to weak staining (+/- to 1+) was observed in the white pulp for two of the three specimens tested.

Staining in the tonsils was predominantly seen in the lymphoid cells in perifollicullar regions. Follicular structures, including the follicular center and mantle zone, were unreactive. Moderate staining was observed on dendritic cells in submucosa, germinal centers, and intraepithelial locations of the tonsil. Squamous epithelial cells were unreactive.

Weak staining was seen on inflammatory cells present in one specimen of the prostate.

All epithelial cells, as well as stratified and squamous epithelia of different organs were found to be unreactive with the test antibody. Reactivity was also not seen in all neuroectodermal tissues tested, including brain and peripheral nerves. Staining of mesenchymal elements, such as skeletal and smooth muscle, fibroblasts, and endothelial cells was negative.

SB-264091: Effect on Host Defense against *Mesocestoides corti* Infection in Mice (CD2005/01090/00)

Methods: This study assessed potential effects of the anti-IL-5 monoclonal antibody, SB-264091, on host defense response to *Mesocestoides corti* (*M. corti*) infection in female mice. SB-264091 is a rat monoclonal antibody (IgG2b) directed against human interleukin-5 (IL-5) and cross-reacts with murine IL-5.

SB-264091 was administered by the intraperitoneal route at doses of 0 (vehicle), 0.5, or 50 mg/kg to female C57BL mice (30/group) on study days 1, 8, 15, 22, 29, 36, and 43 using a dose volume of 5 mL/kg. The vehicle was phosphate-buffered saline, pH 7.4. Mice in the positive control groups received dexamethasone that was administered by the intraperitoneal route on study days -2, 1, 5, and 8, and then twice per week throughout remainder of study. On study Day 2, mice in Groups 2, 4, 5, and 6 were infected by the intraperitoneal route with approximately 760 tetrathyrida (larvae) of *M. corti* in 0.1 mL of 0.9% buffered saline.

Table 49 Study design to assess potential effects of the anti-IL-5 monoclonal antibody, SB-264091, on the host defense response to *Mesocestoides corti* (*M. corti*) infection in female mice.

		SB-264091	Dexamethasone	*M. corti*
Group	Number of Mice	(mg/kg/dose)	(mg/kg/dose)	(# of parasites)
[1]	30	0	0	0
[2]	30	0	0	760
[3]	30	0	50	0
[4]	30	0	50	760
[5]	30	0.5	0	760
[6]	30	50	0	760

(Excerpted from the Sponsor's submission)

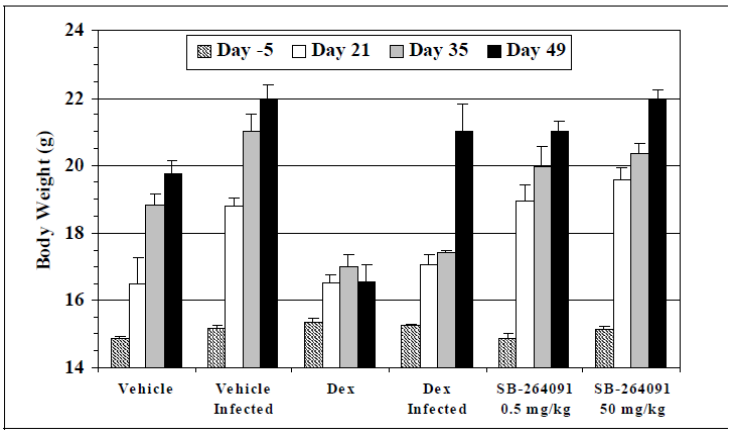
All mice were observed daily for viability. Body weights were measured weekly. One day prior to necropsy for each cohort (i.e., days 20, 34, or 48), blood was collected for measurement of total and differential white cell counts. Ten (10) mice/group were necropsied on Days 21, 35, and 49. The peritoneal cavity was opened, lavaged with 5 mL of heparinized PBS, and the contents were collected. The liver and spleen were removed, weighed, observed for gross pathology, and fixed in 10% neutral-buffered formalin. The parasites were separated from the peritoneal lavage fluid. The peritoneal fluid and contents were fixed with methanol and the parasites were fixed in buffered formal saline. Total and differential white blood cell and *M. corti* counts and parasite viability were determined. The total number of peritoneal cells were counted. Sections of liver from mice infected with *M. corti* and treated with the vehicle or 50 mg/kg SB-264091 and necropsied on Day 49 were compared histologically and scored in a blinded manner using a semi-quantitative technique. The total numbers of parasites were counted. Numbers of parasites surrounded by a fibrous capsule or by inflammatory cells only were also counted. The liver from one *M. corti* infected vehicle treated mouse did not have histologic evidence of a parasitic infection and was excluded from the analysis.

Results:
There were no deaths in the uninfected vehicle-control group 1, *M. corti* infected vehicle-control group 2, or uninfected dexamethasone-treated group 3. In the infected dexamethasone-treated group 4, there were 8/10, 2/10, and 2/10 mice surviving on scheduled euthanasia days 21, 35, and 49, respectively. Cause of death in dexamethasone-treated mice was attributed to impaired host defense against the *M. corti* infections. In group 5 mice infected with *M. corti* and treated with 0.5 mg/kg mepolizumab, there were 2/10 deaths on Day 49. The cause of death in infected mice administered 0.5 mg/kg mepolizumab was uncertain, but the lack of a dose-response relationship (no deaths occurred in infected mice given 50 mg/kg mepolizumab) suggested that it was not related to immunosuppression.

Increased mean body weights were observed on day 21 in the *M. corti* infected vehicle-control (13.9%), 0.5 mg/kg (14.8%), and 50 mg/kg (18.8%) group mice compared to uninfected vehicle-control mice. This trend was also observed on days 35 and 49, although results were only significant for infected mice in the 50 mg/kg group at day 49. Increased body weights were attributed to increases of liver and spleen weights in *M. corti* infected mice (see below).There were no significant differences in day 21 body weights of *M. corti* infected or uninfected mice treated with dexamethasone compared to uninfected vehicle-control. Dexamethasone-treated uninfected mice did not gain weight between days 21 and 49. Data from dexamethasone-treated *M. corti* infected mice on days 35 and 49 were not considered interpretable due to high mortality in this group.

Figure 17 Effect of *M. corti* infection on body weight gain

Figure 1 Effect of *M. corti* Infection on Body Weight

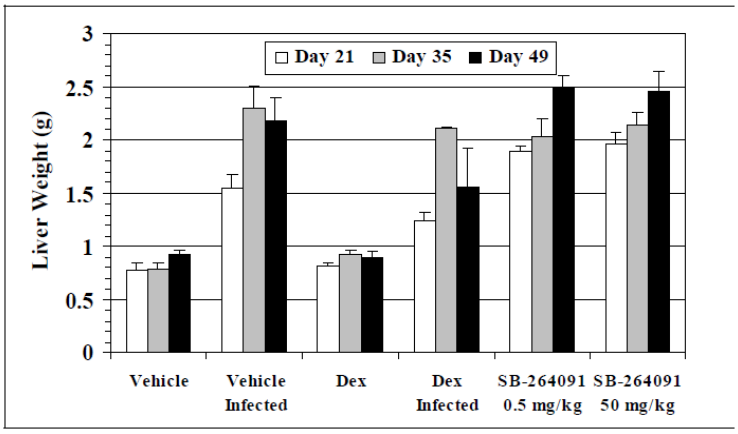


(Excerpted from Sponsor's submission)

During the course of an experimental intraperitoneal infection with *M. corti* in mice, the tetrathyridia have been found to directly invade the liver or spleen, resulting in local inflammation and increased organ weights. In vehicle-control mice infected with *M. corti*, 2- to 3-fold increases of liver weights were observed on days 21, 35, and 49 compared to the uninfected vehicle-control. Similarly in mice treated with 0.5 or 50 mg/kg mepolizumab and infected with *M. corti*, liver weights were increased. In mice infected with *M. corti* and treated with dexamethasone, liver weights on day 21 were increased 1.6-fold relative to uninfected vehicle-control. Dexamethasone had no effect on liver weight in uninfected mice. Data from dexamethasone-treated *M. corti* infected mice on days 35 and 49 were not considered interpretable due to high mortality in this group.

