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APPLICATION NUMBER:

761070Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date: November 1, 2017
From: Timothy J. McGovern, PhD, ODE Associate Director for
Pharmacology and Toxicology, OND IO
BLA: 761070
Agency receipt date: November 16, 2016
Drug: FASENRA (benralizumab) injection, for subcutaneous use
Sponsor: AstraZeneca Pharmaceuticals LP

Indication: Add-on maintenance treatment of patients with severe asthma aged 12 years and older, and with an eosinophilic phenotype.

Reviewing Division: Division of Pulmonary, Allergy, and Rheumatology Products

The primary pharmacology/toxicology reviewer and team leader concluded that the nonclinical data for FASENRA (benralizumab) solution for subcutaneous injection support approval for the indication listed above.

Benralizumab is a humanized, afucosylated IgG1 kappa monoclonal antibody that targets interleukin-5 receptor alpha. The recommended dose of benralizumab is 30 mg administered once every 4 weeks for the first 3 doses, and then once every 8 weeks thereafter. The Established Pharmacologic Class (EPC) for benralizumab is “interleukin-5 receptor alpha-directed cytolytic monoclonal antibody”.

Pivotal nonclinical studies were conducted in Cynomolgus monkeys. In toxicology studies up to 39 weeks duration, NOAELs of 10 mg/kg IV and 30 mg/kg SC were identified. The primary finding at a dose of 25 mg/kg IV was a post-dose reaction in one high-dose female that included bruising in the areas of the eyes, face, chest and lower abdomen, a decrease in platelet counts, and abnormal erythrocytes after the fourth dose. The NOAEL doses were associated with systemic exposures (AUC) that provided 153- to 271-fold exposure margins compared to the recommended clinical dose.

Genetic toxicity studies were not applicable for this therapeutic biologic protein. Carcinogenicity studies were not conducted since rodents are not pharmacologically relevant species. AstraZeneca submitted a carcinogenicity risk assessment that was evaluated by the CDER Executive Carcinogenicity Assessment Committee. The Committee agreed that a carcinogenicity study was not required. Based on a review of the literature by Dr. Robison, the role of eosinophils in tumor development is unclear. The product label will reflect the outcome of this review.

The effects of benralizumab on fertility were evaluated in cynomolgus monkeys in the 39-week toxicity study; no drug-related effects were observed. An enhanced pre- and post-natal development studies were conducted with cynomolgus monkeys to evaluate benralizumab's effects on development. The NOAEL was the high dose of 30 mg/kg IV; associated with exposure that were ~ 310-times the anticipated clinical exposure. Adult

females and infants demonstrated reduced eosinophil levels. Placental transfer was demonstrated by measuring benralizumab levels in the serum of infants *in utero*.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this BLA can be approved from the pharmacology/toxicology perspective. The EPC for benralizumab is appropriate. I have discussed labeling issues with the Division and agree with the proposed text.

APPEARS THIS WAY ON ORIGINAL

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/s/

TIMOTHY J MCGOVERN
11/01/2017

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 OF
EXTRACTABLES AND LEACHABLES FROM THE CONTAINER CLOSURE SYSTEM
(PREFILLED SYRINGE)

Application number: 761070

Supporting document/s: SDN #1 and 22

Applicant's letter date: November 16, 2016 and July 14, 2017

CDER stamp date: November 16, 2016 and July 14, 2017

Product: Benralizumab [Humanized, afucosylated,
immunoglobulin (Ig)G1κ mAb that targets IL-5Rα]

Indication: Asthma

Applicant: AstraZeneca Pharmaceuticals LP
One MedImmune Way
Gaithersburg MD 20878

Review Division: Pulmonary, Allergy, and Rheumatology Products

Reviewer/Team Leader: Timothy W. Robison, Ph.D., D.A.B.T.

Division Director: Badrul Chowdhury, M.D., Ph.D.

Project Manager: Colette Jackson

Template Version: September 1, 2010

Disclaimer

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1 Executive Summary

1.1 Introduction

Benralizumab is a humanized, afucosylated, immunoglobulin (Ig)G1κ monoclonal antibody (mAb) that targets the alpha subunit of the human interleukin-5 receptor (IL-5Rα), which is expressed on eosinophils and basophils. Benralizumab is indicated as an add-on maintenance treatment for patients with severe asthma aged 18 years and older, with an eosinophilic phenotype. It will be administered by the subcutaneous route using a prefilled syringe.

A prefilled syringe (b) (4) (PFS (b) (4)) is the primary container closure system for the Drug Product. (b) (4)

1.2 Brief Discussion of Nonclinical Findings

The (b) (4) glass syringe with a 29-gauge (b) (4) staked-in needle and a rigid needle shield, which is closed with an elastomeric plunger stopper, is a marketed device that is used with other FDA-approved drug products. The manufacturer has done extraction studies with this device.

The Sponsor conducted a safety evaluation of the prefilled syringe components based on a 3-stage, risk-based strategy to ensure that the product contact components do not leach undesirable amounts of potentially harmful compounds into the Drug Product that may adversely impact patient safety (Stage 1: PFS Forced Extraction Study; Stage 2: Prefilled Syringe Buffer Simulation Study; and Stage 3: Drug Product Leachables and Elemental Impurities Studies).

A variety of analytical techniques were used for the identification and measurement of extractables and leachables that included semi-volatile organic compounds, volatile organic compounds, non-volatile organic compounds, and metals.

In forced extraction studies, high temperature and/or harsh solvents were used with the syringe components, listed above, in order to determine the compounds most likely to be extracted from the container closure components. The compounds and elements identified in Stage 1 were then monitored in the Stage 2 simulation study.

The simulation study was conducted using (b) (4) syringes filled with 1.0 mL of Drug Product (b) (4) (20 mM histidine/histidine-HCl, 250 mM trehalose dihydrate, 0.006% w/v PS-20, pH 6.0). For simulation studies using 38-42°C, 23-27°C, and 2-8°C, the only compound observed above the TTC of 1.5 µg/day was (b) (4)

The leachables study tested 3 lots of the Drug Product (Lots 020F15, 021F15, and 004K15). All syringes were stored in a horizontal position at 2-8°C for testing at 0, 6, 12, 24, and 36 months. Leachables testing through the 12 month time point found that levels of elemental impurities were below the dose-adjusted Permissible Daily Exposure

(PDE) levels established in ICH Q3D Guidance. Levels of (b) (4) were less than the TTC of 1.5 µg/day. Levels of (b) (4) were low and did not raise any safety concerns.

The safety assessment was conducted using a conservative approach of estimating safe exposures to potential leachables based upon daily dosing. However, in actuality, the dosing regimen is every 4 weeks for the first 3 doses followed by once every 8 weeks thereafter.

1.3 Recommendations

1.3.1 Approvability

Leachables from the container closure system appear to pose no significant safety concerns to patients. The leachables study was ongoing at the time of this review and results for later time points will be reported over the product shelf life.

1.3.2 Additional Nonclinical Recommendations

None

2 Drug Information

2.1 Drug

CAS Registry Number: 1044511-01-4

Generic Name: Benralizumab

Code Name: Benralizumab (formerly known as MEDI-563, KHK4563, and BIW-8405)

Molecular Weight: Benralizumab is a recombinant humanized afucosylated IgG1k monoclonal antibody of approximately 150 kDa, including oligosaccharides.

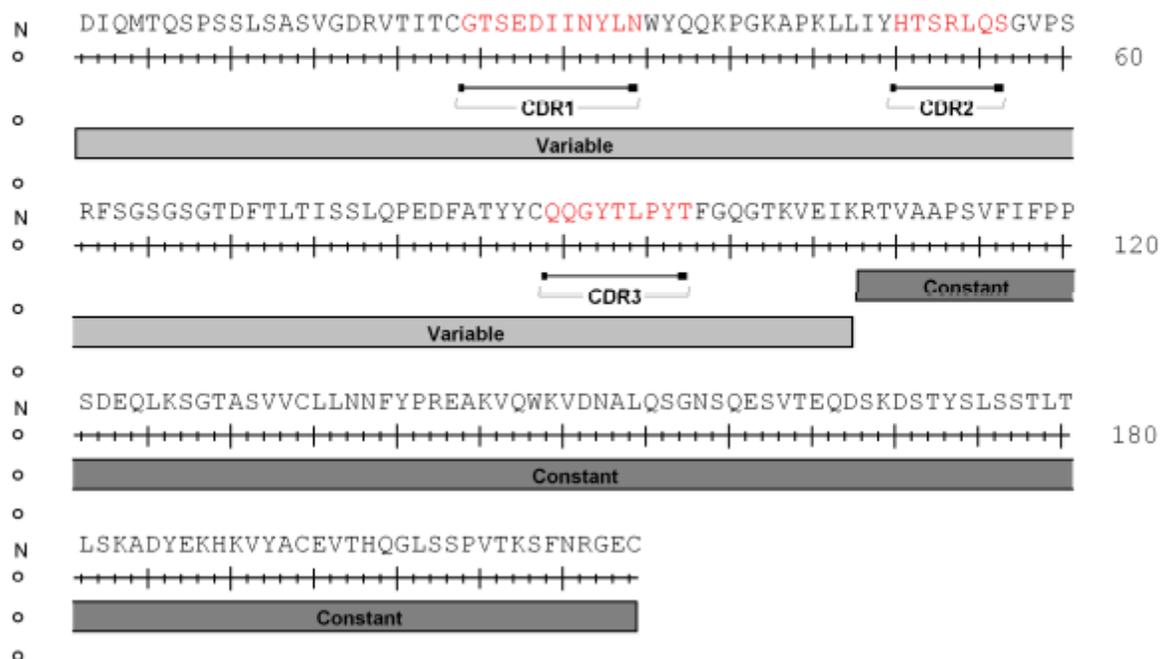
Biochemical description: Benralizumab is recombinantly produced in (b) (4), a Chinese Hamster Ovary (b) (4) cell line which is deficient in α -1,6-fucosyltransferase (FUT8; (b) (4) that is required for the attachment of the monosaccharide fucose to the oligosaccharide chain of benralizumab.

The antibody is composed of two identical heavy chains of approximately 49,400 Da each, and two identical light chains of approximately 23,500 Da each. Benralizumab has primarily N-linked biantennary complex type oligosaccharides attached to each heavy chain at Asn-301, without fucose. As noted above, the expression cell line was engineered to eliminate fucosylation. The average size of the oligosaccharide moiety is approximately 1,500 Da per heavy chain.

Figure 1 Amino acid sequence of benralizumab

Amino Acid Sequence

The amino acid sequence of benralizumab V_L and V_H regions are shown in Figure S.1.2-1 and Figure S.1.2-2, respectively.



Pharmacologic class: Interleukin-5 receptor alpha-directed cytolytic monoclonal antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 100237 (AstraZeneca [MedImmune], benralizumab)

DMFs for primary packaging components:

(b) (4)

Letters of authorization to each DMF were provided in the submission.

2.3 Drug Formulation

The benralizumab Drug Product is a sterile liquid dosage form presented in an accessorized prefilled syringe (APFS) intended for subcutaneous administration. Each syringe contains 30 mg of benralizumab in (b) (4) 1.0 mL volume. The Drug Product contains 30 mg/mL benralizumab in 20 mM histidine/histidine-HCl, 0.25 M trehalose dihydrate, 0.006% w/v polysorbate 20, pH 6.0.

Table 1 Composition of the Drug Product

Ingredient	Concentration	Unit Formula per 30 mg syringe	Purpose	Quality Standard
<i>Active Ingredient</i>				
Benralizumab	30 mg/mL	30 mg	Active	In-house Reference Standard
<i>Excipients</i>				
L-Histidine	9 mM	1.4 mg	(b) (4)	USP/NF; Ph. Eur.; JP
L-Histidine hydrochloride monohydrate	11 mM	2.3 mg		Ph. Eur.; JP
α,α -trehalose dihydrate	0.25 M	95 mg		USP/NF; Ph. Eur.; JP
Polysorbate 20 (b) (4)	0.006% w/v	0.06 mg		USP/NF; Ph. Eur., JP
Water for Injection	Not applicable	Approximately (b) (4) mg		USP/NF; Ph. Eur., JP

JP = Japanese Pharmacopoeia; Ph. Eur. = European Pharmacopoeia; USP/NF = United States Pharmacopoeia/ National Formulary

(Excerpted from the Sponsor’s submission)

(b) (4)

The PFS (b) (4) is the primary container closure system for the Drug Product. (b) (4)

None of the accessories are in contact with the Drug Product solution or part of the fluid path of the delivery system.

(b) (4)

(b) (4)

Table 3 Material components of the (b) (4) syringe barrel

Components	Material of Construction	Additional Characteristics
Syringe barrel	Type I (b) (4) glass	Glass compliant with USP <660> "Container: glass", Ph. Eur 3.2.1 "Glass containers for pharmaceutical use" Type I, and JP 7.01 "Test for glass containers for injection, soluble alkali, test method 1" current editions
Needle	(b) (4)	(b) (4)
Rigid needle shield	(b) (4)	No additional characteristics
Needle shield	(b) (4) elastomer	Formulation is qualified according to biological tests of USP 381. "Elastomeric closures for injection" and Ph. Eur 3.2.9 "Rubber closures, type I" current editions

(b) (4)

(b) (4) JP = Japanese Pharmacopoeia; NF = National Formulary; Ph. Eur. = European Pharmacopoeia; USP = United States Pharmacopoeia

(Excerpted from the Sponsor's submission)

The needle safety shield consists of three components. The needle is covered with a rigid needle shield composed of a (b) (4) elastomeric needle shield (in contact with the needle), which has a rigid (b) (4) outer cap. The (b) (4) elastomeric needle shield complies with USP/Ph. Eur. requirements. The rigid needle shield (b) (4)

Table 4 Material components of the needle safety shield

Components	Material of Construction
Guard	(b) (4)
Body	(b) (4)
(b) (4)	(b) (4)

(Excerpted from the Sponsor's submission)

The extended finger flange (b) (4) material. The plunger rod (b) (4) material. Additional secondary packaging components included the label on the syringe barrel, unit tray, lid stock, package inserts, unit carton, and carton seals.



Figure 3 Accessorized Prefilled Syringe (APFS)



(Excerpted from the Sponsor's submission)

2.4 Comments on Novel Excipients

Trehalose dihydrate is administered at a SC dose of 95 mg (equivalent to (b) (4) mg/m²) in the clinical formulation. See the Review dated July 10, 2017 for a safety assessment of trehalose dihydrate administered by the SC route.

2.5 Comments on Extractables and Leachables

The safety of the prefilled syringe components was evaluated based on a 3-stage, risk-based strategy to ensure that the product contact components do not leach undesirable amounts of potentially harmful compounds into the Drug Product that may adversely impact patient safety (Stage 1: PFS Forced Extraction Study; Stage 2: Prefilled Syringe Buffer Simulation Study; and Stage 3: Drug Product Leachables and Elemental Impurities Studies). A risk assessment was performed at each stage, and the results

were used to inform the next stage. Results from Stage 1 were compared to the available manufacturers' extractables reports.

2.6 Proposed Clinical Population and Dosing Regimen

Benralizumab is an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody indicated as an add-on maintenance treatment for patients with severe asthma aged 18 years and older, with an eosinophilic phenotype. The recommended dose is 30 mg every 4 weeks for the first 3 doses followed by once every 8 weeks thereafter.

2.7 Regulatory Background

A Pre-IND meeting was held with the Sponsor (BioWa, Inc.) in 2005; this meeting was not listed in DARRTS. IND 100237 was submitted to the FDA on June 29, 2006 (received on June 30, 2006).

In a submission dated March 5, 2007, Sponsorship of the IND was transferred to MedImmune (effective March 7, 2007). The product designation was changed from BIW-8405 to MEDI-563.

Comments on the proposed design of the chronic toxicology study with monkeys and enhanced pre-and post-natal development study with monkeys were conveyed to the Sponsor (see Review dated November 28, 2008 and/or Comments conveyed on December 19, 2008).

An EOP2 meeting was held with the Sponsor on February 13, 2013 (see meeting minutes dated March 14, 2013). There was one nonclinical question.

Nonclinical responses for the EOP2 meeting minutes:

Questions for Nonclinical Studies- Pharmacology/Toxicology

Question 3: Does the Agency agree that the nonclinical safety program data to date, including carcinogenicity risk assessments, are sufficient to support initiation of Phase 3 studies and registration of benralizumab for the treatment of adult and adolescent patients as defined in the proposed asthma indication?

FDA Response:

We agree that the nonclinical safety program data to date, including carcinogenicity risk assessments, are sufficient to support initiation of the clinical trials described in the meeting package.

Subjects enrolled in clinical trials should be monitored for potential development of tumors.

It appears premature to discuss registration, although based upon information available at this time, it is unlikely that additional nonclinical studies would be required for the filing of a BLA.

A Pre-BLA meeting was held with the Sponsor on September 20, 2016 (see meeting minutes dated October 27, 2016). There were no nonclinical questions in the meeting package; however, there was one nonclinical comment conveyed to the Sponsor.

Nonclinical comment for the Pre-BLA meeting:

Nonclinical comment:

A safety assessment of leachables (and extractables, as appropriate) with the accessorized prefilled syringe should be included with the BLA.

3 Studies Submitted

3.1 Studies Reviewed

1. FINAL REPORT FOR THE EXTRACTABLES STUDY ON (b) (4) NEEDLE SHIELD ELASTOMERS REVISION 01 (NS-06816856)
2. FINAL REPORT FOR THE FORCED EXTRACTION STUDY ON (b) (4) STOPPERS (NS-06285446)
3. FINAL REPORT FOR THE FORCED EXTRACTION STUDY ON GLASS (b) (4) SYRINGES (NS-06888673)
4. FINAL REPORT FOR AN EXTRACTABLES SIMULATION STUDY ON (b) (4) (b) (4) SYRINGES WITH 1 ML LONG SYRINGE STOPPERS AND (b) (4) RNS (NS-07075186, NS-07169866)
5. STAGE 3 EXTRACTABLES AND LEACHABLES STUDY (EHIVE03-1918845568-19 ED 002)

These study reports were provided in response to an Information Request dated July 10, 2017

3.2 Studies Not Reviewed

None

3.3 Previous Reviews Referenced

Pharmacology and Toxicology Review of BLA 761070 dated July 10, 2017

11 Integrated Summary and Safety Evaluation

The prefilled syringe (b) (4) (PFS (b) (4)) is the primary container closure system for the Drug Product. (b) (4)

(b) (4) None of the accessories are in contact with the Drug Product solution or part of the fluid path of the delivery system. The secondary packaging includes a unit tray and lid stock (b) (4), an opaque paperboard unit carton (b) (4) and package inserts.

The (b) (4) glass syringe with a 29-gauge (b) (4) staked-in needle and a rigid needle shield, which is closed with an elastomeric plunger stopper, is a marketed device that is used with other FDA-approved drug products.

The primary packaging components for the prefilled syringe (b) (4) [(b) (4) 1 mL long syringe] were unchanged between Process 3 Clinical and Process 3 Commercial.

Table 5 Summary of the drug product development

	Process 1	Process 1b	Process 2	Process 3 Clinical	Process 3 Commercial
Dosage form	(b) (4)			Liquid in APFS	
Protein concentration	(b) (4)			30 mg/mL	
Formulation	(b) (4)			20 mM histidine/ histidine hydrochloride, 0.25 M trehalose dihydrate, 0.006% w/v PS-20, pH 6.0	
(b) (4) volume	(b) (4)			1.0 mL	
Primary container	(b) (4)			(b) (4) 1 mL long syringe, 29-gauge thin wall needle, (b) (4) rigid needle shield	
Primary closure	(b) (4)			(b) (4) plunger stopper (b) (4)	
Secondary packaging component or accessory	(b) (4)			(b) (4) needle safety shield, extended finger flange, and plunger rod	

APFS = accessorized prefilled syringe; PS = polysorbate

(Excerpted from the Sponsor's submission)

A material safety assessment was performed on the primary container components by the component manufacturer, (b) (4) certifies that the Drug Product contact materials meet the requirements of the USP/ Ph. Eur./JP and relevant standards.

Syringe Barrel Compliance:

- USP <660>, Containers-glass
- European Pharmacopoeia <3.2.1>, Glass containers for pharmaceutical use
- Japanese Pharmacopoeia <7.01>, Test for glass containers for injection, soluble alkali, test method 1

Needle Compliance:

- ISO 9626 Standard

(b) (4)

- USP <88>. Intracutaneous reactivity. Biological reactivity tests. in vivo

(b) (4)

Needle Shield Elastomer Compliance:

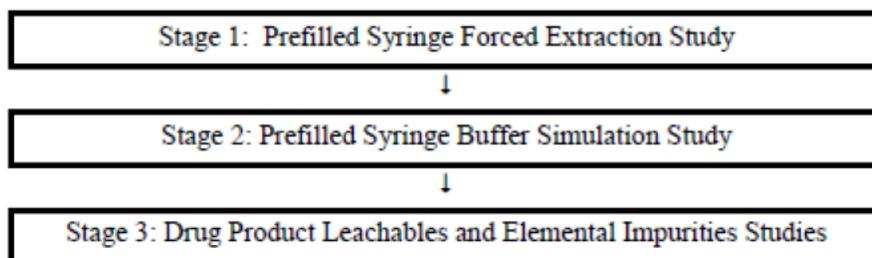
- USP <381>, Elastomeric closures for injection
- European Pharmacopoeia <3.2.9>, Rubber closures, type I

Stopper Elastomer Compliance:

- USP <381>, Elastomeric closures for injection
- European Pharmacopoeia <3.2.9>, Rubber closures, type I

The safety of the prefilled syringe components was evaluated based on a 3-stage, risk-based strategy to ensure that the product contact components do not leach undesirable amounts of potentially harmful compounds into the Drug Product that may adversely impact patient safety (Stage 1: PFS Forced Extraction Study; Stage 2: Prefilled Syringe Buffer Simulation Study; and Stage 3: Drug Product Leachables and Elemental Impurities Studies). A risk assessment was performed at each stage, and the results were used to inform the next stage. Results from Stage 1 were compared to the available manufacturers' extractables reports. These studies were conducted by (b) (4). For each stage, the following analytical techniques were used to identify extractables and leachables: direct injection gas chromatography mass spectrometry (GC/MS) was used for identification and measurement of semi-volatile organic compounds (SVOCs), headspace GC/MS was used for identification and measurement of volatile organic compounds (VOCs), liquid chromatography mass spectrometry (LC/MS) with different detection methods was used for identification and measurement of non-volatile organic compounds (NVOCs), and inductively-coupled plasma (ICP) spectrometry was used for analysis of metals.

Figure 4 Three-stage, risk-based strategy



(Excerpted from the Sponsor's submission)

Stage 1: Prefilled Syringe Forced Extraction Study

Forced extraction studies were performed on the three separate components of the (b) (4) 1 mL long syringes. The components tested were the Type I (b) (4) glass barrel with 29-gauge (b) (4) needle, the syringe tip (b) (4) stainless steel needle adhered to the glass tip of the syringe), (b) (4) plunger stopper, and

(b) (4) elastomeric needle shield. High temperature and/or harsh solvents were used in the studies in order to determine the compounds most likely to be extracted from the container closure components. The analytical techniques described above used to identify and measure extractables in the various extraction solutions. The results were compared to the available manufacturers' extractables reports.

Forced extraction study of the (b) (4) 1 mL long syringe barrel with 29-gauge (b) (4) staked needle: A forced extraction study was performed on the (b) (4) 1 mL long syringe barrel with 29-gauge (b) (4) staked needle (Lots #1305253 and #2262465). The syringes were refluxed for 8 hours in WFI (Water-For-Injection), 0.1% v/v formic acid (pH 2), 0.1 M glycine base (pH 11), 0.2% v/v polysorbate 80 (PS-80), 0.01% polysorbate 20 (PS-20; results not shown in the table below), or 50% ethanol. The solvents selected were chosen to bracket the pH range of the Drug Product and to maximize compounds extracted from the syringe barrels into the extraction solvents. Additionally, a set of syringe tips (Lot #1305253) were separated from the syringes and sonicated in 50% v/v ethanol to facilitate the assessment of (b) (4) and adhesives used in the needle attachment process. The solvents were chosen to bracket the pH range of the Drug Product and to force extraction from the syringes into the solution. The needles (Lot #1305253) were also subjected to a 10% v/v acid matrix composed of 5% concentrated nitric acid and 5% concentrated hydrochloric acid digestion for (b) (4) analysis.

The table below lists the compounds from the extraction solvents having signals greater than the method noise (typically signal to noise ratio greater than (b) (4)). The concentrations were estimated from the method standard and are equivalent to the extrapolated concentration ($\mu\text{g}/\text{dose}$) based on a 1.0 mL dose. No compounds were observed in any extract of either component except in the 50% ethanol sonicate extraction (b) (4)

Only (b) (4) was measured at quantities >TTC and monitored in the Stage 2 simulation s

All Stage 1 results reported in $\mu\text{g}/\text{cm}^2$ or $\mu\text{g}/\text{mL}$ within the reports were converted to $\mu\text{g}/\text{dose}$ by the Sponsor using the total surface area of the individual component listed in the table below. For the results reported in $\mu\text{g}/\text{mL}$, the extraction volume and the total surface area extracted were used to convert to $\mu\text{g}/\text{cm}^2$. The $\mu\text{g}/\text{dose}$ results were reported to show the extractables data in a relevant way to make conclusions about the safety of the container closure system. Benralizumab uses 1 syringe per dose; therefore, results in $\mu\text{g}/\text{syringe}$ equals $\mu\text{g}/\text{dose}$.

Table 6 Prefilled syringe component surface areas

Component	Surface Area (cm ²)
(b) (4) needle shield	(b) (4)
(b) (4) plunger stopper	(b) (4)
Glass barrel with 29-gauge needle	(b) (4)
(b) (4) Stainless steel needle	(b) (4)

(Excerpted from the Sponsor’s submission)

Table 7 Extraction details for the glass barrel and 29-gauge needle

Component	Extraction Volume (mL)	Total Surface Area Extracted (cm ²)	Dilution Factor
Glass barrel with 29-gauge needle	(b) (4)	(b) (4)	(b) (4)
(b) (4) stainless steel needle	(b) (4)	(b) (4)	(b) (4)

(Excerpted from the Sponsor’s submission)

Figure 5 Conversion from µg/mL to µg/dose

$$Result \left(\frac{\mu g}{dose} \right) =$$

$$\frac{Result \left(\frac{\mu g}{mL} \right) \times Extraction Volume (mL)}{Extraction Surface Area (cm^2)} \times Surface Area \left(\frac{cm^2}{syringe} \right) \times Dilution Factor \times 1 \frac{syringe}{dose}$$

(Excerpted from the Sponsor’s submission)

Table 8 Extractables profile of the (b) (4) syringe barrel with 29-gauge staked needle (Forced Extraction Study [Stage 1])

Screening Method	Solvent	Compound *	Extrapolated Concentration (µg/dose)
Headspace gas chromatography – mass spectrometry	NA	ND	NA
Direct injection gas chromatography – mass spectrometry	WFI	ND	NA
	0.1% v/v Formic Acid	ND	NA
	0.1 M Glycine	ND	NA
	0.2% v/v PS-80	ND	NA
	50% v/v Ethanol Sonicate ^b	(b) (4)	(b) (4)
Liquid chromatography/mass spectrometry multimode positive	WFI	ND	NA
	0.1% v/v Formic Acid	ND	NA
	0.1 M Glycine	ND	NA
	0.2% v/v PS-80	ND	NA
	50% v/v Ethanol Sonicate ^b	ND	NA
Liquid chromatography/mass spectrometry multimode negative	WFI	ND	NA
	0.1% v/v Formic Acid	ND	NA
	0.1 M Glycine	ND	NA
	0.2% v/v PS-80	ND	NA
	50% v/v Ethanol Sonicate ^b	ND	NA
High performance liquid chromatography with ultraviolet detection	WFI	ND	NA
	0.1% v/v Formic Acid	ND	NA
	0.1 M Glycine	ND	NA
	0.2% v/v PS-80	ND	NA
	50% v/v Ethanol Sonicate ^b	ND	NA
Inductively coupled plasma – optical emission spectrometry	WFI	(b) (4)	(b) (4)
	0.1% v/v Formic Acid	(b) (4)	(b) (4)

Screening Method	Solvent	Compound *	Extrapolated Concentration (µg/dose)
		(b) (4)	(b) (4)
		(b) (4)	(b) (4)
	0.1 M Glycine	(b) (4)	(b) (4)
	0.2% v/v PS-80	(b) (4)	(b) (4)
	10% v/v Acid Matrix ^c	(b) (4)	(b) (4)

NA = not applicable; ND = not detected above the signal to noise for chromatography methods; PS = polysorbate; WFI = water for injection

^a Compound identification from chromatography screening methods was tentative. Inductively coupled plasma – optical emission spectrometry (ICP-OES) results were positively identified.

^b 50% ethanol sonication was on 29-gauge needles only.

^c 10% v/v acid matrix was a 5% concentrated nitric acid and 5% concentrated hydrochloric acid digestion of the syringe tip. This extract was only analyzed for (b) (4) concentration.

(Excerpted from the Sponsor’s submission)

Forced extraction study of the (b) (4) plunger stopper: The extractables profile of the (b) (4) plunger stopper (Lot #7234860) was determined using high temperature forced extractions. The stoppers were exposed to high temperature only (for headspace analysis) or high temperature reflux in WFI, 0.1% v/v PS-80, or isopropanol (IPA). The tables below list the compounds from the WFI, PS-80, and isopropanol extracts having signals greater than the method noise (typically signal to noise ratio greater than 5). The concentrations were estimated from the method

standard and then compared to the TTC as a worst case. (b) (4)
 (b) (4) two unknowns were detected at concentrations greater than the TTC in WFI and/or the 0.1% v/v PS-80 extracts. (b) (4) were also stated on the Sponsor's potential extractables list. The compounds and elements identified in Stage 1 were then monitored in the Stage 2 simulation study.

Table 9 Extractables profile of the (b) (4) plunger stopper (Forced Extraction Study [Stage 1])

Screening Method	Solvent	Compound *	Extrapolated Concentration (µg/dose)
Headspace gas chromatography – mass spectrometry	NA	ND	NA
	WFI	ND	NA
Direct injection gas chromatography – mass spectrometry	0.1% v/v PS-80	ND	NA
	WFI	Unknown (b) (4)	(b) (4)
Liquid chromatography/mass spectrometry electrospray positive	0.1% v/v PS-80	Unknown (b) (4)	(b) (4)
	WFI	ND	NA
Liquid chromatography/mass spectrometry electrospray negative	0.1% v/v PS-80	(b) (4)	(b) (4)
	WFI	ND	NA
High performance liquid chromatography with ultraviolet detection	0.1% v/v PS-80	ND	NA
	WFI	(b) (4)	(b) (4)
Inductively coupled plasma – optical emission spectrometry	WFI	(b) (4)	(b) (4)
	0.1% v/v PS-80	(b) (4)	(b) (4)

NA = not applicable; ND = not detected above the signal to noise for chromatography methods; PS = polysorbate; WFI = water for injection

* Compound identification from chromatography screening methods was tentative. Inductively coupled plasma – optical emission spectrometry (ICP-OES) results were positively identified. Times reported for unknown compounds are retention times.

(Excerpted from the Sponsor's submission)

From the direct injection GC/MS analysis for analysis of volatile compounds, the three preparations of the isopropanol extraction produced comparable chromatograms and results. All compounds detected above the reporting limit had mass spectra that were common to each other. The main feature of these spectra was the presence of the (b) (4) major ions. This combination of ions, along with the reoccurrence of peaks with this type of spectrum throughout each isopropanol extract, was most likely the result of (b) (4) the stoppers were made from. These unknown compounds could not be classified.

Non-volatile organic compound analysis of isopropanol extracts was conducted by ultraviolet (UV) detection from a photo-diode array detector and by LC/MS in alternating electrospray positive (ES+) mode/electrospray negative (ES-) mode and atmospheric pressure chemical ionization positive mode (APCI+). Several unknown compounds were identified with electrospray positive mode. (b) (4) was detected in all three isopropanol extracts. By HPLC/UV and LC/MS APCI+ mode, no compounds were detected above the reporting limit in all extracts. (b) (4) many unknown compounds were detected in the IPA extract by LC/MS electrospray negative mode. For the ICP analysis of IPA extracts, (b) (4) were detected above the reporting limit.

Table 10 Extractables profile of the [REDACTED] (b) (4) plunger stopper using isopropanol

Screening method	Compounds
Direct injection GC/MS	- None
Headspace GC-MS	- None
LC/MS multi-mode positive	- [REDACTED] (b) (4) - Several unknown compounds
LC/MS multi-mode negative	- [REDACTED] (b) (4) - [REDACTED] - Several unknown compounds
HPLC with ultraviolet detection	- None
ICP metal data analysis	- [REDACTED] (b) (4) - [REDACTED] - [REDACTED]

[REDACTED] APPEARS THIS WAY ON ORIGINAL [REDACTED]

Table 11 Measurements of isopropanol extractables from the plunger stopper using LC/MS with electrospray positive mode (b) (4)

Sample ID	Retention Time (Minutes)	ES ⁺ Major Ions (b) (4) Tentative Identification	Estimated Concentration (µg/cm ²)	Estimated Concentration (µg/g)
(b) (4)	18.9			(b) (4)
Lot 7234860	21.7			
NS-06285448	23.1			
IPA – Prep 1	23.2			
(b) (4)	15.8			
Lot 7234860	16.5			
NS-06285448	18.9			
IPA – Prep 2	21.6			
	21.7			
	23.1			
(b) (4)	16.2			
Lot 7234860	18.9			
NS-06285448	21.6			
IPA – Prep 3	21.7			
	23.2			

Note: All ions observed in the IPA extracts above were used for the extracted ion analysis of the 0.1% Polysorbate 80 extracts. Refer to Table 16 for ions that were observed in the 0.1% Polysorbate 80 extracts based on extracted ion analysis of the ions listed in Table 14 above.

(Excerpted from the Sponsor's submission)

Table 12 Measurements of isopropanol extractables from the plunger stopper using LC/MS with electrospray negative mode (b) (4)

Sample ID	Retention Time (Minutes)	ES Major Ions Tentative Identification (b) (4)	Estimated Concentration (µg/cm ²)	Estimated Concentration (µg/g)
(b) (4)	12.1	(b) (4)	(b) (4)	(b) (4)
Lot 7234860	12.3			
NS-06285448	15.8			
IPA – Prep 1	18.1			
	19.9			
	21.3			
	21.8			
	22.4			
	22.6			
(b) (4)	12.1			
Lot 7234860	12.3			
NS-06285448	15.8			
IPA – Prep 2	18.0			
	19.8			
	21.2			
	21.7			
	22.3			
(b) (4)	12.1	(b) (4)	(b) (4)	(b) (4)
Lot 7234860	12.2			
NS-06285448	13.5			
IPA – Prep 3	15.8			
	18.1			
	19.9			
	20.8			
	21.2			
	21.8			
	22.3			
	22.4			

(Excerpted from the Sponsor’s submission)

Forced extraction studies of the (b) (4) elastomeric needle shield: The extractables profile of the (b) (4) elastomeric needle shield (Lot #1305253 and 0347290) was determined using high temperature forced extractions. The elastomeric needle shield was exposed to high temperature only (for analysis by headspace gas chromatography-mass spectrometry) or high temperature reflux in isopropanol, WFI, or 0.2% w/v polysorbate 80. WFI and 0.2% w/v polysorbate 80 were selected to model the extraction capabilities of aqueous buffer solutions and non-ionic surfactants. The isopropanol was used to create harsh, exaggerated conditions. The same analytical techniques, described above, were used to identify extractables from extraction solutions of the needle shield. The table below lists the compounds from the WFI and PS-80 extracts having signal greater than the method noise (typically signal to noise greater than (b) (4)). The concentrations were estimated from the method standard and are equivalent to the extrapolated concentration (µg/dose) based on a 1.0 mL dose. Extractables exceeding the TTC included (b) (4) and at least 3 unknowns.

Table 13 Extractables profile of the (b) (4) elastomeric needle shield

Table P.2.4.2.2.2-1 (b) (4) Elastomeric Needle Shield Forced Extraction (Stage 1) Study Results

Screening Method	Solvent	Compound *	Extrapolated Concentration (µg/dose)
Headspace gas chromatography – mass spectrometry	NA	(b) (4)	(b) (4)
Direct injection gas chromatography – mass spectrometry	WFI	(b) (4)	(b) (4)
	0.2% w/v PS-S0		
Liquid chromatography/mass spectrometry multi-mode positive	WFI	(b) (4)	(b) (4)
	0.2% w/v PS-S0		
Liquid chromatography/mass spectrometry multi-mode negative	WFI	Unknown (b) (4) min	(b) (4)
		(b) (4)	
	0.2% w/v PS-S0	Unknown (b) (4) min	
		Unknown min	
		(b) (4)	
		Unknown (b) (4) min	
		Unknown min	
		(b) (4)	
High performance liquid chromatography with ultraviolet detection	WFI	Unknown (b) min	(b) (4)
		Unknown (4) min	
		Unknown min	
	0.2% w/v PS-S0	(b) (4)	
		Unknown (b) (4) min	
		Unknown min	

Screening Method	Solvent	Compound *	Extrapolated Concentration (µg/dose)
		Unknown (b) min	(b) (4)
		Unknown (4) min	
		(b) (4)	
Inductively coupled plasma – optical emission spectrometry	WFI	(b) (4)	(b) (4)
	0.2% w/v PS-S0		

NA = not applicable; ND = not detected above the signal to noise for chromatography methods; PS = polysorbate; WFI = water for injection
 * Compound identification from chromatography screening methods was tentative. Inductively coupled plasma – optical emission spectrometry (ICP-OES) results were positively identified. Times reported for unknown compounds are retention times.

(Excerpted from the Sponsor’s submission)

Table 14 Extractables profile of the (b) (4) elastomeric needle shield using isopropanol

Screening method	Compounds
Direct injection GC/MS	- an unknown (b) (4) minutes (b) (4)
Headspace GC-MS	-
LC/MS multi-mode positive	-
	-
	-
	-
LC/MS multi-mode negative	-
	-
	-

	-	(b) (4)
	-	
	-	
HPLC with ultraviolet detection	-	
	-	
ICP metal data analysis	-	none

Prefilled Syringe Buffer Simulation Study

The simulation study was executed using (b) (4) syringes filled with 1.0 mL of Drug Product (b) (4) (20 mM histidine/histidine-HCl, 250 mM trehalose dihydrate, 0.006% w/v PS-20, pH 6.0). These syringes were stored horizontally to maximize exposure to all surfaces and held at 2-8°C, 23-27°C/55-60% RH, or 38-42°C/70-80% RH. The samples were tested at the time points of 0, 1, 3, 6, or 12 months.

Table 15 Prefilled syringe buffer simulation study design

Condition	Testing Intervals (months)
2-8°C	0, 3, 6
23-27°C/55-60% RH	0, 3, 6
38-42°C/70-80% RH	0, 1, 3

RH = relative humidity

(Excerpted from the Sponsor’s submission)

The Drug Product is intended for storage up to (b) (4) months at 2-8°C. An Arrhenius relationship was used to extrapolate the results from 3 months at 38-42°C/70-80% RH to greater than 9 months at 23-27°C/55-60% RH and greater than 36 months at 2-8°C.

The compounds identified from the Stage 1 study and any additional compounds from the manufacturer’s potential extractables list were monitored and results reported in the Stage 2 study. Any new compounds above the signal-to-noise ratio of (b) (4) were also reported. The five analytical methods used in Stage 1 were also implemented for Stage 2 except inductively coupled plasma – optical emission spectrometry (ICP-OES), which was replaced with the more sensitive inductively coupled plasma – mass spectrometry (ICP-MS) method.

The reporting limits were suitable to identify compounds greater than the TTC of 1.5 µg/day. Detectable compounds were extrapolated to exposure concentrations (µg/dose) based on a 1.0 mL dose and compared to the TTC. The Sponsor evaluated the safety of the compounds that were detected above the TTC through a Permissible Daily Exposure (PDE) assessment. The PDE limits were established based on available nonclinical and clinical safety data.

For simulation studies using 38-42°C, 23-27°C, and 2-8°C, the only compound observed above the TTC of 1.5 µg/day was (b) (4). It is noted that metals were excluded from the database of chemicals used to derive the TTC.

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Table 16 Prefilled syringe simulation (Stage 2) study: 38-42°C

Screening Method	Compound ^a	Estimated Concentration ^b (µg/dose)			PDE (µg/day)	
		Initial	1 month	3 months		
Headspace gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA	
Direct injection gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA	
		(b) (4)			NA	
Liquid chromatography/mass spectrometry multi-mode positive	(b) (4)	ND	ND	ND	NA	
		Unknown (b) (4) min	ND	ND	NA	
		Unknown (b) (4) min	ND	ND	ND	NA
Liquid chromatography/mass spectrometry multi-mode negative	(b) (4)	Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
High performance liquid chromatography with ultraviolet detection	(b) (4)	Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA

Screening Method	Compound ^a	Estimated Concentration ^b (µg/dose)			PDE (µg/day)	
		Initial	1 month	3 months		
Inductively coupled plasma mass spectrometry	(b) (4)	Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND	NA

Screening Method	Compound ^a	Estimated Concentration ^b (µg/dose)			PDE (µg/day)
		Initial	1 month	3 months	

(b) (4) NA = not applicable or estimated concentration below TTC; ND = not detected above the signal to noise for chromatography methods or above LOQ for inductively coupled plasma – mass spectrometry (ICP-MS); PDE = permissible daily exposure

^a Compound identification from chromatography screening methods was tentative. ICP-MS results were positively identified. Times reported for unknown compounds are retention times. Unknowns observed in Stage 1 were monitored but not detected in Stage 2 and therefore not identified.