Figure 18 Effect of *M. corti* infection on liver weights

Figure 2 Effect of *M. corti* Infection on Liver Weight

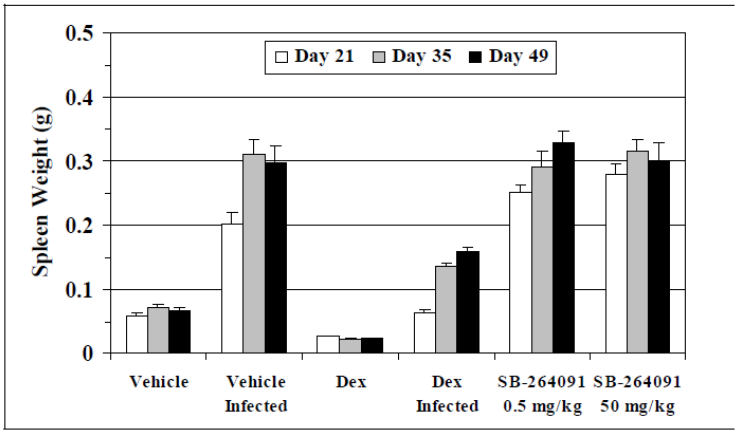


(Excerpted from Sponsor's submission)

Spleen weights were increased (3.3- to 4.4-fold) in *M. corti* infected vehicle-control mice relative to the uninfected vehicle-control on days 21, 35, and 49. Similarly, in mice treated with 0.5 or 50 mg/kg SB-264091 and infected with *M. corti*, spleen weights were increased. In uninfected dexamethasone-treated mice, spleen weights were decreased 50% on Days 21, 35, and 49 relative to uninfected vehicle control. In dexamethasone-treated *M. corti* infected mice there was no increase of spleen weights on day 21. Data from dexamethasone-treated *M. corti* infected mice on days 35 and 49 were not interpretable due to high mortality in this group.

Figure 19 Effect of *M. corti* infection on spleen weights

Figure 3 Effect of *M. corti* Infection on Spleen Weight



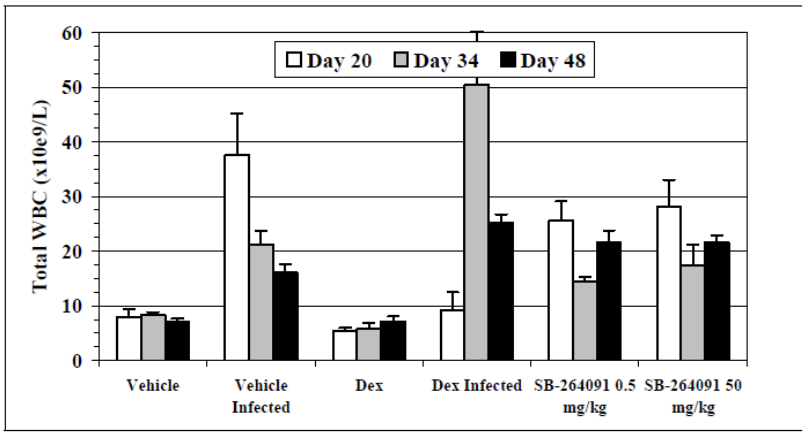
(Excerpted from Sponsor's submission)

In *M. corti* infected vehicle-control and SB-264091 treated mice, white blood cell (WBC) counts were significantly increased 3.2- to 4.8-fold on days 20, 34, and 48 relative to the uninfected vehicle-control. There were no significant differences in WBC counts between the uninfected control vehicle-mice and dexamethasone-treated infected and

uninfected mice on Day 20. Data from dexamethasone-treated *M. corti* infected mice on days 35 and 49 were not interpretable due to high mortality in this group.

Figure 20 Effect of *M. corti* infection on WBC counts

Figure 4 Effect of *M. corti* Infection on Circulating WBCs

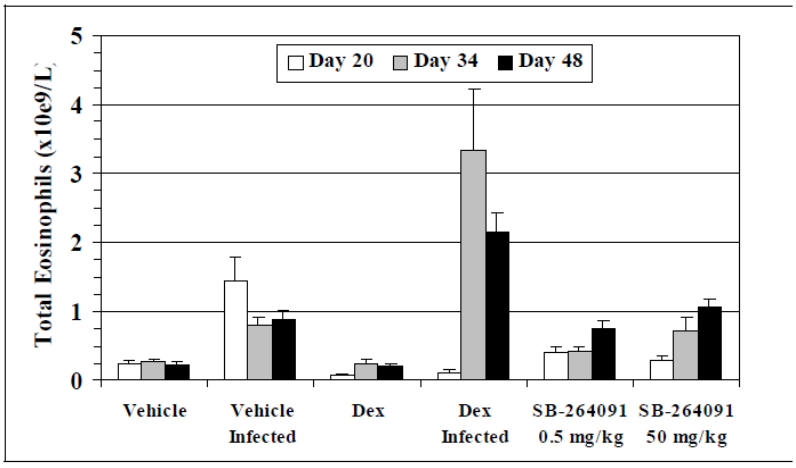


(Excerpted from Sponsor's submission)

Eosinophil counts in *M. corti* infected vehicle-treated mice were increased 6.0-fold on day 20 relative to the uninfected vehicle-control. However, treatment of mice with SB-264091 prevented the infection related eosinophilia resulting in a less than 1.6-fold increase relative to the uninfected vehicle-control. On days 34 and 48, increases of eosinophil counts in *M. corti* infected vehicle-control or SB-264091-treated mice were similar (1.6- to 4.7-fold relative to the uninfected vehicle control). In dexamethasone-treated uninfected mice, eosinophil counts were decreased 66.7% on day 20 relative to the uninfected vehicle-control, but were similar to the uninfected vehicle-controls on days 34 and 48. In dexamethasone-treated *M. corti* infected mice, there were no difference in eosinophil counts compared to uninfected vehicle-control on day 20. Data from dexamethasone-treated *M. corti* infected mice on days 35 and 49 were not interpretable due to high mortality in this group.

Figure 21 Effect of M. corti infection on eosinophil counts

Figure 5 Effect of *M. corti* Infection on Circulating Eosinophils

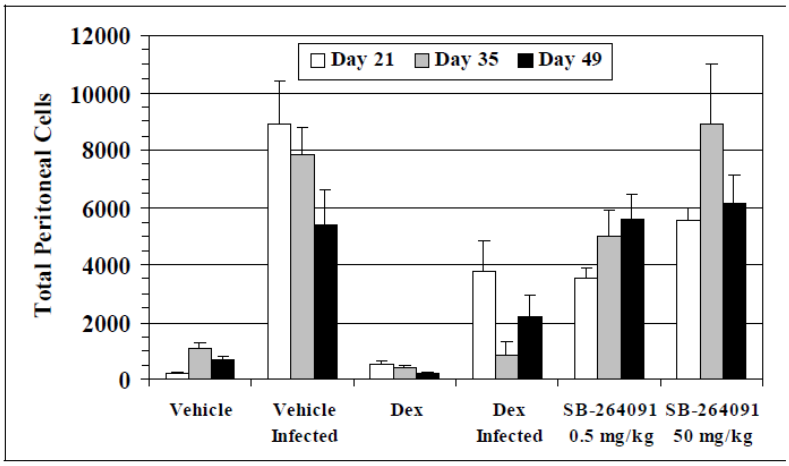


(Excerpted from Sponsor's submission)

In *M. corti* infected mice, peritoneal cell numbers were significantly increased in the vehicle-control, 0.5 mg/kg, and 50 mg/kg groups (43-, 17- and 27-fold, respectively) on day 21 relative to the uninfected vehicle-control. Increases in absolute peritoneal cell numbers were similar in *M. corti* infected vehicle-control and SB-264091 treated mice on days 35 and 49 (4.6- to 8.6-fold). Peritoneal cell count increased significantly (18-fold) on day 21 in *M. corti* infected mice treated with dexamethasone compared to the uninfected vehicle-control.