^b Using method standards.

(b) (4)

(Excerpted from the Sponsor's submission)

Table 17 Prefilled syringe simulation (Stage 2) study: 23-27°C

Screening Method	Compound *	Estimated Concentration ^b (µg/dose)			PDE (µg/day)	
		Initial	3 months	6 months		
Headspace gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA	
		ND	ND	ND	NA	
Direct injection gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA	
		(b) (4)	(b) (4)	(b) (4)	NA	
Liquid chromatography/mass spectrometry multi-mode positive	(b) (4)	ND	ND	ND	NA	
		ND	ND	ND	NA	
		Unknown (b) (4) _{min}	ND	ND	ND	NA
		Unknown (b) (4) _{min}	ND	ND	ND	NA
Liquid chromatography/mass spectrometry multi-mode negative	(b) (4)	Unknown (b) (4) _{min}	ND	ND	ND	NA
		Unknown (b) (4) _{min}	ND	ND	ND	NA
		Unknown (b) (4) _{min}	ND	ND	ND	NA
		(b) (4)	ND	ND	ND	NA
		ND	ND	ND	NA	

Screening Method	Compound *	Estimated Concentration ^b (µg/dose)			PDE (µg/day)
		Initial	3 months	6 months	
	(b) (4)	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
High performance liquid chromatography with ultraviolet detection	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
	Unknown (b) (4) _{min}	ND	ND	ND	NA
Inductively coupled plasma mass spectrometry	(b) (4)	ND	ND	ND	NA
	(b) (4)	(b) (4)	(b) (4)	(b) (4)	NA
	(b) (4)	(b) (4)	(b) (4)	(b) (4)	NA
	(b) (4)	(b) (4)	(b) (4)	(b) (4)	NA
	(b) (4)	ND	ND	(b) (4)	NA
	(b) (4)	ND	ND	(b) (4)	NA
		ND	(b) (4)	NA	

Screening Method	Compound ^a	Estimated Concentration ^b (µg/dose)			PDE (µg/day)
		Initial	3 months	6 months	
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	(b) (4)
	(b) (4)	ND	(b) (4)	ND	NA
	(b) (4)	(b) (4)	ND	ND	NA
	(b) (4)	(b) (4)			(b) (4)
	(b) (4)	ND	ND	ND	NA

(b) (4) NA = not applicable or estimated concentration below TTC; ND = not detected above the signal to noise for chromatography methods or above LOQ for inductively coupled plasma – mass spectrometry (ICP-MS); PDE = permissible daily exposure

^a Compound identification from chromatography screening methods was tentative. ICP-MS results were positively identified. Times reported for unknown compounds are retention times. Unknowns observed in Stage 1 were monitored but not detected in Stage 2 and therefore not identified.

^b Using method standards.

(b) (4)

(Excerpted from the Sponsor’s submission)

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Table 18 Prefilled syringe simulation (Stage 2) study: 2-8°C

Screening Method	Compound ^a	Concentration ^b (µg/dose)			PDE (µg/day)
		Initial	3 months	6 months	
Headspace gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA
Direct injection gas chromatography – mass spectrometry	(b) (4)	ND	ND	ND	NA
		(b) (4)			NA
Liquid chromatography/mass spectrometry multi-mode positive	Unknown (b) (4)	ND	ND	ND	NA
		ND	ND	ND	NA
		ND	ND	ND	NA
		Unknown (b) (4) min	ND	ND	ND

Screening Method	Compound ^a	Concentration ^b (µg/dose)			PDE (µg/day)
		Initial	3 months	6 months	
Liquid chromatography/mass spectrometry multi-mode negative	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
High performance liquid chromatography with ultraviolet detection	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	Unknown (b) (4) min	ND	ND	ND	NA
	(b) (4)	ND	ND	ND	NA
Inductively coupled plasma mass spectrometry	(b) (4)	ND	ND	ND	NA
	(b) (4)	(b) (4)			NA
	(b) (4)	(b) (4)			NA

Screening Method	Compound ^a	Concentration ^b (µg/dose)			PDE (µg/day)	
		Initial	3 months	6 months		
(b) (4)	(b) (4)	(b) (4)			ND	NA
		(b) (4)			(b) (4)	NA
		ND	(b) (4)	(b) (4)	NA	
		ND	ND	ND	NA	
		ND	ND	ND	(b) (4)	
		ND	(b) (4)	(b) (4)	NA	
		(b) (4)	ND	ND	NA	
		(b) (4)	(b) (4)			(b) (4)
		ND	ND	ND	NA	

(b) (4) NA = not applicable or estimated concentration below TTC; ND = not detected above the signal to noise for chromatography methods or above LOQ for inductively coupled plasma – mass spectrometry (ICP-MS); PDE = permissible daily exposure

^a Compound identification from chromatography screening methods was tentative. ICP-MS results were positively identified. Times reported for unknown compounds are retention times. Unknowns observed in Stage 1 were monitored but not detected in Stage 2 and therefore not identified.

^b Using method standards.

(b) (4)

(Excerpted from the Sponsor’s submission)

Drug Product Leachables and Elemental Impurities Studies

Elemental impurities, (b) (4) were monitored in Phase 3 leachables studies. Elemental impurities were monitored in the Stage 3 leachables study as recommended for parenteral drug products in the ICH Q3D Guidance. Elements monitored included (b) (4)

(b) (4) These were all Class 1, Class 2A and Class 3 elements recommended for monitoring with parenteral drug products. Since prefilled syringes were known to potentially have increased amounts of (b) (4) leachables, the Stage 3 Leachables study monitored for the presence of both (b) (4) (b) (4) although neither of these elements were detected above the TTC of 1.5 µg/day in the Stage 2 stimulation study.

Table 19 Elemental impurities limits

Element	Class ^a	Parenteral PDE ^a µg/day	Method LOQ µg/mL
(b) (4)			

- a. From the ICH Q3D Guidance
- b. The method LOQ is determined from the concentration of the lowest method standard and may vary from run-to-run

Table 20 Sponsor's Limits for (b) (4)

Leachable	PDE Limit (µg/day)	J (µg/mL)	Method LOQ (µg/mL)
(b) (4)			

Leachables Study: The leachables study tested 3 lots of the Drug Product (Lots 020F15, 021F15, and 004K15). All syringes were stored in a horizontal position at 2-8°C for testing at 0, 6, 12, 24, and 36 months. Results were provided for 0, 6, and 12 months. The study is still ongoing at the time of this review. The Sponsor has committed to provide results from later time points when available.

Elemental impurities testing conditions and intervals were listed in Table 21 below. Results of leachables testing for 3 commercial lots are listed in Table 22 below.

Leachables testing through the 12 month time point found that levels of elemental impurities were below the dose-adjusted Permissible Daily Exposure (PDE) levels established in ICH Q3D Guidance. Levels of (b) (4) were less than the TTC of 1.5 µg/day. Levels of (b) (4) were low and did not raise any safety concerns. The leachables study was ongoing at the time of this review and results for later time points will be reported over the product shelf life.

Table 21 Time points for measurements of elemental impurities, (b) (4)

Table P.8.1.2.4-2 Elemental Impurity Testing

Tests	Testing Intervals (months)									
	0	1	2	3	6	9	12	18	24	36
Elemental Impurities	•	○	○	○	•	○	•	○	•	•
(b) (4) Testing	•	○	○	○	•	○	•	○	•	•
(b) (4) Testing	•	○	○	○	•	○	•	○	•	•

• = Scheduled; ○ = Not Performed

Table 22 Leachables results for elemental impurities, (b) (4)
from Process 3 commercial lots (Lots 020F15, 021F15, and 004K15) at time points of 0, 6, and 12 months

Table 4-3 Lot 020F15 (30 mg/PFS (b) (4)): Elemental Impurity and Leachables Results

Time Point (months)	Result (µg/mL)
0	(b) (4)
6	(b) (4)
12	(b) (4)

Table 4-4 Lot 021F15 (30 mg/PFS (b) (4)): Elemental Impurity and Leachables Results

Time Point (months)	Result (µg/mL)
0	(b) (4)
6	(b) (4)
12	(b) (4)

Table 4-5 Lot 004K15 (30 mg/PFS (b) (4)): Elemental Impurity and Leachables Results

Time Point (months)	Result (µg/mL)
0	(b) (4)
6	(b) (4)
12	(b) (4)

(Excerpted from the Sponsor's submission)

Risk Assessment:

The prefilled syringe (b) (4) (PFS (b) (4)) is the primary container closure system for the Drug Product. (b) (4)

(b) (4) None of the accessories are in contact with the Drug Product solution or part of the fluid path of the delivery system. The secondary packaging includes a unit tray and lid stock (b) (4) (b) (4) an opaque paperboard unit carton (b) (4) and package inserts.

The (b) (4) glass syringe with a 29-gauge (b) (4) staked-in needle and a rigid needle shield, which is closed with an elastomeric plunger stopper, is a marketed device that is used with other FDA-approved drug products. The manufacturer has done extraction studies with this device.

The Sponsor conducted a safety evaluation of the prefilled syringe components based on a 3-stage, risk-based strategy to ensure that the product contact components do not leach undesirable amounts of potentially harmful compounds into the Drug Product that may adversely impact patient safety (Stage 1: PFS Forced Extraction Study; Stage 2: Prefilled Syringe Buffer Simulation Study; and Stage 3: Drug Product Leachables and Elemental Impurities Studies). A risk assessment was performed at each stage, and the results were used to inform the next stage. Results from Stage 1 were compared to the available manufacturers' extractables reports.

For each stage, the following analytical techniques were used to identify extractables and leachables: direct injection gas chromatography mass spectrometry (GC/MS) was used for identification and measurement of semi-volatile organic compounds (SVOCs), headspace GC/MS was used for identification and measurement of volatile organic compounds (VOCs), liquid chromatography mass spectrometry (LC/MS) with different detection methods was used for identification and measurement of non-volatile organic compounds (NVOCs), and inductively-coupled plasma (ICP) spectrometry was used for analysis of metals. The range of analytical techniques used in these studies appeared to be adequate.

In Phase 1, forced extraction studies were performed on the three separate components of the (b) (4) 1 mL long syringes:

- Type I (b) (4) glass barrel with 29-gauge (b) (4) needle and the syringe tip (b) (4) stainless steel needle adhered to the glass tip of the syringe)
- (b) (4) plunger stopper
- (b) (4) elastomeric needle shield

High temperature and/or harsh solvents were used in the forced extraction studies in order to determine the compounds most likely to be extracted from the container closure

components. The range of solvents in conjugation with high temperature appeared to be adequate.

Extraction studies with the Type I (b) (4) glass barrel with 29-gauge (b) (4) needle and syringe tip identified low levels (b) (4)

(b) (4) Only (b) (4) was measured at quantities >TTC. Extraction studies with the stopper identified low levels of (b) (4) two unknowns at concentrations greater than the TTC in WFI and/or the 0.1% v/v PS-80 extracts. (b) (4)

were also stated on the Sponsor's potential extractables list. Isopropanol extracts identified (b) (4)

Extraction studies with the elastomeric needle shield using isopropanol, WFI, or 0.2% w/v polysorbate 80 identified extractables exceeding the TTC that included (b) (4) at least 3 unknowns. The compounds and elements identified in Stage 1 were then monitored in the Stage 2 simulation study.

The simulation study was conducted using (b) (4) syringes filled with 1.0 mL of Drug Product (b) (4) (20 mM histidine/histidine-HCl, 250 mM trehalose dihydrate, 0.006% w/v PS-20, pH 6.0). These syringes were stored horizontally to maximize exposure to all surfaces and held at 2-8°C, 23-27°C/55-60% RH, or 38-42°C/70-80% RH. The samples were tested at the time points of 0, 1, 3, 6, or 12 months. For simulation studies using 38-42°C, 23-27°C, and 2-8°C, the only compound observed above the TTC of 1.5 µg/day was (b) (4)

The leachables study tested 3 lots of the Drug Product (Lots 020F15, 021F15, and 004K15). All syringes were stored in a horizontal position at 2-8°C for testing at 0, 6, 12, 24, and 36 months. Leachables testing through the 12 month time point found that levels of elemental impurities were below the dose-adjusted Permissible Daily Exposure (PDE) levels established in ICH Q3D Guidance. Levels of (b) (4) were less than the TTC of 1.5 µg/day. Levels of (b) (4) were low and did not raise any safety concerns. The leachables study was ongoing at the time of this review and results for later time points will be reported over the product shelf life.

The safety assessment was conducted using a conservative approach of estimating safe exposures to potential leachables based on daily dosing. However, in actuality, the dosing regimen is every 4 weeks for the first 3 doses followed by once every 8 weeks thereafter. Leachables from the container closure system appear to pose no significant safety concerns to patients. As noted earlier, the container closure system is a marketed product.

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/s/

TIMOTHY W ROBISON
07/28/2017

Pharmacology and Toxicology Secondary Review for BLA 761070

Date: July 19, 2017

To: **BLA 761070**

Benralizumab [Humanized, afucosylated, immunoglobulin (Ig)G1 κ mAb that targets IL-5R α] subcutaneous injection
AstraZeneca Pharmaceuticals LP

From: Carol M. Galvis, PhD

Acting Pharmacology and Toxicology Team Leader
Division of Pulmonary, Allergy, and Rheumatology Products
(DPARP)

Recommendation

I concur with the recommendation of Dr. Timothy Robison that BLA 761070 should be approved from the pharmacology and toxicology perspective (refer to Dr. Robison's review dated July 10, 2017). The nonclinical pharmacology and toxicology profiles of benralizumab have been adequately characterized and the nonclinical program is complete.

Background

AstraZeneca submitted a 351(a) original Biologics License Application (BLA) on November 16, 2016 for benralizumab as an add-on maintenance treatment in patients with severe asthma ages 18 and older, with an eosinophilic phenotype. Benralizumab is a humanized, afucosylated IgG1 κ monoclonal antibody that targets interleukin (IL)-5 receptor alpha (IL-5R α). The proposed clinical dosing regimen is 30 mg [in a 1mL solution for subcutaneous injection in a prefilled syringe (PFS)] every 4 weeks for the first 3 doses, followed by once every 8 weeks thereafter.

Pharmacology/EPC

Benralizumab binds to human and cynomolgus monkey IL-5R α with K_D values of 11 and 42 pM, respectively (as measured by BIAcore). Benralizumab did not cross-react with murine IL-5R α . Benralizumab binds to human and cynomolgus monkey eosinophils with EC₅₀ values of 26 and 40pM, respectively. These data support the selection of cynomolgus monkey as a relevant species for the nonclinical program of benralizumab.

The absence of fucose in the Fc domain of benralizumab results in higher affinity for Fc γ RIIIa receptors (approximately 6-fold compared to the fucosylated parental antibody) on immune effector cells, leading to apoptosis of eosinophils and basophils through enhanced antibody-dependent cell-mediated cytotoxicity (ADCC). The applicant provided adequate data to support the proposed

Established Pharmacological Classification (EPC) of interleukin-5 receptor alpha-directed cytolytic monoclonal antibody.

Toxicology

The toxicity profile of benralizumab was evaluated in cynomolgus monkeys. In the pivotal 39-week toxicity study, cynomolgus monkeys (6/sex/group) received 0 (IV/SC), 10 (IV), 25 (IV), or 30 (SC) mg/kg benralizumab once every 2 weeks for a total of 20 doses. One female in the 25 mg/kg IV dose group experienced a post-dose reaction (clinical signs of bruising/reddened areas around the eyes, face, chest, and lower abdomen; decrease in platelet counts; and abnormal erythrocytes) after the fourth dose on study day 43 that resolved by study day 267 despite continued dosing.

Eosinophil levels were decreased in treated animals at all dose levels, consistent with benralizumab's mechanism of action. No drug-related histopathology findings were observed. The NOAELs were identified at 10 mg/kg IV and 30 mg/kg SC, based on the post-dose reaction described above.

Reproductive and Developmental Toxicology

Male and female fertility parameters were unaffected in sexually mature animals in the 39-week toxicity study with doses up to 25 mg/kg IV and 30 mg/kg SC.

To evaluate the effects of benralizumab in development, an enhanced pre- and post-natal development (ePPND) study was conducted in cynomolgus monkeys. Pregnant cynomolgus monkeys received 0, 10, or 30 mg/kg benralizumab subcutaneously during the period of organogenesis (GD20/22, GD35, and once every 2 weeks thereafter through gestation). Benralizumab was also administered to females on post-partum days 14 and 28.

There was no evidence of maternal toxicity in this study. Eosinophil levels were decreased in treated adult females, consistent with benralizumab's mechanism of action. Eosinophils were decreased through post-partum day 28 and started to increase gradually in most animals. However, a few females had very low eosinophil levels by post-partum day 180, indicating lack of recovery in these animals. No effects were observed in the number of aborted fetuses, stillborn fetuses, or fetal/neonatal survival.

Infants exposed *in utero* to benralizumab had decreased eosinophil levels, which increased gradually over time; except in one infant in the 30 mg/kg group who had eosinophil counts of zero at the end of the study. No effects were observed in infant growth; neurological development; external, visceral, or heart evaluations; skeletal evaluations; TDAR assay; or immunophenotyping (levels of B cells, total T cells, T helper cells, T cytotoxic cells, NK cells, or monocytes). Benralizumab was not teratogenic in cynomolgus monkeys. The NOAEL for maternal toxicity and teratogenicity was identified at 30 mg/kg.

Placental transfer was demonstrated by measuring benralizumab levels in the serum of infants exposed *in utero*.

Carcinogenicity

The applicant submitted a carcinogenicity risk assessment that was discussed with the Executive Carcinogenicity Assessment Committee (ECAC) on January of 2013. The following considerations were discussed with the ECAC: there were no proliferative or pre-neoplastic lesion identified in cynomolgus monkeys after treatment for up to 39 weeks and benralizumab does not bind to murine IL-5R α ; therefore, a 2-year study in rodents was not feasible. The ECAC agreed that rodent carcinogenicity studies are not required for benralizumab. Further, the ECAC agreed that development of a mouse surrogate was not required. Dr. Robison's review dated July 10, 2017 includes an extensive evaluation of the available published literature regarding the role of eosinophils in tumorigenesis. Based on his extensive evaluation, Dr. Robison concluded that the role of eosinophils in tumor development is unclear.

Labeling

Dr. Robison's review dated July 10, 2017 recommended edits to the proposed product labeling for the following sections: Indications and Usage (only the Established Pharmacological Classification), Section 8 Use in Specific Populations (only Sections 8.1 Pregnancy and 8.2 Lactation), Section 12 Clinical Pharmacology (only Section 12.1 Mechanism of Action), and Section 13 Nonclinical Toxicology. I concur with Dr. Robison's proposed edits, which are consistent with current labeling practices and also with the approved anti-IL-5 drugs (NUCALA®, mepolizumab; and CINQAIR®, reslizumab) labels.

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/s/

CAROL M GALVIS
07/19/2017

**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: 761070

Supporting document/s: SDN #1

Applicant's letter date: November 16, 2016

CDER stamp date: November 16, 2016

Product: Benralizumab [Humanized, afucosylated,
immunoglobulin (Ig)G1 κ mAb that targets IL-5R α]

Indication: Asthma

Applicant: AstraZeneca Pharmaceuticals LP
One MedImmune Way
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Review Division: Pulmonary, Allergy, and Rheumatology Products

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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Benralizumab is a humanized, afucosylated, immunoglobulin (Ig)G1 κ monoclonal antibody (mAb) that targets the alpha subunit of the human interleukin-5 receptor (IL-5R α), which is expressed on eosinophils and basophils. The established pharmacological classification is an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody. Benralizumab is indicated as an add-on maintenance treatment for patients with severe asthma aged 18 years and older, with an eosinophilic phenotype. The recommended dose is 30 mg every 4 weeks for the first 3 doses followed by once every 8 weeks thereafter.

1.2 Brief Discussion of Nonclinical Findings

Benralizumab exclusively stained peripheral blood eosinophils and basophils from healthy subjects, which was consistent with the expression of IL-5R α on these cells. Eosinophils expressed an approximate 3-fold higher level of IL-5R α compared to basophils. Further, benralizumab identified a small but specific fraction (approximately 0.9%) of bone marrow mononuclear cells (BMMNCs) that most likely represented the eosinophil/basophil lineage precursors. Thus, eosinophils, basophils, and BMMNCs are the primary targets for the action of benralizumab.

Benralizumab bound to human and Cynomolgus monkey eosinophils with EC₅₀ values of 26 and 40 pM, respectively. K_D values for binding of benralizumab to human and Cynomolgus monkey IL-5R α were 11 and 42 pM, respectively. Benralizumab did not cross-react with murine IL-5R α .

Both benralizumab (fucose negative) and the fucosylated (fucose positive) parent anti-IL-5R α mAb inhibited IL-5-induced proliferation of CTLL-2 cells transfected with recombinant human IL-5R with identical potencies (IC₅₀ = 0.3 nM). The inhibitory potency on IL-5-induced cell growth was 300-fold lower relative to its potency for inducing ADCC activity on eosinophils and basophils (0.5 and 0.9 pM, respectively).

Benralizumab is an afucosylated (fucose negative) IgG1 κ mAb. The absence of the monosaccharide, fucose, on the oligosaccharide core of a human IgG1 has been shown to result in an increased binding affinity to human Fc γ R111a and subsequently increased ADCC. Benralizumab was assessed for binding to Fc γ Rs and ADCC activity. Examinations of the binding affinity of benralizumab to soluble human Fc γ R domains by surface plasmon resonance found that binding to human Fc γ R111a (K_D = 45.5 nM) was increased 6-fold compared with the fucosylated (fucose positive) parental anti-IL-5R α mAb; however, binding was comparable for all other Fc γ Rs. Comparable results were obtained with monkey Fc γ R111a.

The potency of benralizumab (fucose negative) to mediate eosinophil and basophil apoptosis by ADCC *in vitro* was examined. ADCC assays were performed with autologous NK cells, as effector cells, and bone marrow mononuclear cell, as target cells. Cells were analyzed on a flow cytometer and the percentage of Annexin V-

positive eosinophils/basophils was measured; Annexin V binds to apoptotic cells. In the presence, but not absence, of autologous NK effector cells, benralizumab (fucose negative) induced eosinophil and basophil apoptosis, as assessed by means of Annexin V staining, with EC_{50} values of 0.9 and 0.5 pM, respectively. However, when the fucosylated (fucose positive) parental α IL-5R α mAb was used at concentrations 1000 times higher than the benralizumab EC_{50} (in the presence of NK effector cells), it did not induce target cell apoptosis above background levels, although its binding affinity for IL-5R α and its potency to inhibit IL-5-induced cell proliferation were indistinguishable from those of benralizumab (fucose negative). Benralizumab (fucose negative) depleted eosinophils, basophils, and BMMNCs through apoptosis induced by ADCC. The enhanced ADCC was attributed to the absence of the monosaccharide, fucose, on the oligosaccharide core of benralizumab (fucose negative), which results in an increased binding affinity to human Fc γ R11a and subsequently increased ADCC. Thus, an established pharmacological classification as an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody appears appropriate.

In a 39-week toxicology study, Cynomolgus monkeys (6/sex/group) received benralizumab at doses of 0 (IV/SC), 10 (IV), 25 (IV), and 30 (SC) mg/kg once every 2 weeks for a total of 20 doses. Males and females were sexually mature adults to allow for fertility assessments. Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count and indicators of circulating erythrocyte mass that appeared to be reversible. The effects on platelets and erythrocytes (abnormal morphology) suggested a benralizumab-related immune-mediated process (e.g., post-dose reaction) that was considered adverse. It had fully resolved by day 267 despite continued dosing.

Eosinophil counts were decreased for males and females in the 10 and 25 mg/kg IV groups and 30 mg/kg SC group, which could be attributed to the pharmacological action of benralizumab. Eosinophil counts were consistently decreased by Day 63 and a few animals demonstrated an effect as early as Day 3. Lower peripheral eosinophil counts correlated with decreased eosinophil progenitors in the bone marrow. Male and female fertility parameters were unaffected with doses up to 25 mg/kg IV. Histopathological findings were judged to be spontaneous in nature and unrelated to treatment. NOAELs were identified as 10 mg/kg IV and 30 mg/kg SC based upon findings for Female 3501 in the 25 mg/kg IV dose group.

In an enhanced pre- and post-natal development (ePPND) study, pregnant Cynomolgus monkeys received benralizumab by bolus IV injection at doses of 0, 10, or 30 mg/kg on GD20-GD22 (dependent on pregnancy determination), on GD35, and once every 14 days thereafter through gestation. Further, benralizumab was administered to female adult monkeys on postpartum days 14 and 28. A maximum of 14 doses were administered. There was no evidence of maternal toxicity with IV doses of benralizumab at 10 or 30 mg/kg. Complete or near-complete depletion of peripheral blood eosinophils was observed at GD91 in most adult females in the 10 mg/kg group and all females in

the 30 mg/kg group. The depletion of eosinophils continued through PPD 28. Eosinophil counts began to increase gradually in most females in the 10 and 30 mg/kg dose groups by PPDs 91, 136, and 180 with eosinophil counts in a few females approaching the lowest control eosinophil counts by PPD91 and most counts were similar to the control by PPD180. A few females in the 10 and 30 mg/kg groups had peripheral blood eosinophil counts that remained $\leq 10/\mu\text{L}$ through PPD180, indicating a lack of recovery. Decreased eosinophil counts were attributed to the pharmacological action of the benralizumab.

Survival, growth, and neurological development of infants in the ePPND study were unaffected by benralizumab. Benralizumab was not teratogenic in monkeys (no evidence of treatment-related malformations or embryofetal toxicity). Infants exposed *in utero* to benralizumab in the 10 and 30 mg/kg groups had decreased peripheral blood eosinophil counts. Peripheral blood eosinophil counts had risen in most infants by BD180 and BD199 (± 2 days) to counts comparable to controls, except infant # 3141 in the 30 mg/kg group. Peripheral blood eosinophil counts in infant #3141 remained at $0/\mu\text{L}$, which correlated with the bone marrow eosinophil evaluation for this animal. Exposure of infants to benralizumab was demonstrated by detecting benralizumab in the serum of infants. Benralizumab levels observed in infants were consistent with placental transfer from the maternal circulation into the fetal circulation. Benralizumab concentrations in infants on BD180 were below the limit of quantitation.

A carcinogenicity study was not required for benralizumab. There was no evidence for proliferative or pre-neoplastic effects of benralizumab in any GLP-compliant repeat-dose IV or SC toxicologic study in *Cynomolgus* monkeys. Since benralizumab does not bind to murine IL-5R α , direct assessment of carcinogenic risk of benralizumab in a classic 2-year rodent bioassay was not appropriate. Use of a murine surrogate was not required. IL-5- and IL-5R α -deficient mouse models do not appear to be relevant for human risk assessment. The role of eosinophils in tumor development is unclear.

1.3 Recommendations

1.3.1 Approvability

The application is recommended for approval from the nonclinical perspective.

1.3.2 Additional Nonclinical Recommendations

There are no outstanding nonclinical issues.

1.3.3 Labeling

Labeling recommendations were provided for Indications and Usage (under Highlights of Prescribing Information), Section 8.1, Section 8.3, Section 12.1, and Section 13. Additions were denoted as underlined text. Deletions were denoted as ~~text~~.

----- **INDICATIONS AND USAGE** -----

TRADENAME is an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody indicated ^{(b) (4)} add-on maintenance treatment ^{(b) (4)} patients with severe asthma aged ^{(b) (4)} years and older, with an eosinophilic phenotype.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

The data on pregnancy exposure from the clinical trials are insufficient to inform on drug-associated risk. Monoclonal antibodies such as benralizumab are transported across the placenta ^{(b) (4)}

uring the third trimester of pregnancy; therefore, potential effects on a fetus are likely to be greater during the third trimester of pregnancy. In a prenatal and postnatal development study conducted in cynomolgus monkeys, there was no evidence of fetal harm with IV administration of benralizumab throughout pregnancy at doses that produced exposures up to approximately 310 ^{(b) (4)} times the exposure at the maximum recommended human dose (MRHD) of 30 mg SC [see Data]. ^{(b) (4)}

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

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 in the neonate.

Data

Animal Data

In a prenatal and postnatal development study, pregnant cynomolgus monkeys received benralizumab from beginning on GD20 to GD22 (dependent on pregnancy determination), on GD35, once every 14 days thereafter through out the gestation period and 1-month postpartum (maximum 14 doses) at doses that produced exposures up to

approximately 310^{(b) (4)} times that achieved with the MRHD (on an AUC basis with maternal IV doses up to 30 mg/kg once every 2 weeks). Benralizumab did not elicit adverse effects on fetal or neonatal growth (including immune function) up to 6.5 months after birth. There was no evidence of treatment-related external, visceral, or skeletal malformations. Benralizumab was not teratogenic in cynomolgus monkeys. Benralizumab crossed the placenta in cynomolgus monkeys. Benralizumab concentrations were approximately equal in mothers and infants on postpartum day 7, but were lower in infants at later time points. Eosinophil counts were suppressed in infant monkeys with gradual recovery by 6 months postpartum; however, recovery of eosinophil counts was not observed for one infant monkey during this period.

8.2 Lactation

Risk Summary

There is no information regarding the presence of benralizumab in human ^{(b) (4)}
^{(b) (4)} However benralizumab is a humanized monoclonal antibody (IgG1/κ-class) and IgG is present in human milk in small amounts. If benralizumab is transferred into human milk, the effects of local exposure in the gastrointestinal tract and potential limited systemic exposure in the infant to benralizumab are unknown. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for benralizumab and any potential adverse effects on the breast-fed ^{(b) (4)} from benralizumab, or from the underlying maternal condition ^{(b) (4)}

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Benralizumab is a ^{(b) (4)} humanized afucosylated, monoclonal antibody (IgG1, kappa) ^{(b) (4)} that binds to the alpha subunit of the human interleukin-5 receptor (IL-5Rα) with ^{(b) (4)} ~~(a dissociation constant of 11 ^{(b) (4)} pM)~~ ^{(b) (4)}. The IL-5 receptor is ^{(b) (4)} expressed on the surface of eosinophils and basophils. In an *in vitro* setting, ^{(b) (4)} the absence of fucose in the Fc domain of benralizumab ^{(b) (4)} ~~^{(b) (4)} facilitate ^{(b) (4)} binding (45.5 nM) ^{(b) (4)} to ^{(b) (4)} FcγRIII receptors on immune effectors cells, such as natural killer (NK) cells, leading to apoptosis of eosinophils and basophils through ^{(b) (4)} antibody-dependent cell-mediated cytotoxicity (ADCC).~~

Inflammation is an important component in the pathogenesis of asthma. Multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, cytokines) are involved in inflammation. Benralizumab, by

binding to the IL-5R α chain, reduces (b) (4) eosinophils; however, the mechanism of benralizumab action in asthma has not been definitively established.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of benralizumab. Published literature using animal 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 that (b) (4) to (b) (4) IL-5R α (b) (4) such as benralizumab is unknown.

Male and female fertility were unaffected based upon no adverse histopathological findings in the reproductive organs from cynomolgus monkeys treated with benralizumab for 9 months at IV doses up to 25 mg/kg or at SC doses of up to 30 mg/kg once every 2 weeks (approximately 400 (b) (4) and 270 (b) (4) times the MRHD on an AUC basis; (b) (4)

2 Drug Information

2.1 Drug

CAS Registry Number: 1044511-01-4

Trade Name: Under review

Generic Name: Benralizumab

Code Name: Benralizumab (formerly known as MEDI-563, KHK4563, and BIW-8405)

Molecular Weight: Benralizumab is a recombinant humanized afucosylated IgG1k monoclonal antibody of approximately 150 kDa, including oligosaccharides.

Biochemical description: Benralizumab is recombinantly produced in (b) (4), a Chinese Hamster Ovary (b) (4) cell line which is deficient in α -1,6-fucosyltransferase (FUT8; (b) (4) that is required for the attachment of the monosaccharide fucose to the oligosaccharide chain of benralizumab. Thus, benralizumab is an afucosylated antibody to increase affinity for human Fc γ RIII α , which results in ADCC for hIL-5R α -expressing cells, in comparison with the fucosylated parent anti-hIL-5R α mAb (KM8400). (b) (4)

The antibody is composed of two identical heavy chains of approximately 49,400 Da each, and two identical light chains of approximately 23,500 Da each. Benralizumab has

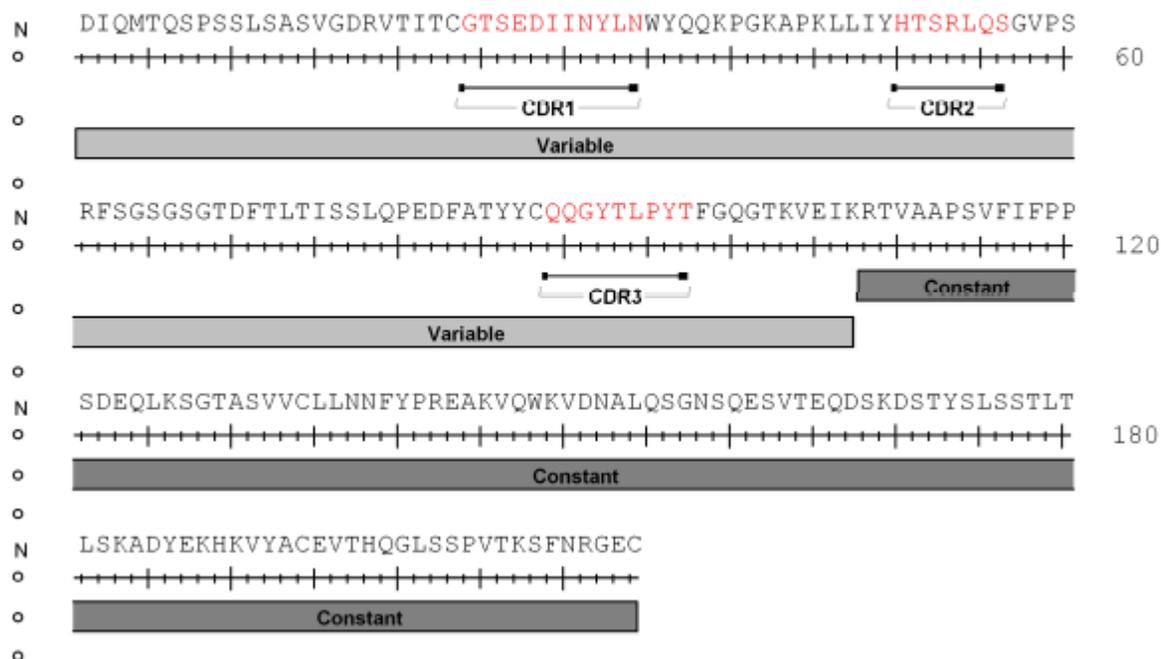
primarily N-linked biantennary complex type oligosaccharides attached to each heavy chain at Asn-301, without fucose. As noted above, the expression cell line was engineered to eliminate fucosylation. The average size of the oligosaccharide moiety is approximately 1,500 Da per heavy chain.

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Figure 1 Amino acid sequence of benralizumab

Amino Acid Sequence

The amino acid sequence of benralizumab V_L and V_H regions are shown in Figure S.1.2-1 and Figure S.1.2-2, respectively.



Pharmacologic class: Interleukin-5 receptor alpha-directed cytolytic monoclonal antibody

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 100237 (AstraZeneca [MedImmune], benralizumab)

2.3 Drug Formulation

The benralizumab Drug Product is a sterile liquid dosage form presented in an accessorized prefilled syringe (APFS) intended for subcutaneous administration. Each syringe contains 30 mg of benralizumab in (b) (4) 1.0 mL volume. The Drug Product contains 30 mg/mL benralizumab in 20 mM histidine/histidine-HCl, 0.25 M trehalose dihydrate, 0.006% w/v polysorbate 20, pH 6.0.

Table 1 Composition of the Drug Product

Table P.1-1 Composition of the Drug Product

Ingredient	Concentration	Unit Formula per 30 mg syringe	Purpose	Quality Standard
<i>Active Ingredient</i>				
Benralizumab	30 mg/mL	30 mg	Active	In-house Reference Standard
<i>Excipients</i>				
L-Histidine	9 mM	1.4 mg	(b) (4)	USP/NF; Ph. Eur.; JP
L-Histidine hydrochloride monohydrate	11 mM	2.3 mg		Ph. Eur.; JP
α,α-trehalose dihydrate	0.25 M	95 mg		USP/NF; Ph. Eur.; JP
Polysorbate 20 (b) (4)	0.006% w/v	0.06 mg		USP/NF; Ph. Eur., JP
Water for Injection	Not applicable	Approximately (b) (4) mg		USP/NF; Ph. Eur., JP

JP = Japanese Pharmacopoeia; Ph. Eur. = European Pharmacopoeia; USP/NF = United States Pharmacopoeia/National Formulary

The APFS uses a prefilled syringe (b) (4) (PFS (b) (4)) as the primary container closure system for the Drug Product. (b) (4)

The Drug Substance undergoes (b) (4)

(b) (4) None of the accessories are in contact with the Drug Product solution or part of the fluid path of the delivery system.

(b) (4)

Figure 3 Accessorized Prefilled Syringe (APFS)

(Excerpted from the Sponsor's submission)

2.4 Comments on Novel Excipients

Trehalose dihydrate is administered at a SC dose of 95 mg (equivalent to (b) (4) mg/m²) in the clinical formulation. Trehalose dihydrate is found as an excipient in the FDA-approved product, COSENTYX[®], at a SC dose of 75.67 mg, which is slightly less than benralizumab, although it is administered more frequently (4 weekly loading doses followed by q4 weeks). Trehalose dihydrate is found in FDA-approved IV and oral products that qualify it with respect to systemic toxicity. This excipient was included in the formulation used in the 9-month toxicology study with monkeys to support the safety qualification of this excipient administered by the SC route, although the study did not include a concurrent control group without trehalose dihydrate for comparison. Monkeys received a SC dose of trehalose at 90 mg/kg (equivalent to 1080 mg/m²) once every 2 weeks. There were no significant adverse findings at SC injection sites in monkeys from the control group. The SC dose in monkeys provides an 18.5-fold exposure margin relative to the clinical dose on a mg/m² basis. It is noted that the general safety concerns for local toxicity of trehalose (two glucose molecules linked by an α,α -1,1-glycosidic bond) at SC injection sites are low.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Benralizumab is an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody indicated as an add-on maintenance treatment for patients with severe asthma aged 18 years and older, with an eosinophilic phenotype. The recommended dose is 30 mg every 4 weeks for the first 3 doses followed by once every 8 weeks thereafter.

2.7 Regulatory Background

A Pre-IND meeting was held with the Sponsor (BioWa, Inc.) in 2005; this meeting was not listed in DARRTS. IND 100237 was submitted to the FDA on June 29, 2006 (received on June 30, 2006).

In a submission dated March 5, 2007, Sponsorship of the IND was transferred to MedImmune (effective March 7, 2007). The product designation was changed from BIW-8405 to MEDI-563.

Comments on the proposed design of the chronic toxicology study with monkeys and enhanced pre-and post-natal development study with monkeys were conveyed to the Sponsor (see Review dated November 28, 2008 and/or Comments conveyed on December 19, 2008).

An EOP2 meeting was held with the Sponsor on February 13, 2013 (see meeting minutes dated March 14, 2013). There was one nonclinical question.

Nonclinical responses for the EOP2 meeting minutes:

Questions for Nonclinical Studies- Pharmacology/Toxicology

Question 3: Does the Agency agree that the nonclinical safety program data to date, including carcinogenicity risk assessments, are sufficient to support initiation of Phase 3 studies and registration of benralizumab for the treatment of adult and adolescent patients as defined in the proposed asthma indication?

FDA Response:

We agree that the nonclinical safety program data to date, including carcinogenicity risk assessments, are sufficient to support initiation of the clinical trials described in the meeting package.

Subjects enrolled in clinical trials should be monitored for potential development of tumors.

It appears premature to discuss registration, although based upon information available at this time, it is unlikely that additional nonclinical studies would be required for the filing of a BLA.

A Pre-BLA meeting was held with the Sponsor on September 20, 2016 (see meeting minutes dated October 27, 2016). There were no nonclinical questions in the meeting package; however, there was one nonclinical comment conveyed to the Sponsor.

Nonclinical comment for the Pre-BLA meeting:

Nonclinical comment:

A safety assessment of leachables (and extractables, as appropriate) with the accessorized prefilled syringe should be included with the BLA.