Figure 22 Effect of M. corti on total peritoneal cell counts

Figure 6 Effect of *M. corti* Infection on Peritoneal Cell Counts



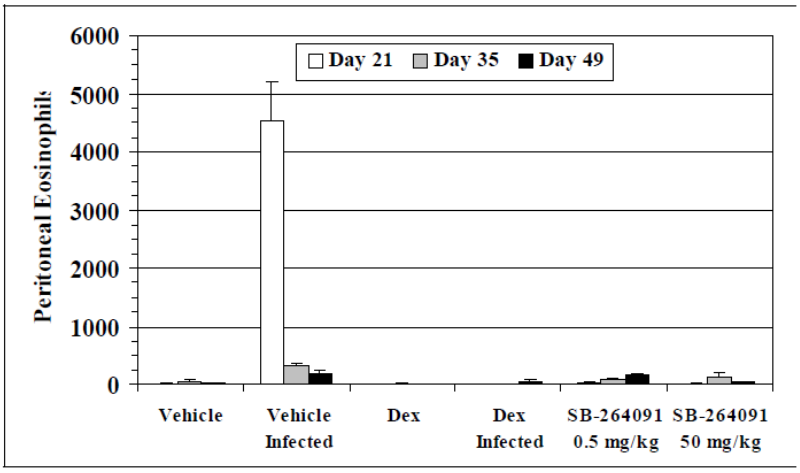
(Excerpted from Sponsor's submission)

Peritoneal eosinophil counts were significantly increased in *M. corti* infected vehicle-control mice at all time points with the largest increase observed on day 21 (4539 peritoneal eosinophils/mouse compared to 10 peritoneal eosinophils/mouse in the uninfected vehicle-control). Peritoneal eosinophils did not increase significantly in

infected mice treated with SB-264091 (36 and 8 eosinophils at 0.5 and 50 mg/kg, respectively, on day 21). Peritoneal eosinophil counts were also reduced in the 0.5 and 50 mg/kg groups on day 35 and the 50 mg/kg group on day 49 relative to the infected vehicle-control group. It can be inferred that eosinophils did not contribute to the increase in total peritoneal cell counts observed in *M. corti* infected mice treated with SB-264091.

Figure 23 Effect of *M. corti* infection on peritoneal eosinophil counts

Figure 7 Effect of *M. corti* Infection on Peritoneal Eosinophil Counts



(Excerpted from Sponsor's submission)

Table 50 Mean peritoneal eosinophil counts on days 21, 35, and 49

Table 7 Mean Peritoneal Eosinophil Counts

	M. corti	SB-264091	Dex	Day 21			Day 35			Day 49		
Group	(# parasites)	(mg/kg)	(mg/kg)	Mean	SEM	N	Mean	SEM	N	Mean	SEM	N
1	0	0	0	10	5	10	60	12	9	14	2	10
2	760	0	0	4539*	665	10	326*	50	10	187*	47	10
3	0	0	50	1*	0	9	12	2	10	5	2	8
4	760	0	50	3**	3	7	2**	2	2	51	37	2
5	760	0.5	0	36**	30	10	72**	22	10	147*	32	8
6	760	50	0	8**	6	10	142**	76	10	42**	20	10

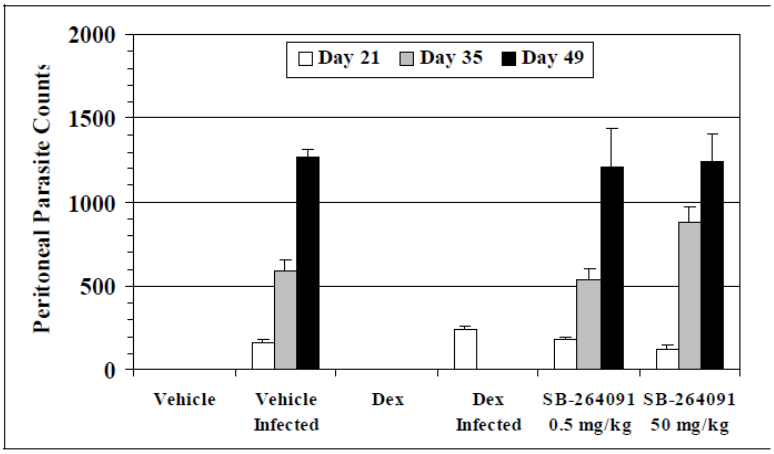
* significantly different compared to Group 1 ($p<0.05$)
** significantly different compared to Group 2 ($p<0.05$)

(Excerpted from Sponsor's submission)

The number of parasites recovered from the peritoneal cavity of mice given SB-264091 was similar to the *M. corti* infected vehicle-control group. However, on day 35 there was a slight, but significant, increase (1.5-fold higher than infected vehicle-control, $p<0.05$) in the number of parasites recovered from mice treated with 50 mg/kg SB-264091, although values were similar to control at day 49. The number of parasites recovered on day 21 from *M. corti* infected dexamethasone-treated mice was comparable to vehicle-control mice.

Figure 24 Effect of *M. corti* infection on peritoneal parasite counts

Figure 8 Effect of *M. corti* Infection on Peritoneal Parasite Counts

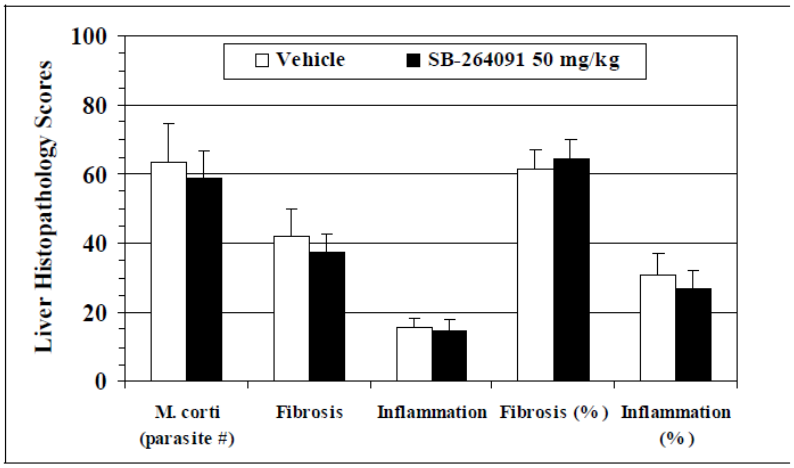


(Excerpted from the Sponsor's submission)

There was no difference in the average number of parasites per liver section present in the vehicle-control and 50 mg/kg SB-264091 groups (63.7 and 58.6 parasites, respectively) on day 49. These numbers were obtained irrespective of the mass or volume of the liver. Numbers of parasites encapsulated or surrounded by inflammatory cells infiltrates were expressed as percentage of the total number of parasites present in individual slides. The average percentage of parasites surrounded by a fibrous capsule or inflammatory cell infiltrates were 61.2% and 30.5% in vehicle-control and 64.5% and 26.8% in the 50 mg/kg SB-264091 group, respectively. There were areas of multifocal-to-coalescing mild-to-moderate areas of coagulative necrosis of the hepatocytes in both the vehicle-control and 50 mg/kg SB-264091 groups on day 49 that were considered a consequence of impediment of the vascular flow due to parasite migration or fibrosis.

Figure 25 Effect of *M. corti* infection on liver pathology in the vehicle-control and 50 mg/kg SB-264091 groups

Figure 9 Effect of *M. corti* Infection on Liver Pathology



(Excerpted from the Sponsor's submission)

Antibodies directed against IL-5, such as SB-264091, have been found to be effective in blocking recruitment, activation, and proliferation of eosinophils. Since recruitment of eosinophils play an important role in host resistance against helminth infections, the potential effects of SB-264091 on host resistance to a model helminth infection, *M. corti*, were evaluated. The data in the present study indicated that SB-264091 treatment significantly reduced the eosinophilic response to *M. corti* infection on day 21, but did not adversely alter other aspects of inflammatory responses, parasite burden in peritoneum or liver, or survival. Thus, host defense was not impaired by treatment with SB-264091. This contrasted to the positive control, dexamethasone, which attenuated the inflammatory responses in *M. corti* infected mice and resulted in significant mortality.

Anti-SB-264091 antibodies were not measured in the present study. Evidence of the pharmacological action of SB-264091 was evident on day 21 based upon the reduced eosinophilic response to infection on day 21; however, the eosinophilic responses on days 34 and 48 were comparable between infected vehicle-control and SB-264091-treated groups. In contrast, numbers of peritoneal eosinophils were reduced in SB-264091-treated groups at all time points relative to the infected vehicle-control group. It was unclear if anti-SB-264091 antibodies reduced or eliminated the pharmacological action of SB-264091 on days 34 and 48.

This study was not conducted with the clinical candidate, mepolizumab, and only provides a general hazard assessment of anti-IL-5 antibodies.

11 Integrated Summary and Safety Evaluation

Mepolizumab (NUCALA™) is a humanized monoclonal antibody (IgG1 kappa), specific for interleukin-5. NUCALA™ is indicated for add-on maintenance treatment in patients aged 12 years and older with severe eosinophilic asthma as identified by blood eosinophils greater than or equal to 150 cells/μL at initiation of treatment or blood eosinophils greater than or equal to 300 cells/μL in the past 12 months.

IL-5 acts on target cells by binding to its specific receptor (IL-5R). The IL-5R consists of a unique α chain (IL-5Rα/CD125) and the common cytokine b-chain (bc/CD131) and is expressed on cells in various lineages, where it transduces signals for multiple functions. The IL-5Rα specifically binds to IL-5 and the b-chain is a molecule shared with other cytokine receptors, including IL-3R and granulocyte/macrophage colony-stimulating factor (GM-CSF) receptor. The b-chain alone does not bind any cytokines, has a relatively long cytoplasmic portion and several functional domains, and is deeply involved in the signal transduction. The cytoplasmic region of IL-5Rα, particularly the membrane-proximal proline-rich sequences, is required for IL-5-induced cellular proliferation and signal transduction.

Figure 26 Signal transduction pathways of IL-5 in eosinophils (From: International Immunology, Vol. 21, No. 12, pp. 1303–1309)

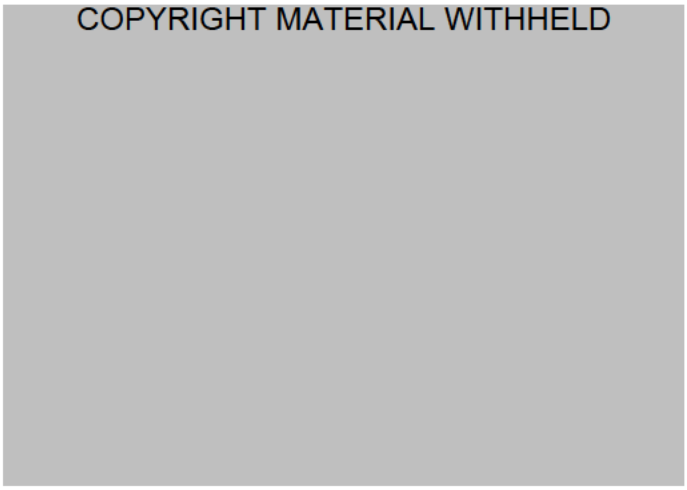


Fig. 1. Signal transduction pathways of IL-5 in the eosinophils. IL-5 and its two receptor chains (IL-5Rα and βc) are shown in the cell membrane, and signals in the cytoplasm and nucleus are indicated. Red ovals represent signaling molecules and purple rectangles show the effect of each signal. STAT* indicates STAT1, STAT3 or STAT5. Molecules specific to IL-5 signaling in B cells or reported only in B cells, such as Btk and Vav, are omitted.

In humans, the biologic effects of IL-5 are best characterized for eosinophils. Eosinophils diverge from hematopoietic stem cells (HSCs). The effects of IL-5 on eosinophils largely fall into four categories, namely differentiation, migration, activation and survival. As for differentiation, IL-5 is not an inducer of eosinophil lineage commitment but rather an enhancing factor for differentiation and proliferation of eosinophil progenitors (EoPs).

Allergic diseases, including asthma, are characterized by inflammation with pronounced infiltration of eosinophils and CD4+ T cells. Regarding the inflammatory cells implicated in asthma, recruitment of CD4+ Th2 cells and eosinophils is a central feature of the late-phase allergic response (LAR). Accumulating evidence indicates that classical Th2-cell-derived cytokines (e.g., IL-3, IL-4, IL-5, IL-9, IL-13 and GM-CSF) together with eotaxin play critical roles in the induction of airway hyper-reactivity and the development of chronic airway wall remodeling.

Pharmacology:
The IL-5R consists of both α and β subunits. The α subunit recognizes IL-5 while the β subunit recognizes the IL-5/α subunit complex and is required for signal transduction. Mepolizumab inhibited binding of IL-5 to its receptor with an IC_{50} value <1.0 nM. The binding affinity of mepolizumab for human IL-5 ranged from 110 to 258 pM.

Inhibition of IL-5-dependent eosinophil differentiation by mepolizumab was measured. Human bone marrow mononuclear cells were incubated with 10 ng/mL IL-5 in the presence of increasing concentrations of mepolizumab over a 14- or 21-day period. There was a dose-dependent increase in the inhibition of eosinophil differentiation in the presence of increasing concentrations of mepolizumab at both time points. At both time points ≥70% inhibition was achieved with 100 pM antibody.

The ability of mepolizumab to inhibit exogenous IL-5-induced proliferation of the murine IL-5/IL-3 dependent LyH7.B13 cell line and human IL-5 responsive TF-1.28 cell line was examined. Recombinant murine and human IL-5 and supernatant from stimulated dog spleen cells induced the proliferation of both LyH7.B13 and human TF-1.28 cells. Supernatant from stimulated rat and rabbit spleen cells induced the proliferation of LyH7.B13 cells. Mepolizumab produced a concentration-dependent inhibition of recombinant human IL-5 induced proliferation (the IC_{50} was between 0.01 and 0.1 nM). For samples from nonclinical test species (i.e., recombinant mouse IL-5 and supernatants from stimulated dog, rat, and rabbit spleen cells) that induced proliferation of either B-13 and or TF-1.28 cells, addition of mepolizumab at concentrations of 1 or 10 nM failed to inhibit cell proliferation. The mouse was judged to not be a pharmacologically relevant species for mepolizumab. Available data with stimulated spleen cell supernatants appeared to suggest that dog, rat, and rabbit were not pharmacologically relevant species.

The ability of mepolizumab to inhibit exogenous IL-5-induced proliferation of the murine IL-5/IL-3 dependent LyH7.B13 cell line and human IL-5 responsive TF-1.28 cell line was examined. Cells were treated with recombinant monkey or human IL-5. The EC_{50} values for both human and monkey IL-5, as measured by the proliferative responses of mouse LyH7.B13 and human TF-1.28 cells, were 0.7-6 pM. In addition, the ability of mepolizumab to inhibit exogenous IL-5-induced differentiation of eosinophils, obtained from the bone marrow of healthy human volunteers or Cynomolgus monkeys, was examined. Both recombinant human and monkey IL-5 were equipotent in causing the differentiation of human and monkey eosinophils from cultures of bone marrow

mononuclear cells with EC_{50} values of 13.3 pM. Mepolizumab inhibited the biological activity of recombinant human and monkey IL-5 in all assays and was equally effective against both cytokines with IC_{50} values of 70-116 pM. It is noted that the amino acid sequence of monkey IL-5 differs from human IL-5 by two conservative substitutions in a region not related to the presumed mepolizumab binding epitope on hIL-5. Thus, it might be expected that mepolizumab would cross react between the two species and inhibit the biological activity of both human and Cynomolgus IL-5. The Cynomolgus monkey was judged to be a pharmacologically relevant species for mepolizumab.

Four female Cynomolgus monkeys received a 1 mg/kg intravenous dose of mepolizumab (SB240563) and approximately three months later a 1 mg/kg subcutaneous dose. Mepolizumab was completely bioavailable following subcutaneous administration. The observed mean terminal half-life (14.5 days) following SC administration was similar to that (13.1 days) obtained following IV administration. Mepolizumab reduced circulating eosinophil count at a dose of 1 mg/kg. A time lag was noted between the plasma mepolizumab concentration-time profile and the eosinophil count data. This observation was consistent with an indirect pharmacologic response. Following subcutaneous administration of mepolizumab, peripheral eosinophil counts decreased in a time dependent manner. The maximum percent decrease (81 to 96%) relative to baseline was observed at three weeks postdose, while maximal concentrations were observed at 2 to 4 days postdose.

The efficacy of mepolizumab was evaluated in an acute model of asthma in Cynomolgus monkeys. Monkeys received either vehicle or 10 mg/kg mepolizumab by intravenous injection on Study Day 1. At 24 hr postdose and 3 and 6 weeks postdose, the monkeys received an aerosol challenge to *A. suum*. The vehicle had no effects on the bronchoconstrictor response or the pulmonary eosinophilia in response to *A. suum* challenge. Mepolizumab at 10 mg/kg had no effect on the bronchoconstrictor response to *A. suum* challenge as measured by R_L and C_{DYN}. Mepolizumab at 10 mg/kg had no effects on pulmonary eosinophilia at 24-hr postdose; however, marked inhibition of pulmonary eosinophilia in response to *A. suum* challenge was observed at 3- and 6-weeks postdose.

Safety pharmacology:
Potential cardiovascular, respiratory, and renal effects of mepolizumab (SB240563) were assessed in three male Cynomolgus monkeys that received intravenous doses of the vehicle (twice) and mepolizumab at 10 and 100 mg/kg. There was one week between each treatment. Mepolizumab at single intravenous doses up to 100 mg/kg had no effects on mean arterial pressure, heart rate, respiratory rate, urinary pH and water, osmolality, sodium, potassium, chloride, and creatinine excretion rates. There were no treatment-related effects on total urinary water, osmolality, sodium, potassium, chloride, and creatinine excretion.