3 Studies Submitted

3.1 Studies Reviewed

STUDY TITLE	STUDY NUMBER
PHARMACOLOGY	
Binding Activity of BIW-8405	Not available
Binding of Benralizumab to Eosinophils and Basophils	Journal of Allergy and Clinical Immunology 125:1344-53, 2010
Binding of BIW-8405 to a Recombinant Cell Line Expressing IL-5R α and Human Eosinophils	Not available
Binding Affinity of BIW-8405 to IL-5R α	Not available
Inhibition of IL-5 Binding to IL-5R α	Not available
Growth Inhibition Activity Against IL-5R α -Expressing Cell Line of KHK4563 (MEDI-563), a Humanized Anti-IL-5R α Monoclonal Antibody	Study d-14-0019 and Journal of Allergy and Clinical Immunology 125:1344-53, 2010
Cross Reactivity of BIW-8405 Against Eosinophils from Non-Human Primates	Not available
Cross Reactivity of Non-Labeled BIW-8405 with Eosinophils from Non-Human Primates	Not available
Binding of Benralizumab (MEDI-563) to Human and Cynomolgus Monkey IL-5R α Expressed on Peripheral Blood-Derived Eosinophils and to Soluble Human and Cynomolgus Monkey IL-5R α	MedImmune Research Report RIA563-0001 and Journal of Allergy and Clinical Immunology 125:1344-53, 2010
Benralizumab (MEDI-563) Does Not Cross-React with Murine IL-5 Receptor Alpha	MedImmune Research Report RIA563-0003
Identification of the Binding Epitope for Benralizumab on IL-5R α	Journal of Allergy and Clinical Immunology 125:1344-53, 2010
Antibody-Dependent Cellular Cytotoxicity (ADCC) of BIW-8405 Against a Recombinant Cell Line	Not available
ADCC Against Human Eosinophils - Release of Eosinophil Granule Proteins	Not available
Induction of Apoptosis in Eosinophils	Not available
MEDI-563 Mediates Eosinophil Apoptosis In Vitro Through Enhanced ADCC	Journal of Allergy and Clinical Immunology 125:1344-53, 2010
Benralizumab (MEDI-563) Does Not Induce Complement Dependent Cytotoxicity of Primary Human Eosinophils	MedImmune Research Report RIA563-0002
Binding Activity of Biotinylated KM8407 (B-KM8407), a Humanized Anti-Human IL-5 Receptor Alpha Chain (hIL-5R) Antibody (IgG1), to Peripheral Blood Leukocytes	Report number: 4-1-06-00-006
Effects of KM8407, a Humanized Anti-IL-5 Receptor Monoclonal Antibody, on IL-5-Induced Peripheral Blood Eosinophilia Model	Report number: 4-1-06-00-007
Evaluation of KM8407, a Humanized Anti-IL-5 Receptor Monoclonal Antibody, in a Monkey Asthma Model	Report number: 4-1-06-00-009
ADME/TOXICOKINETICS	
A Toxicokinetic Study of MEDI-563 Administered Subcutaneously in Male Cynomolgus Monkeys	Non GLP Study No. (b) (4) 263.02
TOXICOLOGY	
Repeat Dose Intravenous Toxicity Study of BIW-8405 in Cynomolgus Monkeys with an 18-Day Recovery Period	Study no.: (b) (4) 112.01
A Fifteen Week Repeat Dose Subcutaneous Toxicity Study of MEDI-563 in Cynomolgus Monkeys Followed by a 12-Week Recovery	Study no.: (b) (4) 263.04

Period	
A 9-Month Intravenous and Subcutaneous Dose Toxicity, Toxicokinetic, and Immunogenicity Study of MEDI-563 in Cynomolgus Monkeys with a 12-Week Recovery Period	Testing Facility Study No. AAO00095
REPRODUCTIVE TOXICOLOGY	
Maternal, Embryo-Fetal and Neonatal Toxicity Study of MEDI-563 Administered Bi-Weekly by Intravenous Injection to Pregnant Cynomolgus Monkeys, Including a 6.5 Month Postnatal Evaluation	Testing Facility Study No. AAO00036
SPECIAL TOXICOLOGY STUDIES	
Cross-Reactivity Study of F-BIW-8405 with Normal Human and Cynomolgus Monkey Tissues	(b) (4) Study Numbers IM1231 and IM1232

3.2 Studies Not Reviewed

STUDY TITLE	STUDY NUMBER
ADME/TOXICOKINETICS	
Assay Qualification of "Capture Binding ELISA of BIW-8405 in Plasma"	TEST PROTOCOL NUMBER: 30421QP
Assay Qualification of "ELISA Assay for Anti-BIW-8405 in Plasma"	TEST PROTOCOL NUMBER: 30424QP
Validation Report for the Detection and Titration of anti MEDI-563 Antibodies in Cynomolgus Serum by ELISA	Doc No.: CTVR-0029
Validation of the ELISA for the Quantification of MEDI-563 in Cynomolgus Monkey Serum	Doc No.: CTVR-0032
SPECIAL TOXICOLOGY STUDIES	
Tolerance Study in Rabbits After a Single Injection of KHK4563 (MEDI-563) by the Subcutaneous Route (Conducted by	Test Facility Study No. 517668, Report No. 31342

3.3 Previous Reviews Referenced

1. Pharmacology and Toxicology Review of IND 100237 dated July 25, 2006
2. Pharmacology and Toxicology Review of IND 100237 dated August 23, 2006
3. Pharmacology and Toxicology Review of IND 100237 dated March 5, 2008
4. Pharmacology and Toxicology Review of IND 100237 dated November 12, 2008
5. Pharmacology and Toxicology Review of IND 100237 dated November 28, 2008
6. Pharmacology and Toxicology Review of IND 100237 dated July 8, 2011
7. Pharmacology and Toxicology Review of IND 100237 dated April 24, 2013
8. Pharmacology and Toxicology Review of IND 100237 dated March 11, 2014
9. Pharmacology and Toxicology Review of IND 100237 dated March 13, 2014

4 Pharmacology

4.1 Primary Pharmacology

Activated eosinophils are the cellular source of granule associated basic proteins, reactive oxygen species, and lipid mediators; which collectively can damage surrounding cells and induce airway hyperresponsiveness and mucus hypersecretion.

IL-5 is the principal cytokine mediating eosinophil mobilization, maturation, activation, and survival. In human subjects, the IL-5 receptor (IL-5R) is expressed exclusively on eosinophil and basophil progenitors in the bone marrow (BM) and on mature eosinophils and basophils. Enhanced eosinophil survival has been considered critical for accumulation of eosinophils in the lungs of asthmatics. Apoptosis of both blood and tissue eosinophils was found to be delayed in patients with asthma (≥ 14 days) when compared to apoptotic rates in healthy individuals (8-18 hr in blood, 2-5 days in tissue). IL-5 was identified as an eosinophil survival-prolonging cytokine and considered to be a strong contributing factor towards initiation and maintenance of eosinophilic airway inflammation in asthma. Furthermore, increased numbers of eosinophils in the airways and peripheral blood of subjects with asthma have been shown to correlate with asthma severity. Neutralization of IL-5 in murine and nonhuman primate models of asthma resulted in reduction of eosinophil counts, which was associated with improved lung pathology.

The following anti-IL-5R antibodies were used in *in vitro* and *in vivo* studies that composed the nonclinical program. The *in vivo* toxicology studies only used benralizumab.

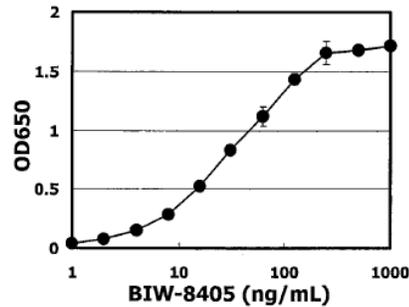
Table 2 Characteristics and nomenclature of benralizumab and benralizumab relevant antibodies

Antibody	Description
KM1259	Mouse anti-hIL-5R α IgG1 antibody
KM8400	Humanized anti-hIL-5R α IgG1 fucosylated variant
KM8407	Humanized afucosylated anti-hIL-5R α IgG1 derived from stable transfected cell pool (<i>FUT8</i> deficient CHO cells).
Benralizumab (MEDI-563, BIW-8405, KHK4563)	Humanized afucosylated anti-hIL-5R α IgG1 derived from stable transfected cell line, (b) (4) <i>FUT8</i> deficient CHO cell line).

(Excerpted from the Sponsor's submission)

Mechanism of action:

Binding Activity of BIW-8405: Binding activity of BIW-8405 was evaluated in an ELISA format using immobilized soluble recombinant IL-5R α protein (sIL-5R α) and an anti-IL-5R α monoclonal antibody (KM1257, mouse IgG1) that recognizes a different epitope in IL-5R α , from that of BIW-8405, as a capture antibody. KM1257 was immobilized onto multi-well plastic plates. Recombinant sIL-5R α protein was captured on to the immobilized KM1257. BIW-8405 was added to wells at various concentrations (1 to 1000 ng/mL). Bound BIW-8405 was measured using alkaline phosphatase (ALP) labeled anti-human IgG monoclonal antibody. BIW-8405 showed reactivity against immobilized sIL-5R α protein in a dose dependent manner.

Figure 4 Binding ELISA of BIW-8405**Figure 1 Binding ELISA of BIW-8405**

Recombinant sIL-5R α protein was immobilized on an immuno assay plate through a capture Mab, KM1257, and the binding activity of increasing concentrations of BIW-8405 was evaluated. Representative data are shown. Data is expressed as mean \pm SD of the OD at 650 nm of three wells for each point.

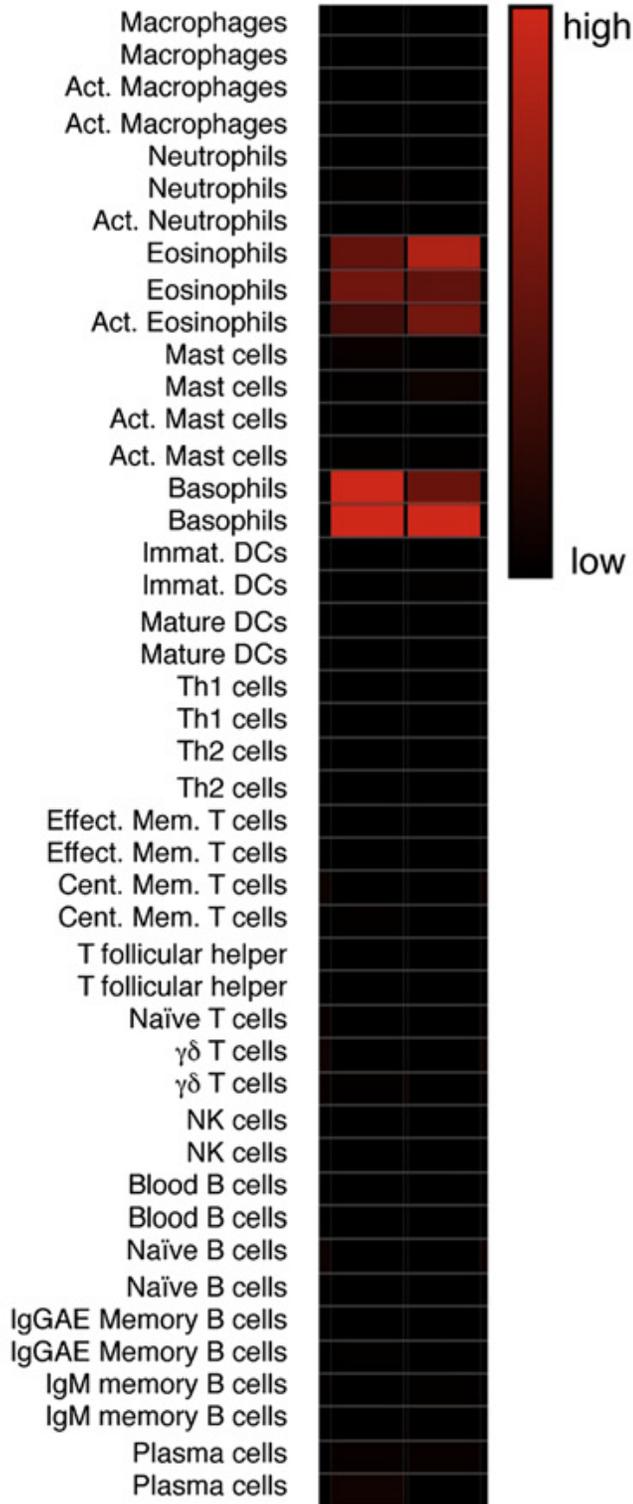
(Excerpted from the Sponsor's submission)

Binding of Benralizumab to Eosinophils and Basophils (Kolbeck et al., Journal of Allergy and Clinical Immunology 125:1344-53, 2010): Consistent with the expression of IL-5R α on human eosinophils and basophils, MEDI-563 exclusively stained peripheral blood eosinophils and basophils from healthy subjects. Eosinophils expressed an approximate 3-fold higher level of IL-5R α compared to basophils as quantified based on median fluorescence intensity. Further, MEDI-563 identified a small but specific fraction (approximately 0.9%) of bone marrow mononuclear cells (BMMNCs) that most likely represented the eosinophil/basophil lineage precursors.

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Table 3 IL5R α mRNA expression on human immune cells

A



(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

Binding of BIW-8405 to a Recombinant Cell Line Expressing IL-5R α and Human

Eosinophils: To evaluate the binding activity of BIW-8405 to IL-5R α protein expressed on the cell surface, flow cytometric analysis was performed on a recombinant KC1270 cell line expressing IL-5R α and human eosinophils purified from healthy volunteers. For the flow cytometry study, BIW-8405 and human IgG1 were labeled with the biotin moiety. Biotinylated BIW-8405 showed a clear reactivity against KC1270 cells and human eosinophils. However, the relative fluorescence intensity of human eosinophils was significantly lower than that of KC1270 cells. Human eosinophils expressed 10 times less IL-5R α protein compared with KC1270 cells.

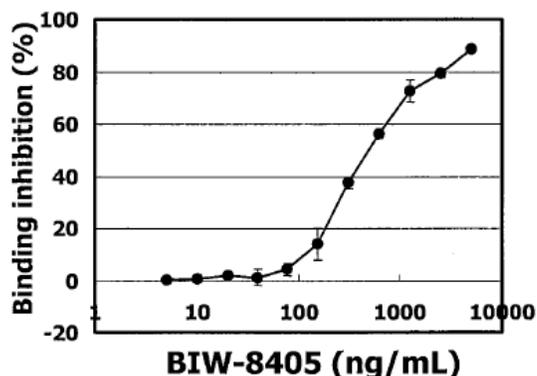
Binding Affinity of BIW-8405 to IL-5R α : BIW-8405 was captured on to a coated sensor chip and recombinant sIL-5R α protein flowed at various concentrations over the mAb. Generated sensograms were analyzed using software for BIAcore equipment to yield on rates (K_{on}), off rates (K_{off}), and dissociation constants (K_D). The average K_D of BIW-8405 was determined to be 2.9 nM, which suggested a high affinity binding of the mAb for the receptor.

Table 4 BIAcore analysis of BIW-8405

Experimental condition	$K_{on}(M^{-1}s^{-1})$	$K_{off}(s^{-1})$	K_D (nM)
Low density BIW-8405 surface	7.60×10^5	0.00226	2.97
High density BIW-8405 surface	7.31×10^5	0.00206	2.82
Average	7.4×10^5	0.0021	2.9

(Excerpted from the Sponsor's submission)

Inhibition of IL-5 Binding to IL-5R α : In a ligand binding inhibition assay, inhibition of binding of IL-5 to immobilized recombinant IL-5R α protein by BIW-8405 (MEDI-563) was evaluated. Inhibitory activity of BIW-8405 in the ligand binding was confirmed by an ELISA using recombinant IL-5R α protein and an anti-IL-5R α monoclonal antibody (KM1257, mouse IgG1), which recognized a different binding epitope in IL-5R α from that of BIW-8405, as the capture antibody. IL-5 binding to its receptor was progressively inhibited by increasing concentrations of BIW-8405 as shown in the figure below. The IC_{50} value in the assay was between 500 and 1000 ng/mL.

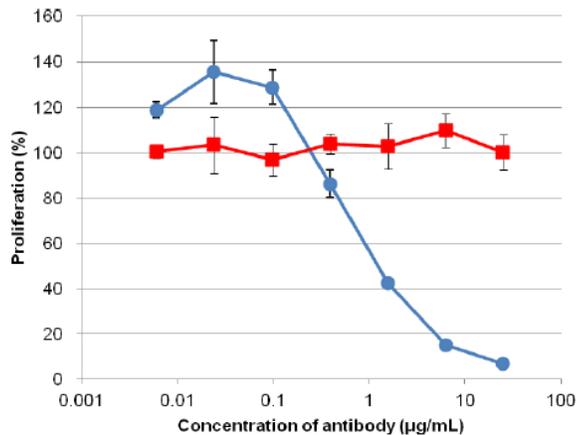
Figure 5 Ligand binding inhibition by BIW-8405**Figure 3 Ligand binding inhibition by BIW-8405.**

The sIL-5R α protein was immobilized on an immunoassay plate employing a mouse anti-IL-5R α Mab, KM1257, which recognizes a different epitope in IL-5R α from that of BIW-8405.

Recombinant IL-5 binding to immobilized receptor protein was detected using biotinylated anti-IL-5 monoclonal antibody. Representative data are shown. Results are expressed as mean \pm SD of the inhibition percentages of three wells for each point.

Growth Inhibition Activity Against IL-5R α -Expressing Cell Line of KHK4563 (MEDI-563), a Humanized Anti-IL-5R α Monoclonal Antibody (Study d-14-0019 and Kolbeck *et al.*, *Journal of Allergy and Clinical Immunology* 125:1344-53, 2010): To evaluate the inhibitory activity of BIW-8405 (MEDI-563) on IL-5-dependent cell growth, cell lines expressing recombinant hIL-5R α , KC1270 and CTLL-2, and responding to IL-5 were used.

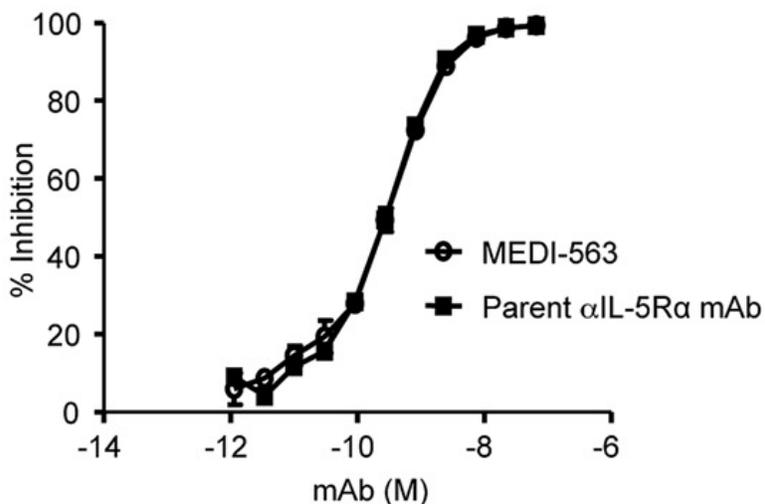
BIW-8405 showed inhibitory activity against IL-5 dependent cell growth of KC1270 cells expressing recombinant IL-5R α in a dose-dependent manner (circle), while control human IgG1 did not affect the cell growth (square).

Figure 6 Proliferation of IL-5R α -expressing cell line**Figure 1 Proliferation of IL-5R α -expressing cell line**

IL-5R α -expressing cell line (KC1270) was incubated with KHK4563 (MEDI-563) (●) and anti-DNP antibody (negative control) (■) at the concentrations of 0.006, 0.024, 0.098, 0.391, 1.563, 6.250 and 25 µg/mL and the growth was measured using WST-1 assay. Data points are mean \pm S.D. of triplicate.

(Excerpted from the Sponsor's submission)

Both MEDI-563 and the fucosylated parent anti-IL-5R α mAb inhibited IL-5-induced proliferation of CTLL-2 cells transfected with recombinant human IL-5R with identical potencies (IC_{50} = 0.3 nmol/L). The inhibitory potency on IL-5-induced cell growth was 300-fold lower relative to its potency for inducing ADCC activity on eosinophils and basophils.

Figure 7 Inhibition of IL-5-induced CTLL-2 cell proliferation

(Excerpted from the Sponsor's submission)

Cross Reactivity of BIW-8405 Against Eosinophils from Non-Human Primates:

Reactivity of BIW-8405 (MEDI-563) to eosinophils in non-human primates was tested by flow cytometric analysis using biotinylated BIW-8405. Peripheral blood from healthy human volunteers or Cynomolgus monkeys was incubated with 1 µg biotinylated BIW-8405 or biotinylated human IgG1 for 1 hr at room temperature in the presence of 6.25 mg of human γ -globulin as a Fc receptor blocker. Biotin-labeled BIW-8405 showed binding activity against human eosinophils from three donors. Biotin-labeled BIW-8405 showed positive reactivity against 2 of 3 tested monkeys. The reason for failure of biotinylated BIW-8405 to bind to the eosinophils from one monkey was unknown. The reactive expression levels of IL5R α on monkey eosinophils were estimated to be in the same range as human eosinophils.

Cross Reactivity of Non-Labeled BIW-8405 with Eosinophils from Non-Human Primates:

A study was conducted to determine if eosinophils from all Cynomolgus monkeys would bind unmodified BIW-8405 (MEDI-563). Blood samples from 15 Cynomolgus monkeys were analyzed by flow cytometry. Peripheral blood samples (100 µL) from Cynomolgus monkeys were incubated with 20.1 µg unmodified BIW-8405 or a control antibody, human IgG1, for 1 hr at room temperature in the presence of 20.1 µg of normal mouse IgG. Non-labeled BIW-8405 bound to monkey eosinophils from all animals. The reason for the lack of reactivity of biotinylated BIW-8405 against some monkey eosinophils was unknown.

Table 5 Binding activity of non-labeled BIW-8405 against monkey eosinophils**Table 5 Binding activity of non-labeled BIW-8405 against monkey eosinophils.**

Animal #	Sex	BIW-8405 (MFI*)	hIgG1 (MFI)	Animal #	Sex	BIW-8405 (MFI*)	hIgG1 (MFI)
1	M	245.91	13.76	9	F	253.02	16.42
2	M	147.82	13.49	10	F	139.91	16.73
3	M	227.82	14.83	11	F	142.10	30.51
4	M	259.23	15.40	12	F	468.64	20.27
5	M	217.90	19.00	13	F	227.60	14.39
6	M	156.43	25.31	14	F	265.86	36.38
7	M	130.41	18.62	15	F	86.80	14.77
8	M	265.79	11.91				

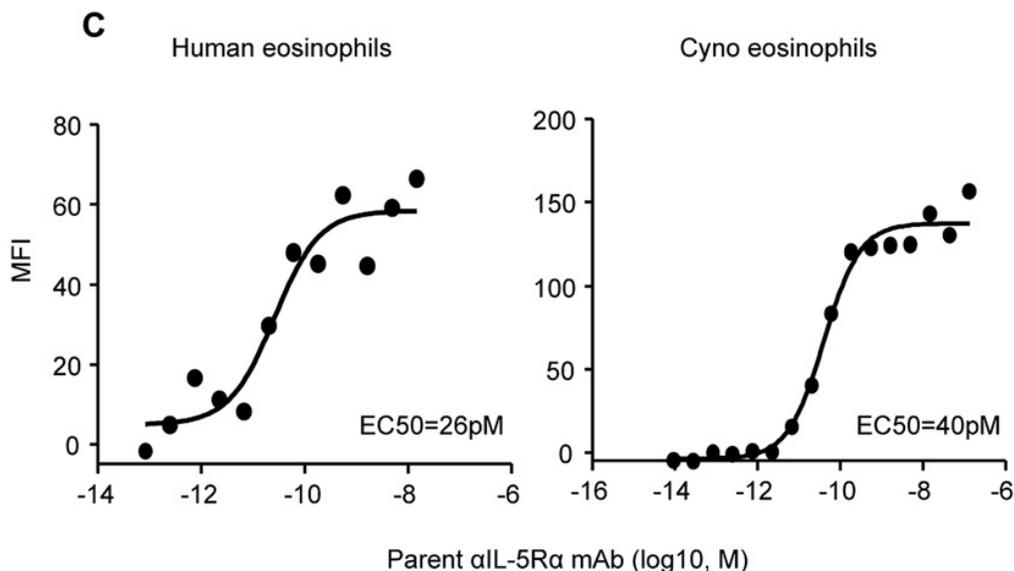
*MFI: Mean Fluorescence Intensity

(Excerpted from the Sponsor's submission)

Binding of Benralizumab (MEDI-563) to Human and Cynomolgus Monkey IL-5R α Expressed on Peripheral Blood-Derived Eosinophils and to Soluble Human and Cynomolgus Monkey IL-5R- α (MedImmune Research Report RIA563-0001 and Kolbeck et al., Journal of Allergy and Clinical Immunology 125:1344-53, 2010): Binding of MEDI-563 to human and Cynomolgus monkey eosinophils and soluble human and Cynomolgus monkey IL-5R α was studied by using flow cytometry and BIAcore methods, respectively.

An indirect binding flow cytometry assay was used to determine the binding EC_{50} values of parental fucosylated MEDI-563 to human and Cynomolgus monkey blood eosinophils. The unlabeled parental fucosylated form of MEDI-563 (8400) was used for binding studies to avoid nonspecific background binding to CD16 (Fc γ Receptor III) expressed on eosinophils. Whole blood (100 μ L) from human or Cynomolgus monkey was mixed with parental fucosylated MEDI-563 at final concentrations ranging from 0.00001 to 10 nM. Tubes were incubated at RT for 1 hr. Allophycocyanin (APC)-labeled goat anti-human IgG Fc γ specific F(ab')₂ fragment was added to cell pellets to label MEDI-563. Flow cytometric data acquisition was performed on a Becton Dickinson LSRII flow cytometry instrument. Eosinophils were identified in the granulocyte gate as cells with high autofluorescence in the PE channel and high side scatter. EC_{50} values of parental fucosylated MEDI-563 binding to eosinophils from humans and Cynomolgus monkeys were 26 and 40 pM, respectively.

Table 6 Binding of parent anti-IL-5R α mAb to human and Cynomolgus monkey (Cyno) eosinophils (MFI, Mean fluorescence intensity)



(Excerpted from the Sponsor's submission / Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

The kinetic rate (k_{on} , k_{off}) constants for the binding of MEDI-563 to recombinant human and Cynomolgus monkey IL-5R α were measured using a BIAcore 3000 instrument. Human and Cynomolgus monkey IL-5R α were immobilized at low density onto separate flow cells on a CM5 sensor chip. BIAcore analyses determined the kinetic rate (on, off) constants from which the apparent K_D was then calculated as k_{off}/k_{on} . The kinetic rate/binding constants of MEDI-563 against human and monkey IL-5R α are shown in the table below. Reduced binding (kinetic rate/binding constant) was observed with the MEDI-563 F(ab) fragment against human and monkey IL-5R α .

Table 7 Kinetic Rate and Binding Constants of MEDI-563 to Human and Cynomolgus monkey Interleukin-5 Receptor Alpha

	MEDI-563			MEDI-563 F(ab)		
	K_{on} ($1/ms \times 10^5$) \pm SEM	K_{off} ($1/s \times 10^{-3}$) \pm SEM	K_D (nmol/L) \pm SEM	K_{on} ($1/ms \times 10^5$) \pm SEM	K_{off} ($1/s \times 10^{-3}$) \pm SEM	K_D (nmol/L) \pm SEM
Human IL-5R α	43.6 \pm 0.5	0.048 \pm 0.02	0.011 \pm 0.005	15.8 \pm 2.6	1.92 \pm 0.01	1.26 \pm 1.0
Cyno IL-5R α	252 \pm 141	0.818 \pm 0.301	0.042 \pm 0.035	15.7 \pm 2.1	31.8 \pm 1.25	20.5 \pm 1.95

Cyno, Cynomolgus monkey; K_D , dissociation constant; K_{off} , off rate; K_{on} , on rate; SEM, standard error of the mean.

(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

Benralizumab (MEDI-563) Does Not Cross-react with Murine IL-5 Receptor Alpha (MedImmune Research Report RIA563-0003): Murine IL-5R α protein shares 68% homology with human IL-5R α . Binding of benralizumab to murine IL-5R α was characterized by flow cytometry using human embryonic kidney (HEK) 293F cells that expressed full length murine IL-5R α on the cell surface.

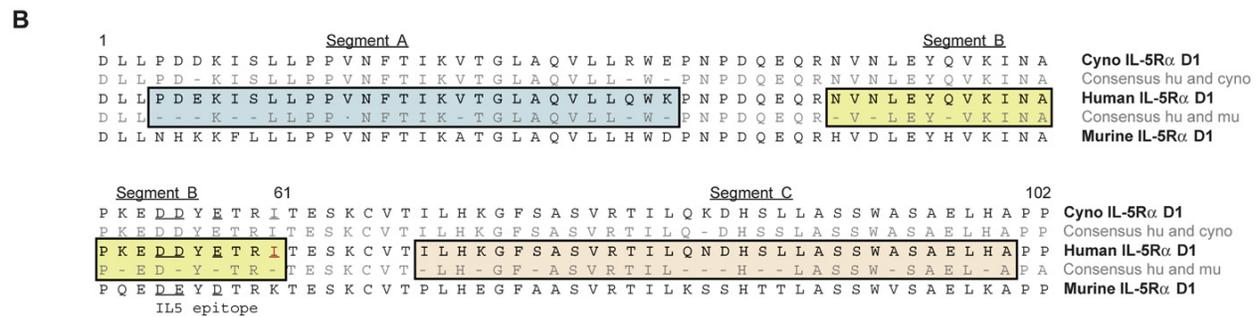
About 1×10^6 HEK293F cells transfected with murine or human IL-5R α constructs were incubated with benralizumab (10 μ g/mL) for 30 minutes. Cells were subsequently incubated with 1 μ g/mL anti-human IgG antibody conjugated to fluorescein isothiocyanate (FITC). Expression of murine and human IL-5R α was monitored with the use of goat anti-mouse and anti-human IL-5R α polyclonal antibodies, respectively, and followed by anti-goat IgG antibody conjugated to FITC. Samples were analyzed using a LSRII flow cytometer.

Full-length murine or human IL-5R α proteins were transiently expressed on the cell surface of HEK293 cells. There was no detectable binding of benralizumab to murine IL-5R α -expressing cells. Benralizumab displayed binding to human IL-5R α -expressing cells as expected. Benralizumab did not cross-react with murine IL-5R α .

Identification of the Binding Epitope for Benralizumab on IL-5R α (Kolbeck et al., Journal of Allergy and Clinical Immunology 125:1344-53, 2010): The lack of binding of benralizumab to murine IL-5R α was used to assist in the identification of the human IL-5R α receptor epitope recognized by benralizumab. Extracellular human IL-5R α domains 1 (D1), 2 (D2), and 3 (D3) were replaced with corresponding murine IL-5R α domain sequences to create knockout variants or the reverse to create knock-in variants using transfected HEK293F cells. The expression levels of all variants were monitored with anti-human or anti-mouse IL-5R α polyclonal antibodies using flow cytometry. Benralizumab bound only to constructs containing the human IL-5R α domain 1. Alignment of human, Cynomolgus monkey, and murine IL-5R α D1 amino acid sequences identified differences between the receptors and these areas were targeted to further characterize the binding epitope. Only swap mutants encoding human segment B were recognized by benralizumab, thus identifying the region containing the binding epitope. Further refinement of the epitope was performed by swapping amino acids in segment B not conserved between the human and murine sequences. Substituting amino acids N40, N42, Q46, D56, and E58 in the human IL-5R α segment B

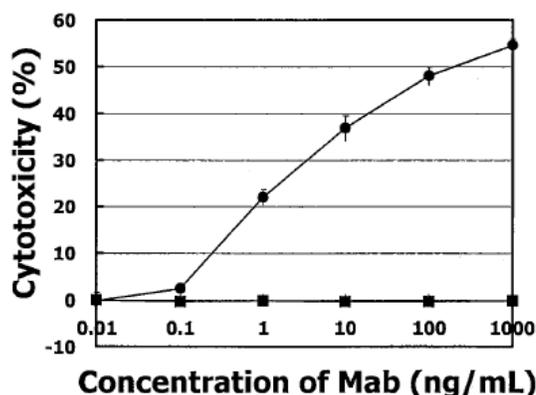
with the corresponding murine residues had no effect on benralizumab binding to human IL-5R α . However, a single amino acid change to isoleucine at position 61 (I61) was sufficient to confer the benralizumab binding to murine IL-5R α . Conversely, benralizumab binding to human IL-5R α was obliterated by replacing I61 with the murine lysine residue at position 61 (K61), ultimately identifying amino acid I61 from the human IL-5R α as the critical residue binding. Segment B (amino acids 40-61) in D1 (including I61) was found to be 100% conserved between humans and Cynomolgus monkeys, thus explaining the cross reactivity of benralizumab with Cynomolgus monkey IL-5R α .

Figure 8 Sequence alignments of murine, human, and Cynomolgus monkey (Cyno) IL-5R α Domain 1 (differences are shown as dashes; segments A [SA], B [SB], and C [SC] are boxed)



(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

Antibody-Dependent Cellular Cytotoxicity (ADCC) of BIW-8405 Against a Recombinant Cell Line: ADCC activity of BIW-8405 (MEDI-563) was evaluated using a recombinant cell line, KC1270, as a target cell and human peripheral blood mononuclear cells (PBMCs) as effector cells. The cytotoxic activity of BIW-8405 by ADCC was evaluated by measuring the amount of the intracellular enzyme, lactate dehydrogenase (LDH), activity in the supernatants, which was released from lysed cells. BIW-8405 exhibited ADCC activity against the target cell line, KC1270 cells, based upon LDH release. The IC₅₀ was approximately 100 ng/mL.

Figure 9 ADCC Activity of BIW-8405**Figure 5 ADCC Activity of BIW-8405**

ADCC activity of BIW-8405 (circle) was measured using human PBMC as the effector cells and KC1270 cells expressing recombinant IL-5R α as the target cells. Human IgG₁ (square) was used as a control substance. ADCC activity was evaluated by measuring the enzymatic activity of LDH, an intracellular protein, in the supernatants. Results are expressed as mean \pm SD of cytotoxicity percentages of three wells for each point.

(Excerpted from the Sponsor's submission)

ADCC Against Human Eosinophils - Release of Eosinophil Granule Proteins: The main target cells of BIW-8405 will be human eosinophils, which express approximately 10-fold less hIL-5R α protein on their surface than the recombinant cell line, KC1270. Peripheral blood mononuclear cells (PBMCs, effector cells) and eosinophils (target cells) were obtained from the same healthy volunteers. To induce the ADCC reaction, purified PBMCs (1×10^6 cells/well) and eosinophils (4×10^4 cells/well) were incubated with BIW-8405 ($1 \mu\text{g/mL}$) for 20 hr at 37°C . After the incubation period, supernatants were recovered from the reaction mixture to evaluate the release of eosinophilic granule proteins, eosinophil derived neurotoxin (EDN), and eosinophil peroxidase (EPO), through the ADCC reaction.

When purified human eosinophils were incubated with autologous PBMCs as a source of the effector cells in the presence of BIW-8405 for 20 hr, eosinophil specific granule proteins were not released into the supernatant. Lack of release of either EDN or EPO suggested there was no cellular necrosis and reduced concerns that these toxic agents might be released in the *in vivo* setting.

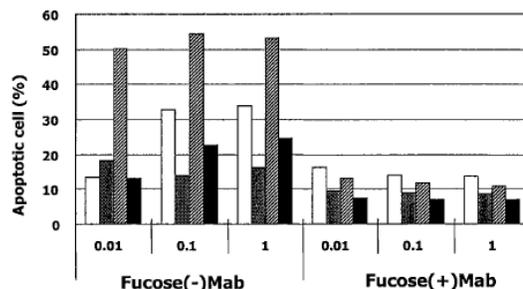
Table 8 Evaluation of eosinophil granule protein release resulting from ADCC activity

Condition	EDN (% release to total)	EPO (% release to total)
Control	9.7 ± 1.5	9.0 ± 0.5
BIW-8405 (1 µg/mL)	10.9 ± 0.5	8.3 ± 2.4

Release of the eosinophil granule proteins, eosinophil derived neurotoxin (EDN) and eosinophil peroxidase (EPO), was assessed following ADCC assay by measuring EDN and EPO in the supernatants. Total released proteins were determined by treating cells with detergent and measuring proteins in the supernatant. Values are expressed as mean ± SD of eosinophil granule protein release percentages representing the two independent experiments.

(Excerpted from the Sponsor's submission)

Induction of Apoptosis in Eosinophils: ADCC-induced apoptosis of human eosinophils was compared between afucosylated (fucose negative) BIW-8405 and fucosylated (fucose positive) BIW-8405 using flow cytometric analysis. To induce ADCC, purified PBMCs (1×10^6 cells/well), as effector cells, and eosinophils (4×10^4 cells/well), as target cells, were incubated with either afucosylated (fucose negative) or fucosylated (fucose positive) BIW-8405 at final concentrations of 0.01, 0.1, and 1 µg/mL in the presence of IL-5 (1 ng/mL) for 20 hr. Afucosylated (fucose negative) BIW-8405 was expressed in the FUT8 gene deficient CHO cell line and fucosylated (fucose positive) BIW-8405 was expressed in the normal CHO cell line. After the incubation period, cells were stained with FITC-labeled Annexin V. Annexin V is known to bind to apoptotic cells. Cells were also stained with PE labeled anti-CD3, anti-CD14, and anti-CD16 monoclonal antibodies to distinguish eosinophils from other white blood cells in samples. CD3⁺CD14⁻CD16⁻ cells having forward and side scatter pattern of eosinophils were analyzed on a flow cytometer for FITC-labeled Annexin V fluorescence. Based upon binding of Annexin V, afucosylated (fucose negative) BIW-8405 induced apoptosis in the human eosinophils while fucosylated (fucose positive) BIW-8405, having lower ADCC activity, exhibited much less apoptosis under the same conditions.

Figure 10 Induction of apoptosis in human eosinophils by ADCC**Figure 6 Induction of apoptosis in human eosinophils via ADCC.**

Eosinophils and PBMC from four volunteers (open, shaded, hatched, and filled column) were cultured with either BIW-8405, fucose (-), or the same Mab produced in a fucose (+) CHO cell. Cells were cultured with various concentrations (0.01, 0.1, and 1 µg/mL) in the presence of IL-5 (1 ng/mL) for 20 h. After the culture period, cells were stained with FITC-labeled Annexin V and analyzed on a flow cytometer. The percentage of Annexin V positive cells in the eosinophils fraction was expressed as a percentage of the apoptotic cells.

(Excerpted from the Sponsor's submission)

MEDI-563 Mediates Eosinophil Apoptosis In Vitro Through Enhanced ADCC (Kolbeck et al., Journal of Allergy and Clinical Immunology 125:1344-53, 2010):

The absence of the monosaccharide, fucose, on the oligosaccharide core of a human IgG1 has previously been shown to result in an increased binding affinity to human FcγRIIIa and subsequently increased ADCC. MEDI-563 (fucose negative) was assessed for binding to FcγRs and ADCC activity relative to the fucosylated parental anti-IL-5Rα mAb.

Examinations of the binding affinity of MEDI-563 (fucose negative) to soluble human FcγR domains by surface plasmon resonance found that binding to human FcγRIIIa ($K_D = 45.5$ nM) was increased 6-fold compared with the fucosylated (fucose positive) parental anti-IL-5Rα mAb; however, binding was similar for all other FcγRs. Similar results were obtained with monkey FcγRIIIa.

Table 9 Binding affinities of MEDI-563 and parent anti-IL-5Ra mAb to human and cynomolgus monkey Fc receptors assessed by means of surface plasmon resonance

	Human FcγRI, K_D (nmol/L) ± SEM	Human FcγRIIIa, K_D (nmol/L) ± SEM	Human FcγRIIb, K_D (nmol/L) ± SEM	Human FcγRIIIa(V), K_D (nmol/L), ± SEM	Cyno FcγRI, K_D (nmol/L)	Cyno FcγRIIIa, K_D (nmol/L)	Cyno FcγRIIb, K_D (nmol/L)	Cyno FcγRIIIa, K_D (nmol/L) ± SEM
MEDI-563	18.5 ± 2.5	1,280 ± 10	4,580 ± 1,150	45.5 ± 0.5	1	3,170	2,070	23 ± 3
Parent αIL-5Rα mAb	18	1,170	3,890	275.5 ± 0.5	1	2,970	1,720	195.5 ± 10.5

Cyno, Cynomolgus monkeys; K_D , dissociation constant; SEM, standard error of the mean.

*Note the 6- and 8-fold higher affinity of MEDI-563 for human and cynomolgus monkey FcγRIIIa, respectively, without affecting the affinity for other Fcγ receptors.

(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

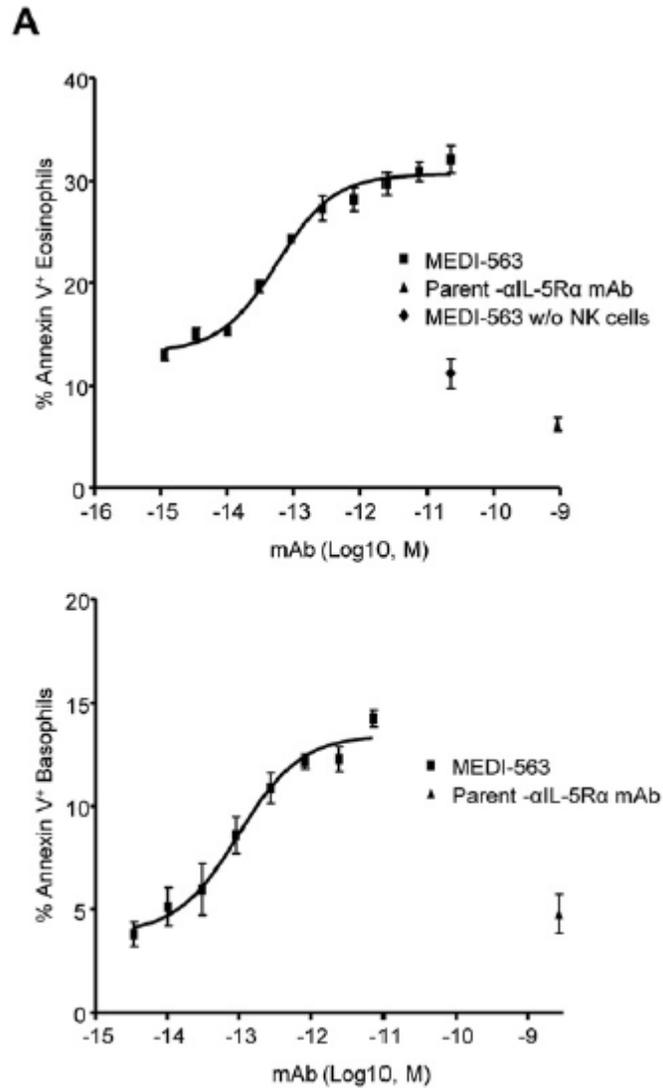
The potency of MEDI-563 (fucose negative) to mediate eosinophil and basophil apoptosis by ADCC *in vitro* was examined. ADCC assays were performed with autologous NK cells as effector cells and bone marrow mononuclear cell (BMMNCs; approximately 1% IL-5R α 1 cells) as target cells. The number of IL-5R α 1 cells was determined by flow cytometry with anti-IL-5R α mAb (KM1257, 10 mg/mL) and phycoerythrin-labeled goat anti-mouse IgG F(ab₂)₂. For ADCC assays with peripheral blood-derived eosinophils or basophils, 10⁴ NK cells and 10⁴ eosinophils or basophils were co-incubated in 96-well plates in the presence of serial dilutions of MEDI-563 (fucose negative) or fucosylated (fucose positive) parental anti-IL-5R α mAb for 22 hr. Annexin V Alexa 647 at 1:500 dilution was added. Cells were analyzed on a flow cytometer (LSRII, BD Biosciences) and the percentage of Annexin V-positive eosinophils/basophils was measured. Eosinophils were identified based on their granularity (high side scatter) and basophils based on Fc ϵ R1 α expression. ADCC was determined by gating of Annexin V-positive target cells. The recovery of target cells was quantitative (approximately 20% of the total cell number). Supernatants were collected at the end of ADCC assays to measure eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) levels by using an ELISA. Total ECP and EDN levels were determined by means of eosinophil lysis with 1% Triton X-100 (100% degranulation), and a mixture of the cytokines, RANTES (13 nmol/L), eotaxin (12 nmol/L), and IL-33 (6 nmol/L) was used as a positive control.

In the presence, but not absence, of autologous NK effector cells, MEDI-563 (fucose negative) induced eosinophil and basophil apoptosis, as assessed by means of Annexin V staining, with EC₅₀ values of 0.9 and 0.5 pM, respectively. However, when the fucosylated (fucose positive) parental α IL-5R α mAb was used at concentrations 1000 times higher than the MEDI-563 (fucose negative) EC₅₀ (in the presence of NK effector cells), it did not induce target cell apoptosis above background levels, although its binding affinity for IL-5R α (data not shown) and its potency to inhibit IL-5-induced cell proliferation were indistinguishable from those of MEDI-563 (fucose negative). MEDI-563 (fucose negative) also depleted human IL-5R α 1 BMMNCs when co-cultured with NK effector cells, whereas an irrelevant afucosylated (fucose negative) isotype control mAb was ineffective.

In contrast to stimulation with a mixture of cytokines (RANTES, eotaxin, and IL-33), eosinophil apoptosis induced by MEDI-563 (fucose negative) was not associated with release of EDN or ECP, indicating a lack of significant eosinophil degranulation and no cellular necrosis.

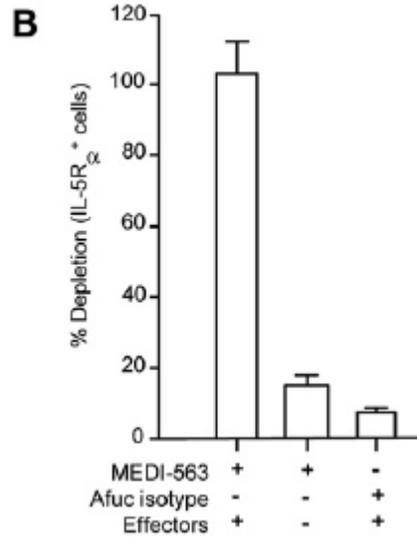
The data suggested an enhanced MEDI-563 (fucose negative) ADCC potency to deplete IL-5R α -expressing eosinophils, basophils, and BMMNCs *in vitro* resulted from fucose deficiency.

Figure 11 MEDI-563-mediated ADCC of human eosinophils ($EC_{50} = 0.9$ pmol/L, $n = 5$) and basophils ($EC_{50} = 0.5$ pmol/L, $n = 5$)



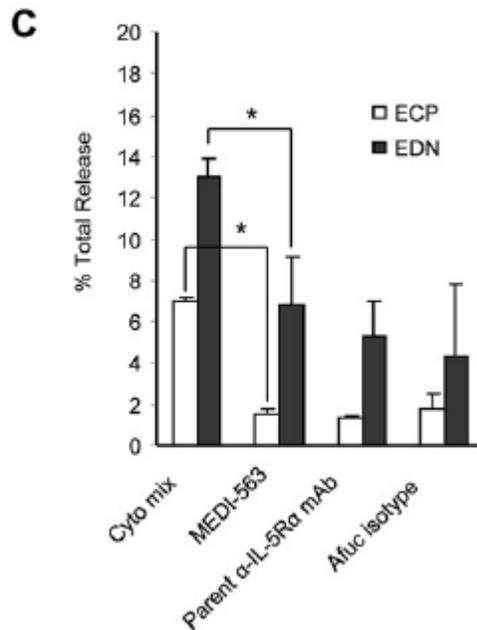
(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

Figure 12 Depletion of human IL-5R α 1 BMMNCs by MEDI-563 in vitro (n = 2)



(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

Figure 13 ADCC of eosinophils was not associated with ECP and EDN release (n=2). [Afuc isotype (Afucosylated isotype antibody control); Total ECP and EDN release induced by Triton X-100 was set to 100%; CYTO mix consisted of RANTES, eotaxin, and IL-33]



(Excerpted from the Journal of Allergy and Clinical Immunology 125:1344-53, 2010)

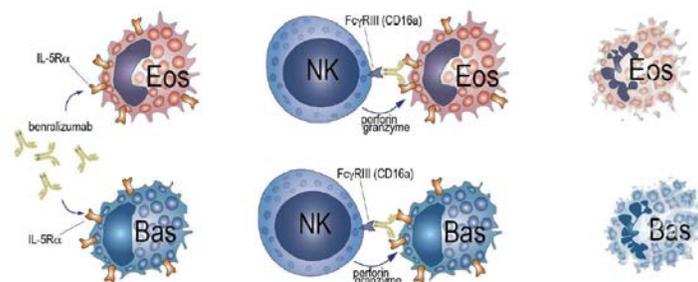
Figure 14 Target cell depletion by benralizumab through enhanced ADCC

Figure 2.6.1-1 Target Cell Depletion by Benralizumab Through Enhanced ADCC

Benralizumab binds to IL-5R α -expressing target cells (eosinophils, basophils) in the circulation and tissues. Effector cells (NK cells, macrophages) express Fc γ RIIIa (CD16a) which binds to benralizumab Fc with high affinity, thereby replacing non-specific low-affinity IgG (fucosylated) from Fc γ RIIIa. Fc γ RIIIa activation results in the release of cytotoxic mediators (perforin, proteases, granzyme) which mediate target cell apoptosis.

(Excerpted from the Sponsor's submission)

Benralizumab (MEDI-563) Does Not Induce Complement Dependent Cytotoxicity of Primary Human Eosinophils (MedImmune Research Report RIA563-0002): The ability of benralizumab and its wild-type, afucosylated form [KM8400; produced in a Chinese hamster ovary (CHO)-^{(b) (4)} cell line deficient in α -1,6-fucosyl transferase (FUT8)] to induce complement dependent cytotoxicity (CDC) of primary human eosinophils was evaluated.

Eosinophils were isolated from whole blood collected from healthy volunteers. To determine if benralizumab could induce CDC, human eosinophils were plated at 20,000-50,000 cells per well in U-bottom 96-well plate in normal human serum media (NHSM), as the source of complement, or heat inactivated human serum media (HIHSM) as a negative control. Benralizumab, the wild type parental antibody (KM8400), or negative control antibodies (R347aFuc or Rituximab) were titrated down from 10 μ g/mL and co-incubated with cells under NHSM or HIHSM conditions. Plates containing cells and antibody with NHSM or HIHSM were incubated at 37°C for 4 hr. Thereafter, cells were washed in PBS and stained with fixable blue live/dead stain. After quenching the dye with PBS + 5% bovine serum albumin (FACS buffer), cells were fixed with 4% paraformaldehyde. Finally, cells were washed and resuspended with FACS buffer, and the number of live/dead cells was visualized by an LSRII flow cytometer.

Rituximab induced CDC of Daudi cells in the presence of complement provided by NHSM; maximal cytotoxicity was 60-80% and was dose dependent. CDC activity was absent when cells and antibody were incubated in HIHSM.

Benralizumab and KM8400 used at concentrations more than 1000-fold greater than the EC₅₀ for ADCC did not demonstrate CDC activity with eosinophils purified from three different human donors.

The lack of CDC activity of benralizumab with eosinophils was attributed to the low expression of IL-5R α on primary human NK eosinophils, which varies from 200 to 1000

copies/cell. In contrast, Daudi cells have about 500-fold more copies of CD20/cell, which explains the CDC activity of rituximab on these cells.

Figure 15 Lack of benralizumab-induced CDC activity with human eosinophils

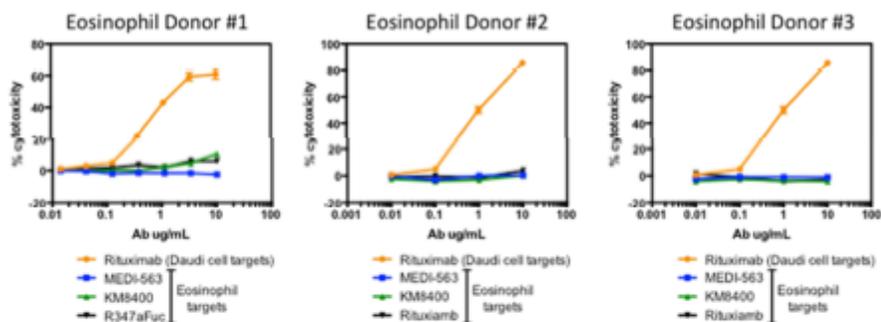


Figure 12-1 Benralizumab (MEDI-563) Does Not Have Complement-dependent Cytotoxicity Activity on Human Eosinophils

Daudi or purified eosinophil target cells were incubated in the presence of increasing concentrations of antibody and normal human serum for 4 hours at 37°C. The extent of cytotoxicity was measured by FACS staining with live/dead fixable blue dye and calculated based on the total number of cells per well. Each data point represents the mean \pm standard deviation of 2-3 replicate wells.

In this figure benralizumab is referred to as MEDI-563. KM8400 = fucosylated parent anti-IL-5R α mAb; R347aFuc = afucosylated isotype control mAb; Rituzimab = anti-CD20 antibody; Rituximab (Daudi cell targets) = Rituximab was incubated with Daudi cells which was used as a positive control.

(Excerpted from the Sponsor's submission)

Binding Activity of Biotinylated KM8407 (B-KM8407), a Humanized Anti-human IL-5 Receptor Alpha Chain (hIL-5R) Antibody (IgG1), to Peripheral Blood Leukocytes (Report number: 4-1-06-00-006): KM8407 was an anti-hIL-5R antibody expected to bind eosinophils expressing IL-5R α . A biotinylated version of KM8407 was prepared to evaluate binding to eosinophils, neutrophils, lymphocytes, and monocytes obtained from the blood of 3 healthy Japanese adult humans and 3 healthy male Cynomolgus monkeys. Monkeys were 5 years 7 months to 5 years 9 months old. A biotinylated human IgG1 served as an isotype control. Flow cytometry was used to evaluate cells. The histograms of B-KM8407 binding to lymphocytes, monocytes, and neutrophils of humans and Cynomolgus monkeys were shown to be essentially identical to those using the isotype control. These results indicated that B-KM8407 did not bind to lymphocytes, monocytes, and neutrophils of human and Cynomolgus monkeys. B-KM8407 specifically stained eosinophils from all 3 human volunteers and 2 out of 3 monkeys (positive ratio, from 47.6% to 85.9%) demonstrating that B-KM8407 bound to most eosinophils of humans and Cynomolgus monkeys. These results showed that B-KM8407 specifically bound to peripheral eosinophils of both humans and Cynomolgus monkeys without binding to lymphocytes, monocytes, and neutrophils. B-KM8407 did not exhibit significant binding activity to eosinophils from 1 of 3 Cynomolgus monkeys.

Drug activity related to proposed indication:

Effects of KM8407, a Humanized Anti-IL-5 Receptor Monoclonal Antibody, on IL-5-Induced Peripheral Blood Eosinophilia Model (Report number: 4-1-06-00-007):

It has been reported that intraperitoneal injection of IL-5 into BALB/c mice induced eosinophilia and the injection of an anti-mIL-5R α mAb prevented IL-5 induced eosinophilia. Based on this information, an IL-5-induced monkey eosinophilia model was established and the efficacy of BIW-8405 was evaluated in this model. Cynomolgus monkeys received subcutaneous administration of recombinant human IL-5 (1 μ g/kg/day for 20 days) and the number of eosinophils in peripheral blood was monitored. A single administration of BIW-8405 was given by the intravenous route into these animals at doses of 0.01 or 0.3 mg/kg on day 10 after induction of IL-5-induced eosinophilia. Two animals were used as controls. The number of eosinophils in the peripheral blood of the monkeys that received IL-5 was significantly increased. Eosinophil counts in animals administered a single dose of 0.3 mg/kg BIW-8405 were significantly lower than in the controls. In 2 of 3 animals that received a dose of 0.01 mg/kg, eosinophil counts were also significantly lower than in the controls. Eosinophil counts were decreased in a dose-related manner. BIW-8405 displayed pharmacological activity in monkeys indicating that it was a relevant species for toxicological testing.

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Figure 16 Recombinant human IL-5 (rhIL-5)-induced peripheral blood eosinophilia in cynomolgus monkeys

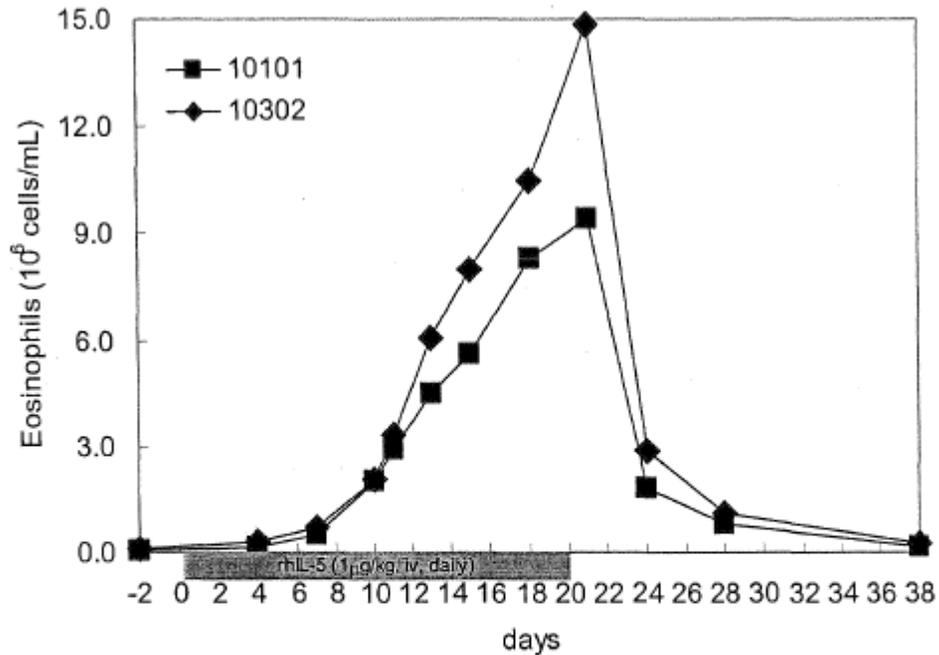


Figure 1 Recombinant human IL-5 (rhIL-5)-induced peripheral blood eosinophilia in cynomolgus monkeys. Two monkeys (animal ID: 10101, 10302) were subcutaneously injected with 0.001 mg/kg of rhIL-5 once a day for 20 days. The number of peripheral blood eosinophils was measured by ADVIA120 on 2 days before and 4, 7, 10, 11, 13, 15, 18, 21, 24, 28 and 38 days after the first rhIL-5 injection.

(Excerpted from the Sponsor's submission)

Figure 17 Effect of KM8407 (0.01 mg/kg) on rhIL-5-induced peripheral blood eosinophilia in Cynomolgus monkeys

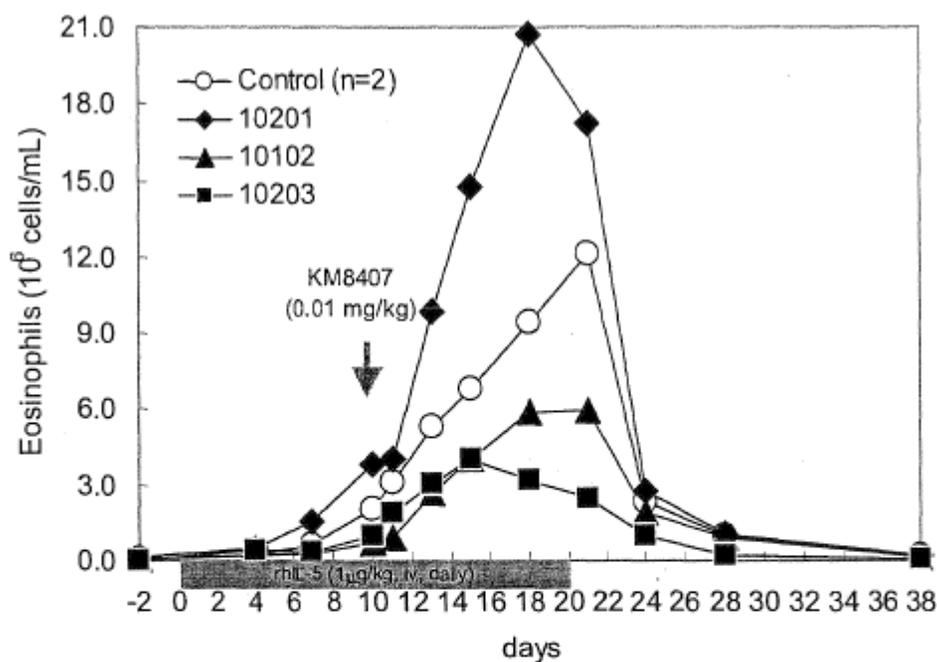


Figure 2 Effect of KM8407 (0.01 mg/kg) on rhIL-5-induced peripheral blood eosinophilia in cynomolgus monkeys. Three monkeys (animal ID: 10101, 10201, 10203) were subcutaneously injected with 0.001 mg/kg of rhIL-5 once a day for 20 days. KM8407 (0.01 mg/kg) was given by intravenous injection at the 10th day of rhIL-5 treatment. The number of peripheral blood eosinophils was measured by ADVIA120 on 2 days before and 4, 7, 10, 11, 13, 15, 18, 21, 24, 28 and 38 days after the first rhIL-5 injection. The symbol of control group represents the mean of two monkeys (animal ID: 10101, 10302).

(Excerpted from the Sponsor's submission)

Figure 18 Effect of KM8407 (0.3 mg/kg) on rhIL-5-induced peripheral blood eosinophilia in Cynomolgus monkeys

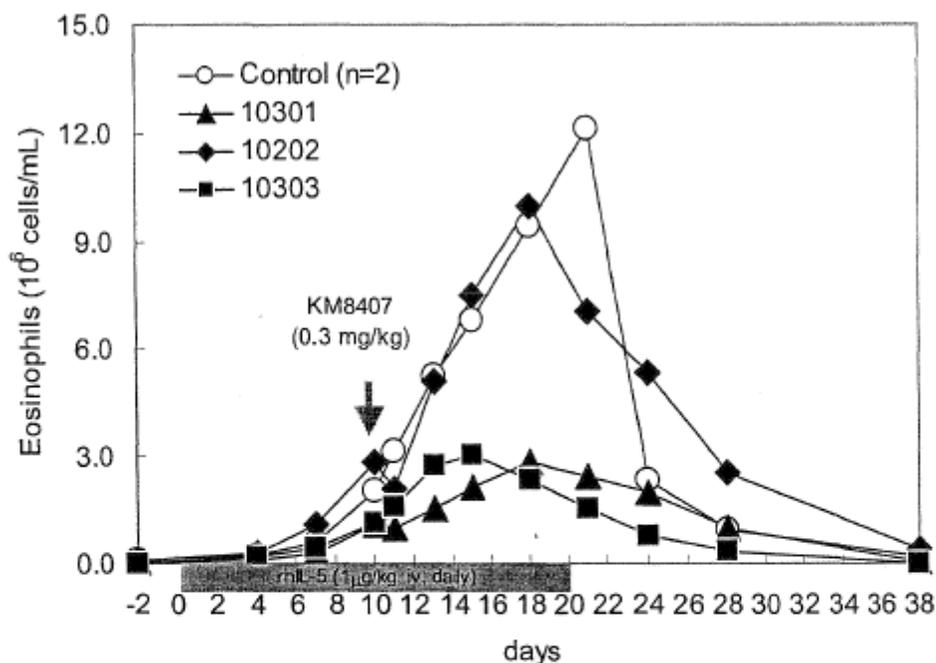


Figure 3 Effect of KM8407 (0.3 mg/kg) on rhIL-5-induced peripheral blood eosinophilia in cynomolgus monkeys. Three monkeys (animal ID: 10202, 10301, 10303) were subcutaneously injected with 0.001 mg/kg of rhIL-5 once a day for 20 days. KM8407 (0.3 mg/kg) was given by intravenous injection at the 10th day of rhIL-5 treatment. The number of peripheral blood eosinophils was measured by ADVIA120 on 2 days before and 4, 7, 10, 11, 13, 15, 18, 21, 24, 28 and 38 days after the first rhIL-5 injection. The symbol of control group represents the mean of two monkeys (animal ID: 10101, 10302).

(Excerpted from the Sponsor's submission)

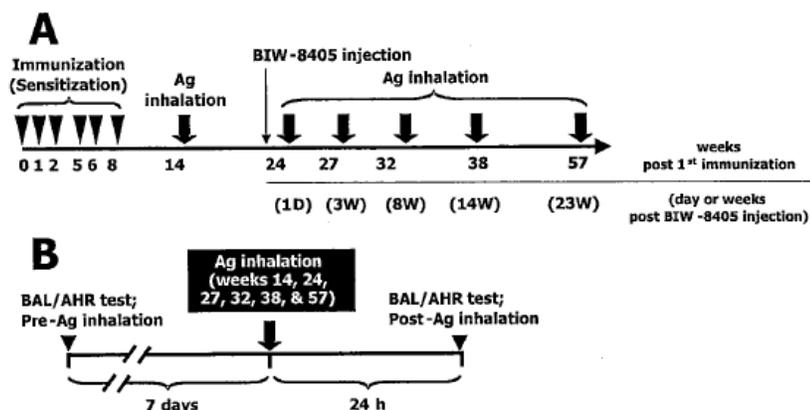
Evaluation of KM8407, a Humanized Anti-IL-5 Receptor Monoclonal Antibody, in a Monkey Asthma Model (Report number: 4-1-06-00-009):

A monkey model actively immunized (sensitized) to *Ascaris suum* antigen was developed and employed to evaluate the anti-asthmatic activity of BIW-8405. Five Cynomolgus monkeys that had no skin reaction to subcutaneous injection of *Ascaris* antigen were immunized with six injections of antigen, administered four times by the intramuscular route and two times by inhalation route. At week 14 after the initial antigen sensitization, airway hyperreactivity (AHR, evaluated by measuring reactivity to methacholine) and eosinophil counts in the bronchoalveolar lavage (BAL) were determined before and after inhalation challenge with antigen. To assess the effects of BIW-8405 on AHR and BAL, monkeys received BIW-8405 by the intravenous route at a dose of 1 mg/kg at 24 hr prior to

antigen challenge during week 24. During subsequent weeks following the single dose of BIW-8405, AHR and BAL were evaluated as shown in the diagram below.

Figure 19 Study design for active sensitization of monkeys, treatment of monkeys with BIW-8405, and antigen challenge

Figure 12 Asthma model: Scheme for monkey active sensitization.



A. Study design. Five male monkeys were immunized (sensitized) with *Ascaris suum* Ag extract (filled triangle). The Ag was administered intramuscularly four times and on two occasions by inhalation. At week 14 after the initial Ag sensitization, AHR and levels of eosinophils in lung (bronchoalveolar lavage [BAL]) were determined both before and after Ag inhalation (as per scheme in Figure 12B). To test the effects of BIW-8405 on AHR and BAL, animals were injected i.v. with 1 mg/kg of Mab 24 h before antigen challenge at week 24. On subsequent weeks following Mab administration, AHR and BAL were evaluated (as per scheme in Figure 12B).

B. Scheme for BAL tests and measurements of AHR to MCh aerosol. Seven days prior to each of the inhalation antigen challenges, BAL testing and AHR measurements were performed. BAL and AHR tests were again performed on each monkey 24 h after antigen challenge.

- Increased eosinophil counts were observed in the BAL following *Ascaris* antigen challenge. A single intravenous dose of BIW-8405 at 1 mg/kg administered to monkeys during week 24 significantly decreased BAL eosinophil counts. The effect of BIW-8405 on eosinophil counts persisted over a 14-week observation period after dosing. As the KM8407 concentration in plasma decreased and became undetectable in all monkeys, the infiltration of eosinophils was observed again after antigen challenge in 3 monkeys (#1, 7, and 15). However, the infiltration of eosinophils remained suppressed 23 weeks after the KM8407 administration in Animals #8 and 10.

Figure 20 Number of eosinophils in the airways of sensitized monkeys

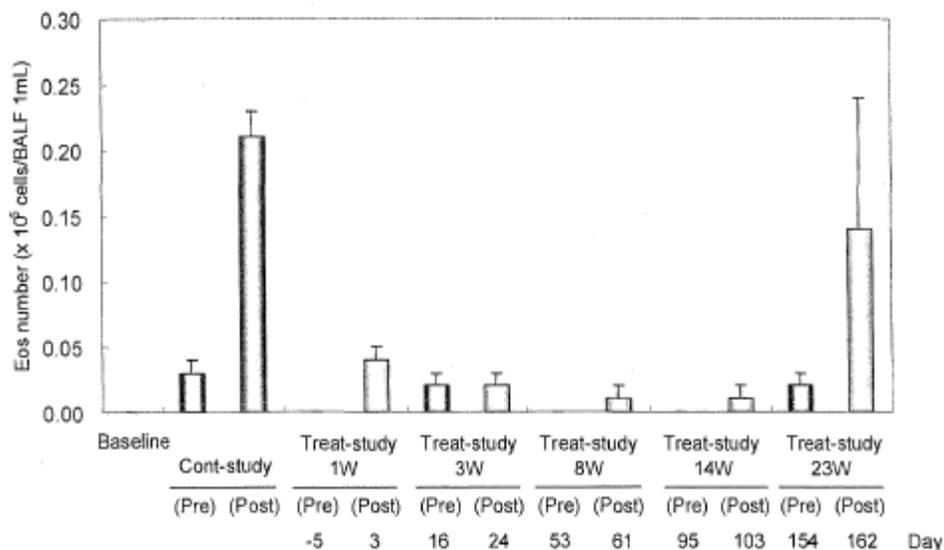


Fig.3 The number of eosinophils in the airways of sensitized monkeys. Samples were collected before (Pre, blue columns) and 24 hours after (Post, red columns) the antigen challenge. KM8407-treatment study (Treat-study) was performed eight weeks after the vehicle-treatment study (Cont-study). KM8407 was administered by single intravenous injection on Day 1. Baseline indicates the value in intact animals. Each column and vertical bar represents the mean + SEM of 5 monkeys. Eos: Eosinophils.

(Excerpted from the Sponsor's submission)

Figure 21 Numbers of eosinophils in the airways of individual, sensitized monkeys

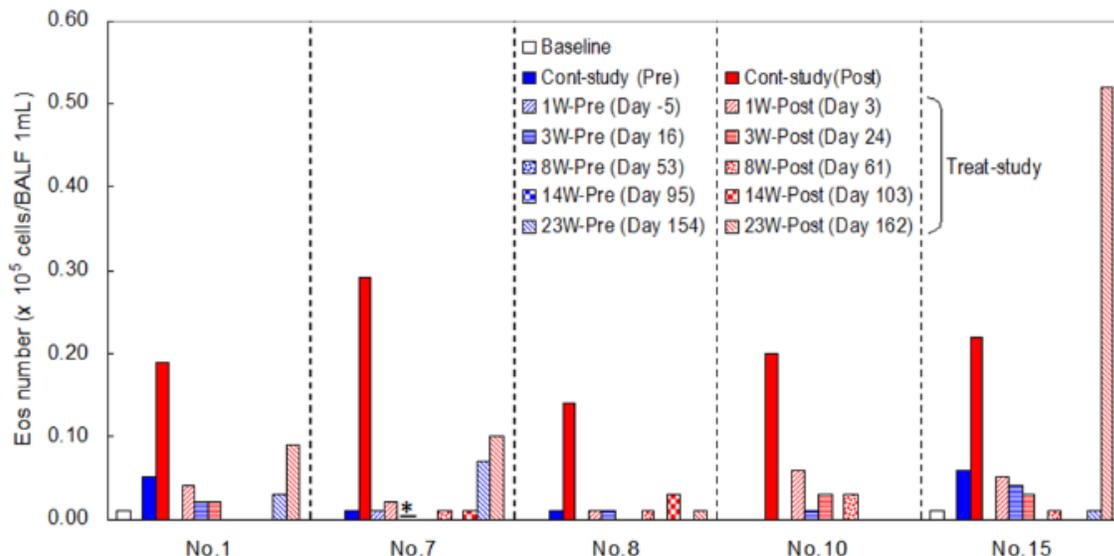


Fig.4 The number of eosinophils in the airways of sensitized monkeys (each individual animal). Samples were collected before (Pre, blue columns) and 24 hours after (Post, red columns) the antigen challenge. KM8407-treatment study (Treat-study) was performed eight weeks after the vehicle-treatment study (Cont-study). KM8407 was administered by single intravenous injection on Day 1. Baseline indicates the value in intact animals. Each column represents the individual data of 5 monkeys. Eos: Eosinophils. (Excerpted from the Sponsor's submission)

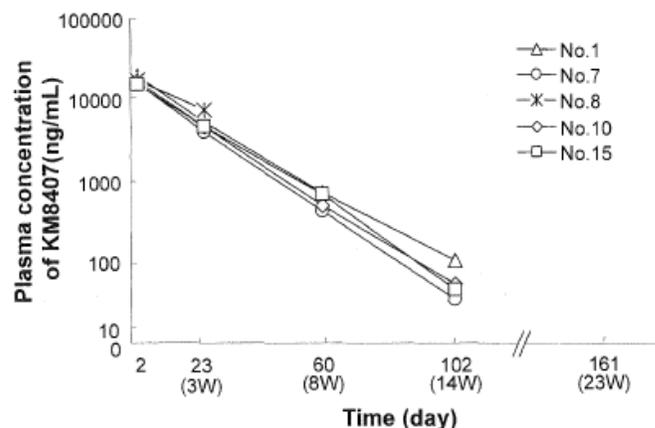
Figure 22 Plasma concentrations of KM8407

Fig.6 Plasma concentration of KM8407 in sensitized monkeys. Each line represents one animal. Only one animal (No. 8, blue line) was unable to maintain a measurable plasma concentration of KM8407 at Day 60 (8W). Animals were given 1 mg/kg of KM-8407.

(Excerpted from the Sponsor's submission)

- Development of airway hyperreactivity was evaluated by measuring reactivity to MCh aerosol inhalation by anesthetized animals before and 24 hr after inhalation of antigen during week 14. PC150, the concentration of inhaled MCh that caused a 50% increase in respiratory system resistance, was used as the indicator for evaluation. Development of AHR was expressed as the decrease in MCh PC150 after antigen inhalation in the figure below (PostPC150/PrePC150). Only 2 (#7 and 8) of 5 immunized animals showed the development of AHR upon Ag challenge. AHR was evaluated on the two animals that tested positive for antigen reactivity. Tests performed at 1 day postdose and at weeks 3, 8, 14, and 23 postdose found that BIW-8405 suppressed the development of AHR in 1 of the 2 animals over a 23-week observation period. The value of Post PC150/Pre PC150 of monkeys #7 and #8 decreased to 55% and 39% upon Ag inhalation. These AHR values were almost as the same as those of the monkeys in the naturally immunized *Ascaris* model. KM8407 completely suppressed the AHR value of monkey #7 at least for 8 weeks (until day 61). In monkey #8, the effect of KM8407 on the AHR suppression was detected 2 days after its administration. However, the significant AHR suppression was not detected thereafter except in week 8

Figure 23 Airway hyperresponsiveness (AHR; %, Post PC 150/Pre PC 150) induced by antigen inhalation in sensitized monkeys

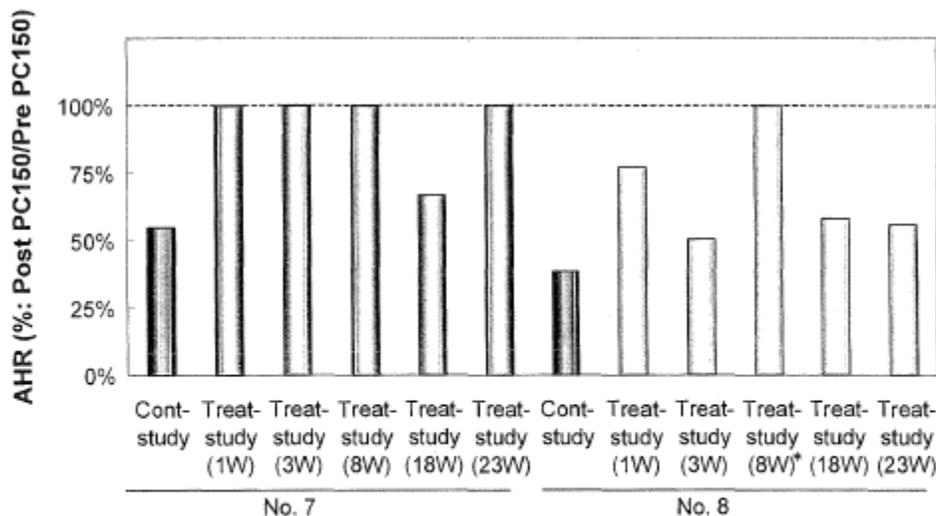


Fig.5 Airway hyperresponsiveness (AHR; %, Post PC150/Pre PC150) induced by antigen inhalation in sensitized monkeys.

Only two monkeys observed AHR in Control-Study were evaluated (Inhibition%: max100%)

*: Low Pre PC150 compared with usual (data not shown)

(Excerpted from the Sponsor's submission)

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted with benralizumab.

4.3 Safety Pharmacology

No standalone safety pharmacology studies were conducted with benralizumab. Observations of safety pharmacology parameters (CNS, respiratory, and cardiovascular function) were incorporated into general toxicology studies (see below).

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

No nonclinical ADME studies were conducted with benralizumab. Toxicokinetic parameters were assessed in general toxicology studies (see below).

5.2 Toxicokinetics

A Toxicokinetic Study of MEDI-563 Administered Subcutaneously in Male Cynomolgus Monkeys (Non GLP Study No. (b) (4) 263.02)

Methods: Three male Cynomolgus monkeys received a single subcutaneous dose of MEDI-563 at 30 mg/kg. A concurrent control group was not included. Observations of

injection sites and clinical signs were conducted twice daily. Body weights were measured on last day of acclimation and on day 22. Blood and urine samples for determination of hematology and urinalysis parameters, respectively, were collected on days -3, 3, and 10. Blood samples for determination of plasma concentrations of MEDI-563 using an ELISA method were collected at 4, 12, 24, 36, 48, 72, 96, 144, 192, 240, 288, 384, and 504 hr postdose. Blood samples for determination of plasma titers of anti-MEDI-563 antibodies using an ELISA method were collected once during acclimation and on days 15 and 22. Monkeys were returned to the stock colony following completion of the study.

Results: Body weight for male #2 declined by approximately 10% from days 1 to 22; however, body weights of the other two males were essentially unchanged. There were significant declines of white blood cell, neutrophil, eosinophil, and monocyte counts from days -3 to 10 that were similar to findings in the 9-week intravenous toxicology study. Animal #2 had red-tinged urine observed in the cage pan on days 21 and 22. Urinalysis results for this animal indicated prominent hematuria and proteinuria of unknown cause. However, there was no evidence of hematuria on days 3 and 10. Animal #4 had evidence of hematuria on day 3, but not day 10. Animal #6 had evidence of hematuria on day 10. Toxicokinetic parameters are shown in the table below. The mean $t_{1/2}$ was 9.73 days in monkeys. The immunogenicity screening assay did not detect the presence of anti-MEDI-563 antibodies during acclimation or days 15 and 22.

Table 10 Hematology parameters - Group means following a single SC dose of MEDI-563

Parameter	Day -3	Day 3	Day 10
White blood cells, $10^3/\mu\text{L}$	15.03	11.51	9.94
Neutrophils, $10^3/\mu\text{L}$	6.45	4.39	2.37
Eosinophils, $10^3/\mu\text{L}$	0.15	0.04	0.01
Monocytes, $10^3/\mu\text{L}$	0.60	0.51	0.41

Table 11 Toxicokinetic parameters of MEDI-563 in Cynomolgus monkeys following a single SC dose

Table 10-1 Noncompartmental TK Parameters of MEDI-563 in Cynomolgus Monkey

Group	30 mg/kg
T_{max} (d)	3 (1.5 - 3)
C_{max} ($\mu\text{g/mL}$)	213 (20.8)
$AUC_{(0-4)}$ (d· $\mu\text{g/mL}$)	2750 (108)
AUC_{inf} (d· $\mu\text{g/mL}$)	3630 (476)
CL/F (mL/d/kg)	8.37 (1.04)
$T_{1/2}$ (d)	9.73 (2.90)

- Parameters are shown as Mean (Standard Deviation), except for T_{max} , which is shown as median (range).
- All parameters are shown in 3 significant figures.

(Excerpted from the Sponsor's submission)

6 General Toxicology

6.1 Single-Dose Toxicity

No single dose GLP toxicology studies were conducted.

6.2 Repeat-Dose Toxicity

Study title: Repeat Dose Intravenous Toxicity Study of BIW-8405 in Cynomolgus Monkeys with an 18-Day Recovery Period.

Key study findings:

- In a 9-week intravenous toxicology study, monkeys received BIW-8405 at doses of 0, 0.1, 1, 10, and 30 mg/kg every 3 weeks for a total of 4 doses (Days 1, 22, 43, and 64).
- For female #25 at 30 mg/kg, there were observations of lameness in the hind limbs from days 22 to 67. There were potentially corresponding histopathological findings, of fibrosis of the perineurium of the sciatic nerve, which may have been responsible for observed clinical signs of lameness. There were observations of liquid feces for this female throughout the treatment period. It is noted that female #25 in the 30 mg/kg group showed positive anti-BIW-8405 responses in plasma samples collected on days 43 and 64; however, lameness and other clinical signs preceded findings of anti-BIW-

8405 antibodies. These findings were judged to be unlikely related to the drug given that no similar findings were evident in longer IV/SC toxicology studies.

- Increased breathing rates were observed for 3 of 10 animals in the 30 mg/kg group at various time points during the treatment period. These increases were generally sporadic, although no similar changes were observed for control and lower dose groups.
- Blood pO₂ levels for male #28 in the high dose group was decreased to 69.9 mm Hg on day 64 (Mean pO₂ levels for the control and high dose groups were 99.3 and 88.0 mm Hg, respectively).
- Heart rates for male #34 and female #36 in the 30 mg/kg group on day 1 decreased to 40 and 36 beats/min, respectively. There were no extreme decreases of heart rate at later time points for these two monkeys.
- Eosinophil counts were decreased in all treatment groups. In the bone marrow, there were parallel decreases of eosinophil precursors. These changes of eosinophil counts in the blood and eosinophil precursors in the bone marrow were attributed to the pharmacological action of BIW-8405.
- Decreased white blood cell counts, due to declines of neutrophil counts, were observed for males and females in the 30 mg/kg group during the treatment period. Recovery of white blood cell and neutrophil counts was generally evident before the end of the treatment period.
- Histopathological findings related to the pharmacological action of BIW-8405 were observed in the bone marrow and lymph nodes. There were no or decreased eosinophils in bone marrow from the femur and sternum. Decreased eosinophils were observed in the lymph nodes from treated animals.
- Cynomolgus monkey anti-BIW-8405 antibody was detected in plasma samples from 2 of 24 animals receiving test article. Male #14 in the low dose group and female #25 in the high dose group showed positive anti-BIW-8405 responses in plasma samples collected on days 64 and days 43 and 64, respectively.
- C_{max} and AUC values for BIW-8405 increased in a dose-related manner on days 1 and 43. Half-lives for BIW-8405 ranged from 189 to 419 hr.
- The NOAEL was identified as the high dose of 30 mg/kg. Findings of lameness and sciatic nerve damage for female #25 in the 30 mg/kg group were judged to be unlikely related to the drug given no similar findings in longer studies. Clinical signs consisting of increased breathing rate, decreased heart rate, and decreased pO₂ for other monkeys at 30 mg/kg were judged to be monitorable in a clinical setting.