ADME:
For Cynomolgus monkeys, mepolizumab following IV administration had a half-life ranging from 8.97 to 13.9 days. Volume of distribution values ranged from 65.7 to 74.9 mL/kg, which were slightly greater than the plasma volume in monkeys (45 mL/kg).

Mean clearance values ranged from 0.156 to 0.219 mL/hr/kg and slightly decreased with increasing dose of mepolizumab. Bioavailability of mepolizumab following subcutaneous administration was high (close to 100%).

General Toxicology:
In a 1-month toxicology study, Cynomolgus monkeys received IV doses of mepolizumab at 0.05, 0.5, 5, and 50 mg/kg on days 1 and 29. Monkeys were followed for 120 days after the first dose. Administration of mepolizumab at doses of 5 and 50 mg/kg produced significant suppression (89-94%) of eosinophil counts by day 13 or 27. Following the second dose of mepolizumab on day 29, eosinophil counts in monkeys that received 5 or 50 mg/kg remained suppressed (82-88%) through day 77. Eosinophil counts by day 99 had returned to pre-drug values in most monkeys that received mepolizumab at 5 or 50 mg/kg. However, mean eosinophil counts for some monkeys that received 50 mg/kg remained decreased compared to baseline and concurrent controls. The pharmacodynamic action of mepolizumab (decreased eosinophil counts) persisted for more than 5 half-lives (>95% elimination of drug), although evidence of reversibility was demonstrated. As noted in the 6-month toxicology study, mepolizumab appeared to act in the bone marrow to block maturation and/or release of eosinophils from the bone marrow and not depletion by mepolizumab of eosinophil lineage cells. Mepolizumab at doses ≥0.5 mg/kg suppressed recombinant human IL-2-induced eosinophilia. These monkeys were not sacrificed, but allowed a "6-month recovery period" and used in the 6-month toxicology study.

In a 6-month toxicology study, Cynomolgus monkeys received mepolizumab (SB240563) at a subcutaneous dose of 10 mg/kg or an intravenous dose of 10 or 100 mg/kg once every 4 weeks for a total of 7 doses (i.e., administration of mepolizumab on days 1, 29, 59, 85, 114, 141, and 169). Control animals received both an intravenous dose and a subcutaneous dose of the vehicle at each time point. Eosinophil counts were decreased (up to 95% inhibition) for all male and female treatment groups from days 29 through 169. Neutrophil counts were increased for male treatment groups from days 29 through 169; however, dose-response relationships were not present or were flat at all time points. Neutrophil counts were increased for females in the 100 mg/kg IV group on days 114, 141, and 169. Evaluation of bone marrow suggested a block of maturation and/or release of eosinophils from the bone marrow and not depletion by mepolizumab of eosinophil lineage cells. Histopathological examinations of organs and tissues did not identify any target organs of toxicity. Mepolizumab at IV doses up to 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks had no effects on male or female fertility based upon no adverse findings from histopathological examinations of male and female reproductive organs. C_{max} and AUC values for the 10 and 100 mg/kg IV groups were dose proportional. C_{max} and AUC values for the 10 mg/kg SC and 10 mg/kg IV groups were generally comparable suggesting high bioavailability following subcutaneous administration. SB240563 was detectable in the bronchoalveolar fluid from all treatment groups. Three control monkeys (M1044, M1046, and F1048) were detected with anti-SB240563 antibodies on day 169. The NOAEL was identified as the IV dose of 100 mg/kg q4 weeks or SC dose of 10 mg/kg q4 weeks based upon no treatment-related histopathological findings in any organs or tissues. Systemic exposure

at 100 mg/kg q4 weeks was 808975 µg·hr/mL. This exposure provides a large safety margin.

Table 51 Safety margins for a clinical dose of 100 mg SC q4weeks

26-week toxicology study with monkeys (SB240563/RSD-100X0L/1)	AUC µg*hr/mL	Safety margins for a clinical dose of 100 mg q4 weeks (AUC = 357 µg*day/mL or 8568 µg*hr/mL)[1]
NOAEL = 100 mg/kg q4 weeks	808975	94.4

[1.] From the Clinical Pharmacologist, Dr. Yunzhao Ren, only population PK analysis was available for the SC dose of mepolizumab at 100 mg. The estimated systemic clearance ranged from 0.280 to 0.326 L/day for subjects weighing 70 kg. This can be translated into an $AUC_{0\text{-}inf}$, following a single SC dose of mepolizumab at 100 mg, to 0.307 to 0.357 g*day/L (or *1000 for µg*day/mL) for subjects weighing 70 kg.

Genetic Toxicity:
Not applicable for a therapeutic biological protein produced by recombinant DNA technology.

Carcinogenicity:
The carcinogenic potential of mepolizumab and the need for a carcinogenicity study were discussed with Drs. David Jacobson-Kram, Abby Jacobs, and Paul Brown of the Executive Carcinogenicity Assessment Committee in emails dated May 22 and 23, 2008.

Principles issues of the discussion were as follows:

1. In a 6-month toxicology study with Cynomolgus monkeys that received mepolizumab by the intravenous or subcutaneous route, there was no evidence of pre-neoplastic or neoplastic lesions.

2. The species specificity of mepolizumab was restricted to human and nonhuman primate IL-5 only. Mepolizumab did not bind to mouse or rat IL-5. Thus, a rodent carcinogenicity study with mepolizumab did not appear to be technically feasible.

3. Per the ICH S6 (R1) Guidance (finalized in 2011), a study with a murine surrogate anti-IL-5 antibody was not required. Results of the 6-month toxicology study with monkeys do not suggest the need for such a study.

4. IL-5 knockout mice are reported to be viable and generally healthy. The IL-5 knockout mouse has not been systemically studied for the development of tumors (animals have not been followed on a lifetime basis to assess development of tumors). Further, the relevance of this model was questioned given that these animals still possess high levels of eosinophils although their functionally is altered compared to the wild-type mice. In contrast, monkeys and humans treated with mepolizumab shown significant reductions of eosinophil counts.

5. Evaluation of the published scientific literature regarding the roles for IL-5 and eosinophils in cancer

Although recent publications have proposed roles for IL-5 and eosinophils in immune tumor surveillance; conversely, other references have reported a tumor-supporting role for eosinophils. Further, eosinophils themselves can express transforming growth factor-α (TGF-α) and potentially contribute to tumor-associated pathological responses.

- Innate non-T IL-5–producing cells localized most abundantly in the lung and proliferated and upregulated IL-5 production in response to IL-25 and IL-33. IL-33 was more effective than IL-25. These cells contribute to maintaining sufficient numbers of lung eosinophils and are important for eosinophil recruitment mediated by IL-25 and IL-33. Given that eosinophils are shown to possess antitumor activity, lung tumor metastasis was studied in IL-5/Venus KI mice and it was found that innate IL-5-producing cells were increased in response to tumor invasion, and their regulation of eosinophils was critical to suppress tumor metastasis. Genetic blockade or neutralization of IL-5 impaired eosinophil recruitment into the lung and resulted in increased tumor metastasis. Conversely, exogenous IL-5 treatment resulted in suppressed tumor metastasis and augmented eosinophil infiltration. These results suggested that innate IL-5–producing cells play a role in tumor surveillance through lung eosinophils and may contribute to development of novel immunotherapies for cancer (The Journal of Immunology 188: 703–713, 2012).

- Two recently-published papers have proposed roles for IL-5 and eosinophils in immune tumor surveillance (Journal of Immunology 160: 345-350, 1998; Journal of Leukocyte Biology 79: 1131-1139, 2006). These publications suggest that eosinophils are part of an early inflammatory reaction at the site of tumorigenesis and, when recruited into tumors, can very effectively eradicate transplantable tumors. It was also found that methylcholanthrene (MCA)-induced tumor incidence and growth were significantly attenuated in IL-5 transgenic mice (over-expression of IL-5) of both the BALB/c strain and C57BL/6 background. Histological examination revealed that the protective effect of IL-5 was associated with massively enhanced numbers of eosinophils within and surrounding tumors.

- Conversely, it was reported that the plasmacytoma, J558L, and the mammary adenocarcinoma, TS/A, were transfected with an expression vector encoding the mouse gene for IL-5. Injection of parietal cells, mock transfectants, and IL-5 producing cells into syngeneic mice showed that local IL-5 secretion indeed induced rapid tumor infiltration by eosinophils as evidenced by immunohistochemical staining, but did not alter the tumor growth kinetics of IL-5 transfectants. It was concluded that the presence of both IL-5 and eosinophils did not suppress tumor growth (European Journal of Immunology 23: 992-995; 1993).