Study no.: (b) (4) 112.01

Volume #, and page #: Volumes 15, 16, and 17, Pages 1-951

Conducting laboratory and location: (b) (4)

Date of study initiation: September 26, 2005

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity:

Methods

Doses: Monkeys received BIW-8405 by the intravenous bolus administration once every 3 weeks over a 9-week treatment period (Days 1, 22, 43, and 64).

Table 12 Study Design of 9-week toxicology study with monkeys

Treatment Group/ Color Code	Dose Level (mg/kg)	Dose Conc. (mg/mL)	Dose Volume (mL/kg) ^c	Number of animals		Study Specific Animal Numbers	
				Females	Males	Females	Males
1 / white	0.0	Vehicle	6.0	3 ^a + 2 ^b	3 ^a + 2 ^b	1 ^a , 3 ^a , 5 ^a , 7 ^b , 9 ^b	2 ^a , 4 ^a , 6 ^a , 8 ^b , 10 ^b
2 / yellow	0.1	4.96	0.02	2 ^a	2 ^a	11 ^a , 13 ^a	12 ^a , 14 ^a
3 / green	1.0	4.96	0.2	3 ^a	3 ^a	15 ^a , 17 ^a , 19 ^a	16 ^a , 18 ^a , 20 ^a
4 / blue	10.0	4.96	2.0	2 ^a	2 ^a	21 ^a , 23 ^a	22 ^a , 24 ^a
5 / red	30.0	4.96	6.0	3 ^a + 2 ^b	3 ^a + 2 ^b	25 ^a , 27 ^a , 29 ^a , 31 ^b , 33 ^b	26 ^a , 28 ^a , 30 ^a , 32 ^b , 34 ^b

Note: Total dose volume (mL) was calculated based on the most recent body weight.

a: Terminal Necropsy, Day 67

b: Recovery Necropsy, Day 85

c: Dose volume was adjusted with a correction factor of 1.008.

Species/strain: Purpose-bred Cynomolgus monkeys (*Macaca fascicularis*) of Indonesian origin were used for this study.

Number/sex/group or time point (main study): The control, 1 mg/kg, and 30 mg/kg groups had 3 monkeys/sex/group. The 0.1 and 10 mg/kg groups had 2 monkeys/sex/group.

Route, formulation, volume, and infusion rate: Vehicle and BIW-8405 solution were administered by intravenous bolus injection. Dose volume ranged from 0.02 to 6.0 mL/kg. This was followed by a 1.0 mL flush of vehicle. Due to the low dose volume, after the first dose, Group 2 animals were dosed using needles and syringes without any flush. The control article was a Tween 80 containing citrate buffered sodium chloride formulation (10 mM Citrate buffer (pH 6.0), 150 mM NaCl, and 0.02% polysorbate 80).

Satellite groups used for toxicokinetics or recovery: The control and 30 mg/kg groups had 2 monkeys/sex/group for an 18-day recovery period.

Age: At acclimation, animals were 2 to 9 years old.

Weight: At acclimation, the body weight range for male and female monkeys was 2.276 to 5.879 kg.

Unique study design or methodology (if any): Potential toxicity of BIW-8405 was evaluated in Cynomolgus monkeys when administered once every 3 weeks for 9 weeks.

Observation and Times:

Clinical signs: Clinical observations, including additional central nervous system and injection site observations, were performed twice daily. Physical examinations were performed on all animals once during acclimation, on treatment days 1, 22, 43, and 65, and once prior to necropsy.

Body weights: Animals were weighed twice during acclimation, weekly during the treatment period, and on the day of necropsy.

Food consumption: Animals were offered up to 10 biscuits twice per day. Estimated food consumption was recorded twice daily for all animals in the morning and evening as counts of remaining biscuits.

Ophthalmoscopy: Ophthalmic examinations were conducted once during the acclimation period and on treatment days 1 and 64 at 4 to 8 hr after dosing.

ECG: Heart rate was measured on acclimation day 2, treatment days 1, 22, 43, and 64, and recovery day 14. Measurements of blood pressure and ECG parameters were conducted once during acclimation and on treatment days 22 and 64. Animals were sedated during measurements. The study cardiologist performed qualitative analysis of the leads I, II, aVR, aVL, and aVF and quantitative analysis of heart rate in beats/min and RR, PR, QRS, and QT intervals and QTc in seconds. On days of treatment, collections of heart rate and ECG measurements in relationship to the time of dosing were not clear. A rectal probe was used to measure body temperature.

Hematology: Blood samples for measurement of hematology and coagulation parameters were collected on acclimation days 4 and 11, treatment days 4, 25, 46, and 64, and prior to recovery necropsy.

Clinical chemistry: Blood samples for measurement of serum chemistry parameters were collected on acclimation days 4 and 11, treatment days 4, 25, 46, and 64, and prior to recovery necropsy. Blood samples for flow cytometric immunophenotyping of lymphocytes using antibodies to CD3, CD4, CD8, CD16, and CD20 were collected on acclimation days 4 and 11, treatment days 8 and 57, and recovery day 12.

Urinalysis: Urine was collected at necropsy by puncture of the urinary bladder using a disposable syringe.

Gross pathology: Surviving animals were euthanized on day 67 and recovery day 19 and submitted to necropsy examination. Tissues were preserved in 10% neutral buffered formalin with the exception of the eyes, which were fixed in a mixture of formaldehyde and glutaraldehyde.

Organ weights: Absolute and relative organ weights were determined for the adrenals, brain, heart, kidneys, liver, lungs (including bronchi), ovaries, spleen, testes, and thymus.

Histopathology: Tissues for histopathologic examination were collected from all animals at necropsy. Hematoxylin and eosin stain and other stains were used as necessary. The sponsor stated that as treatment-related findings were not observed in histopathological examination, immunohistochemistry examination was not conducted.

Toxicokinetics: Blood samples for measurement of plasma concentrations of BIW-8405 were collected on acclimation days 4 and 11 and on treatment days 1 and 43 at pre-dose; at 0.083, 0.5, 4, 8, 254, 72, 120, 168, 240, 336, and 504 hr postdose; and once prior to recovery necropsy. Plasma samples were shipped [REDACTED] (b) (4) for analysis.

Blood samples for measurement of immune response to BIW-8405 were collected from all animals on acclimation day 4, treatment day 22, 43, and 64, and prior to the recovery sacrifice on recovery day 12. Plasma samples were shipped [REDACTED] (b) (4) for analysis.

Other: Respiratory rate was recorded during physical examinations once during acclimation, post-dosing on treatment days 1, 22, 43, and 64, and on recovery day 14 for animals.

Blood was collected from all animals for blood gas measurements (pO₂ and pCO₂) at pre-dose and 2-4 hr postdose on days 22 and 64.

Results:

Mortality: None.

Clinical signs: For female #25 at 30 mg/kg, there were observations of lameness in the hind limbs from days 22 to 67. There were potentially corresponding histopathological findings, of fibrosis of the perineurium of the sciatic nerve, which may have been responsible for observed clinical signs of lameness.

For female #25 at 30 mg/kg, there were observations of liquid feces on acclimation days 9 to 11 and treatment days 1, 25-30, 34, 36, 41, 42, 44, 52, 54, 56, 61, 62, and 64. Soft feces were observed on acclimation days 3, 8, and 12-14 and treatment days 1, 2, 8-11, 22-27, 32-41, 44-46, 52-55, and 58-67. Bloody feces were observed on acclimation day 14 and treatment days 1, 29, 30, 38, and 39. Mucous feces were observed on treatment days 37 to 39. This animal received treatment with lactobacillus and Metamucil.

It is noted that female #25 in the 30 mg/kg group showed positive anti-BIW-8405 responses in plasma samples collected on days 43 and 64; however, lameness and other clinical signs preceded findings of anti-BIW-8405 antibodies. Relationships between lameness, clinical signs, and sciatic nerve damage and treatment cannot be ruled out.

Increased breathing rates were observed for 3 of 10 animals in the 30 mg/kg group at various time points during the treatment period. These increases were generally sporadic, although no similar changes were observed for control and lower dose groups. For male #34, breathing rates were increased on days 1, 22, and 43 to 188, 60, and 68 breaths/min, respectively. For male #30, breathing rate on day 22 was increased to 60 breaths/min. For female #29, breathing rate on day 1 was increased to 168

breaths/min. Mean breathing rates for male and female control groups ranged from 27 to 38 and 29 to 54 breaths/min, respectively.

Blood pO₂ levels for male #28 in the high dose group was decreased to 69.9 mm Hg on day 64 (Mean pO₂ levels for the control and high dose groups were 99.3 and 88.0 mm Hg, respectively).

There were no treatment-related effects on body temperature.

Physical examinations found no treatment-related effects on cardiovascular, integument, oral/dental, and urogenital systems. Abnormal muscular/skeletal observations were reported on treatment day 22 for 1 female in the control group, 2 of 3 females in the 1 mg/kg group, 1 of 2 males and 2 of 2 females in the 10 mg/kg group, and 3 of 5 males and of 4 of 5 females in the 30 mg/kg group; however, there were generally no similar findings at other time points (i.e., days 1, 43, and 64). Abnormal gastrointestinal observations were observed for 1 male and 1 female in the 30 mg/kg group on day 64; however, there were no similar observations at other time points.

Body weights: There were no treatment-related effects on body weight gain from acclimation day 14 to treatment day 63.

Food consumption: There were no treatment-related effects on food consumption during the treatment period.

Ophthalmoscopy: There were no treatment-related ophthalmic findings on days 1 and 64.

ECG:

Heart rate: Heart rates for male #34 and female #36 in the 30 mg/kg group on day 1 decreased to 40 and 36 beats/min, respectively. Heart rates for males in the 30 mg/kg group on day 1 were decreased to 72.3% of the control (166 beats/min). Mean heart rates for male and female control groups on day 1 were 166 ± 22 and 172 ± 15 beats/min, respectively. There were no extreme decreases of heart rate, as observed for these two monkeys, at later time points.

Heart rates for males in the 10 and 30 mg/kg groups on day 64 were decreased to 78 and 70% of the control (186 beats/min), respectively. RR intervals for males in the 10 and 30 mg/kg on day 64 were conversely increased.

Heart rates for females in the 10 and 30 mg/kg group on day 43 were increased to 135.8 and 135.2% of the control (162 beats/min), respectively. There were no similar changes at later time points.

Systolic and Diastolic Blood Pressure: Systolic blood pressure for female #31 in the 30 mg/kg group on day 22 was decreased to 69% of the control (133 mm Hg). There were no treatment-related changes of systolic blood pressure for female

treatment groups on day 64. There were no treatment-related changes of diastolic blood pressure for female treatment groups on day 22 or 64. There were no treatment related changes of systolic and diastolic blood pressures for male treatment groups on days 22 or 64.

ECG Parameters: There were no treatment-related changes of ECG parameters on day 22 or 64.

Hematology: Eosinophil counts were decreased in all treatment groups. In the bone marrow, there were parallel decreases of eosinophil precursors, eosinophilic myelocytes, eosinophilic metamyelocytes, eosinophilic band cells, and segmented eosinophils. These changes of eosinophil counts in the blood and eosinophil precursors in the bone marrow were attributed to the pharmacological action of BIW-8405.

Additional changes in bone marrow smears included increased promonocytes for males in the 1, 10, and 30 mg/kg groups and increased plasma cells for females in the 30 mg/kg group.

Decreased white blood cell counts, due to declines of neutrophil counts, were observed for males and females in the 30 mg/kg group during the treatment period. Recovery of white blood cell and neutrophil counts was generally evident before the end of the treatment period (see table showing white blood cell and neutrophil counts for individual males and female monkeys in the 30 mg/kg group).

Lymphocyte counts were increased for female treatment groups on day 25; however, these increases were not evident at later time points.

Monocyte counts were increased for females in the 10 and 30 mg/kg groups.

Fibrinogen levels for male #26 and female #29 in the 30 mg/kg group on day 25 were increased to 215.5 and 192.7% of control values (322 and 302 mg/dL), respectively.

Reticulocyte counts were increased for male and female control and treatment groups as the study progressed due to extensive blood collection for measurements of hematology, serum chemistry, and toxicokinetic parameters.

Changes of other bone marrow cell populations beyond eosinophil precursors were observed at the terminal and recovery sacrifices as shown in the table below. The toxicological significance of these changes was unclear. There did not appear to be any significant changes of neutrophil precursors that might explain observed changes of neutrophil counts.

Table 13 Hematology parameters for male monkeys on acclimation phase day 11, treatment days 4, 25, 46, and 64, and recovery day 12

Hematology parameters	Time	0	0.1	1	10	30
Eosinophils x 10 ³ /μL	AP11	0.25	0.09	0.19	0.25	0.11
	DP4	0.33	0.01	0	0.10	0.02
	DP25	0.32	0	0	0	0
	DP46	0.36	0	0	0	0
	DP64	0.31	0	0	0	0
	R12	0.21	-	-	-	0
White blood cell counts x 10 ³ /μL	AP11	13.16	8.99	11.41	10.29	12.21
	DP4					
	DP25	12.72	8.05	9.87	12.70	7.69 (60.5%)
	DP46	11.73	10.63	9.96 (85%)	9.19 (78%)	9.65 (82%)
	DP64	16.65	16.90	15.18	11.61 (70%)	12.05 (72%)
Neutrophils x 10 ³ /μL	AP11	6.67	4.61	4.78	3.52	6.77
	DP4					
	DP25	5.83	3.34	4.84	6.23	2.77 (48%)
	DP46	5.57	5.84	4.92 (88%)	3.70 (66%)	4.31 (77%)
	DP64	10.69	13.39	10.76	3.94 (37%)	7.16 (67%)

AP = acclimation period, DP = dosing period, R = recovery

Table 14 Hematology parameters for female monkeys

Hematology parameters	Time	0	0.1	1	10	30
Eosinophils x 10 ³ /μL	AP11	0.11	0.35	0.30	0.39	0.30
	DP4	0.14	0.06	0	0.01	0.10
	DP25	0.16	0	0	0	0
	DP46	0.19	0	0.01	0	0.01*
	DP64	0.12	0.01	0	0	0.02*
	R12	0.06	-	-	-	0
White blood cell counts x 10 ³ /μL	AP11	9.15	12.12	11.72	12.00	12.00
	DP4	9.90	11.05	10.60	12.48	9.60

	DP25	9.07	10.40	10.63	13.33	10.46
	DP46	11.34	10.32	9.46	7.94 (70%)	9.42 (83%)
	DP64	14.15	12.12 (86%)	12.05 (85%)	9.54 (67%)	10.32 (73%)
Neutrophils x 10 ³ /μL	AP11	3.93	4.08	5.46	6.73	5.91
	DP4	3.44	3.92	4.07	7.61	4.70
	DP25	4.37	3.37	4.56	6.42	3.54 (81%)
	DP46	5.86	3.32 (57%)	3.72 (64%)	3.51 (60%)	4.94 (84%)
	DP64	10.42	7.37 (71%)	7.17 (69%)	6.37 (61%)	6.38 (61%)
Lymphocytes x 10 ³ /μL	D25	4.07	6.46 (159%)	5.52 (136%)	6.11 (150%)	6.27 (154%)
	D25	0.28	0.49	0.36	0.63 (225%)	0.55 (196%)

AP = acclimation period, DP = dosing period, R = recovery

* Female #25 in the high dose group developed anti-BIW-8405 antibodies. There was evidence of increased eosinophil counts in this animal with the decline of plasma concentrations of BIW-8405.

Table 15 Hematology parameters for the 30 mg/kg group

Hematology parameters	Time	Males					Females				
		#26	#28	#30	#32	#34	#25	#27	#29	#31	#33
White blood cell counts x 10 ³ /μL	AP11	8.52	10.04	16.00	8.45	18.04	11.08	11.63	12.11	9.01	16.18
	DP4	7.35	12.18	12.97	9.99	22.87	8.96	10.56	8.29	8.52	11.69
	DP25	1.96	8.86	7.77	5.75	14.13	5.91	8.94	18.09	7.94	11.41
	DP46	2.80	10.19	8.52	10.31	16.45	5.04	9.70	9.39	6.67	16.31
	DP64	6.80	11.25	12.21	13.04	16.96	13.58	9.41	8.91	5.47	14.23
Neutrophils x 10 ³ /μL	AP11	6.40	5.55	8.38	3.78	9.74	4.75	6.76	7.45	4.19	6.79
	DP4	5.09	7.07	8.75	5.05	10.58	3.47	5.60	4.30	3.32	3.43
	DP25	0.18	4.26	2.35	1.73	5.32	1.24	4.38	6.51	2.14	11.07
	DP46	0.88	5.46	3.24	3.58	8.40	1.01	5.18	4.62	2.84	8.93
	DP64	4.38	8.05	6.52	9.44	7.41	8.56	5.66	5.99	2.77	2.32

AP = acclimation period, DP = dosing period

Figure 24 Peripheral blood eosinophil depletion in Cynomolgus monkeys by MEDI-563

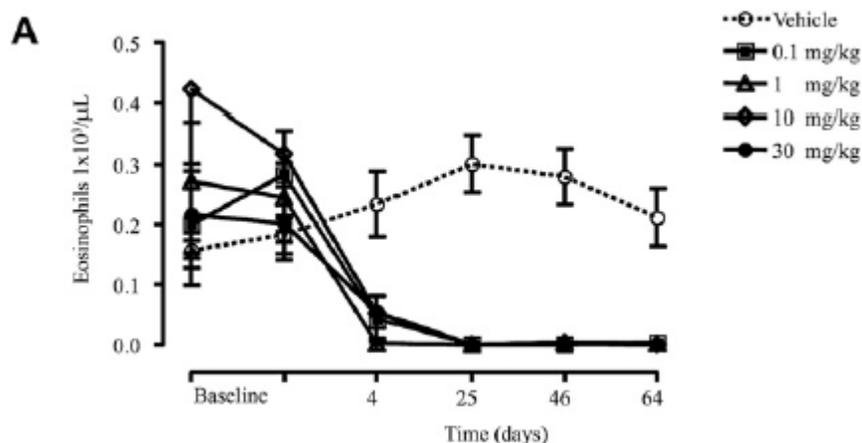


Table 16 Differential count of bone marrow smears at the terminal sacrifice (units were not specified)

Feature	Males					Females				
	0	0.1	1	10	30	0	0.1	1	10	30
Eosinophilic myelocyte	2.67	1.50	0	0	0	1.00	0	0.33	0	0
Eosinophilic metamyelocytes	2.67	1.50	0	0	0	1.00	0	0	0	0
Eosinophilic band	3.67	1.00	0	0	0	2.33	0	0	0	1.00 (43%)
Segmented eosinophil	5.33	1.00	0	0	0	7.00	0	0	0	0.67 (9.6%)
Rubricyte	96	134.5 (140%)	142 (148%)	130.5 (136%)	135.67 (141%)					
Promonocyte	2	2	5.33 (267%)	6.0 (300%)	8.0 (400%)					
Prorubricyte						13.67	12.50	10.67 (78%)	9 (66%)	6 (44%)
Promyelocyte						6.00	8.50	8.33	15.50 (258%)	12.33 (206%)
Plasma cell						7.00	5.00	10.33 (148%)	9.50 (136%)	16.33 (233%)

Blank cells indicate that there were no differences between control and drug-treated groups

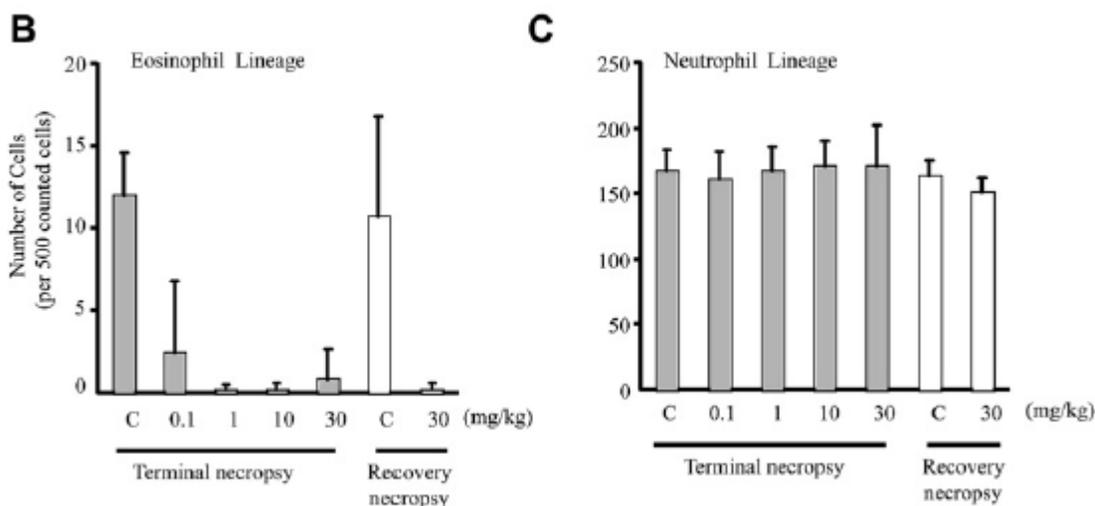
Table 17 Differential count of bone marrow smears at the recovery sacrifice (units were not specified)

Feature	Males		Females	
	0	30	0	30
Eosinophilic myelocyte	2.00	0.00	1.00	0.00
Eosinophilic metamyelocytes	0.50	0.00	0.50	0.00

Eosinophilic band	3.00	0.00	3.50	0.00
Segmented eosinophil	7.00	0.00	3.50	0.50
Basophilic myelocyte	1.00	0.00		
Basophilic metamyelocytes	0.50	0.00	0.00	1.00
Basophilic band	0.00	1.00		
Segment basophil	0.00	0.50	0.00	0.50
Promonocyte	3.50	2.00 (57%)		
Plasma cell	10.00	14.00 (140%)		
Mature lymphocyte	77.00	97.50 (126.6%)		
Promegakaryocyte			0.00	1.50
Megakaryoblast			0.50	0.00

Blank cells indicate that there were no differences between control and drug-treated groups

Figure 25 Depletion of BM eosinophil precursors (B) and neutrophil precursors (C) [Data indicate the average of 3 to 10 monkeys per group and error bars represent the SD]



Clinical chemistry: Globulin levels were slightly elevated for males and females in the 10 and 30 mg/kg groups on days 25, 46, and 64. These elevations may be due to administered IgG, which is detected by the assay. Globulin levels for male #22 in the 10 mg/kg group on day 4 were increased to 198.0 g/dL; however, elevated levels were not observed at later time points. Globulin levels for control female #9 on day 4 were increased to 194 g/dL.

IgG levels were increased for males in the 30 mg/kg group on day 46 and recovery day 12. These elevations may be due to administered BIW-8405, which is detected by the assay.

Male #24 in the 10 mg/kg group on day 4 was found to have a creatine kinase activity value of 15803 U/L as compared to the control mean of 144 U/L; however, there were no similar changes in the 30 mg/kg group and values for this animal were within the control range at later time points.

Table 18 Clinical chemistry [Acclimation days 4 (A4) and 11 (A11), Treatment days 4, 25, 46, and 64, and Recovery day 12 (R12)]

Parameter	Day	Males					Females				
		0	0.1	1	10	30	0	0.1	1	10	30
Globulin g/dL	4	3.1	2.9	2.9	100.5	3.5	41.5	3.1	3.5	3.2	3.2
	25	3.2	2.9	3.1	3.3	3.9 (122%)	3.2	3.3	3.7 (116%)	3.5 (109%)	3.7 (116%)
	46	3.4	3.2	3.2	3.5	4.6 (135%)	3.5	3.4	3.8 (109%)	3.7 (106%)	3.9 (111%)
	64	3.1	2.8	3.1	3.6 (116%)	4.0 (129%)	3.1	3.1	3.6 (116%)	3.4 (110%)	3.4 (110%)
IgG mg/dL	46	1360.2	1017.5	1298.7	1363.5	1923.6 (141%)					
	R12	1156.0				1569.0 (136%)					
Triglyceride mg/dL	25	37	44	32	29	54 (146%)					

Blank cells indicate that there were no differences between control and drug-treated groups

Urinalysis: There were no treatment-related changes of urinalysis parameters on day 67 or recovery day 19.

Gross pathology: For one female (#27) in the 30 mg/kg group, there were findings of a moderate amount of gas in an expanded colon. Microscopic examination found that the colon was flattened without any abnormal histopathological changes. For one male (#26) in the 30 mg/kg, water was observed in the lungs; however, this judged to be an artifact given that exsanguination was performed in a sink with running water. Absolute and relative lung weights for male #26 were significantly higher than any other animal in the study.

Organ weights: Absolute and relative spleen weights were increased for males in the 10 and 30 mg/kg groups and females in 0.1, 10, and 30 mg/kg groups. A dose-response relationship was not evident for female treatment groups suggesting that observed differences were not likely related to treatment. There were no apparent corresponding histopathological findings in the spleen.

Table 19 Absolute and relative organ weights at the end of the treatment and recovery period

Organ	Time	Males					Females				
		0	0.1	1	10	30	0	0.1	1	10	30
Spleen g	Term	7.667	6.4900	7.9867	10.170 (133%)	10.620 (139%)	4.8333	8.455 (175%)	5.20 (108%)	5.9750 (124%)	7.6767 (159%)
	Rec	5.2950				8.9350 (169%)	4.7950				5.5450 (116%)
Spleen %BW	Term	0.1534	0.1515	0.1666	0.2407 (157%)	0.2191 (143%)	0.1555	0.1817 (117%)	5.20 (124%)	0.2035 (131%)	0.2545 (164%)
	Rec	0.1395				0.2808 (201%)	0.1877				0.2280 (122%)
Spleen %BrW	Term	12.155	9.9594	12.228	15.443 (127%)	14.609 (120%)	8.5852	13.970 (163%)	9.2108 (107%)	9.7571 (114%)	12.751 (146%)
	Rec	7.9514				14.446 (182%)	8.0406				9.8095 (122%)

%BW = relative to body weight, %BrW = relative to brain weight

Histopathology: Histopathological findings were observed in several organs and tissues.

Findings related to the pharmacological action of BIW-8405 were observed in the bone marrow and lymph nodes. There were no (completely absent/depleted) or decreased eosinophils observed in bone marrow from the femur and sternum of drug-treated monkeys; eosinophils were observed in the bone marrow of control monkeys. Decreased eosinophils were observed in the lymph nodes from treated animals.

For 1 female (#25) in the 30 mg/kg group, there were observations of fibrosis of the perineum of the sciatic nerve. This animal was observed with lameness during the treatment period.

Mononuclear cell infiltration was observed in several organs and tissues; however, incidences and severity generally displayed no relationship to treatment (i.e., no dose-response). For the left and medial lobes of the liver, mononuclear cell infiltration was observed for almost all control and treated animals. Mononuclear cell infiltration was considered an adaptive response to various types of commonly observed parasitic infections, which were evident in several animals in control and treatment groups. Lack of dose-response relationships for incidence and severity suggested that observed findings were not related to treatment.

Immature testes and epididymides observed in the 10 and/or 30 mg/kg were attributed to the ages of male monkeys.

There were several findings generally observed for single animals in the high dose group with no clear relation to treatment.

Table 20 Histopathological findings at the terminal sacrifice, day 67

Tissue/Organ	Sex	0	0.1	1	10	30
Bone marrow (femur, left) -no/decreased eosinophils	M F	0/2 0/2	1/2 2/2	1/1 2/2	1/1 2/2	3/3 2/3
Bone marrow (sternum) -no/decreased eosinophils	M F	0/3 0/3	1/2 2/2	3/3 2/2	2/2 2/2	3/3 2/3
-increased granulopoiesis	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 0/3
Lymph node (mandibular, left) -eosinophil sinus	M F	1(+)/3 3(±)/3	0/2 0/2	0/3 0/3	0/2 0/2	0/3 1(±)/3
Lymph node, mesenteric -eosinophil, sinus	M F	1(+)/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	0/3 0/3
Sciatic nerve (cross and longitudinal section) -fibrosis, perineurium	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	0/3 1(+)/3
Lungs -brown pigment deposition	M F	0/3 1(±)/3	1(±)/2 0/2	0/3 0/3	0/2 0/2	2(±)/3 1(±)/3
-mononuclear cell infiltration	M F	0/3 0/3	1(±)/2 0/2	1(±)/3 0/3	0/2 0/2	1(±)/3 0/3
Bone (femur with knee joint, right) -mononuclear cell infiltration	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 0/3
Pituitary gland -mononuclear cell infiltration, pars intermedia	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 0/3
Submandibular gland -mineralization duct	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 0/3
-mononuclear cell infiltration, interstitium	M F	0/3 1(±)/3	1(±)/2 2(±)/2	3(±)/3 3(±)/3	2(±)/2 0/2	1(±)/3 2(±)/3
Thyroid -ectopic thymus	M F	0/3 0/3	1/2 0/2	1/3 1/3	0/2 0/2	1/3 0/3
-mononuclear cell infiltration, interstitium	M F	1(±)/3 0/3	0/2 1(±)/2	2(±)/3 1(±)/3	0/2 0/2	2(±)/3 1(±)/3
Adrenals -mononuclear cell infiltration, cortex	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	0/3 1(±)/3
Brain (cerebellum) -mononuclear cell infiltration, perivascular	M F	0/3 0/3	0/2 1(±)/2	0/3 0/3	2(±)/2 0/2	0/3 2(±)/3
Brain (cerebrum, parietal)						

lobe) -mononuclear cell infiltration, perivascular	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	0/3 1(±)/3
Brain (cerebrum, diencephalon) -mineralization, parenchyma	M F	0/3 0/3	1(±)/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 0/3
Eyeball (optic nerve, left) -mononuclear cell infiltration, ciliary body	M F	0/3 0/3	0/2 1(±)/2	0/3 1(±)/3	0/2 0/2	0/3 1(±)/3
Eyeball (optic nerve, right) -mononuclear cell infiltration, ciliary body	M F	1(±)/3 0/3	0/2 0/2	0/3 1(±)/3	0/2 0/2	0/3 2(±)/3
Heart (left ventricle) -mononuclear cell infiltration	M F	1(±)/3 0/3	1(±)/2 0/2	0/3 0/3	0/2 0/2	1(±)/3 1(±)/3
Kidney (left) -mononuclear cell infiltration, pelvic epithelium	M F	3(±)/3 0/3	1(±)/3 0/2	3(±)/3 0/3	2(±)/2 0/2	3(±)/3 1(±)/3
Kidney (right) -mononuclear cell infiltration, pelvic epithelium	M F	2(±)/3 1(±)/3	1(±)/2 2(±)/2	1(±)/3 3(2±, 1+)/3	1(±)/2 1(±)/2	3(±)/3 3(±)/3
Liver (right lobe, medial lobe) -mononuclear cell infiltration	M F	2(±)/3 0/3	1(±)/2 1(±)/2	3(±)/3 1(±)/3	0/2 1(±)/2	3(±)/3 2(±)/3
Liver (left lobe, medial lobe) -mononuclear cell infiltration	M F	3(±)/3 3(±)/3	1(±)/2 2(±)/2	1(±)/3 3(±)/3	0/2 1(±)/2	3(±)/3 2(±)/3
Pancreas -mononuclear cell infiltration, interstitium	M F	0/3 0/3	0/2 1(+)/2	0/3 0/3	0/2 0/2	0/3 1(±)/3
Urinary bladder -mononuclear cell infiltration, submucosa	M F	0/3 0/3	0/2 0/2	0/3 1(+)/3	0/2 1/2	1(±)/3 1(±)/3
-mononuclear cell infiltration, muscular layer	M F	0/3 0/3	0/2 0/2	0/3 1(+)/3	0/2 0/2	0/3 1(±)/3
Epididymides -immature	M	0/3	0/2	0/3	1/2	0/3
-mononuclear cell infiltration, interstitium	M	0/3	0/2	0/3	0/2	1(±)/3
Testes -immature	M	0/3	0/2	0/3	1/2	1/3
Spleen -eosinophilic substance, germinal center	M F	0/3 0/3	0/2 1(±)/2	1(±)/3 1(±)/3	0/2 1(±)/2	1(±)/3 0/3
Trachea -bone formation, cartilage	M F	0/3 0/3	0/2 0/2	0/3 0/3	0/2 0/2	1/3 0/3
Ovaries -brown pigment deposition	F	0/3	0/2	0/3	0/2	1(±)/3

Grading: - :No abnormal changes, ± :Minimal, + :Mild, 2+ :Moderate, 3+ :Severe, P : Non-graded change, and U :Unexamined (no section)

Table 21 Histopathological findings at the recovery sacrifice

Organ/Tissue	Sex	0	30
Bone marrow (femur, left) -No/decreased eosinophils	M F	0/2 0/2	2/2 2/2
Bone marrow (sternum) -No/decreased eosinophil	M F	0/2 0/2	2/2 2/2
Ovaries -corpus luteum	F	0/2	2/2
-vacuolated corpus luteum	F	0/2	2/2
Liver (left lobe, medial lobe) - parasitic egg	M F	0/2 0/2	0/2 1/2
Liver (right lobe, medial lobe) -brown pigment deposition	M F	0/2 0/2	0/2 1(±)/2
-mononuclear cell infiltration	M F	1(±)/2 1(±)/2	2(±)/2 2(±)/2
-parasitic egg	M F	0/2 0/2	0/2 1/2

Grading: ± :Minimal

Toxicokinetics: Cynomolgus monkey anti-BIW-8405 antibody was detected in plasma samples from 2 of 24 animals receiving test article. Male #14 in the low dose group and female #25 in the high dose group showed positive anti-BIW-8405 responses in plasma samples collected on days 64 and days 43 and 64, respectively; antibody titers on these days were 1.8, 1.7, and 6.1 µg/mL, respectively. Declining plasma concentrations of BIW-8405 in female #25 after test article administration on day 43 are shown in the figure below.

C_{max} and AUC values for BIW-8405 increased in a dose-related manner on days 1 and 43. C_{max} and AUC values on days 1 and 43 were relatively comparable. Half-lives for BIW-8405 ranged from 189 to 419 hr. It is noted that the half-life for the 30 mg/kg group on day 43 was prolonged to 419 hr. If this group is excluded, half-lives ranged from 189 to 296 hr. Clearance values for BIW-8405 ranged from 0.158 to 0.274 mL/hr/kg. The clearance value for the 30 mg/kg/day group on day 43 was elevated to 0.274 mL/hr/kg due to the value of 0.902 mL/hr/kg for female #25, which had a positive anti-BIW-8405 antibody response. If this group is excluded, clearance values ranged from 0.158 to 0.187 mL/kg/hr, which were generally consistent with the clearance of IgG. Volume of distribution values ranged from 50.52 to 177.12 mL/kg. The volume of distribution value for the high dose group on day 43 was elevated to 177.12 mL/kg due to the value of 736 mL/kg for female #25. If this group is excluded, volume of distribution values ranged

from 50.52 to 78.66 mL/kg, which were generally consistent with the blood volume and suggested that the distribution of BIW-8405 was limited to the blood volume.

The concentration versus time profile for animal #14 (Group 2) declined very rapidly and erratically. Thus, $t_{1/2}$ and clearance could not be assessed for animal #14. The presence of anti-BIW-8405 antibody altered toxicokinetic parameters in this animal.

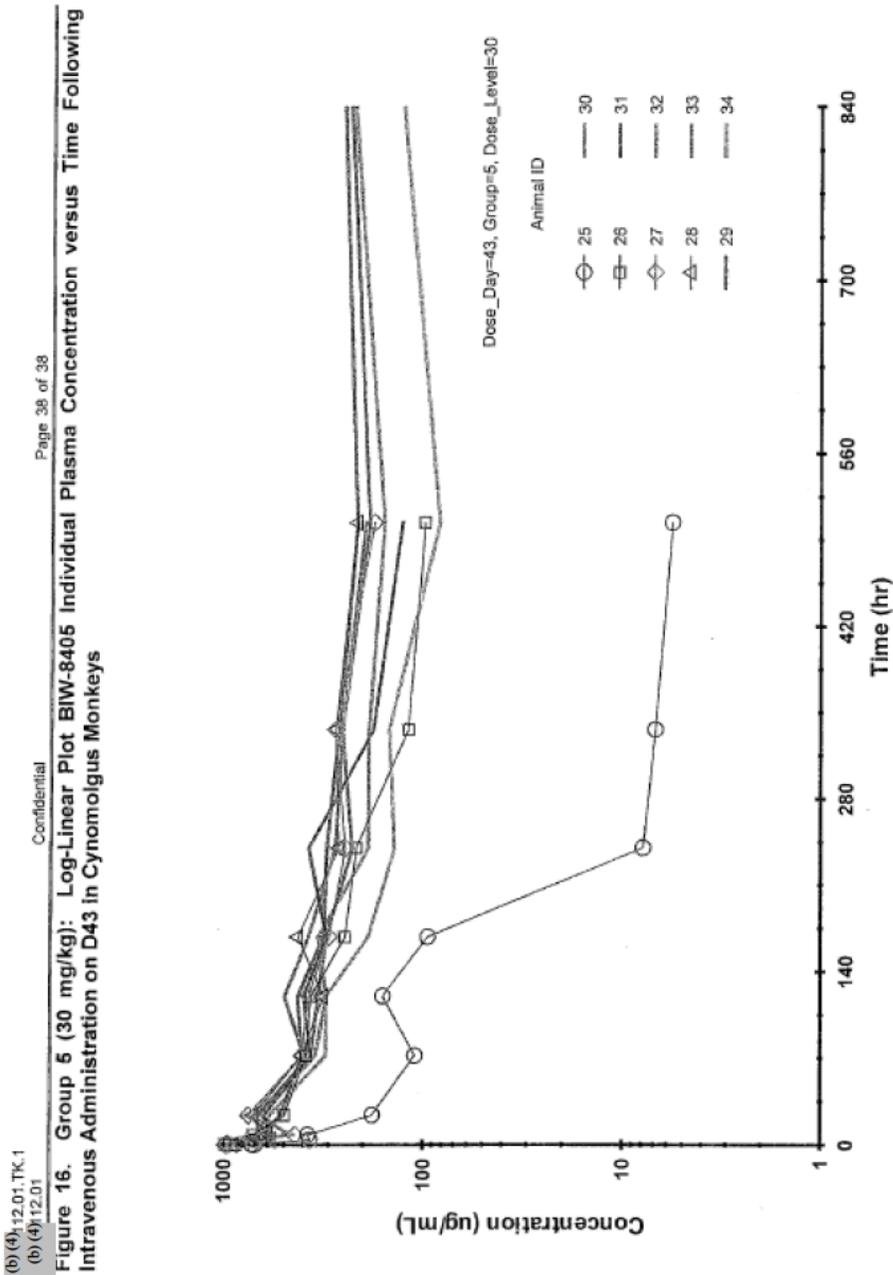
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Table 22 Toxicokinetic parameters in the 9-week toxicology study with monkeys**Summary of Mean Toxicokinetic Parameters**

Dose (mg/kg)	Day		C ₀ (µg/mL)	C _{max} (µg/mL)	T _{max} (hr)	AUC ₀₋₅₀₄ (hr*µg/mL)	AUC _{0-∞} (hr*µg/mL)	t _{1/2} (hr)	Cl (mL/hr/kg)	V _z (mL/kg)
0.10	1	N	4	4	4	4	4	4	4	4
		Mean	3.36	3.27	0.187	471	557	189	0.187	50.52
		SD	0.91	0.78	0.208	102	127	18	0.042	10.45
	43	N	4	4	4	4	3	3	3	3
		Mean	3.02	3.10	2.062	534	670*	254	0.158**	54.76
		SD	0.67	0.50	3.959	311	186	98	0.045	12.19
1.0	1	N	6	6	6	6	5	5	5	5
		Mean	34.18	32.67	0.083	4231	5981	296	0.174	71.48
		SD	6.27	5.43	0.000	586	1286	78	0.038	10.86
	43	N	6	6	6	6	6	5	6	5
		Mean	39.98	38.83	0.152	5606	5606*	283	0.183**	78.66
		SD	4.16	3.97	0.170	1064	1064	31	0.032	14.45
10	1	N	4	4	4	4	3	3	3	3
		Mean	288.17	290.00	0.187	45583	56231	238	0.179	60.75
		SD	19.44	8.16	0.208	6546	5742	37	0.019	3.49
	43	N	4	4	4	4	4	3	4	3
		Mean	387.11	380.00	2.062	58692	58692*	262	0.172**	67.08
		SD	65.68	40.82	3.959	6748	6748	81	0.0222	26.45
30	1	N	10	10	10	10	10	10	10	10
		Mean	817.19	802.00	0.166	124218	167817	295	0.184	73.74
		SD	55.37	44.67	0.176	11313	29346	116	0.036	19.50
	43	N	10	10	10	10	10	10	10	10
		Mean	872.45	934.00	0.375	138255	138255*	419	0.274**	177.12
		SD	114.83	86.44	0.201	42199	42199	133	0.223	197.99

* Values reported for Day 43 are AUC_{0-t}** Cl reported is Cl_{ss} (steady-state clearance)

Figure 26 Loss of drug exposure for male #25 in the 30 mg/kg group due to ADA



Other:**Table 23 Histopathology inventory of the 9-week IV toxicology study with monkeys**

Study	9-week intravenous toxicology study
Species	Cynomolgus monkeys
Adrenals	X*
Aorta	X
Bone Marrow smear	
Bone (femur) + marrow	X
Brain	X*
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymis	X
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	X
Jejunum	X
Kidneys	X*
Lachrymal gland	X
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X (w/thyroid)
Peripheral nerve	
Pharynx	
Pituitary	X
Prostate	X
Rectum	X
Salivary gland	X
Sciatic nerve	X

Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Sternum + bone marrow	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X (w/parathyroid)
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

X, histopathology performed

*, organ weight obtained

Study title: A Fifteen Week Repeat Dose Subcutaneous Toxicity Study of MEDI-563 in Cynomolgus Monkeys Followed by a 12-Week Recovery Period

Key study findings:

- In a 15-week subcutaneous toxicology study, monkeys (6/sex/group) received MEDI-563 at subcutaneous doses of 0, 1, 10, and 30 mg/kg once every 2 weeks for a total of 8 doses. After the 15-week dosing period, 3 monkeys/sex/group were sacrificed on day 101 for examination. The remaining 3 monkeys/sex/group were allowed a 12-week recovery period before sacrifice on day 183.
- Eosinophil counts in the peripheral blood and bone marrow were unaffected with subcutaneous doses up to 30 mg/kg administered once every 2 weeks over the entire dosing period. This contrasts to the 9-week intravenous toxicology study, in which significant suppression of eosinophil counts were observed in the peripheral blood and bone marrow with doses up to 30 mg/kg administered once every 3 weeks over the entire dosing period and extending into the recovery period. However, it was noted in the current study that eosinophil counts were low at baseline (Day -5) for all groups.
- The incidence of mononuclear cell infiltration in the prostate was increased for males in the 30 mg/kg group at the end of the dosing period; however, there was no evidence of any injury or lesion in the surrounding tissue of the prostate that accompanied the cellular infiltration. The relationship of this finding to treatment was questionable. Further, this finding was not observed in the 9-week intravenous toxicology study with monkeys that received doses up to 30 mg/kg once every 3 weeks although the treatment period was shorter and dosing was less frequent as compared to the present study.