- In another study, MCA205 cells were transfected with the IL-5 gene, resulting in MCA205-IL5. IL-$5^{-/-}$ and wild-type mice were injected with 2.5 x 10^5 cells/mouse of the bulk culture. Analysis of the tumor growth showed no change in the tumorigenicity between parental and IL-5 transduced tumor cells in either wild-type or IL-$5^{-/-}$ mice (Journal of Immunology 160: 345-350, 1998).

- There are no known reports of IL-5 deficient mice developing an increased rate or risk of neoplasia as compared to wild-type control mice ((b) (4) however, there is no publication reporting that IL-5 knockout mice have been systemically studied for the development of tumors (i.e., knockout mice have not been followed on a lifetime basis to assess development of tumors versus wild-type mice). Mice deficient in IL-5, although lacking in eosinophilia, exhibit normal antibody and cytotoxic T-cell responses, indicative that if anti-tumor CTL responses are needed, they would likely be functional even in the absence of systemic IL-5 (Immunity 4: 15-24, 1996).

- It has been reported that host-derived IL-5 promoted malignant pleural effusions (MPE) in C57BL/6 mice following intraperitoneal injections of Lewis lung cancer (LLC) or colon (MC38) adenocarcinoma cells using wild-type (IL-$5^{+/+}$) and IL-5-deficient (IL-$5^{-/-}$) mice. Exogenous IL-5 promoted MPE formation in both IL-$5^{+/+}$ and IL-$5^{-/-}$ mice while anti-IL-5 antibody treatment limited experimental MPE in IL-$5^{+/+}$ mice (Stathopoulos et al., American Journal of Respiratory and Critical Care Medicine 2010).

- Studies have reported both favorable and unfavorable progress for patients with tumors exhibiting tumor-associated tissue eosinophilia (TATE). In attempting to elucidate the potential role of eosinophils in squamous cell carcinoma development, a carcinogen (7,12-dimethylbenz(a)anthracene [DMBA])-induced hamster oral cancer model was utilized. Eosinophils were determined to progressively infiltrate into this model, and when ablated by the use of an anti-interleukin-5 monoclonal antibody, resulted in a smaller tumor burden and delayed onset of tumor development as compared with control animals. It was proposed that eosinophils may have a tumor-promoting role in that eosinophils have been demonstrated to express TGFα. Transgenic mice overexpressing TGFα develop mammary and lacrimal tumors as well as demonstrating accelerated development of DMBA-induced mammary tumors (Oral Oncology 35: 496-501, 1999; American Journal of Pathology 137: 1425-1434, 1990; Cancer Research 52: 389-393, 1992). It has also been reported that human eosinophils also express TGF-α and may contribute to physiological, immunological, and pathological responses (Journal of Experimental Medicine 172: 673-681, 1990).

- IL-5 has been reported to show activity on B-cells in mice, but not in humans (McKenzie et al 1991). Murine and human IL-5 polypeptides exhibit 70% sequence homology and display species-specific activity to some extent. While

the cytokine (IL-5) induces eosinophilic production and activation in both species, murine IL-5 has additional activity on B-cells in mice. To this end, even though the interactions of IL-5 with its receptor may be similar between mouse and human, differences in downstream activity may make the mouse of more limited relevance for the assessment of human safety.

• Literature references indicate, based principally on the mechanism of action and/or pharmacologic/toxicologic properties of IL-5, that certain differences in activities of IL-5 exist between mice and humans suggesting that the mouse model may have limited relevance regarding the assessment of projected safety in humans.

It was concluded that a carcinogenicity study with mepolizumab was not required. Key issues were the lack of pre-neoplastic or neoplastic findings in the 6-month toxicology study with monkeys and given that the species specificity of mepolizumab was limited to humans and Cynomolgus monkeys, a rodent carcinogenicity study did not appear to be technically feasible.

Reproductive Toxicity:
Reproductive toxicity studies included assessments of effects on male and female fertility in the 6-month toxicology study with mepolizumab in Cynomolgus monkeys; male and female fertility in CD-1 mice treated with SB264091, a rat anti-human IL-5 surrogate monoclonal antibody that cross reacts with human and murine IL-5; pre- and post-natal development in a study using pregnant female Cynomolgus monkeys treated with mepolizumab; and an embryofetal development in a study using male and female CD-1 mice treated with SB264091. The literature for IL-5 knockout mice was also evaluated.

Potential effects of mepolizumab on fertility were assessed on adult male and female monkeys treated for 6 months. Mepolizumab at IV doses up to 100 mg/kg q4 weeks or a SC dose of 10 mg/kg q4 weeks had no effects on male or female fertility based upon no adverse findings from histopathological examinations of male and female reproductive organs.

Potential effects on fertility were also evaluated with male and female CD-1 mice treated with SB264091, a rat anti-human IL-5 surrogate monoclonal antibody (lgG2b), which cross reacts with human and murine IL-5. Male and female mice were treated with IV doses of SB-264091 at 0, 0.5, and 50 mg/kg/week. Male mice (F_0) were dosed on day 1 of the study and continued on a once-weekly basis until termination (for a total of 5 to 6 weekly doses). Female mice (F_0) were dosed 1 time per week for 2 weeks preceding cohabitation, 1 time per week during cohabitation (2 weeks maximum) until mated, and on gestation days 0, 7, and 14 (for a total of 5 to 6 weekly doses). Mating and pregnancy incidences were unaffected with doses up to 50 mg/kg/week.

In a pre- and postnatal development study, 12 pregnant female Cynomolgus monkeys/group received mepolizumab (SB240563) at intravenous doses of 0, 10, or 100 mg/kg once per month on gestation days 20, 50, 80, 110, and 140. These animals

were allowed to naturally deliver their offspring. The development of offspring was followed for 9 months after birth. Surviving animals and their offspring were returned to the primate colony at the end of the study. There was no evidence of treatment-related maternal toxicity or effects on pregnancy outcome or natural delivery. Eight or nine monkeys from each group gave birth to live offspring (1 infant per mother). Offspring were passively exposed to mepolizumab *in utero* through transplacental transfer or consumption of breast milk after birth, and drug was detectable in infant plasma through 6 months post-partum. There was no evidence of treatment-related effects on physical, hematologic, or immunologic development of offspring. Following immunization with Tripedia®, there were no treatment-related effects on development of specific titers to the vaccine components. Anti-mepolizumab antibodies were detected in one mother and its infant from the 10 mg/kg group. Mepolizumab at intravenous doses ≤100 mg/kg administered to mothers had no effects on the pre- and postnatal development of their offspring up to 9 months after birth. The present study is considered adequate; however, it should be noted that this study was small with only 8 or 9 infant monkeys per group for assessment. Visual and physical examinations of infants did not identify any malformations. Infants were not examined for skeletal or visceral malformations and variations. The results of this study should be judged with caution given the limitations in study design and resources.

Table 52 Safety margins for a clinical dose of 100 mg SC q4weeks relative to the dose of 100 mg/kg q4 week in the PPND study with monkeys

PPND Study in Cynomolgus monkeys (CD2003/01020/00)	AUC µg*hr/mL	Safety margins for a clinical dose of 100 mg q4 weeks (AUC = 357 µg*day/mL or 8568 µg*hr/mL)
NOAEL = 100 mg/kg q4 weeks	254111	30

Fertility and embryofetal development were evaluated with male and female CD-1 mice treated with SB264091, a rat anti-human IL-5 surrogate monoclonal antibody (lgG2b), which cross reacts with human and murine IL-5. Male and female mice were treated with IV doses of SB-264091 at 0, 0.5, and 50 mg/kg/week. Male mice (F_0) were dosed on day 1 of the study and continued on a once-weekly basis until termination (for a total of 5 to 6 weekly doses). Female mice (F_0) were dosed 1 time per week for 2 weeks preceding cohabitation, 1 time per week during cohabitation (2 weeks maximum) until mated, and on gestation days 0, 7, and 14 (for a total of 5 to 6 weekly doses).

Circulating concentrations of SB264091 as well as formation of anti-SB264091 antibodies in male and female mice that received up to 5 or 6 weekly doses were not examined. The only available data was to extrapolate from measurements of ADA titer and eosinophil counts collected from female mice that received 1, 2, or 3 weekly doses of 5 or 50 mg/kg/week in a dose range finding study (SB240563/RSD-100MZP/2). No data was available for the dose of 0.5 mg/kg/week. For female mice in the 5 or 50 mg/kg/week groups that had received 1 to 3 doses of drug (on days 1, 8, and 15), eosinophil counts were decreased on days 8, 15, and 29. The pharmacodynamic (PD) effect (i.e., decreased eosinophils) persisted through day 29, although the last dose of SB264091 was on day 15. It can generally be inferred that the PD effect for the 50 mg/kg/week group persisted through at least day 29, which was judged to be adequate.