- Anti-MEDI-563 antibodies were detected for female #15 in the 1 mg/kg group, male #36 in the 10 mg/kg group, and females #37 and #43 in the 30 mg/kg group. Female #15 in the 1 mg/kg group and female #43 in the 30 mg/kg group lost exposure to the drug. Female #13 in the 1 mg/kg group also lost exposure to the drug; so it was presumed that anti-MEDI-563 antibodies were generated although they were not detected in the Sponsor's assay. Male #36 in the 10 mg/kg group and female #37 in the 30 mg/kg group maintained drug exposure despite the presence of anti-MEDI-563 antibodies.

- The NOAEL was identified as the high dose of 30 mg/kg.

Study no.: (b) (4) 263.04

Volume #, and page #: Volumes 2-5, Pages 1-997

Conducting laboratory and location: (b) (4)

Date of study initiation: October 9, 2007

GLP compliance: Yes

QA report: yes (X) no ()

Drug, lot #, and % purity: MEDI-563, Lot numbers TL3594-90L (1 mg/mL) and TL-3594-90M (10 mg/mL), and TL3594-90H (30 mg/mL)

The test article, MEDI-563, was provided in 10 mM histidine with 300 mM glycine and 0.02% polysorbate 20 at pH 6.0.

Methods

Doses: MEDI-563 was administered by the subcutaneous route at doses of 0, 1, 10, and 30 mg/kg once every 2 weeks for a total of 8 doses (days 1, 15, 29, 43, 57, 71, 85, and 99).

Table 24 Study design of the 15-week SC toxicology study in monkeys

Table 1: Study design

Group	Dose Level (mg/kg)	Number of Animals (Male/Female)	Necropsy (Male/Female)	
			Terminal	Recovery
1	0	6/6	3/3	3/3
2	1	6/6	3/3	3/3
3	10	6/6	3/3	3/3
4	30	6/6	3/3	3/3

Species/strain: Purpose-bred, naïve Cynomolgus monkeys (*Macaca fascicularis*) were used in this study. Animals were of Cambodian origin.

Number/sex/group or time point (main study): 3 monkeys/sex/group

Route, formulation, volume, and infusion rate: Vehicle (10 mM histidine with 300 mM glycine and 0.02% polysorbate 20 at pH 6.0) and test article solutions were administered by the subcutaneous route using a dose volume of 1 mL/kg. The injections

were alternated between the left and right quadrants of the intrascapular regions of each animal.

Satellite groups used for toxicokinetics or recovery: An additional 3 monkeys/sex/group were included in each group for a 12-week recovery period.

Age: Males and females were 3-5 years old at the pre-study physical examination.

Weight: Body weight ranges at the pre-study physical examination were 3.5-5.0 kg for males and 2.5-4.5 kg for females.

Unique study design or methodology (if any): Doses were essentially identical to those used in the 9-week intravenous toxicology study.

Observation and Times:

Clinical signs: Clinical observations were conducted twice daily. Injection sites were scored, using the Draize dermal irritation scoring system, daily on days 2-7, days (within 2 hr postdose), daily on days 30-35, day 57 (within 2 hr postdose), and daily on days 58-63.

Body weights: Body weights were measured weekly.

Food consumption: Food consumption was qualitatively evaluated on a daily basis.

Ophthalmoscopy: Ophthalmic examinations were conducted once during the acclimation (day -9) and once prior to each scheduled necropsy (days 99 and 183).

ECG: Electrocardiographic parameters were recorded and blood pressures were measured during acclimation (day -12), during week 1 (day 3), and once in the week prior to each scheduled necropsy (days 99 and 183).

Hematology: Blood samples for determination of hematology and coagulation parameters were collected on days -5, 3, 28, 70, and the day of each scheduled necropsy (days 101 and 183).

Clinical chemistry: Blood samples for determination of serum chemistry parameters were collected on days -5, 3, 28, 70, and the day of each scheduled necropsy (days 101 and 183).

Urinalysis: Urine samples for assessment of urinalysis parameters were collected overnight on days -5, 3, 28, and prior to scheduled necropsies (days 101 and 183).

Gross pathology: Terminal and recovery sacrifices were conducted on days 101 and 183, respectively. Each animal was submitted to a necropsy examination. Bone marrow smears from the sternum were prepared, fixed, stained, and shipped to Madison Toxicologic Pathologists, LLC, for evaluation.

Organ weights: Absolute and relative organ weights were measured for the brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate/seminal vesicles, spleen, thyroids (including parathyroids), testes, thymus, and uterus.

Histopathology: Organs and tissues were processed to paraffin blocks and shipped to Experimental Pathology Laboratories, Inc. of Sterling, VA. Paraffin blocks were sectioned, stained with hematoxylin and eosin, and examined by light microscopy.

Toxicokinetics: Blood samples for measurements of serum concentrations of MEDI-563 were collected on day 1 (predose and 12 hr postdose), day 2 (24 and 36 hr postdose), days 3, 4, 5, 7, 9, 11, 13, 17, and 21, day 99 (pre-dose and 12 hr postdose), day 100 (24 and 36 hr postdose), and days 101, 102, 103, 106, 110, 113, 120, 127, 134, 162, 178, and 183. Samples were shipped overnight to MedImmune, Inc., Mountain View, CA, for analysis. Serum concentrations of MEDI-563 were measured using an ELISA

method (This information was provided in an e-mail dated November 3, 2008 following a request for information).

Anti-product antibody analysis: Blood samples for measurement of serum concentrations of anti-product antibodies were collected on days -5, 70, 101 (terminal animals only), 127, and 183. Samples were shipped overnight to MedImmune, Inc., Mountain View, CA, for analysis. Serum concentrations of anti-MEDI-563 antibodies were measured using an ELISA method.

Results:

Mortality: None.

Clinical signs: There were no treatment-related clinical observations.

There were transient observations of increased severity of erythema and edema for treatment groups on days 3 and 57, respectively although these observations appeared to have no toxicological significance. In general, there were no significant differences of injection site observations between control and treatment groups.

Table 25 Injection site observations

Observation	Day	Males				Females			
Erythema formation	3	0.17	0.17	0.83	0.83	0.2	0	1	0.67
Edema formation	57	0	0	0	0.17	0.17	0	0.67	0.5

Body weights: Body weight gain over the dosing period was slightly decreased for males in the 30 mg/kg group although there appeared to be little or no toxicological significance.

Body weights for males in the 0, 1, 10, and 30 mg/kg groups on day 98 were increased by 9.6, 9.1, 8.0, and 5.0% of body weights on day -1, respectively. Body weights for females in the 0, 1, 10, and 30 mg/kg groups on day 98 were increased by 11.1, 9.4, 10.9, and 8.9% of body weights on day -1, respectively.

Body weight gains were unaffected during the recovery period.

Food consumption: Qualitative food consumption was unaffected during the dosing and recovery periods.

Ophthalmoscopy: No treatment-related ophthalmic effects were observed at the end of the dosing and recovery period.

ECG: There were no treatment-related changes of heart rate, RR, PR, QRS, QT, and QTc intervals, and systolic and diastolic blood pressure during the dosing and recovery periods.

Hematology: Eosinophil counts were unaffected with subcutaneous doses up to 30 mg/kg administered once every 2 weeks over the entire dosing period. This contrasts to the 9-week intravenous toxicology study, in which significant suppression of eosinophil counts were observed with doses up to 30 mg/kg administered once every 3 weeks over the entire dosing period and extending into the recovery period. It is noted in the current study that eosinophil counts were low at baseline (Day -5) for all groups. Examination of bone marrow smears at the end of the dosing and recovery periods revealed no evidence of decreases of eosinophil precursors as observed in the 9-week intravenous toxicology study with monkeys.

Neutrophil counts for females #43 and #47 in the 30 mg/kg group on day 70 were elevated to 15.57 and 11.13 x 10³/μL, respectively. Neutrophil counts for female #25 in the 10 mg/kg group and female #43 in the 30 mg/kg group were lowered to 0.64 and 0.41 x 10³/μL, respectively. The significance of these differences was not clear.

The monocyte count for female #37 in the 30 mg/kg group on day 101 was elevated to 0.62 x 10³/μL although the significance of this difference was not clear.

The fibrinogen level for female #43 in the 30 mg/kg group on day 101 was elevated to 446 mg/dL although the significance of this difference was not clear.

The reticulocyte count and percentage for female #25 in the 10 mg/kg were elevated to 0.472 x 10⁶/μL and 10.5%, respectively.

Table 26 Hematology parameters (Days -5, 3, 28, 70, 101, and 183)

Parameter	Day	Males				Females			
		0	1	10	30	0	1	10	30
Eosinophils 10 ³ /μL	-5	0.06	0.02	0.02	0.01	0.03	0.03	0.04	0.04
	3	0.13	0.17	0.10	0.09	0.05	0.10	0.30	0.04
	28	0.06	0.01	0.02	0.05	0.05	0.01	0.28	0.00
	70	0.09	0.04	0.05	0.07	0.06	0.02	0.27	0.07
	101	0.09	0.07	0.09	0.10	0.07	0.03	0.38	0.07
	183	0.06	0.07	0.26	0.12	0.06	0.08	0.58	0.07
Neutrophils 10 ³ /μL	-5					6.18	3.89	7.37	6.02
	70					2.67	3.94	5.59	7.70
	101					4.65	3.51	4.18	2.89
Monocytes 10 ³ /μL	-5					0.25	0.18	0.22	0.20
	101					0.26	0.26	0.29	0.34
Fibrinogen mg/dL	-5					227	267	256	301
	101					205	221	230	305

Reticulocytes %	-5					1.4	0.9	1.2	0.9
	70					1.1	1.1	2.5	0.9
Reticulocyte 10 ⁹ /μL	-5					0.079	0.048	0.069	0.049
	70					0.062	0.057	0.119	0.051

Blank cells indicate that there were no differences between control and drug-treated groups

Clinical chemistry: Slight elevations of AST and triglyceride were observed during the dosing period. These changes appeared to have little or no toxicological significance and are monitorable in a clinical setting.

AST values for males #40, 42, and 44 on day 101 were elevated to 70, 86, and 64 U/L, respectively, as compared to a concurrent control range of 29 to 46 U/L. There were no corresponding histopathological findings in the liver. AST values are monitorable in a clinical setting.

Triglyceride levels were slightly elevated for males and females in the 30 mg/kg group on day 28 and for males in the 10 and 30 mg/kg groups on day 70. These elevations were transient as they were not evident at later time points.

C reactive protein levels were generally below the lower limit of linearity for most animals. However, elevated C reactive protein levels were measured for male #38 and female #45 in the 30 mg/kg group on day 28 with values of 2.6 and 2.4 mg/dL, respectively, and male #46 and female #43 in the 30 mg/kg group on day 101 with values of 2.3 and 10.9 mg/dL, respectively. No elevations were evident on day 70. The significance of these elevated values was not clear.

Table 27 Serum chemistry parameters (Days -5, 3, 28, 70, 101, and 183)

Parameter	Day	Males				Females			
		0	1	10	30	0	1	10	30
AST U/L	-5	40	45	46	43				
	101	37	52	40	56				
Triglyceride mg/dL	-5	27	34	37	39	42	41	41	35
	28	39	45	47	51 (131%)	56	57	52	73 (130%)
	70	39	44	56 (144%)	52 (133%)	54	50	43	42
	101	25	27	29	38				
	183	40	40	47	46				

Blank cells indicate that there were no differences between control and drug-treated groups

Urinalysis: There was no evidence of hematuria or proteinuria during the 15-week dosing and 12-week recovery periods as compared to the study in which 3 male monkeys received a single subcutaneous dose of 30 mg/kg.

Gross pathology: Observed gross pathological findings at end of the dosing and recovery periods displayed no dose-response relationships.

Organ weights: Observed differences of organ weights between control and treatment groups displayed no apparent relationships to observed histopathological findings. Observed differences might be attributed to variations of sexual maturity and body weight between monkeys.

Histopathology: The incidence of mononuclear cell infiltration in the prostate was increased for males in the 30 mg/kg group at the end of the dosing period; however, there no evidence of any injury or lesion in the surrounding tissue of the prostate that accompanied the cellular infiltration. The relationship of this finding to treatment might be questionable. Further, this finding was not observed in the 9-week intravenous toxicology study with monkeys that received doses up to 30 mg/kg once every 3 weeks although the treatment period was shorter and dosing was less frequent as compared to the present study.

The incidence of mononuclear cell infiltration in the prostate was increased for males in the 30 mg/kg group at the end of the dosing period. The published background incidence of this finding was reported to be 34.4% (76/221). There no evidence of any injury or lesion in the surrounding tissue of the prostate that accompanied the cellular infiltration. These findings were judged to have no toxicological significance.

Corpora hemorrhagica was observed in the ovaries for 2 of 3 females in the 30 mg/kg group. This finding appears to suggest recent ovulation in these animals and appears to have no relation to treatment.

Minimal centrilobular vacuolation was reported for 1 female in the 10 mg/kg group and 1 male and 1 female in the 30 mg/kg group at the end of the dosing period. The published background incidence of this finding for male and female monkeys was reported to be 20.4% (45/221) and 21.3% (47/221), respectively, suggesting that observed findings in the present study were spontaneous in nature.

There were several histopathological findings with incidences confined to one animal primarily in the 30 mg/kg group at the end of the dosing or recovery period. Relationships of these findings to treatment, if any, were unclear. Mononuclear cell infiltration is known to be a common background finding in several tissues including the liver, lung, urinary bladder, and tongue with published background incidences up 80, 28, 15, and 18%, respectively. Further, these findings of mononuclear cell infiltration are known to be common responses to parasitic infections found spontaneously in monkeys.

The Sponsor reported that observed histopathological findings in the duodenum (hemorrhage), stomach (erosion, hemorrhage, inflammation), cecum (hemorrhage), and jejunum (inflammation, dilatation of lymphatics in villi) with incidences confined to single

monkeys were unusual; however, these findings appeared to be unrelated to treatment as dose-response relationships were not present.

Table 28 Histopathological findings at the end of the dosing and recovery periods for monkeys that received MEDI-563 at subcutaneous doses of 0, 1, 10, and 30 mg/kg

Organ/Tissue	Sex	Dosing Period				Recovery Period			
		0	1	10	30	0	1	10	30
Prostate -mononuclear cell infiltration, minimal	M	0/3	1/3	1/3	3/3	1/3	1/3	0/3	1/3
Ovaries -corpora hemorrhagica	F	0/3	0/3	0/3	2/3	0/3	0/3	0/3	0/3
Liver -vacuolation, centrilobular, minimal -mononuclear cell infiltration, peribiliary	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	1/3	0/3	0/3	0/3	0/3
	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Duodenum -hyperplasia, lymphoid, minimal -hemorrhage	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Mandibular LN -erythrocytosis, minimal-mild	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	1/3	0/3	0/3	0/3	0/3
Lungs/Bronchi -inflammation, chronic, minimal	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Mesenteric LN -histiocytosis, mild	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Urinary bladder -degeneration, mucosa, minimal -lymphoid hyperplasia, submucosa, minimal -mononuclear cell infiltration, submucosa	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
Kidneys -mineralization, minimal -pyelitis, chronic, minimal	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
Tongue -mononuclear cell infiltration, muscle, minimal	M	0/3	0/3	0/3	1/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

Stomach -erosion, mild	M	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-hemorrhage, mild	M	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
-hemorrhage/inflammation, acute	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Cecum -hemorrhage/minimal	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Jejunum -inflammation, acute, lamina propria	M	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-lymphatic, dilatation, villi, minimal	M	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Spleen -fibrosis, capsule, minimal- mild	M	0/3	0/3	0/3	0/3	0/3	0/3	1/3	1/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3
Thyroids/Parathyroid -hyperplasia, C-cell, Minimal	M	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3

Toxicokinetics: Absorption on day 1 was delayed as mean T_{max} values were not achieved until 3 to 3.5 days postdose; however, absorption was more rapid on day 99 as mean T_{max} values were obtained on 1 to 2.5 days postdose. C_{max} and AUC values on days 1 and 99 increased in an approximate dose proportional manner. C_{max} and AUC values on day 99 were greater than values on day 1 indicating accumulation occurred to achieve steady-state. Steady-state was achieved by day 99 as AUC_{0-14d} values were comparable to AUC_{INF} values. Half-lives following administration on day 1 ranged from 11.6 to 14 days.

Table 29 Toxicokinetic parameters of MEDI-563 in Cynomolgus monkeys on Day 1**Table 6-1 Noncompartmental TK Parameters of MEDI-563 in Cynomolgus Monkey****First Dose (Day 1, All Animals)**

Group	2 (1 mg/kg)	3 (10 mg/kg)	4 (30 mg/kg)
T_{max} (d)	3.5 (2 - 8)	3 (1.5 - 6)	3 (1.5 - 10)
C_{max} (µg/mL)	10.5 (1.40)	80.3 (10.7)	250 (76.8)
AUC_(0-12d) (d·µg/mL)	99.3 (11.6)	756 (88.9)	2250 (503)
AUC_{inf} (d·µg/mL)	223 (44.7)	1560 (306)	5150 (1290)
CL/F (mL/d/kg)	4.65 (0.965)	6.67 (1.5)	6.13 (1.38)
T_{1/2} (d)	12.6 (3.43)	11.6 (3.13)	14 (4.31)

Table 30 Toxicokinetic parameters of MEDI-563 in Cynomolgus monkeys on Day 99 (with recovery animals)**Last Dose (Day 99, Recovery Animals)**

Group	2 (1 mg/kg)	3 (10 mg/kg)	4 (30 mg/kg)
T_{max} (d)	2.5 (1 - 3)	2.5 (0.5 - 4)	1 (1 - 11)
C_{max} (µg/mL)	21.2 (5.61)	151 (30.9)	419 (222)
AUC_(0-14d) (d·µg/mL)	224 (64.9)	1650 (267)	4360 (2290)

- Parameters are shown as Mean (Standard Deviation), except for T_{max}, which is shown as median (range).
- All parameters except for T_{max} are shown in 3 significant figures.

Anti-MEDI-563 antibodies were detected for female #15 in the 1 mg/kg group, male #36 in the 10 mg/kg group, and females #37 and #43 in the 30 mg/kg group. Female #15 in the 1 mg/kg group and female #43 in the 30 mg/kg group lost exposure to the drug. Female #13 in the 1 mg/kg group also lost exposure to the drug; so it was presumed that anti-MEDI-563 antibodies were generated although they were not detected in the

Sponsor's assay. Male #36 in the 10 mg/kg group and female #37 in the 30 mg/kg group maintained drug exposure despite the presence of anti-MED-563 antibodies.

False positive responses were evident for male #52 in the control group, female #21 in the 1 mg/kg group, females #25 and #51 in the 10 mg/kg group, and females #42 and #47 in the 30 mg/kg group.

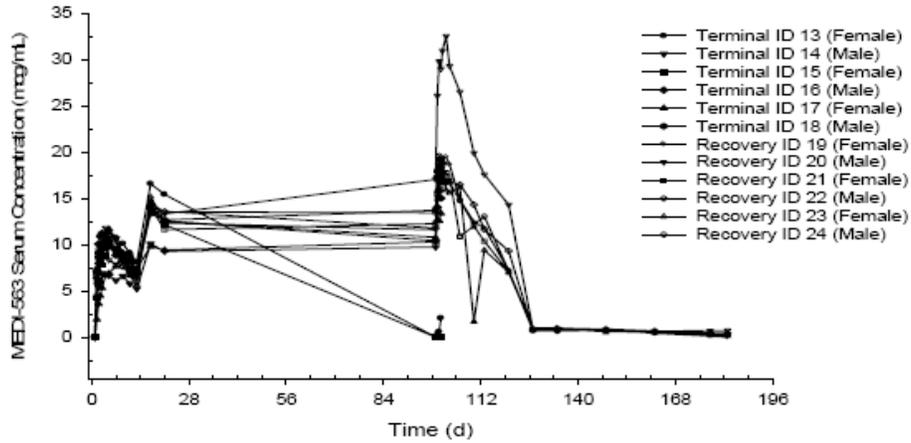
Table 31 Anti-MEDI-563 antibodies in Cynomolgus monkeys

Group	Animal #	Day	Result	Titer
Control	52/M	Predose	Positive	10
		Day 70	Negative	<10
		Day 101	Negative	<10
1 mg/kg	15/F	Day 70	Positive	20480
		Day 101	Positive	81920
1 mg/kg	21/F	Predose	Positive	10
		Day 70	Negative	<10
		Day 127	Negative	<10
		Day 183	Negative	<10
10 mg/kg	25/F	Predose	Positive	10
		Day 70	Negative	<10
		Day 101	Negative	<10
10 mg/kg	36/M	Day 183	Positive	320
10 mg/kg	51/F	Predose	Positive	10
		Day 70	Positive	20
		Day 101	Negative	<10
30 mg/kg	37/F	Day 70	Positive	10
		Day 101	Positive	20
30 mg/kg	42/F	Predose	Positive	10
		Day 70	Negative	<10
		Day 101	Negative	<10
30 mg/kg	43/F	Day 70	Positive	40960
		Day 127	Positive	327680
		Day 183	Positive	655360
30 mg/kg	47/F	Predose	Negative	<10
		Day 70	Positive	10
		Day 127	Negative	<10
		Day 183	Negative	<10

Figure 27 Loss of drug exposure for animals #13 and 15 in Group 2 (1 mg/kg)

Appendix 2 Individual Serum MEDI-563 Concentration-Time Profiles

Figure A2-1. Individual Serum MEDI-563 Concentration - Time Profiles for Group 2 (1 mg/kg)



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Figure 28 Drug exposure for animals in Group 3 (10 mg/kg)

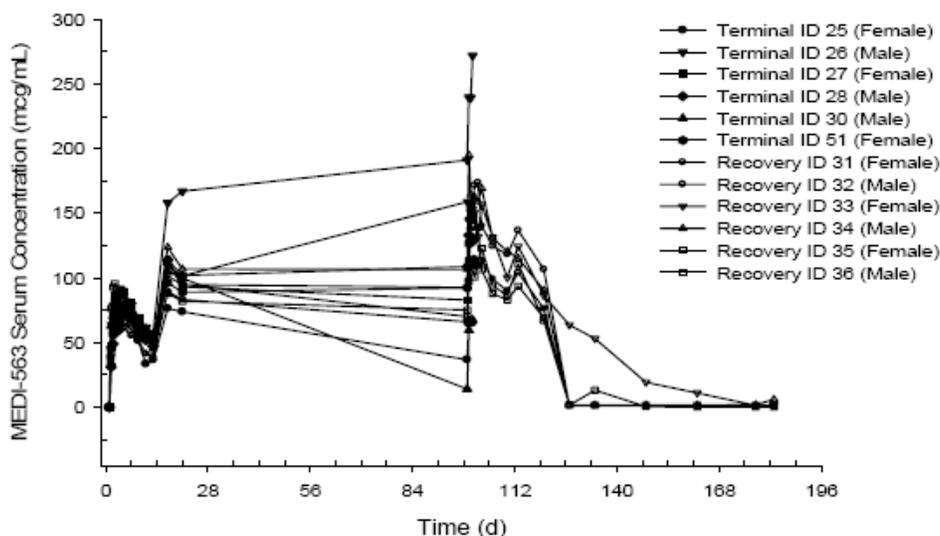
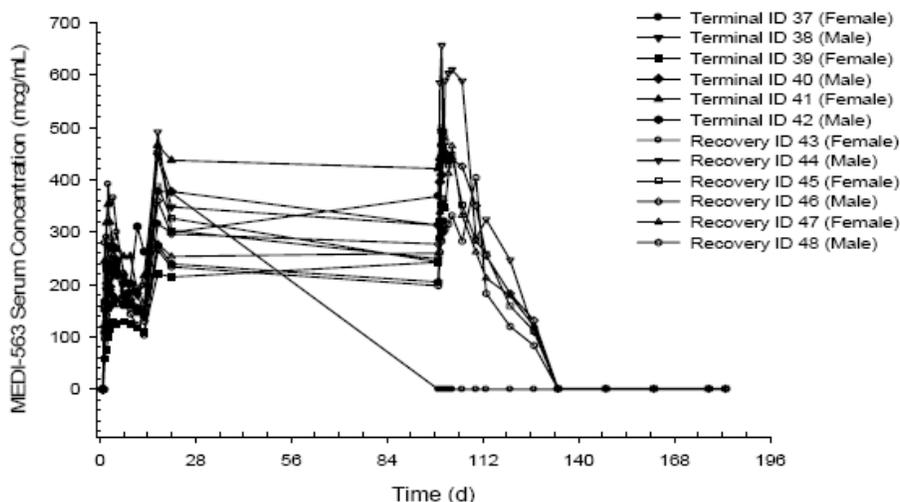


Figure 29 Loss of drug exposure for animal #43 in Group 4 (30 mg/kg)

Figure A2-3. Individual Serum MEDI-563 Concentration - Time Profiles for Group 4 (30 mg/kg)



Histopathology inventory (optional)

Study	15-week subcutaneous toxicology study
Species	Cynomolgus monkeys
Adrenals	X*
Aorta	X
Bone Marrow smear	X
Bone (femur)	
Brain	X*
Cecum	X

Cervix	w/Uterus
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Ileum	X
Injection site	X
Jejunum	X
Joints (knee)	X
Kidneys	X*
Lachrymal gland	
Larynx	
Liver	X*
Lungs	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X (Females)
Nasal cavity	
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X* (w/Thyroids)
Peripheral nerve	Sciatic
Pharynx	
Pituitary	X*
Prostate	X* (w/SV)
Rectum	X
Salivary gland	X
Sciatic nerve	X
Seminal vesicles	X* (w/prostate)
Skeletal muscle	X
Skin	X (Mammary)
Spinal cord	X
Spleen	X*
Sternum	X
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X* (w/PT)
Tongue	X
Trachea	X
Urinary bladder	X
Uterus	X*
Vagina	X

Zymbal gland
 X, histopathology performed
 *, organ weight obtained

Study title: A 9-Month Intravenous and Subcutaneous Dose Toxicity, Toxicokinetic, and Immunogenicity Study of MEDI-563 in Cynomolgus Monkeys with a 12-Week Recovery Period

Study no.: Testing Facility Study No. AAO00095
 Study report location: EDR
 Conducting laboratory and location: (b) (4)

Date of study initiation: February 9, 2009
 GLP compliance: Yes
 QA statement: Yes
 Drug, lot #, and % purity:

Dose Group	Lot No.	Nominal Concentration (mg/mL)	Actual Concentration (mg/mL)	Receipt Date
2	NDL4517.042	2	2.15	07 Apr 2009
	CNF5186.48		2.11	30 Jul 2009
3	NDL4517.042	5	5.55	07 Apr 2009
	CNF5186.49		5.26	30 Jul 2009
4	NDL4517.042	30	33.6	07 Apr 2009

Purity ≥99.3% monomer

Key Study Findings

- Comments on the proposed design of the chronic toxicology study with monkeys were conveyed to the Sponsor on December 19, 2008.
- In a 39-week toxicology study, Cynomolgus monkeys (6/sex/group) received MEDI-563 at doses of 0 (IV/SC), 10 (IV), 25 (IV), and 30 (SC) mg/kg once every 2 weeks for a total of 20 doses. Selected animals (3/sex/group) were allowed a 12-week recovery period. Males and females were sexually mature adults to allow for fertility assessments.
- Systemic drug exposure was highest in the 25 mg/kg IV group. Exposure for the 30 mg/kg SC group was intermediate between the 10 and 25 mg/kg IV groups.
- One male in the 25 mg/kg IV group was euthanized on day 47 due to a right wrist anomaly; this finding was judged to have no relationship to treatment.
- Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count and indicators of circulating erythrocyte mass that appeared to be reversible. Female 3501 was not dosed on day 57 due to these adverse clinical signs. After dosing resumed on day 71, platelet count decreased on day 87 and then gradually increased to baseline levels by day 267. Indicators of circulating erythrocyte mass were only minimally affected on day 87 and were greater

than prestudy by day 183. The effects on platelets and erythrocytes (abnormal morphology) suggested that a MEDI-563-related immune-mediated process (e.g., potential post-dose reaction) had occurred, but fully resolved by day 267 despite continued dosing. The findings were considered to be adverse. Immunogenicity results for Group 3 female #3501 showed a detectable anti-MEDI-563 titer from samples taken near Dose 14 that would be considered immunopositive. However, titers were just above the assay LLOQ (<1:10) and were considered to be false positive results. There was no evidence of an altered TK profile for this animal as might be expected following generation of ADAs.

- Eosinophil counts were decreased for males and females in the 10 and 25 mg/kg IV groups and 30 mg/kg SC group, which could be attributed to the pharmacological action of MEDI-563. The decreases were most pronounced for males in the 25 mg/kg IV groups. Eosinophil counts were consistently decreased by Day 63 and a few animals demonstrated an effect as early as Day 3. Lower peripheral blood eosinophil counts correlated with decreased eosinophils in the bone marrow.
- Treatment-related bone marrow effects consisted of lower percentages of eosinophils for monkeys that received 10 or 25 mg/kg IV or 30 mg/kg SC. Decreased eosinophil counts was an expected pharmacologic effect of the test article.
- Male and female fertility parameters were unaffected with doses up to 25 mg/kg IV.
- Histopathological findings were judged to be spontaneous in nature and unrelated to treatment. There were findings of necrosis or fibrosis in hearts from animals in both control and treatment groups that were judged to be unrelated to treatment. There were no treatment-related ECG effects. Monkeys used in this study (males >6 years old and females >4 years old) were generally older than animals used in published and test laboratory historical control databases, which complicated the analysis; however, findings of necrosis and fibrosis were found to occur spontaneously in control animals.
- NOAELs were identified as the 10 mg/kg IV and 30 mg/kg SC based upon findings for Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count and indicators of circulating erythrocyte mass that appeared to be reversible.
- Systemic exposures for the 10 mg/kg IV group (2320 µg·day/mL) and 30 mg/kg SC group (4110 µg·day/mL) were significantly lower than the 25 mg/kg IV group (6100 µg·day/mL).

Methods

Doses: 0 (IV/SC), 10 (IV), 25 (IV), and 30 (SC) mg/kg once every 2 weeks

Frequency of dosing: Once every 2 weeks for a total of 20 doses (i.e., Days 1, 15, 29, 43, 57, 71, 85, 99, 113, 127, 141, 155, 169, 183, 197, 211, 225, 239, 253, and 267)

Route of administration: IV or SC
Groups 1, 2, and 3: IV infusion in the cephalic or saphenous vein
Groups 1 and 4: SC in the upper dorsal area

Dose volume: IV (5 mL/kg given as a 30-min IV infusion) or SC (1 mL/kg)

Formulation/Vehicle: 20 mM histidine, 9% trehalose, 0.004% polysorbate 20 at pH 6.0

Species/Strain: Cynomolgus monkeys (*Macaca fascicularis*) were obtained (b) (4)
(b) (4) The country of origin was (b) (4)
Monkeys were purpose-bred and experimentally naive at the outset of the study.

Number/Sex/Group: 3 monkeys/sex/group for 9-month treatment (Sacrificed day 270)

Age: 6.3 to 12.9 years of age for males and 4.4 to 6.2 years of age for females

Weight: 4.8 to 10.1 kg for males and 2.5 to 3.4 kg for females on Day -1 of the study

Satellite groups: 3 monkeys/sex/group for a 12-week recovery period

Unique study design: Basis of dose selection: In a previous repeat dose (once every three weeks for 4 total administrations) IV study in Cynomolgus monkeys (Study No. (b) (4) 112.01), the no-observed-adverse-effect level (NOAEL) for MEDI-563 was ≤ 30 mg/kg, the highest dose tested. The neutrophil change noted in 2 of 10 animals given 30 mg/kg was mild and transient, while the intended depletion of eosinophils was noted at all dose levels tested (0.1 to 30 mg/kg/dose range). In a previous repeat dose (once every two weeks for 8 total administrations) SC study in Cynomolgus monkeys (Study No. (b) (4) 263.04), the NOAEL for MEDI-563 was ≤ 30 mg/kg, the highest dose tested.

Fertility assessments were included in the study

as both males and females were sexually mature.

Deviation from study protocol: Deviations did not impact the integrity of the study.

Table 32 Design of the 39-week toxicology study with Cynomolgus monkeys

Group No.	Number of M/F	Route	Nominal Dose Level (mg/kg)	Dose Volume (mL/kg)	Nominal Dose Solution Concentration (mg/mL)	Number Necropsied (M/F):	
						Terminal (Day 270)	Recovery (Day 351)
1	6/6	IV/SC	0 (control)	5.0/1.0	0	3/3	3/3
2	6/6	IV	10	5.0	2	3/3	3/3
3	6/6	IV	25	5.0	5	3/3	2 ^a /3
4	6/6	SC	30	1.0	30	3/3	3/3

^a Animal 3004 was necropsied early on Day 47.

IV = Intravenous infusion

SC = Subcutaneous

M/F = Males/Females

Observations and Results

Mortality: Animals were observed for morbidity/mortality twice daily.

One male (#3004) in the 25 mg/kg IV group was euthanized on day 47 due to a right wrist anomaly; this finding was judged to have no relationship to treatment.

Male 3004 in the 25 mg/kg IV MEDI-563 dose group was euthanized on Day 47 due to observed discomfort from progression of a subclinical right wrist anomaly. X-ray of the wrist confirmed dislocated radial-carpal joint with associated torn ligaments. Euthanasia of this animal was recommended as the animal would no longer fulfill the study objectives. At necropsy, a joint deformity was observed (right carpal with no evidence of recent hemorrhage, swelling or edema; the wrist was deviated laterally with protrusion of the distal ulna; the wrist had lack of mobility). Cytologic examination of bone marrow smears on Day 47 revealed that the percentage of eosinophils (4.1%) was similar to the average percentage of eosinophils (4.6%) in control males at the end of the dosing period (Day 270). Increases in CD3+ T-lymphocytes (165%), CD3+/CD4+ T-helper lymphocytes (148%), and CD3+/CD8+ T-cytotoxic lymphocytes (213%); and a reduction in CD3-/CD16+ NK cells (75%) were observed at Day 47 when compared to the prestudy high or low value, respectively. A relationship of these observations to intravenous administration of MEDI-563 was considered unlikely.

Clinical Signs: Cageside observations were conducted twice daily. Observations were made prior to dosing on dosing days and at approximately the same time each week (\pm 1.5 hours). Observations of infusion/injection sites were conducted on days 1 (Dose 1), 57 (Dose 5), 127 (Dose 10), 197 (Dose 15), and 267 (Dose 20): prior to dosing, within 2 to 4 hr after dosing, and 24, 48, 72, and 96 hours after dosing. If irritation scores of Grade 1 or higher occurred, injection site observations continued daily until the score was 0.

With the exception of Female 3501 in the 25 mg/kg IV group (discussed below), there were no test article-related clinical signs. Femoral bruising was observed that was attributed to venipuncture for dosing and/or blood sample collection. Other clinical signs that occurred during the dosing and recovery periods were sporadic across all groups including the control group.

Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on Day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count (lowest platelet count [2,000/ μ L] on Day 55) and indicators of circulating erythrocyte mass (hemoglobin [11.8 g/dL], hematocrit [39.4], red blood cell count [4.82×10^6 / μ L], RDW (increased to 17.0%), MCHC [30.0 g/dL], Reticulocytes [increased to 2.55×10^5 / μ L] on day 63) that appeared to be reversible. Female 3501 was not dosed on Day 57 due to adverse clinical signs (petechiae and ecchymosis) and low platelet values on Days 52, 55, and 57.

All blood smears through Day 71 were examined. On Day 52, reduced platelet density was noted and on Days 55 and 57, reduced platelet and RBC density, spherocytes (suggestive of immune-mediated red cell destruction), and schistocytes (suggestive of red cell trauma) were present. Platelet and RBC density appeared to be within normal limits by Day 63, although spherocytes and schistocytes were still observed. On Day 69, only rare spherocytes and schistocytes were noted. Evaluation of blood smears from prestudy, Day 1 (predose), and Day 3 (48 hours post dose) was unremarkable.

After dosing resumed on Day 71, platelet count decreased to 47,000/ μ L (0.12X prestudy) on Day 87 and then gradually increased despite continued dosing, finally reaching 443,000/ μ L (1.15X prestudy) on Day 267. Indicators of circulating erythrocyte mass were only minimally affected on Day 87 and were greater than prestudy by Day 183. The effects on platelets and erythrocytes (abnormal morphology) suggest a MEDI-563-related immune-mediated process (e.g., potential post-dose reaction) had occurred, but had fully resolved by Day 267 despite continued dosing. However, the moderate to marked decreases in these parameters during the dosing phase were considered to be adverse.

Based on adverse clinical signs (petechiae and ecchymosis) and low platelet values on Days 52 and 55, Group 3 Female 3501 (25 mg/kg IV) had clinical pathology blood samples collected on Day 57. Because platelet count remained low on Day 57, this animal was given a dosing holiday on Day 57. Blood samples for possible complement evaluation were also collected on Day 57, Days 71 (predose), and then again 48 hours after dosing on Day 71.

Blood samples for possible complement evaluation were processed as follows:

- 1.5 mL (Day 57) or 1 mL (all other time points) of blood was collected and processed to serum, and the resulting serum was retained for possible CH50 analysis.

- 1 mL of blood was collected into ethylenediaminetetraacetic acid (EDTA) tube and processed to plasma, and the resulting plasma was retained for possible C3a analysis.

These samples were maintained frozen at $\leq -65^{\circ}\text{C}$. However, samples were discarded after the completion of the in-life phase of the study after analyses were deemed not necessary following review of available in-life data.

In addition to scheduled clinical pathology blood sample collection, samples were also collected from this animal on Day 69.

Serum chemistry samples collected from this animal on Days 57, 63, and 69 were also analyzed for C-reactive protein (CRP).

Additional assessments for CRP on days 55, 57, and 63 (below the limit of detection), and fibrinogen starting on day 57 were within normal limits. None of these changes in red blood cell mass or platelet count in Female 3501 was reflected in the bone marrow cytology that was collected at necropsy on day 270.

Table 33 Hematology parameters for female #3501 in the 25 mg/kg IV group on days 47, 52, 55, 57, 69, and 94

Red Blood Cell Count - $10^6/\mu\text{L}$

Group 3: 25 mg/kg

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	5.34	----	----	----	----	----
3501	F	----	4.88	3.37	2.68	5.03	4.98

Hemoglobin Concentration - g/dL

Group 3: 25 mg/kg

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	13.0	----	----	----	----	----
3501	F	----	12.6	8.9	6.8	12.4	12.2

Hematocrit - %**Group 3: 25 mg/kg**

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	38.4	----	----	----	----	----
3501	F	----	37.5	26.0	21.5	39.8	39.2

Red Cell Distribution Width - %**Group 3: 25 mg/kg**

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	14.1	----	----	----	----	----
3501	F	----	14.6	16.1	19.7	15.0	13.6

Reticulocyte Count - 10⁵/uL**Group 3: 25 mg/kg**

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	0.37	----	----	----	----	----
3501	F	----	0.88	1.95	4.45	1.01	1.59

Platelet Count - 10³/uL**Group 3: 25 mg/kg**

Animal Number	Sex	Day 47	Day 52	Day 55	Day 57	Day 69	Day 94
		Unscheduled Clinical Pathology					
3004	M	377	----	----	----	----	----
3501	F	----	12	2	7	453	148

Immunogenicity results for Group 3 female #3501 showed a detectable anti-MEDI-563 titer from samples taken near Dose 14 that would be considered immunopositive. However, titers were just above the assay LLOQ (<1:10) and were considered to be false positive results. There was no evidence of an altered TK profile for this animal as might be expected following generation of ADAs.

Physical Examination: Physical examinations that included measurements of heart rate, respiration rate, and body temperature were conducted during weeks -1, 3, 7, 11, 15, 19, 23, 27, 31, 35, 39, 43, and 47.

There were no treatment-related changes of heart rate, respiration rate, and body temperature.

Body Weights: Body weights were measured weekly.

Body weight gains of male and female treatment groups were unaffected by treatment with MEDI-563.

Feed Consumption: Food consumption was qualitatively evaluated by observing the number of biscuits remaining from the previous days feed ration.

Food consumption of male and female treatment groups was unaffected by treatment with MEDI-563.

Ophthalmoscopy: Ophthalmic examinations were conducted during the prestudy period, week 39 (week before terminal necropsy), and during the week before recovery necropsy (week 51).

There were no findings of treatment-related ophthalmic effects at weeks 39 or 51.

ECG: Electrocardiograms and blood pressure measurements were recorded using Leads I, II, III, aVR, aVL, and aVF during the prestudy period, on days 1 and 267 (within 45 min of the completion of dosing), on day 269, and during the week before recovery

necropsy (Day 350, Week 51). Animals were restrained, but not sedated during recordings.

According to the sponsor's consultant (b) (4) all electrocardiograms evaluated were qualitatively considered normal for Cynomolgus monkeys. No test article related abnormalities in rhythm or waveform morphology were found. There were no abnormal electrocardiographic findings attributable to the administration of MEDI-563.

Systolic, diastolic, and mean arterial blood pressures were unaffected for male and female treatment groups.

Hematology: Blood samples for measurement of a complete panel of hematology parameters were collected on days -8/-7, 1, 3, 63, 87, 183, 267, 270, 281, and 350. Blood samples for lymphocyte immunophenotyping (measured by flow cytometry) were collected once during week -1 and on days 3, 183, 267, 270, and 350.

Eosinophil counts were decreased for males and females in the 10 and 25 mg/kg IV groups and 30 mg/kg SC group, which could be attributed to the pharmacological action of MEDI-563. The decreases were most pronounced for males in the 25 mg/kg IV groups. Eosinophil counts were consistently decreased by Day 63 and a few animals demonstrated an effect as early as Day 3. Lower peripheral eosinophil counts correlated with decreased eosinophils in the bone marrow.

Following a 12-week recovery period, most animals that exhibited decreased peripheral eosinophil counts at the end of the dosing phase (Day 267) continued to exhibit low eosinophil counts through Day 350 (statistically significant in Groups 2 and 4 females on Day 281). Reversal of decreased eosinophil counts by Day 350 was not observed for males in the 25 mg/kg IV and 30 mg/kg SC groups and females in the 30 mg/kg SC group.

Two animals (2002 and 3504) developed anti-drug antibodies (ADA) prior to day 183. Animal 2002 demonstrated transiently decreased eosinophil counts that resolved to greater than prestudy by Day 183 and Animal 3504 demonstrated no effect on eosinophil counts over the course of the study.

Decreased white blood cell, neutrophil, and unclassified cell counts were observed for male treatment groups although the relationships to treatment were questionable.

Table 34 Hematology parameters (changes from control are denoted in bold)

Parameter	Day	Males				Females			
		0	10	25	30-SC	0	10	25	30-SC
Eosinophils cells/ μ L	-8/-7	164.3	92.8	74.7	91.3	276.8	98.8	194.8	145.7
	1	191.5	187.3	78.5	94.2	308.3	170.2	153.7	109.3

Parameter	Day	Males				Females			
		0	10	25	30-SC	0	10	25	30-SC
	3	133.0	50.0	82.7	85.7	169.3	109.3	63.7	73.7
	63	206.5	57.7	0.0	24.0	206.2	6.0	52.7	10.7
	87	130.8	61.8	1.6	15.2	267.2	6.5	58.3	5.8
	183	244.8	98.7	2.0	34.5	270.2	20.7	47.5	18.0
	267	304.0	89.8	8.4	26.0	278.5	45.3	84.3	40.8
	270	128.0	138.7	3.6	32.3	154.5	24.7	99.8	23.0
	281	282.3	207.3	0.0	40.3	189.0	50.0	82.7	8.3
	350	271.0	175.0	11.0	94.7	199.3	147.3	183.3	15.7
WBC 10 ³ /μL	-8/-7	12.50	16.70	11.97	12.03	11.28	8.40	9.80	9.55
	1	12.87	11.23	11.48	10.00	10.12	10.05	9.80	12.27
	3	12.72	11.47	12.92	11.17	11.02	8.20	8.32	9.82
	63	13.80	11.37	11.76	10.10	10.65	9.07	12.37	10.43
	87	12.12	11.12	10.34	9.80	11.13	9.57	9.93	9.67
	183	15.17	13.05	12.18	9.80	11.72	9.52	9.60	10.97
	267	13.60	13.03	11.06	10.22	9.70	9.52	9.08	9.90
	270	12.93	13.83	9.80	11.82	9.68	8.38	8.93	8.05
	281	17.90	15.97	12.10	13.80	11.87	9.70	10.20	11.67
	350	16.80	11.07	10.80	10.23	11.67	9.77	10.57	9.17
Neutrophils cells/μL	-8/-7	6352.2	11521.5	5781.0	6240.5	5040.2	3524.3	4526.2	4560.0
	1	6590.8	4985.8	4814.5	4562.0	4017.2	4961.7	4949.3	7446.5
	3	7658.8	6403.3	7885.7	6441.7	5877.5	3382.8	4428.2	5500.3
	63	7100.7	5633.8	5071.2	4876.3	4657.8	3156.5	5113.5	4622.7
	87	6319.2	4918.7	3762.6	4799.8	3794.0	3204.7	3344.0	3877.7
	183	7540.3	6513.0	4387.0	4225.2	4117.2	2968.5	3450.3	4499.0
	267	6007.3	7470.8	4439.6	5243.5	3341.0	3569.8	3522.8	4005.0
	270	7773.0	8437.2	4602.4	7943.2	4947.8	3886.3	4339.0	4277.8
	281	9378.0	11373.0	6258.0	8620.7	6953.7	5633.3	5493.7	6666.3
	350	8071.3	5219.7	4866.0	4928.7	5169.3	4358.7	3853.7	4456.0
Unclassified	-8/-7	73.8	64.3	67.5	63.2	77.7	66.3	58.0	56.2

Parameter	Day	Males				Females			
		0	10	25	30-SC	0	10	25	30-SC
Cells cells/ μ L	1	78.5	86.8	80.7	63.3	60.0	68.2	45.5	58.3
	3	65.2	84.5	70.3	63.8	58.8	54.7	46.0	62.2
	63	81.8	94.2	67.6	74.3	62.3	82.7	110.5	67.3
	87	105.7	87.3	90.4	66.7	96.2	91.5	86.3	67.3
	183	67.0	91.7	55.2	40.2	47.0	46.7	44.7	76.7
	267	59.5	72.0	48.0	30.8	44.3	37.8	32.7	57.3
	270	62.0	78.0	47.6	27.8	40.3	43.5	41.0	31.8
	281	109.3	67.0	63.0	51.0	45.7	47.0	40.7	42.3
	350	82.7	82.7	53.0	42.0	54.0	48.3	49.0	51.0

Figure 30 9-month toxicology study: Eosinophil counts in male control and drug-treated groups

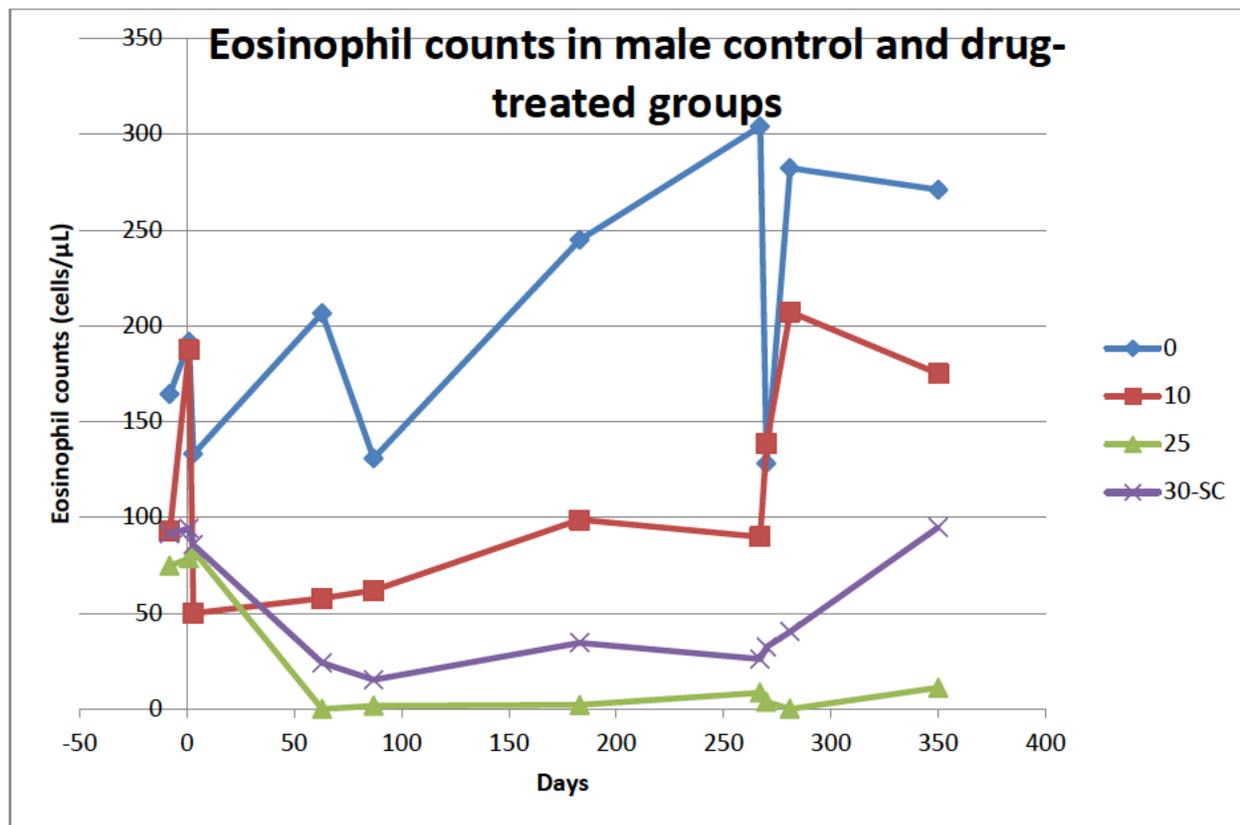
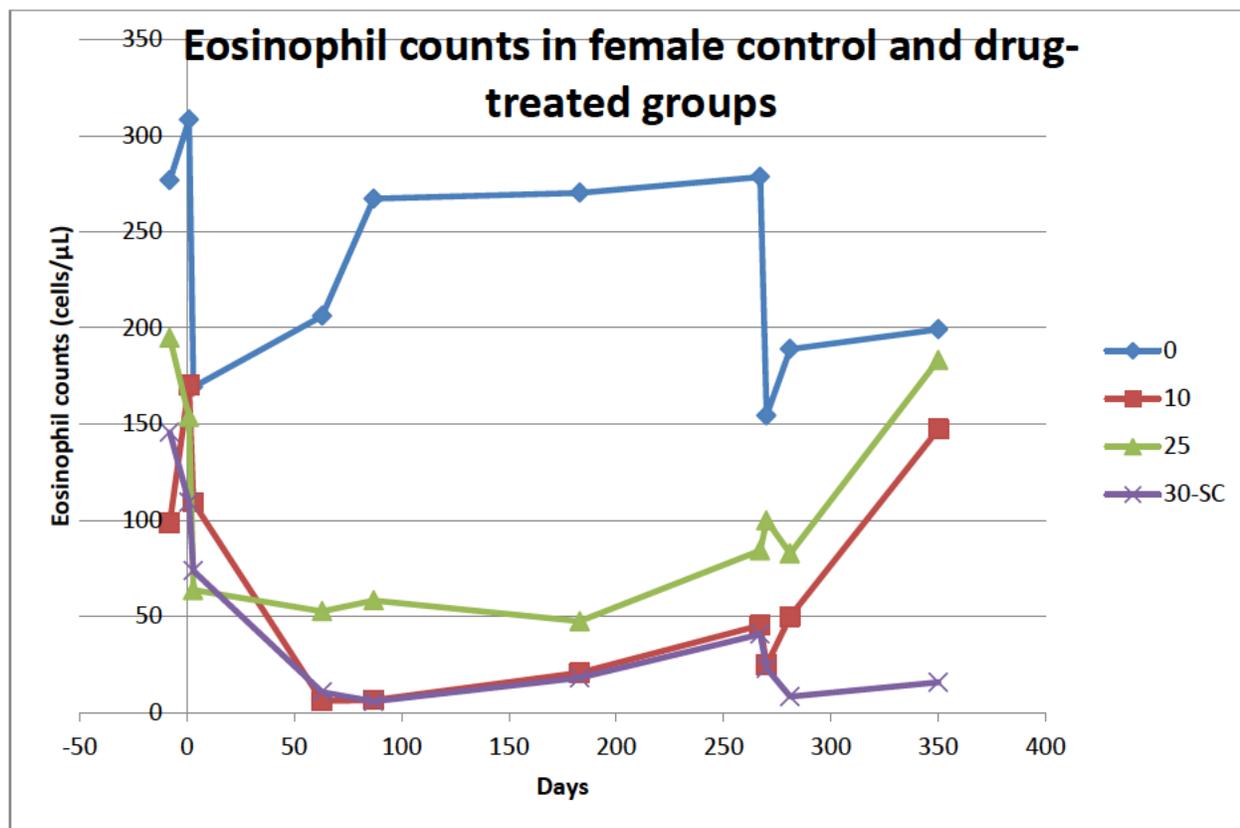


Figure 31 9-month toxicology study: Eosinophil counts in female control and drug-treated groups



Increased CD3+ T-lymphocytes, CD3+/CD4+ T-helper lymphocytes, and CD3+/CD8+ T-cytotoxic lymphocytes were observed for males in the 25 mg/kg IV group on day 183; however, these increases were relatively small and transient as increases were not evident at later time points.

Reductions of CD3-/CD16+ NK cells and CD3-/CD159a+ NK cells were observed in most animals dosed with MEDI-563 from days 3 to 270. The magnitude of these reductions was variable between animals and groups. These reductions were of questionable relationship to treatment as reductions of similar magnitude and frequency were identified in control article-dosed monkeys when compared to their individual prestudy baseline values.

Table 35 Immunophenotyping of lymphocytes by Flow Cytometry

Lymphocytes	Day	Males				Females			
		0	10-IV	25-IV	30-SC	0	10	25	30-SC
CD3+ per μL	183	3997.33	3800.00	5111.80 (128%)	3779.00	NC	NC	NC	NC
CD3+CD4+ per μL	183	1071.00	1133.00	1604.60 (150%)	1176.60	NC	NC	NC	NC
CD3+CD8+ per μL	183	1043.50	1075.50	1445.60 (139%)	1305.00	NC	NC	NC	NC

CD3-CD16+ per μ L	3	535.00	411.67 (77%)	283.00 (52.9%)	330.83 (61.8%)	338.33	109.83 (32%)	179.33 (53%)	164.33 (49%)
	183	1113.00	702.83 (63.2%)	549.80 (49.4%)	349.67* (31.4%)	836.00	362.33	342.33	286.67
	267	1059.50	806.67 (76%)	536.40 (51%)	439.83* (42%)	694.17	359.83	359.00	342.67
	270	800.50	627.83 (78%)	399.60 (49.9%)	312.50 (39%)	451.00	190.50 (42%)	222.83 (49%)	182.17 (40%)
CD3-CD159a+ per μ L	3	695.00	655.67	439.50 (63%)	480.17 (69%)	464.00	154.33 (33%)	262.00 (56%)	263.00 (57%)
	183	1250.67	903.67	720.40	530.83 (42%)	905.67	420.83	527.00	419.00
	267	1038.17	884.33	659.40 (64%)	548.17 (53%)	711.17	345.00	462.00	423.17
	270	798.00	789.00	541.00 (68%)	409.50 (51%)	479.17	204.50 (43%)	297.83 (62%)	249.83 (52%)

NC = no change

Bone Marrow Evaluation: Two bone marrow smears were collected from the seventh rib of each animal at necropsy. Bone marrow cytologic preparations were evaluated from all terminal (Day 270) and recovery (Day 351) animals (including Male No. 3004 that was euthanized early), and a myeloid:erythroid ratio was determined and quantified for each monkey. Lymphocytes were counted and presented as a percentage of cells per 200 myeloid and erythroid cells counted. In addition, the smears were evaluated for morphologic or maturation abnormalities.

Treatment-related bone marrow effects consisted of lower percentages of eosinophils for monkeys that received 10 or 25 mg/kg IV or 30 mg/kg SC. Decreased eosinophil counts was an expected pharmacologic effect of the test article. There were no effects on the myeloid to erythroid ratio or percentages of lymphocytes.

At day 270, the average percentage of eosinophils was lower in all groups administered MEDI-563, except for females in the 30 mg/kg SC group. Eosinophils were not observed during a 200-cell count in 2/3 males and 1/3 females in the 10 mg/kg IV group, 3/3 males and 3/3 females in the 25 mg/kg IV group, and 2/3 males and 1/3 females dosed at 30 mg/kg SC. Lower numbers of eosinophils quantified in the bone marrow generally correlated with lower peripheral blood eosinophil counts on Day 270 in most animals administered 10 or 25 mg/kg IV or 30 mg/kg SC. Eosinophils observed in bone marrow cytologic preparations from animals administered MEDI-563 exhibited normal maturation and morphology.

Following a 12-week dose-free period, the average percentage of eosinophils was lower in some animals in all groups administered MEDI-563 when compared to control animals. Eosinophils were not observed during a 200-cell count in 1/3 males in the 10 mg/kg IV group, 2/2 males in the 25 mg/kg IV group, and 1/2 males and 3/3 females in the 30 mg/kg SC group. Additionally, eosinophil percentages of <1% were observed in 3 animals (2005, 2506, and 3506) and were considered to be related to administration of MEDI-563. Decreased eosinophils quantified in the bone marrow generally correlated with lower peripheral blood eosinophil counts on Day 350.

Table 36 Barrow Marrow Evaluations at the end of the treatment (day 270) and recovery (day 351) periods

Parameter	Day	Males				Females			
		0	10	25	30-SC	0	10	25	30-SC
Eosinophil Percentage	270	4.6%	2.8%	0.0%	0.3%	8.2%	1.9%	0.0%	3.3%
	351	7.0%	3.0%	0.0%	2.4%	7.8%	3.7%	5.0%	0.0%

Clinical Chemistry: Blood samples for measurement of a complete panel of clinical chemistry parameters were collected once during week -1 and on days 1, 3, 63, 87, 183, 270, 281, and 350.

There were no treatment-related changes of clinical chemistry parameters.

Urinalysis: Urinalysis was not performed.

There were no histopathological findings or changes of clinical chemistry parameters that would indicate renal injury.

Gross Pathology:

One Group 3 male, Animal 3004, was necropsied early on Day 47. Twenty-four animals (3/sex/group) were euthanized 3 days after the last dose (Day 270) for terminal necropsy. The remaining 23 animals (3/sex/group, Groups 1, 2, and 4; 2 males and 3 females, Group 3) were continued on study without further dosing, and terminated approximately 12 weeks after the last dose (Day 351; recovery necropsy). At termination, a full necropsy was conducted on all animals, and tissues were collected, preserved, processed, and examined microscopically by a Study Pathologist certified by the American College of Veterinary Pathologists (ACVP).

There were no treatment-related gross pathological findings at the end of the treatment period (day 270) or at the end of the recovery period (day 351).

Organ Weights: Absolute and relative organ weights were measured for the adrenals, brain, epididymides, heart, kidneys, liver, lungs, ovaries, pituitary, prostate, seminal vesicles, spleen, testes, thymus, thyroids and parathyroids, and uterus.

Organ weight differences were evident for heart, liver, spleen, pituitary, adrenals, and uterus. With the potential exception of the heart, there were no corresponding histopathological changes to these observed differences of organ weights. Heart weights (absolute and relative to brain weight) were increased for male treatment groups. There were potential corresponding histopathological changes that included necrosis and fibrosis of the heart papillary muscle.

Table 37 Organ weights at the end of the treatment and recovery periods

Organ weight	Day	Males				Females			
		0	10-IV	25-IV	30-SC	0	10-IV	25-IV	30-SC
Heart g	270	18.8587	21.5160 (114%)	25.6737 (136%)	22.6553 (120%)	NC	NC	NC	NC
Heart g/kg BW	270	2.8833	3.6800 (127%)	3.3467 (115%)	2.9067 (101%)	NC	NC	NC	NC
Heart g/g BrW	270	0.2833	0.3467 (122%)	0.3833 (135%)	0.3500 (124%)	NC	NC	NC	NC
Liver g	270	100.6547	110.9373 (110.2%)	116.4117 (115.65%)	116.3757 (115.6%)	NC	NC	NC	NC
Liver g/kg BW	270	15.4800	18.9167 (122%)	14.9600 (96%)	14.9733 (96%)	NC	NC	NC	NC
Liver g/g BrW	270	1.5067	1.7733 (118%)	1.7300 (115%)	1.8167 (121%)	NC	NC	NC	NC
Spleen g	270	3.7110	7.4663 (201%)	5.3380 (144%)	5.2030 (140%)	2.9477	2.9307	2.8127	3.9013 (132%)
Spleen g/kg BW	270	0.5793	1.2813 (221%)	0.6837 (118%)	0.6567 (113%)	0.8907	0.9763	0.8357	1.1953 (134%)
Spleen g/g BrW	270	0.0557	0.1173 (211%)	0.0793 (142%)	0.0820 (147%)	0.0467	0.0477	0.0460	0.0573 (123%)
Pituitary g	270	0.1127	0.0660 (59%)	0.0750 (67%)	0.0867 (77%)	NC	NC	NC	NC
Pituitary g/kg BW	270	0.0178	0.0114 (64%)	0.0097 (54%)	0.0116 (65%)	NC	NC	NC	NC
Pituitary g/g BrW	270	0.0017	0.0010 (59%)	0.0011 (65%)	0.0014 (82%)	NC	NC	NC	NC
Adrenals g	270	NC	NC	NC	NC	0.5933	0.5450	0.4567 (77%)	0.4123 (70%)
Adrenals g/kg BW	270	NC	NC	NC	NC	0.1753	0.1810	0.1383 (79%)	0.1263 (72%)
Adrenals g/g BrW	270	NC	NC	NC	NC	0.0093	0.0090	0.0073 (79%)	0.0060 (65%)
Uterus g	270					2.7073	3.0180	2.5920	1.9817 (73%)
Uterus g/kg BW	270					0.8133	1.0020	0.7793	0.6123 (75%)
Uterus g/g BrW	270					0.0433	0.0497	0.0430	0.0290 (67%)

NC = no change

Histopathology:

Adequate Battery: A full panel of organs and tissues was submitted to histopathological examination. For all animals necropsied, the tissues listed in the table below (except tattoos) were embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E), and examined by a Study Pathologist certified by the ACVP.

Table 38 Tissues collected for histopathological examination

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

Tissues Collected	
Lymph Nodes	Other
Axillary	Animal Number Tattoo
Mesenteric	Gross Lesions
Inguinal	Injection Sites (cephalic vein and dorsal areas)
Mandibular	

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

Peer Review: The histopathology findings were peer reviewed by a Sponsor Pathologist. Tissues from selected control and high dose (or other dose group exposed to the Test Article) animals were examined by the review pathologist. Target organs, neoplasms, and/or suspected neoplasms, and other anatomic findings considered unusual, based on the initial examination by the study pathologist, were also examined microscopically by the review pathologist. Pertinent data tables and reports/narratives were also reviewed. Except as indicated on Anatomic Findings Consensus Sheets or Individual Animal Pathology Tables, the study pathologist and review pathologist reached consensus agreements regarding terminology for findings reviewed and the incidence/severity of findings. The pathology report/narrative reflects final diagnoses reached as a result of this review.

Histological Findings: Histopathological findings with potential relevance to treatment were observed in the heart, thyroid, lung, liver, bone marrow, mandibular LN, adrenals, eye, kidneys, sciatic nerve, pituitary, prostate, rectum, trachea, brain, ovary, pancreas, subcutaneous injection site, left cephalic vein injection site, and left saphenous vein injection site. After assessment of these results as described below, the histopathological findings were judged to be spontaneous in nature and unrelated to treatment.

In an information request dated June 15, 2011, the Sponsor was asked to provide historical control incidences from the test laboratory (for animals of comparable age and in studies of comparable duration) and assessments of toxicological significance for histopathological findings in the heart, thyroid, lung, liver, and bone marrow that were described below. In the Sponsor's response, it was noted that the inclusion of sex, age, and study duration criteria similar to the current study severely limited the number of control animals which met all criteria, namely due to the fewer number of chronic, 9 month duration studies using older males (Study No. AAO00095; 6.3 to 12.9 years of age) and females (Study No. AAO00095; 4.4 to 6.2 years of age).

Systemic exposure to MEDI-563 was highest in the 25 mg/kg IV group. In general, histopathological findings only observed in the 30 mg/kg SC group were generally considered background in nature as findings were not confirmed in the 25 mg/kg IV group where higher drug exposures were achieved.

After analysis of histopathology findings, the only finding judged to be of potential concern was multifocal fibrosis of the interstitium at the base of the papillary muscle within the interventricular septum and thickening of the intima of a single medium to large caliber artery in male #3001 in the 25 mg/kg IV group at the end of the treatment period.

Heart: Several histopathological findings were observed in the heart at the end of the treatment and/or recovery periods. Minimal to mild necrosis of the papillary muscle was observed for one male in each of the three treatment groups at the end of the treatment period (i.e., 2003 at 10 mg/kg IV, 3003 at 25 mg/kg IV, and 4001 at 30 mg/kg SC). Necrosis consisted of hyalinization and disruption of myocardiocyte sarcoplasm with loss of cross-striations. Minimal change consisted of one or few necrotic myofibers, while mild change included a focally extensive region of myofiber necrosis, with hemorrhage. The incidence of this finding was not dose-responsive and none of these males had evidence of concurrent fibrosis. Further, these findings were not observed in female treatment groups. Multifocal fibrosis of the interstitium at the base of the papillary muscle within the interventricular septum and thickening of the intima of a single medium to large caliber artery was observed for one male (#3001) in the 25 mg/kg IV group at the end of the treatment period. Multifocal fibrosis of the interstitium of the papillary muscle was observed for one male in the 30 mg/kg SC group at the end of the recovery period; however, there were no findings in this group at the end of the treatment period. Focal fibrosis of the interstitium was observed for one male in each of the control and treatment groups at the end of the recovery period. Perivascular

granulomatous inflammation was observed in the left atrium and left atrial adventitia for one male (#3003) in the 25 mg/kg IV group at the end of the treatment period. Granulomatous inflammation is often associated with parasitic infection.

Both necrosis and fibrosis findings were associated with papillary muscle myofiber hypertrophy (not separately diagnosed) characterized by enlarged, elongated myofiber nuclei, and increased sarcoplasm. Hypertrophy of the papillary muscle myofibers was also commonly seen in both male and female controls and was considered incidental in *Cynomolgus* monkeys of this age at this anatomic location. The Sponsor contended that these changes reflected a continuum of myocardial damage, with the fibrotic changes in the one male (#3001) reflecting the chronicity of repair of previously damaged myofibers and the hypertrophy reflecting compensatory change of surviving cardiomyocytes. Myocardial degeneration/necrosis has been reported in control *Cynomolgus* monkeys, primarily of Indonesian and Mauritanian origin, at incidences ranging from 4 to 13% (Toxicologic Pathology 34: 67-74, 2006; Toxicologic Pathology 38: 297-302, 2010) although animals in the present study were of Chinese origin. Chamanza *et al.* (Toxicologic Pathology 34: 357-363, 2006) reported that myocardial fibrosis occurred with an incidence of 0.1% (3/2050) in control male and female *Cynomolgus* monkeys combined. It is noted that these animals in this publication were 12-30 months old; however, monkeys, in the present study, were a minimum of 6.3 and 4.4 years old for males and females, respectively. Chamanza *et al.* (Toxicologic Pathology 38: 642-657, 2010) reported that myocardial degeneration/fibrosis occurred with an incidence of 5.6% (32/570) in *Cynomolgus* monkeys with origin from Mauritania, China, or Vietnam and an age range of 12 to 36 months. There were no abnormal ECG findings in any animals on study.

The Sponsor was asked to provide historical control incidences with respect to findings in the heart that consisted of fibrosis, interstitium, multifocal, minimal-mild; fibrosis, interstitium, focal, minimal-mild; necrosis, papillary muscle, minimal-mild; thickening, artery, intima; and inflammation, granulomatous, perivascular, left atrium, minimal. Minimal focal fibrosis in the myocardium, papillary muscle, or epicardium was observed for 6 of 1649 males and 5 of 1625 females from studies of unspecified duration (findings in 6 studies with a total of 32 males, 2.3 to 5.6 years old and 5 studies with 27 females 2.7 to 4.9 years old). Minimal to mild multifocal fibrosis was observed for 3 of 1625 females from studies of unspecified duration (findings in 3 studies with a total of 15 females, 2.2 to 5.3 years); however, no males were reported with this finding. Minimal to mild focal or multifocal necrosis/degeneration of myocardium, cardiomyocyte, myofiber, or papillary muscle was observed for 15 of 674 males and 12 of 683 females in studies with duration ≥ 3 months (findings in 15 studies with a total of 64 males; 1.0 [ePPND] and 2.6 to 10.2 years and 9 studies with a total of 51 females; 1.0 [ePPND] and 2.5 to 8.8 years old). Minimal focal proliferation of the arterial intima was observed for 2 of 683 males in studies with duration ≥ 3 months (findings in 2 studies with a total of 12 males; 3.4 to 11.3 years). Minimal to mild focal to multifocal thickening of the intima or coronary artery was observed for 3 of 674 females in studies with duration ≥ 3 months (findings in 3 studies with a total of 17 females; 3.2 to 5.9 years old).

The incidence of the finding of papillary muscle necrosis in male treatment groups was not dose responsive and not associated with concurrent fibrosis. No females in treatment groups were observed with this finding. This finding was observed at a low incidence in control males and females from the testing laboratory. Thus, this finding was judged to be background in nature.

Given the overall occurrences of focal and multifocal fibrosis, these findings were judged to be unrelated to treatment. These findings are known to occur spontaneously based upon published literature and historical control data from the testing laboratory. The males (6.3 to 12.9 years of age) and females (4.4 to 6.2 years of age) used in the study were older than animals assessed in the published literature.

Thyroid: In the thyroid, multifocal proliferation of the follicular epithelium was observed for one male in the 25 mg/kg IV group at the end of the treatment period.

The Sponsor was asked to provide historical control incidences with respect to this finding in the thyroid. Minimal focal or multifocal follicular cell hyperplasia was observed for 1 of 674 males and 2 of 683 females in studies with duration ≥ 3 months (findings in 1 study with a total of 6 males; 3.2 to 3.5 years old and 2 studies with a total of 7 females; 3.1 to 3.7 years old).

The relationship of this finding to treatment remains unclear.

Lung: In the lung, mononuclear cell infiltrate in the pleura was observed for one female each in the 10 and 25 mg/kg IV groups at the end of the treatment period. A polyp (well differentiated cuboidal epithelial cells supported by fine fibrovascular stroma observed within perivascular alveolar space) was observed for one male (#3006) in the 25 mg/kg IV group at the end of the 12-week recovery period. There was no comparable finding at the end of the drug treatment period.

The Sponsor was asked to provide historical control incidences with respect to the finding of a lung polyp. Minimal to mild multifocal hyperplasia of the alveolar epithelium was observed for 1 of 674 males and 1 of 683 females from studies with duration ≥ 3 months (1 study with 6 males, 3.7 to 5.7 years old and 1 study with 6 females, 4.2 to 5.2 years old).

The MedImmune peer review pathologist commented that lung polyps are a common incidental finding in *Cynomolgus* monkeys, especially in the peripheral parts of lung lobes, and are often associated with parasite migration. However, the treatment relationship remains unclear.

Sato *et al.* (Journal of Toxicology and Pathology 25: 63-101, 2012) reported that focal hyperplasia of the alveolar epithelium with fibrous thickening of the alveolar wall is rarely seen.

Liver: In the liver, cytoplasmic vacuolation was observed in midzonal hepatocytes for one male in the 25 mg/kg IV group at the end of the treatment period. A diffuse, flocculent, cytoplasmic alteration was observed in hepatocytes from one male in the 25 mg/kg IV group at the end of the recovery period.

From the testing laboratory, minimal to moderate diffuse, focal, or multifocal lipidosis or cytoplasmic vacuolation was observed in 23 of 674 males and 38 of 683 females from studies with duration ≥ 3 months (18 studies with 91 males; 2.2 to 7.8 years and 26 studies with 121 females; 0.9 to 11.3 years old).

From the testing laboratory, minimal to moderate diffuse cytoplasmic rarefaction or flocculent cytoplasmic alteration was observed for 82 of 674 males and 74 of 683 females from studies with duration ≥ 3 months (29 studies with 143 males; 0.9 to 8.8 years and 26 studies with 131 females; 2.5 to 11.3 years old).

Based upon historical control incidences of these findings from the test laboratory, these two findings were considered to be unrelated to treatment.

Bone marrow: In the bone marrow, increased incidences of lymphoid infiltrates (aggregates and/or follicles) were observed for male and/or female treatment groups at the end of the treatment period and males and females in the 25 mg/kg IV group at the end of the recovery period. Published background incidences of lymphoid follicles for males and females were reported to be 8.6 and 12.2%, respectively.

From the testing laboratory, minimal to mild focal or multifocal lymphoid aggregates or follicles were observed for 16 of 674 males and 17 of 683 females in studies with duration ≥ 3 months (11 studies with a total of 42 males; 2.9 to 7.8 years old and 17 studies with a total of 66 females, 0.4 to 7.9 years).

Based upon the published incidence and historical control incidence from the testing laboratory, this finding was considered unrelated to treatment.

Mandibular LN: In the mandibular LN, subcapsular sinus histiocytosis was observed for 1 male in the 25 mg/kg IV group at the end of the treatment period. The relationship of this finding to treatment was unclear. Neutrophilia in the medullary cord was observed for 1 female each in the 25 mg/kg IV and 30 mg/kg SC groups at the end of the treatment period. It was unclear if this finding might be related to potential infection.

Sciatic nerve: In the sciatic nerve, multifocal axonal degeneration was observed for 1 male in the 25 mg/kg IV group at the end of the treatment period. This finding is unlikely related to treatment.

Pituitary: In the pituitary, perivascular, lymphoplasmacytic inflammation in the pars nervosa was observed for 1 female in the 25 mg/kg IV group at the end of the treatment period. The relationship of this finding to treatment was unclear.

Prostate: In the prostate, increased incidences of multifocal chronic active inflammation were observed in all male treatment groups at the end of the treatment period. The published spontaneous incidence of mononuclear cell infiltration in the prostate is 34.4% (76/221). Chamanza et al. (Toxicologic Pathology 38: 642-657, 2010) reported an incidence of inflammatory cell infiltrates at 3.2% (9/285). The findings in the present could be considered background in nature.

Rectum: In the rectum, multifocal submucosal hemorrhage was observed for 1 male in the 25 mg/kg IV group at the end of the treatment period. The relationship of this finding to treatment was unclear.

Trachea: In the trachea, mixed cell infiltrate in the submucosa and neutrophil infiltrate in the lumen were observed in the male 25 mg/kg IV group at the end of the treatment period. The relationship of this finding to treatment was unclear.

Ovaries: In the ovaries, unilateral large follicles were observed for 3 of 3 females in the 30 mg/kg SC group at the end of the recovery period. The unilateral nature of the finding suggests that it is probably not related to treatment.

Pancreas: In the pancreas, lymphoid infiltrates (aggregates and/or follicles) in the interstitium was observed for one female in the 25 mg/kg IV group at the end of the recovery period. The relationship of this finding to treatment was unclear.

SC injection sites: At subcutaneous injection sites, there were increased incidences of perivascular mononuclear cell infiltrates in the subcutis, hemorrhage in the subcutis, and perivascular mixed inflammation in the dermis for males and/or females in the 30 mg/kg SC group. These findings should be monitorable in a clinical setting.

IV injection sites: Findings in intravenous injection sites consisted of low incidences of perivascular neutrophil infiltrate in the dermis, myocyte degeneration/regeneration, and perivascular mononuclear cell infiltrate in the subcutis. These findings should be monitorable in a clinical setting.

Table 39 Histopathological findings at the end of the treatment and recovery periods

Organ/Tissue	Sex	End of treatment period				End of recovery period			
		0	10-IV	25-IV	30-SC	0	10-IV	25-IV	30-SC
Heart									
-fibrosis, interstitium, multifocal, minimal-mild	M	0/3	0/3	1/3	0/3	0/3	0/3	0/3	1/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-fibrosis, interstitium, focal, minimal-mild	M	0/3	0/3	0/3	0/3	1/3	1/3	1/3	1/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-thickening, artery, intima	M	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-inflammation, granulomatous, perivascular, left atrium, minimal	M	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-necrosis, papillary muscle, minimal-mild	M	0/3	1/3 _(Min)	1/3 _(Mid)	1/3 _(Min)	0/3	0/3	0/3	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Thyroid									
-proliferation, follicular epithelium, multifocal	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Lung									
-infiltrate, mononuclear cell, pleura, focal	M	0/3	0/3	0/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3
-polyp	M	0/3	0/3	0/3	0/3	0/3	0/3	1/2	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Liver									
-vacuolation, cytoplasm, discrete round, hepatocyte, midzonal	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-alteration, cytoplasm, flocculent, hepatocyte, diffuse	M	0/3	0/3	0/3	0/3	0/3	0/3	1/2	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
Bone marrow, Sternum									
-lymphoid infiltrate, aggregates, and/or follicles	M	0/3	1/3	1/3	0/3	0/3	0/3	1/2	0/3
	F	0/3	0/3	1/3	2/3	0/3	0/3	2/3	0/3
LN Mandibular									
-sinus histiocytosis, subcapsular	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
-neutrophilia, medullary cord	M	0/3	0/3	0/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	1/3	1/3	0/3	0/3	0/3	0/3
Nerve, Sciatic									
-degeneration, axonal, multifocal	M	0/3	0/3	0/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Pituitary									
-inflammation, lymphoplasmacytic, pars nervosa, perivascular, diffuse	M	0/3	0/3	0/3	0/3	0/3	0/3	0/2	0/3
	F	0/3	0/3	1/3	0/3	0/3	0/3	0/3	0/3
Prostate									
-inflammation, chronic active, gland, multifocal	M	0/3	1/3	2/3	1/3	0/3	0/3	0/2	0/3

Rectum										
-hemorrhage, submucosa, multifocal	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3	
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	
Trachea										
-infiltrate, mixed cell, submucosa, focally extensive	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3	
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	
-infiltrate, neutrophil, lumen	M	0/3	0/3	1/3	0/3	0/3	0/3	0/2	0/3	
	F	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3	
Ovary										
-large follicle, unilateral	F	0/3	0/3	0/3	0/3	0/3	1/3	0/3	3/3	
Pancreas										
-lymphoid, infiltrates, aggregates, and /or follicles, interstitium	M	0/3	0/3	0/3	0/3	0/3	0/3	0/2	0/3	
	F	0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/3	
Injection Site, Subcutaneous										
-infiltrate, mononuclear cell, subcutis, perivascular	M	0/3	-	-	2/3	0/3	-	-	0/3	
	F	1/3	-	-	3/3	0/3	-	-	1/3	
-hemorrhage, subcutis	M	0/3	-	-	1/3	0/3	-	-	0/3	
	F	0/3	-	-	0/3	0/3	-	-	0/3	
-inflammation, mixed, dermis, perivascular	M	0/3	-	-	0/3	0/3	-	-	0/3	
	F	0/3	-	-	1/3	0/3	-	-	0/3	
Injection Site, Left Cephalic Vein										
-infiltrate, neutrophil, dermis, perivascular	M	0/3	0/3	1/3	-	0/3	0/3	0/2	-	
	F	0/3	0/3	0/3	-	0/3	0/3	0/3	-	
-degeneration/regeneration, myocyte, focally extensive	M	0/3	0/3	0/3	-	0/3	0/3	0/2	-	
	F	0/3	0/3	1/3	-	0/3	0/3	0/3	-	
Injection Site, Left Saphenous Vein										
-infiltrate, mononuclear cell, subcutis, perivascular	M	0/3	0/3	0/3	-	0/3	0/3	0/2	-	
	F	0/3	0/3	1/3	-	0/3	0/3	0/3	-	

Special Evaluations:

Assessment of Male Fertility:

- Testicular measurements were conducted for all males twice during the prestudy period and on days 64, 120, and 269.

Testicular volume was unaffected by treatment with MEDI-563.

- Semen samples for analysis of total sperm count, sperm motility, and sperm morphology were collected (if possible) by penile electro-ejaculation twice during the prestudy period, during weeks 10 and 18, and after the last dose during the week before scheduled terminal necropsy (Week 39). If no semen sample was available upon the initial attempt for each time point, additional attempts were made on subsequent days in the week, up to three attempts per time point.

There were no treatment-related effects on sperm motility, concentration, or counts per ejaculate for male treatment groups that received MEDI-563.

- Male hormones [Testosterone, Luteinizing Hormone (LH), and Follicle-Stimulating Hormone (FSH)] were measured once during weeks -2 and -1 of the prestudy period, and on days 63, 119, 268, and 345.

Levels of testosterone, LH, and FSH were unaffected for male treatment groups that received MEDI-563. Observed variability of levels were generally within the ranges observed during the prestudy periods and for the control group over the course of the study.