In the 50 mg/kg/week group, 1 female (#7823) was found dead on day 28, one day after the 3rd dose, and another female (#7820) was found dead on GD 7, one day after the 5th dose. The Sponsor attributed these deaths to anaphylactoid reactions, although no investigations were conducted to definitively determine causes of death. Body weights were unaffected in male and female mice that received doses up to 50 mg/kg/week. Mating and pregnancy incidences were unaffected with doses up to 50 mg/kg/week. Estrous cycle duration was unaffected with doses up to 50 mg/kg/week. Weights of male reproductive organs and sperm concentration and motility were unaffected in male mice that received doses up to 50 mg/kg/week. SB-264091 was not teratogenic in CD-1 mice that received a dose of 50 mg/kg/week. It must be noted that SB-264091 is not the clinical candidate (mepolizumab). This surrogate anti-IL-5 monoclonal antibody provides a qualitative hazard assessment; however, it would not be appropriate to extrapolate exposure multiples relative to the clinical exposure.

Colbert et al. (Contemporary Topics in Laboratory Animal Science 44: 53-55, 2005) reported decreased size and increased mortality rates in IL-5-deficient mice versus IL-5 heterozygous and wild-type mice (i.e., for IL-5 deficient mice nursed by IL-5 deficient mothers, there was suppression of body weight gain and increased mortality that appeared to be linked to impaired maturation of the mammary glands). Mammary tissue from IL-5-deficient dams appeared to have fewer terminal end buds, less-well developed branching of the mammary ducts, and lower overall density of mammary gland structures. In a telephone conversation with Dr. James Lee, the senior author of the paper, these histopathological changes in IL-5 knockout mice were evident in the mammary gland during the pre-lactation period. The pre- and postnatal developmental study with monkeys covers this period and there were no apparent abnormalities of lactation. Thus, these findings in IL-5 knockout mice were not considered relevant to the clinical situation.

Other Toxicity Studies:
The distribution of mepolizumab binding to normal human tissues was assessed by immunocytochemical analysis. Staining was observed with bone marrow; weak to moderate staining was observed on cells of granulocyte lineages (eosinophils and/or neutrophils) present in the samples. These findings are generally consistent with the results of the 6-month toxicology study with monkeys. Staining was observed throughout the sections of lymph nodes tested. In the spleen, staining was observed in reticular cells or macrophages in the red pulp, and intense staining was seen in granulocytes (eosinophils and/or neutrophils). Equivocal to weak staining was observed in the white pulp. Staining in the tonsils was predominantly seen in the lymphoid cells in perifollicullar regions. Moderate staining was observed on dendritic cells in submucosa, germinal centers, and intraepithelial locations of the tonsil.

Antibodies directed against IL-5, such as SB-264091, have been found to be effective in blocking recruitment, activation, and proliferation of eosinophils. Since recruitment of eosinophils plays an important role in host resistance against helminth infections, the potential effects of SB-264091 on host resistance to a model helminth infection,

Mesocestoides corti (*M. corti*), were evaluated. SB-264091 is a rat monoclonal antibody (IgG2b) directed against human interleukin-5 (IL-5) and cross-reacts with murine IL-5. SB-264091 was administered by the intraperitoneal route at doses of 0 (vehicle), 0.5, or 50 mg/kg to female C57BL mice (30/group) on study days 1, 8, 15, 22, 29, 36, and 43. Mice in the positive control groups received dexamethasone that was administered by the intraperitoneal route on study days -2, 1, 5, and 8, and then twice per week throughout remainder of study. On study Day 2, mice in Groups 2, 4, 5, and 6 were infected by the intraperitoneal route with approximately 760 tetrathyrida (larvae) of *M. corti* in 0.1 mL of 0.9% buffered saline. The data in the present study indicated that SB-264091 treatment significantly reduced the eosinophilic response to *M. corti* infection on day 21, but did not adversely alter other aspects of inflammatory responses, parasite burden in peritoneum or liver, or survival. Thus, host defense was not impaired by treatment with SB-264091. This contrasted to the positive control, dexamethasone, which attenuated the inflammatory responses in *M. corti* infected mice and resulted in significant mortality. Anti-SB-264091 antibodies were not measured in the present study. Evidence of the pharmacological action of SB-264091 was evident on day 21 based upon the reduced eosinophilic response to infection on day 21; however, the eosinophilic responses on days 34 and 48 were comparable between infected vehicle-control and SB-264091-treated groups. In contrast, numbers of peritoneal eosinophils were reduced in SB-264091-treated groups at all time points relative to the infected vehicle-control group. It was unclear if anti-SB-264091 antibodies reduced or eliminated the pharmacological action of SB-264091 on days 34 and 48. This study was not conducted with the clinical candidate, mepolizumab, and only provides a general hazard assessment of anti-IL-5 antibodies.

IL-5 Knockout Mice:
Kopf et al. (Immunity 4: 15-24, 1996) generated mice deficient in interleukin-5 (IL-$5^{-/-}$ mice) by gene targeting in embryonal stem cells. No role for IL-5 was evident in the regulation of conventional B (B-2) cells, in normal T cell–dependent antibody responses, or in cytotoxic T cell development. However, CD5+ B cells (B-1 cells) in the peritoneal cavity were reduced by 50-80% in 2-week-old IL-$5^{-/-}$ mice, but returned to normal by 6-8 weeks of age. The IL-5 mice did not develop blood and tissue eosinophilia when infected with the helminth, *Mesocestoides corti*, but basal levels of eosinophils with normal morphology were produced in the absence of IL-5. IL-5 deficiency did not affect the worm burden of infected mice, suggesting that increased eosinophils did not play a significant role in the host defense with this parasite model. IL-5 was essential for the eosinophil parasite-induced eosinophilia, but not for the steady-state production of eosinophils.

Eosinophils have been reported to be a predominant feature of parasitic infections and are thought to play a central role in host defense. However, a search of the literature for IL-5 deficient mice found reports of no changes in the clearance of parasitic infections as compared to wild-type controls, whereas other reports suggested impaired clearance of parasitic infections in IL-5 deficient mice as compared to wild-type controls. The reported differences in clearance of parasitic infections might be related to the specific pathogen. As noted above, Kopf et al. reported no differences in the clearance of *M.*

corti between IL-5 deficient mice and wild-type controls. Similarly, for schistosomiasis infection in wild-type and IL-5 deficient mice, IL-5 regulated processes played only a small role in the development of Th2 responses, susceptibility to primary infections, and the ability to resist subsequent infections (Infect. Immun. 67: 3014-3018, 1999). Vallance et al. (American Journal of Physiology 277 (2 Pt 1): G400-G408, 1999) infected IL-5-deficient mice and their wild-type controls with the nematode, *Trichinella spiralis*. Intestinal parasites and eosinophils were counted, and jejunal longitudinal muscle contractility was assessed. During infection, IL-5 gene expression increased significantly in wild-type mice and was accompanied by significant intestinal eosinophilia in wild-type but not IL-5-deficient mice. Although both strains developed increased muscle contractility during infection, contraction was significantly less in the IL-5-deficient mice at days 16 and 21 postinfection. In addition, parasite expulsion was transiently delayed at day 16 in IL-5-deficient mice. Thus, in the nematode-infected mouse, IL-5 appears essential for intestinal eosinophilia and contributes to, but is not essential for, the development of muscle hypercontractility. IL-5 also appears to play a minor role in expelling a primary *T. spiralis* infection from the gut (American Journal of Physiology 277: G400-G408, 1999 and Parasite Immunology 22: 487-492, 2000). Peripheral and tissue eosinophilia is a prominent feature of enteric nematode infections, such as T. spiralis. Infection of wild-type or IL-5 deficient mice with *T. spiralis* resulted in expulsion of the parasite by day 21. In response to secondary infection, IL-5 deficient mice had little increase in eosinophil numbers within the intestine in comparison with wild-type mice. However, during the course of infection, IL-5 deficient mice developed larger worm burdens and the kinetics of elimination of the parasite were slower than those observed in wild-type mice. Thus, in response to secondary infection with *T. spiralis*, IL-5 played a major role in regulating eosinophilia and contributed to parasite elimination, demonstrating an important role for IL-5 in host defense against this parasite. By contrast, a detrimental role for IL-5 was reported in the early phases of infection with *Toxoplasma gondii* (Infect. Immun. 69: 1044-1052, 2001). After oral administration of this parasite, IL-5 deficient mice were less susceptible to infection, had reduced mortality rates, and less severe morphologic changes in the small intestine.

Recommendation: From the nonclinical perspective, the application is recommended for approval. No additional nonclinical studies are recommended. There are no outstanding nonclinical issues.

Labeling:

Indications and Usage:

Sponsor's Recommended Labeling:

NUCALA is a (b) (4), indicated for add-on maintenance treatment in patients aged 12 years and older with severe (b) (4) asthma (b) (4)

(b) (4)

Reviewer's Evaluation: The established pharmacological classification (EPC) was discussed with Dr. Paul Brown by email on June 17, 2015. The most appropriate EPC for mepolizumab was judged to be interleukin-5 antagonist. Relevant examples in Dr. Brown's email included secukinumab (interleukin-17A antagonist), siltuximab (interleukin-6 antagonist), anakinra (interleukin-1 receptor antagonist), tocilizumab (interleukin-6 receptor antagonist), (b) (4) (b) (4), and basilixumab (interleukin-2 receptor blocking antibody).

Recommended labeling:

NUCALA is a humanized (b) (4) interleukin-5 antagonist (IgG1 kappa), indicated for add-on maintenance treatment in patients aged 12 years and older with (b) (4) eosinophilic asthma (b) (4).

8.1 Pregnancy

Sponsor's Recommended Labeling:

Pregnancy Registry

(b) (4)

(b) (4)

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(b) (4) Risk Summary

(b) (4)

(b) (4)

Data

(b) (4)

Animal Data: (b) (4)

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(b) (4)

Reviewer's Evaluation: Section 8.1 was modified in Consultation with the Maternal Health Team to comply with the Pregnancy and Lactation Labeling Review.

For the 100 mg/kg group in the PPND study with monkeys, SB240563 concentrations in milk were measured in 6 of 7 mothers on postpartum day 14 and found to range from 67 to 328 ng/mL. These concentrations were 0.006 and 0.028% of the plasma concentration (C_{24hr} = 1159 µg/mL) after the 5th dose.

Recommended labeling:

Pregnancy Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to NUCALA during pregnancy. Healthcare providers can enroll patients or encourage patients to enroll themselves by calling 1-877-xxx-xxxx or visiting www.nucalapregnancyregistry.com.

(b) (4)

(b) (4) Risk Summary

There are no data with NUCALA use in pregnant women to inform a drug associated risk. Monoclonal antibodies, such as mepolizumab, are transported across the placenta in a linear fashion as pregnancy progresses; therefore, potential effects on a fetus are likely to be greater during the second and third trimester of pregnancy. In an animal pre- and post-natal development study conducted in cynomolgus monkeys, there was no evidence of fetal harm with administration of intravenous mepolizumab throughout pregnancy at doses that produced exposures up to approximately 30 times the exposure at the maximum recommended human dose (MRHD) of 100 mg *[see Data]*. Consider the benefits and risks of NUCALA when prescribing NUCALA to a pregnant woman.

In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

(b) (4)

Clinical Considerations

Disease-associated maternal and/or embryo-fetal risk

In women with poorly or moderately controlled asthma, evidence demonstrates that there is an increased risk of preeclampsia in the mother and prematurity, low birth weight and small-for-gestational age for the neonate. The level of asthma control should be closely monitored in pregnant women and treatment adjusted as necessary to maintain optimal control.

(b) (4)

Data

Animal data

In a pre- and post-natal development study, pregnant cynomolgus monkeys received mepolizumab from gestation days 20 to 140 at doses that produced exposures up to approximately 30 times that achieved with the MRHD (on an AUC basis with maternal intravenous doses up to 100 mg/kg once every 4 weeks). Mepolizumab did not elicit adverse effects on fetal or neonatal growth (including immune function) up to 9 months after birth. Examinations for internal or skeletal malformations were not performed. Mepolizumab crossed the placenta in cynomolgus monkeys. Concentrations of mepolizumab were approximately 2.4 times higher in infants than in mothers for several months post-partum. Mepolizumab was quantifiable in infant plasma samples continuing through day 178 postpartum. Levels of mepolizumab in milk were 0.006 to 0.028% of maternal serum concentration.

In a fertility, early embryonic and embryofetal development study, pregnant CD-1 mice received an analogous antibody, which inhibits the activity of murine IL-5, at an intravenous dose of 50 mg/kg once per week throughout gestation. The analogous antibody was not teratogenic in mice. Embryofetal development of IL-5 deficient mice has been reported to be generally unaffected relative to wild-type mice.


(b) (4)

(b) (4)

Sponsor's Recommended Labeling:

Risk Summary

(b) (4)

Reviewer's Evaluation: Section (b) (4) was modified in Consultation with the Maternal Health Team to comply with the Pregnancy and Lactation Labeling Review.

Recommended labeling:

Risk Summary

There is no information regarding the presence of mepolizumab in human milk, the effects on the breastfed infant, or the effects on milk production. However, mepolizumab is a humanized monoclonal antibody (IgG1 kappa), and immunoglobulin G (IgG) is present in human milk in small amounts. Mepolizumab is present in the milk of cynomolgus monkeys *[see Use in Specific Populations (8.1)].* The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for NUCALA and any potential adverse effects on the breastfed infant from NUCALA or from the underlying maternal condition.

(b) (4)

11 DESCRIPTION

Sponsor's Recommended Labeling:

(b) (4)

Reviewer's Evaluation: The first sentence of Section 11 was modified to be consistent with the EPC (see above).

Recommended labeling:

NUCALA (mepolizumab) is a (b) (4) ~~IL~~interleukin-5 antagonist.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Sponsor's Recommended Labeling:

(b) (4)

Reviewer's Evaluation: The labeling was modified to include the dissociation constant for mepolizumab. It is noted that the effects of mepolizumab on cells expressing the IL-5 receptor were primarily conducted using the murine IL-5/IL-3 dependent LyH7.B13 cell line and human IL-5 responsive TF-1.28 cell line. A few experiments were conducted using human and Cynomolgus monkey eosinophils to examine the effects of mepolizumab on differentiation. This was considered sufficient to include eosinophils in the labeling (i.e., Mepolizumab binds to IL-5, with a dissociation constant of 100 pM, inhibiting the bioactivity of IL-5 by blocking its binding to the alpha chain of the IL-5 receptor complex expressed on the eosinophil cell surface.)

Recommended labeling:

(b) (4) Mepolizumab binds to IL-5, with a dissociation constant of 100 pM, inhibiting the bioactivity of IL-5 (b) (4) –by blocking (b) (4) –its binding (b) (4) –to the alpha chain of the IL-5 receptor complex expressed on the eosinophil cell surface.,– (b) (4) Mepolizumab, by inhibiting IL-5 signaling, (b) (4) reduces (b) (4) the production and survival of eosinophils. (b) (4) (b) (4)

13 NONCLINICAL TOXICOLOGY

Sponsor's Recommended Labeling:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of mepolizumab. (b) (4)

(b) (4)

(b) (4)

Reviewer's Evaluation: In consultation with the Executive Carcinogenicity Assessment Committee by emails on June 18 and 19, 2015, the labeling in the first paragraph was modified (b) (4) An alternative option might be to just include the first sentence; however, Drs. Brown, Jacob, and Davis-Bruno expressed opinions that the labeling (b) (4)

(b) (4)

(b) (4)

Recommended labeling:

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of mepolizumab. (b) (4)
Published literature using mouse models suggests that IL-5 and eosinophils are part of an early inflammatory reaction at the site of tumorigenesis and can promote tumor rejection. However, other reports indicate that eosinophil infiltration into tumors can promote tumor growth. Therefore, the malignancy risk in humans from an antibody to IL-5 such as mepolizumab is unknown (b) (4)

Male and female fertility were unaffected based upon no adverse histopathological findings in the reproductive organs from Cynomolgus monkeys treated with mepolizumab for 6 months at intravenous doses up to 100 mg/kg once every 4 weeks (approximately 30 times the maximum recommended human dose on an AUC basis). Mating and reproductive performance were unaffected in male and female CD-1 mice treated with an analogous antibody, which inhibits the activity of murine IL-5, at an intravenous dose of 50 mg/kg once per week.

(b) (4)

(b) (4)

12 Appendix/Attachments

None

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

/s/

TIMOTHY W ROBISON
06/29/2015

MARCIE L WOOD
06/29/2015
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 125526 **Applicant: GSK** **Stamp Date: November 4, 2014**

Drug Name: Mepolizumab (NUCALA) **BLA Type: Original BLA**

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		
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).			Not applicable
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 submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?			Not applicable

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

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
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		
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)		X	Will work with CMC Reviewer as needed.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable
12	If this BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable

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

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant. **None**

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

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

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

/s/

TIMOTHY W ROBISON
12/16/2014