Assessment of Female Fertility:

- Vaginal swabs were collected from all females on a daily basis beginning on day -64 and continuing through the termination of the in-life phase. Menstrual cycle lengths were determined.

Menstrual cycle length was unaffected for female treatment groups that received MEDI-563. Group mean menstrual cycle lengths ranged from 30.6 ± 4.9 to 31.8 ± 6.5 days during the prestudy phase (all groups), 30.3 ± 3.8 to 31.8 ± 5.5 during the dosing phase (all groups), and 28.3 ± 11.5 to 32.8 ± 7.5 days during the recovery period (all groups), which were within the normal range of variability for Cynomolgus monkeys at the Testing Facility (30.6 ± 4.5 days, n=725 cycles).

Table 40 Females with absent menses

Instances of Absent Menses

Female	Phase	Cycle Length	Adjusted Cycle Length	Cycle Start (Day)	End 1st Cycle	End 2nd Cycle	End 3rd Cycle
1506	Dosing	125	31/31/32	-6	24	55	86
1505	Recovery	58	29/29	273	301	Not applicable	Not applicable
3504	Dosing	74	25/24	14	38	62	Not applicable
	Dosing	50	25/25	88	112	Not applicable	Not applicable
3506	Dosing	57	29	22	50	Not applicable	Not applicable

- Female hormones (progesterone, estradiol, Luteinizing Hormone (LH), and Follicle-Stimulating Hormone) were measured twice weekly for the following periods: days -64 to -12 of the prestudy period and days 91 to 143 and days 196 to 249 of the treatment period.

All females showed evidence of regular ovulation (i.e., preovulatory estradiol, luteinizing, and follicle stimulating hormone surges, followed by incremental progesterone production) during the prestudy and dosing periods. There was significant variability of hormonal levels between animals of the same group and between different groups.

Female #2506 in the 10 mg/kg IV group did not show evidence of ovulation during the first reproductive hormone collection during the dosing phase (Days 112 to 175). During this time, serum estradiol concentrations were in their early to mid follicular range (generally ≤ 200 pg/mL) without evidence for ovulation or progesterone secretion and no vaginal bleeding. However, evidence of ovulation was present during the second reproductive hormone collection during the dosing phase (Days 196 to 249). Some cycles were longer than the prestudy average (up to 63 days during dosing vs. 32 days prestudy). Since hormone patterns indicative of ovulation occurred during the latter part of the study and vaginal bleeding consistent with cycling was also present; the single instance of altered hormone pattern (anovulatory) was unlikely to be test article-related. Anovulatory cycles have been reported to occur in both young as well as mature cycling Cynomolgus monkeys. In one study, 10.6% anovulatory cycles of a total of 104 cycles were reported from a colony of Cynomolgus monkeys where the ovulatory status was established (Journal of Reproductive Fertility Supplement 22: 25-31, 1975). Further, no abnormal histologic observations were evident in the reproductive tissues of this animal.

Female #3504 in the 25 mg/kg IV group was observed with serum concentrations of estradiol, LH and FSH that indicated normal follicular and preovulatory patterns from Day 91 to 112, although there was no evidence of subsequent progesterone secretion. However, it appears that the ovarian cycle restarted immediately with a normal menstrual cycle with ovulation completed in the next 28 days. There were no abnormal histologic observations in the reproductive tissues of this animal.

Female #4506 in the 30 mg/kg SC group had menstrual cycle lengths of 31 days during prestudy, and 28 to 37 days during dosing. This female had an apparent 9 day cycle at the beginning of the recovery phase based on the menstruation duration (4 days beginning on Day 265 and 6 days beginning on Day 278; dosing stopped at Day 267). The subsequent 2 menstrual cycles (36 and 32 days) during recovery were of lengths that were comparable to those observed for the female prestudy and during dosing. In the absence of reproductive hormone data during this period (Days 269 through 278), it could not be determined if this female experienced prolonged menstruation (15 days), which would result in the subsequent cycle to be 45 days (rather than 36 days). An association with test article cannot be excluded. This occurred in only one animal at the start of the recovery period; the previous (8 during dosing phase) and subsequent menstrual cycles (2 during recovery) were comparable to prestudy menstrual cycle lengths. There were no abnormal histologic observations in the reproductive tissues in this animal. Further, there were no such findings in the 25 mg/kg IV group that achieved higher systemic drug exposures.

Toxicokinetics: Blood samples for measurement of serum concentrations of MEDI-563 were collected on day 1 at predose, immediately postdose, and 12 hr postdose, day 2 (24 hr postdose), day 3 (48 hr postdose), day 4 (72 hr postdose), day 6 (120 hr postdose), day 8 (168 hr postdose), day 15 (336 hr postdose/predose), day 57 (predose), day 127 (predose), day 197 (predose), day 253 at predose and 12 hr postdose, day 254 (24 hr postdose), day 255 (48 hr postdose), day 256 (72 hr postdose), day 258 (120 hr postdose), day 260 (168 hr postdose), day 263 (240 hr postdose), day 267 (predose), day 269 (48 hr postdose), day 270 (72 hr postdose), day

274 (168 hr postdose), day 281 (336 hr postdose), day 288, day 295, day 302, day 316, day 330, and day 351.

AUC and C_{max} values for MEDI-563 following intravenous administration increased in a dose proportional manner from 10 to 25 mg/kg. Following SC administration of MEDI-563, peak serum concentrations were attained between 12 hours and 10 days postdose. Accumulation ratios in the process to achieve steady-state exposures for each group were approximately 2 suggesting a two-fold systemic accumulation following bi-weekly dosing. Elimination half-lives (10.3 to 15.6 days) were similar for each dose group after Dose 1 and Dose 19. SC bioavailability of MEDI-563 in Cynomolgus monkeys after Dose 1 and Dose 19 was 61% and 58%, respectively. Anti-drug antibodies were detected in 2 of the 36 (4.2%) animals treated with MEDI-563.

Table 41 Toxicokinetic parameters for doses 1, 19, and 20

Table 5.1.2-1 Summary of Mean Toxicokinetic Results of MEDI-563 in Cynomolgus Monkeys following Bi-Weekly IV and SC Administration of MEDI-563 (Parameters Presented for Dose 1, 19 and 20 Only).

Group (Dose)	Dose Number	Toxicokinetic Parameters ^{a,b}						
		T_{max} (d)	C_{max} (µg/mL)	AUC _(0-14d) (µg·d/mL)	AUC _{inf} (µg·d/mL)	CL ^c (mL/d/kg)	$t_{1/2}$ (d)	AR
2 (10 mg/kg, IV)	1 st	0.0208 (0.0208-0.500)	269±41.5	1430±146	2680±657	3.94±0.945	13.7±4.23	1.62±0.458
	19 th	0.500 (0.500-1.00)	287±77.4	2320±700	N/A	N/A	15.2±5.33	
	20 th	N/A	N/A	N/A	N/A	N/A	13.9±0.857	
3 (25 mg/kg, IV)	1 st	0.0208 (0.0208-0.0208)	627±116	3270±439	5560±776	4.57±0.593	12.3±2.29	1.93±0.648
	19 th	0.500 (0.500-1.00)	834±140	6100±1170	N/A	N/A	10.3±2.62	
	20 th	N/A	N/A	N/A	N/A	N/A	15.6±0.797	
4 (30 mg/kg, SC)	1 st	3.00 (2.00-10.0)	218±80.9	2170±656	4500±982 ^d	6.96±1.52 ^d	13.4±4.21 ^d	2.12±1.26
	19 th	2.00 (0.500-5.00)	406±149	4110±1480	N/A	N/A	13.7±8.58	
	20 th	N/A	N/A	N/A	N/A	N/A	12.5±0.989	

TK Parameters and AR were rounded to 3 significant figures after calculations were performed.

Parameters are shown as Mean±SD, except for T_{max} , which is shown as median (range).

^a n=11, Animal (2002) from Group 2 excluded from summary statistic calculations due to formation of Anti-Drug Antibodies.

^b n=10, Animal (3504) from Group 3 excluded from summary statistic calculations due to formation of Anti-Drug Antibodies; Animal (3004) from Group 3 excluded from summary statistic calculation due to early termination from the study.

^c Apparent clearance (CL/F) calculated for SC administered animals (Group 4).

^d n=11, Terminal phase for Animal (4002) from Group 4 could not be accurately estimated; TK parameters (AUC_{inf}, CL/F, $t_{1/2}$) could not be determined.

AUC_(0-14d): area under the concentration-time curve from 0 to 14 days postdose; Accumulation ratio (AR) determined using AUC_{(0-14d), Dose 19}/AUC_{(0-14d), Dose 1}.

Table 42 Trough concentrations for doses 2, 5, 10, 15, 19, and 20

Average^a Trough^b Concentrations (µg/mL) for Cynomolgus Monkeys Following Bi-weekly IV and SC Administration of MEDI-563

Group (dose, route)	Dose					
	2	5	10	15	19	20
2 (10 mg/kg, IV)	62.5±12.3	119±44.6	124±43.0	144±68.7	123±58.8	118±47.0
3 (25mg/kg, IV)	134±24.2	214±70.1	260±67.9	285±63.2	269±66.0	263±54.8
4 (30 mg/kg, SC)	117±23.5	182±39.5	225±66.6	246±84.9	245±132	210±87.5

Average trough concentrations were rounded to 3 significant figures after calculations were performed.

Data are shown as mean ± standard deviation (SD)

^a n=11 Group 2 - Animal (2002) excluded from summary statistic calculations due to formation of ATA; n=10

Group 3 - Animal (3504) excluded from summary statistic calculations due to formation of ATA; Animal (3004) excluded from summary statistic calculation due to early termination from the study; n=12 (Group 4).

^bTroughs are minimum concentration values determined from sample collections just prior to dosing (predose).

Blood samples for measurement of anti-drug antibodies (ADA) were collected on days 1, 183, 270, and 350.

One male animal (2002) from Group 2 and one female animal (3504) from Group 3 tested positive for anti-MEDI-563 antibodies. The onsets of ADAs were observed prior to Dose 15 of MEDI-563 for both animals 2002 and 3504. Consistent with an anti-MEDI-563 antibody response, a decrease in serum MEDI-563 levels for these animals were observed. Due to the formation of ADAs to MEDI-563 it was not possible to accurately characterize exposure in these animals. Exposure to MEDI-563 was maintained in all other animals where ADAs were not detected.

Immunogenicity results for one male animal (4003) from Group 4 (30 mg/kg, SC) showed a detectable anti-MEDI-563 titer from samples taken predose on Day 1 that would be considered immunopositive. However, the titers (1:40) were just above the assay LLOQ (<1:10) and as a result, considered to be a false positive value. This is supported by all subsequent results for this animal being ADA negative and a lack of observed impact to the animal's TK profile.

Immunogenicity results for one Group 1 female (1501), and two Group 3 females (3501 and 3505) showed a detectable anti-MEDI-563 titer from samples taken near Dose 14 that would be considered immunopositive. However, the titers were just above the assay LLOQ (<1:10) and were considered to be false positive results. There was no evidence of altered TK profiles for these animals as might be expected following generation of ADAs.

Table 43 Immunogenicity results for Cynomolgus monkeys following bi-weekly IV or SC administration of MEDI-563**Summary of Immunogenicity Results in Adult Cynomolgus Monkeys following Bi-weekly IV Administration of MEDI-563**

Group	Dose, Route	Immunopositive	Percentage Positive	Animal IDs (sex)
1	0 mg/kg, IV/SC	0 of 12	0%	N/A
2	10 mg/kg, IV	1 of 12	8%	2002 (male)
3	25 mg/kg, IV	1 of 12	8%	3504 (female)
4	30 mg/kg, SC	0 of 12	0%	N/A

N/A - not applicable

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Table 44 Immunogenicity titers for ADA positive Cynomolgus monkeys**Table 5.1.3-1 Immunogenicity Titers for ADA Positive Cynomolgus Monkeys**

Group	Animal ID (M/F)	Study Day / Dose Number			
		Pre-dose Phase	183 / Dose 14	267 / Dose 20	350
1 (n=12)	1501 (F)	—	QNS	80	NS
2 (n=12)	2002 (M)	—	81920	81920	NS
3 (n=12)	3504 (F)	—	327680	327680	1310720
	3505 (F)	—	20	—	—
4 (n=12)	4003 (M)	40	—	—	NS

M - Male; F - Female; NS - No Scheduled Sample; QNS - Quantity Not Sufficient;

ADA negative samples (titers below the LLOQ <1:10) are represented by the symbol (—).

Dosing Formulation Analysis: Residual volumes of the dose formulations at Days 1, 99 (Group 2 only), 127 (Group 2 only), and Day 267 were analyzed to verify the concentrations of MEDI-563. With respect to concentration verification, actual MEDI-563 concentrations in dosing formulations were generally within 100 to 110% of theoretical concentrations. However, two exceptions were noted.

- For the Group 4 residual on Day 267, the actual concentration (24.9 mg/mL) was only 83% of the theoretical concentration (30 mg/mL). It was noted for the stability assessment conducted at the end of the study for the Group 4 dosing formulation that the actual concentration (33.6 mg/mL) was approximately 110% of the theoretical concentration (30 mg/mL). This appears to allay concerns that animals might have not been adequately dosed.
- For the Group 2 residual on Day 267, the actual concentration was 3.03 mg/mL, approximately 50% higher than the theoretical concentration of 2 mg/mL. The results were verified, and it is unclear why the results at this time point were approximately 50% higher than the theoretical concentration given the results of other residual samples for this group.

Stability of MEDI-563 in dosing formulations was evaluated at the completion of the study. Actual concentrations ranged from 100 to 110% of the theoretical concentrations indicating that stability was acceptable.

Table 45 MEDI-563 concentrations for dose formulations used in this study

MEDI-563 concentrations for dose formulations used on this study are presented below:

Group ID – sample time point	Lot #	Theoretical Concentration (mg/mL)	Actual Concentration (mg/mL)	Purity (%)
1 - Week 1	KP487.037	0	<LLOQ	NA
2 - Week 1	NDL4517.042	2	2.03	99.4
3 - Week 1	NDL4517.042	5	5.55	99.4
4 - Week 1	NDL4517.042	30	33.1	99.3
2 - Day 99 (Week 15)	NDL4517.042	2	2.13	99.5
2 - Day 127	CNF5186.048	2	2.04	99.4
3 - Day 267	NDL4517.042	5	5.61	99.4
2 - Day 267	CNF5186.048	3	3.03	99.4
3 - Day 267	CNF5186.049	5	5.25	99.4
4 - Day 267	NDL4517.042	30	24.9	99.3
1 - Day 267	KP487.037	0	<LLOQ	NA

LLOQ = Lower Limit of Quantification NA = Not Applicable

Table 46 Stability of MEDI-563 dose formulations after the completion of the study

The stability of the MEDI-563 dose formulations after the completion of the study was determined by the Sponsor and the results obtained after completion of the study are listed in the following table.

Group ID	Lot #	Theoretical Concentration (mg/mL)	Actual Concentration (mg/mL)	Purity (%)
1	KP487.037	0	None detected	-
2	NDL4517.042	2	2.15	99.3
3	NDL4517.042	5	5.55	99.3
4	NDL4517.042	30	33.6	99.3
2	CNF5186.048	2	2.11	99.1
3	CNF5186.049	5	5.26	99.2

7 Genetic Toxicology

Genetic toxicology studies are not required for a protein produced by recombinant DNA technology.

8 Carcinogenicity

The Sponsor provided a rationale for not conducting a carcinogenicity study with benralizumab in the EOP2 meeting package. The rationale focused on toxicology studies conducted with the test article in monkeys, rodents were not a pharmacologically relevant species for studying the effects of the test article, use of a murine-reactive surrogate or targeted deletion and transgenic mouse models, and evaluating the potential role of eosinophils in cancer.

No studies evaluating the carcinogenic potential of benralizumab were conducted. As benralizumab is intended for chronic treatment of asthma, its long term effects were assessed in GLP-compliant toxicity studies conducted in Cynomolgus monkeys following repeated IV or SC administrations. Repeated IV and SC administrations (every other week for 39 weeks for a total of 20 doses) of benralizumab at dose levels up to 30

mg/kg SC and 25 mg/kg IV revealed no adverse changes in organ weights or any histologic findings suggestive of pre-neoplastic lesions.

Affinity for binding of benralizumab to IL-5R α on murine, human, and Cynomolgus monkey eosinophils was determined by surface plasmon resonance (MedImmune Research Report RIA563-001). Benralizumab bound to IL-5R α on human and Cynomolgus monkey eosinophils with similar affinities; it did not bind to murine IL-5R α . Benralizumab does not bind to murine IL-5R α . Therefore, rodents are not suitable species for direct evaluation of carcinogenic potential of benralizumab.

Use of a murine-reactive surrogate or targeted deletion and transgenic mouse models were considered. The ICH S6 (R1) Guidance does not require development of a murine surrogate under these circumstances. The Sponsor judged that any suitable surrogate should elicit the enhanced ADCC effector function exhibited by benralizumab in humans. An ADCC is a lytic attack on antibody-targeted cells following binding of the antibody's Fc region to appropriate Fc γ receptors (Fc γ R). In humans, Fc γ IIIa, an Fc γ R expressed mainly on natural killer cells, which interacts well with IgG1 isotype antibodies like benralizumab, is responsible for ADCC activation-based destruction of target cells. The enhanced ADCC effector function of benralizumab results from its intentional afucosylation that improves binding to human Fc γ IIIa and, thereby, enhances depletion of eosinophils. In mice, Fc γ IV is the equivalent to human Fc γ IIIa, which is not expressed on natural killer cells but on neutrophils, monocytes, and dendritic cells. Another key difference is that, unlike human Fc γ IIIa, mouse Fc γ IV does not bind to IgG1 isotype antibodies; it binds to IgG2a and IgG2b isotype antibodies. Given these species-based differences in Fc γ R mediation of ADCC effector functions, it was concluded that it would not be feasible to create a rodent surrogate that mimics the enhanced ADCC effector function exhibited by benralizumab in humans.

Consideration was also given to the ability of targeted deletion and transgenic mouse models to provide risk assessment of carcinogenicity of benralizumab. Mice that are genetically targeted for eosinophil deficiency were available (Δ dbl GATA deficient mice and EPO-DTA [PHIL] transgenic mice; Yu et al, 2002; Lee et al, 2004). However, these mice were not considered an appropriate model since their derivation was based on modulation of genes other than IL-5R α or its ligand. Therefore, they may have other potential physiological liabilities that would impact the results and interpretation of study and its clinical relevance.

Knock-out strains, such as IL-5-deficient and IL-5R α -deficient mice, were considered. Interleukin-5-deficient mice (Kopf et al, 1996) have normal levels of eosinophils with normal morphology in the bone marrow and periphery. Although systemic eosinophilia in IL-5-deficient animals was absent, during infection with *Mesocestoides corti*, eosinophils in this model still homed to sites of infection, suggesting that they might have normal function. 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 IL-5 systemically (Immunity 4: 15-24, 1996). 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 exist (Personal communication, (b) (4)). 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. Therefore, it could be concluded that the IL-5-deficient knockout mouse model was not fully relevant to the human physiology and for assessing the risk of carcinogenicity of benralizumab following clinical administration.

IL-5R α was expressed on eosinophils and basophils in both mice and humans; however, mice constitutively express IL-5R α on B-1 cells. In addition, expression of IL-5R α in mice can be induced on stimulated B-2 cells (Takatsu, 2009). This results in a physiological outcome of decreased peritoneal B-1 cells noted in IL-5R α -deficient mice, along with low serum concentrations of IgM and IgG3 (Yoshida, 1996). There was considerable evidence for a role of B-1 cells in cancer (Kasaian and Casali, 1993; Hardy, 2006). The TCL1 gene was identified from translocations from T-cell leukemias and was expressed in human B-cell neoplasms. Mice expressing this gene under control of the E μ enhancer develop a pre-leukemic expansion of B cells of a B-1 phenotype. As the mice age, CLL-like expansions develop that have a phenotype similar to normal CD5+ B-1 B cells (Bichi, 2002). Mice deficient for SPA-1, a Rap1 GTPase-activating protein, develop lupus like autoimmune disease (CD5+ B-1 cells can transfer the disease) and sporadically progress to B-cell leukemia with hemolytic autoantibody, suggesting that the Rap1 signal plays a role in maintaining self-tolerance (Ishida, 2006). Finally, transgenic mice bearing human CLL derived low-affinity polyreactive B-cell receptors (expression of polyreactive autoantibodies) had transgene-positive B cells in the peritoneal cavity with a B-1a phenotype (memory-type that were possibly selected by cross-reactivity to mouse self-antigens) that were readily induced to secrete Ig by nonspecific activation (Widhopf, 2004). Given the proposed role of B-1 cells in carcinogenesis, this difference in expression of IL-5R α between mice and humans suggests that standard mouse 2 year bioassays were unlikely to provide clinically meaningful information with respect to risk of carcinogenicity of benralizumab.

Since benralizumab targets eosinophils for destruction, it is important to understand the impact of eosinophil depletion in relation to carcinogenicity risk. While eosinophils have been found in association with solid tumors, especially tumors of epithelial origin (breast and colon; Samoszuk *et al.*, 1996), the role eosinophils may have in tumor growth, if any, remains unclear. While some clinical studies have suggested the presence of eosinophils may be a positive prognostic indicator of cancer patient survival, a definitive link has not yet been established (Lowe *et al.*, 1981; Hogan, 2007).

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.

- 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 MCA-induced tumor incidence and growth were significantly attenuated in IL-5 transgenic mice of both the BALB/c strain and C57BL/6 background. Histological examination revealed that the protective effect of IL-5 was associated with significantly increased 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 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 2.5×10^5 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 wildtype or IL-5^{-/-} mice (Journal of Immunology 160: 345-350, 1998).

- 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 (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 TGF α . Transgenic mice overexpressing TGF α 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.

The Sponsor's rationale for not conducting a carcinogenicity study with benralizumab was discussed with Executive Carcinogenicity Assessment Committee by email on January 30 and 31, 2013. It was agreed that a carcinogenicity study was not required for benralizumab. The key points of the discussion included:

1. To date, there is no evidence for proliferative or pre-neoplastic effects of benralizumab in any GLP-compliant repeat-dose IV or SC toxicologic study in Cynomolgus monkeys.
2. Since benralizumab does not bind to murine IL-5R α , direct assessment of carcinogenic risk of benralizumab in a classic 2-year rodent bioassay was not appropriate.
3. A murine surrogate is not required.
4. IL-5- and IL-5R α -deficient mouse models do not appear to be relevant for human risk assessment.

Based on the lack of suitable rodent models for assessment of carcinogenic risk of benralizumab, the Sponsor plans to use results from the completed repeat-dose toxicologic studies in Cynomolgus monkeys to provide nonclinical risk assessment regarding carcinogenic potential of benralizumab following its chronic administration.

The role of eosinophils in tumor development is unclear. Subjects in clinical trials should be monitored for potential development of tumors. To mitigate the potential risk of malignancies, the Sponsor has included eligibility criteria in clinical study protocols to exclude vulnerable subjects and subjects in studies will be monitored through standard pharmacovigilance activities, including routine monitoring of TEAEs/TESAEs in clinical studies. The Medical Officer will assess the acceptability of the Sponsor's plans.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

An assessment of fertility was included in the 9-month toxicology study with adult male and female Cynomolgus monkeys. There were no drug-related effects on male or female fertility.

9.2 Embryonic Fetal Development

Pregnant female monkeys were treated with benralizumab during the period of organogenesis (gestation days 20-50) in the enhanced pre-and post-natal development study with monkeys (drug treatment started at approximately gestation day 20 and continued up to 1-month post-partum) described below.

9.3 Prenatal and Postnatal Development

Study title: Maternal, Embryo-Fetal and Neonatal Toxicity Study of MEDI-563 Administered Bi-Weekly by Intravenous Injection to Pregnant Cynomolgus Monkeys, Including a 6.5 Month Postnatal Evaluation

Study no.: Testing Facility Study No. AAO00036
Study report location: EDR
Conducting laboratory and location:  (b) (4)

Date of study initiation: Experimental Start Date: 27 Oct 2008
Start of Dosing: 19 Nov 2008
Start of Necropsy (BD199 ± 2): 02 Nov 2009
End of In-Life: 30 Jul 2010
Experimental Completion Date: 14 Apr 2011

GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: MEDI-563 in 20 mM histidine, 9% trehalose, 0.004% polysorbate 20 at pH 6.0
Lot Numbers: KP4277.154 (6.4 mg/mL for 10 mg/kg/dose) and KP4277.155 (16.2 mg/mL for 30 mg/kg/dose)

Key Study Findings

- Comments on the proposed design of the enhanced PPND study with monkeys were conveyed to the Sponsor on December 19, 2008.
- Pregnant Cynomolgus monkeys received MEDI-563 by bolus IV injection at doses of 0, 10, or 30 mg/kg on GD20-GD22 (dependent on pregnancy determination), on GD35, and once every 14 days thereafter through gestation. Further, MEDI-563 was administered to female adult monkeys on postpartum days 14 and 28. A maximum of 14 doses were administered.

- There was no evidence of maternal toxicity with IV doses of MEDI-563 at 10 or 30 mg/kg.
- Complete or near-complete depletion of peripheral blood eosinophils was observed at GD91 in most adult females in the 10 mg/kg group and all females in the 30 mg/kg group. The depletion of eosinophils continued through PPD 28. Eosinophil counts began to increase gradually in most females in the 10 and 30 mg/kg dose groups by PPDs 91, 136, and 180 with eosinophil counts in a few females approaching the lowest control eosinophil counts by PPD 91 and most counts were similar to the control by PPD180. A few females in the 10 and 30 mg/kg groups had peripheral blood eosinophil counts that remained $\leq 10/\mu\text{L}$ through PPD180, indicating a lack of recovery. Decreased eosinophil counts were attributed to the pharmacological action of benralizumab.
- Incidences of aborted, stillborn fetuses, and fetal/neonatal survival appeared to be unaffected by treatment with benralizumab. Incidences of stillborn fetuses were higher in drug-treated group; however, no dose-response relationship was evident.
- Infants exposed *in utero* to MEDI-563 in the 10 and 30 mg/kg groups had decreased peripheral blood eosinophil counts. Peripheral blood eosinophil counts had risen in most infants by BD180 and BD199 (± 2 days) to counts comparable to controls for all infants except infant #3141 in the 30 mg/kg group. Eosinophil counts in infant #3141 remained at $0/\mu\text{L}$, which correlated with the bone marrow eosinophil evaluation for this animal.
- Growth and neurological development of infants were unaffected by benralizumab. External, visceral, and heart evaluations of infants in the control and drug-treated groups on BD199 were normal.
- Skeletal evaluations were generally normal. However, infant # 3091 in the 30 mg/kg group had an abnormal lumbar vertebra where the right side of the 4th lumbar vertebra was malformed (wedge-shaped) resulting in scoliosis and deviation of the spinal column to the left.
- There were no benralizumab-related changes observed in anti-KLH IgM and anti-KLH IgG titer values in infants from the 10 and 30 mg/kg groups as compared to the control group.
- Infants exposed *in utero* to MEDI-563 in the 10 and 30 mg/kg groups had measurable levels of serum IgM and IgG comparable to control infants at all time points.
- There were no changes of B lymphocytes, Total T lymphocytes, T-helper lymphocytes, T-cytotoxic/suppressor lymphocytes, NK cells, or monocytes in infants attributed to *in utero* exposure to benralizumab in the 10 or 30 mg/kg groups.
- Exposure of infants to MEDI-563 was demonstrated by detecting MEDI-563 in the serum of infants. MEDI-563 levels observed in infants were consistent with placental

transfer from the maternal circulation into the fetal circulation. MEDI-563 concentrations in infants on BD180 were below the limit of quantitation.

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Methods

Doses: 0, 10, or 30 mg/kg

Frequency of dosing: MEDI-563 was administered by bolus IV injection on GD20-GD22 (dependent on pregnancy determination), on GD35, and once every 14 days thereafter through gestation and 1 month postpartum (maximum 14 doses).

Dose volume: 1.6 or 1.9 mL/kg

Route of administration: Bolus IV Injection (over at least 2 minutes) by the saphenous vein using a butterfly catheter. The cephalic vein was used if necessary.

Formulation/Vehicle: 20 mM histidine, 9% trehalose, 0.004% polysorbate 20 at pH 6.0

Species/Strain: Purpose-bred, sexually mature, and experimentally naive adult female Cynomolgus monkeys (*Macaca fascicularis*) were obtained from (b) (4). The animals' country of origin was China. Ages of female monkeys ranged from 4.4 to 9.7 years at the time of mating. Body weight ranged from 2.3 to 5.0 kg at the outset (GW-1)

Number/Sex/Group: 14 to 21 pregnant monkeys per group

Satellite groups: None

Study design: Pregnant females were randomly assigned to dose groups. For the 2 abortions that occurred prior to GD50, 2 additional pregnant females were assigned to study. Six additional pregnancies were added to Group 3 (30 mg/kg) based on experimental outcomes, to ensure adequate group size for data interpretation. Ultrasonography evaluations for pregnancy determination were conducted on sedated adult females once between GD18 to GD20, and confirmed within 2 to 4 days between GD20 to GD22. MEDI-563 was administered by bolus IV injection on GD20-GD22 (dependent on pregnancy determination), on GD35, and once every 14 days thereafter through gestation and 1 month postpartum (maximum 14 doses). Fetuses were evaluated by ultrasound evaluations during the gestation period. Neonates were observed daily. When a maternal postpartum death occurred (Adult Female No. 3503), a lactating colony female was assigned to study as a foster mother (Adult

Female No. 3603). Infants were maintained with their mothers during the postnatal period up to BD199 \pm 2 days. The mothers were released from the study once their infant was euthanized on approximately Birth Day (BD) 199 (\pm 2 days). Additional details of the study are provided below.

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

Table 47 Design of enhanced PPND study with monkeys

Pregnant cynomolgus monkeys were assigned to the following dose groups:

Group No.	No. of Pregnant Females	Nominal Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Formulation Concentration (mg/mL)
1	15 ^a	0 (Control)	1.9	0
2	14	10	1.6	6.4
3	21 ^b	30	1.9	16.2

^a Includes 1 female that was enrolled onto study to replace early pregnancy loss prior to GD50.

^b Includes 1 female that was enrolled onto study to replace early pregnancy loss prior to GD50 and 6 females that were enrolled onto study to ensure adequate group size for data interpretation.

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Observations and Results

Mothers

Table 48 Blood collection time points from adult females for assessments of immunophenotyping, hematology parameters, immunoglobulins, serum benralizumab, anti-test article antibody titers, and serum chemistry parameters

Gestation Day or Postpartum Day	Time Points – Relative to Dosing	Samples Collected ^a
GD18-GD22	Predose I (at least one day prior to Predose II sample)	Flow
GD20-GD22	Predose (Predose II for Flow)	Hem, Flow, Ig, TK, ATA
GD20-GD22	2 ± 2 minutes post dose	TK
GD21-GD23	24 hours post dose	TK
GD23-GD25	72 hours post dose	TK
GD27-GD29	168 hours post dose	TK
GD34-GD35	336 hours post dose (for those dosed on GD20-GD21) and 312 hours post dose (for those dosed on GD22). Prior to dosing on GD35, as applicable.	TK
GD63	Predose	TK, ATA
GD91	Predose	Hem, Flow, Ig
GD105	Predose	TK, ATA
GD133	Predose	TK, ATA
GD133	2 ± 2 minutes post dose	TK
GD134	24 hours post dose	TK
GD136	72 hours post dose	TK
GD140	168 hours post dose	TK
GD147	336 hours post dose (prior to dosing on GD147)	TK
PPD7	Not applicable	Hem, Flow, Ig, TK, ATA
PPD14	Predose	Hem, Ig, TK, ATA
PPD28	Predose	Hem, Flow, Ig, TK, ATA
PPD91	Not applicable	Hem, Ig, TK, ATA
PPD136	Not applicable	Hem
PPD180 ± 1	Prior to infant KLH challenge	Hem, Flow, Ig, TK, ATA
As applicable	Day of confirmed abortion or day of stillbirth	Chem, Hem, Flow, TK, ATA
As applicable	Day of unscheduled necropsy	Chem, Hem, Flow, TK, ATA

^a ATA – anti-therapeutic antibody; Chem – serum chemistry; Flow – flow cytometry; Hem – hematology; Ig - immunoglobulin, TK – toxicokinetic

(Excerpted from the Sponsor's submission)

Mortality: Mothers were observed for signs of morbidity and/or mortality twice daily.

Adult Female #3503 in the 30 mg/kg group was found dead on PPD10 and Adult Female #1504 in the control group was found moribund and euthanized on PPD67. The death of Female #3503 in the 30 mg/kg group was attributed to a retained placenta with subsequent uterine perforation and systemic sepsis. The death did not appear to have any relation to treatment with benralizumab.

Adult Female #3503 (30 mg/kg, mother of Infant No. 3036) was found dead on PPD10. Clinical records indicated that the female retained the placenta on PPD1 and was in the process of passing the placenta on PPD3. The female was treated with oxytocin on PPD1 and PPD3 to aid passing of the placenta, which finally passed on PPD4. The primary finding from the gross necropsy examination was focal uterine perforation with fibrinous adhesions to the jejunum. The retained placenta in this female likely predisposed it to development of systemic illness and sepsis. Other gross findings considered secondary to the uterine perforation and systemic sepsis included an enlarged mottled liver, enlarged red adrenal glands, red discolored lungs with an abscess, and an accentuated follicular pattern in the spleen. Pale kidneys observed grossly were attributed to postmortem autolysis. There was no histologic correlation to thickening of the urinary bladder. The primary finding from the histopathological examination was coagulative necrosis of the uterus (infarction) at the site of perforation, with septic thrombi and intralesional bacteria. Septic thrombi were also seen in the heart and lung and microabscesses were scattered throughout the liver. The lung also had abscessation with intralesional bacteria and infarction. These findings were consistent with systemic sepsis. Since the uterine perforation was due to coagulative necrosis, this was likely secondary to the sepsis with multifocal thrombosis. The retained placenta was the likely cause of the infection leading to development of disseminated sepsis with the greatest severity in the uterus. Lymphoid tissues (lymph nodes, spleen, and thymus) had lymphoid depletion; lymph nodes and spleen had decreased size/number germinal centers. The lymphoid depletion may have been secondary to systemic sepsis. The adrenal cortices of this animal were markedly hypertrophied, which might have also been secondary to systemic sepsis. The bone marrow of this adult female had generalized hypercellularity, with an increase in myeloid precursors (myeloid hypercellularity), and a mild depletion of mature and band neutrophils. Hematology data collected on PPD7 revealed a high neutrophil count (resulting in a high total white blood cell count) suggestive of inflammation. These changes in the hematopoietic population were considered an appropriate and expected response to systemic sepsis. This death was likely due to complications related to the retained placenta.

The Infant (#3036) of Adult Female #3503 (30 mg/kg) was cross-fostered to a lactating colony female (Adult Female #3603); however, this infant was found dead on BD14. Further details are described below.

Adult Female #1504 in the control group was found moribund and euthanized on PPD67. The adult female was found prostrate on the bottom of its cage the morning of PPD67, dehydrated, and with a distended abdomen/bloated. Body temperature was

88.1°F. Veterinary care included warming the animal and administration of lactated Ringer's solution. Body temperature increased slowly to 96.5°F; however, the animal remained inactive. A radiograph of the abdominal area revealed moderate obstipation with lower bowel gas and severe upper bowel distention with abnormal abdominal architecture. This animal's clinical observations had been normal for the previous 1-2 weeks with the exception of low food consumption noted approximately 10 days prior to its death and body weight decrease from 3.7 kg on PPD28 to 3.2 kg on PPD56. The primary necropsy finding was torsion of the colon at the base of the mesentery, with dark discoloration of the mucosa of the affected region and general distension of the entire colon. The histopathological examination of the mucosa of the region of the colon affected by the torsion found diffuse, marked hemorrhage, and coagulative necrosis. The colon away from the area of torsion exhibited diffuse, mild edema of the submucosa. At necropsy, the left lateral lobe of the liver was discolored red and was firm, correlating to marked sinusoidal congestion histologically, suggesting possible liver lobe torsion near the time of death (torsion was not present at necropsy). Other findings were generally considered related to the illness of the animal (including congestion of the lungs and stomach, adrenal cortical hyperplasia, decreased germinal centers of the lymph nodes and spleen, and lymphoid depletion in the thymus).

Infant No. 1046 (control) was born on GD172, but was euthanized on BD71 following lack of ability to continue independently on study after its mother (Adult Female No. 1504) became moribund and was euthanized on PPD67. Further details are described below.

Clinical Signs: After pregnancy confirmation, mothers were examined at least twice daily throughout the study (Day of initial dosing until PPD199 ± 2 days) for changes in general appearance and behavior.

There were no treatment-related clinical signs for adult female monkeys.

Abortions: There were 2 (13.3%) abortions in the control group and 3 (14.3%) abortions in the high dose group at 30 mg/kg. Abortions were not attributed to treatment with benralizumab.

Two abortions, one in the control group and one in the 30 mg/kg group (triplet pregnancy), occurred on GD46, near the end of the first trimester (organogenesis). Fetuses were nonviable by ultrasound.

The second abortion in the 30 mg/kg group occurred on GD52 shortly after the period of organogenesis. The female showed evidence of red vaginal discharge on GD51 and red vaginal discharge with red material inside and under the cage on GD52. The red material was considered evidence of fetal tissue.

The second abortion in the control group occurred for Female #1506 on GD102. The female did not have any clinical signs that were considered indicative of a difficult pregnancy prior to the day of abortion.

The third abortion occurred in the 30 mg/kg group on GD138, near the end of the third trimester. Infants born on \geq GD140 would generally be expected to be viable. Female #3519 in the 30 mg/kg group did not have any clinical signs that were considered indicative of a difficult pregnancy prior to the day of abortion, but lost 0.2 kg from GD130 (GW19) to GD137 (GW20).

Stillbirths: Stillbirths were observed for 1 fetus in the control group, 3 fetuses in the 10 mg/kg group, and 4 fetuses in the 30 mg/kg group. It was noted that breech fetuses occurred for 1 female in the 10 mg/kg group and 2 female in the 30 mg/kg group. Stillbirths are discussed in more detail below.

The adult females that had clinical pathology samples collected on the day of confirmation of abortion/stillbirths were found to have decreased eosinophil counts (Adult Female Nos. 2510 [GD160], 2512 [GD140], 3506 [GD144], 3507 [GD151], and 3510 [GD155]), which were also evident at GD91.

Maternal rejection of infants: Two female controls (#1507 and 1513) rejected their infants on BD1 and BD3, respectively. Infant #1071 of Mother #1507 was hand-reared from BD1 to BD3. Given the difficulties of hand-rearing an infant, a decision was made to euthanize the animal on BD3. Similarly, infant #1136 of Mother #1513 was euthanized on BD3.

Table 49 Pregnancy Outcome

Parameter	Control	10 mg/kg	30 mg/kg
Total # of pregnant females	15	14	21
# of infants (M/F)	12 (5/7)	11 (8/3)	14 (8/6)
# of pregnancy losses (M/F/U)	3 (1/0/2)	3 (1/2/0)	7 (3/2/2)
Pregnancy losses prior to GD 50 [1 st trimester] (M/F/U)	1	0	1
Pregnancy losses GD50 to GD99 [2 nd trimester] (M/F/U)	0 (0/0/0)	0 (0/0/0)	1 (0/0/1)
Pregnancy losses \geq GD 100 [3 rd trimester] (M/F/U)	2 (1/0/1)	3 (1/2/0) ^a	5 (3/2/0) ^a

- a. Breech fetuses occurred for 1 female in the 10 mg/kg group and 2 females in the 30 mg/kg group.

Body Weight: Body weights of mothers were measured on (Gestation Day (GD)0, Gestation Week (GW)-1), GD10 (GW2), GD18 (GW3), GD25 (GW4) and weekly thereafter until delivery. After parturition, body weights of mothers were measured on Postpartum Day (PPD)1, PPD14, PPD28, PPD56, PPD84, PPD112, PPD140, PPD168, and PPD199 \pm 2 days.

Body weight gains were slightly decreased for mothers in the 10 and 30 mg/kg groups from GW-1 to GW23 relative to the control group. Body weight gains for mothers in the 10 and 30 mg/kg groups were unaffected from PPD1 to PPD199.

Table 50 Body weight gains of mothers from GW -1 to GW 23 and PPD1 to PPD 199

Parameter	Control	10 mg/kg	30 mg/kg
BW (kg) at GW -1	3.23	3.49	3.46
BW (kg) at GW 23	4.73	4.81	4.71
▲ (kg)	1.50	1.32	1.25
BW gain as % of GW -1	46.4%	37.8%	36.1%
PPD1 to PPD199			
BW (kg) at PPD1	3.98	4.09	4.22
BW (kg) at PPD199	3.90	4.14	4.17
▲ (kg)	-0.08	0.05	-0.05
BW gain as % of PPD1	-2.0%	1.2%	-1.2%

Feed Consumption: Food consumption was qualitatively assessed at least once daily (a.m.) throughout the study. For the Final Report, observations were tabulated starting on the last day of mating (GD1) until PPD199 ± 2 days.

There were no treatment-related effects on qualitative food consumption.

Hematology: Blood samples for measurements of hematology parameters were collected from adult females on GD20-22, GD91, PPD7, PPD14, PPD28, PPD91, PPD136, and PPD180±1.

With the exception of Adult Females #2512 and 2513 in the 10 mg/kg group, complete or near-complete depletion of peripheral blood eosinophils was observed at GD91 in all adult females in the 10 mg/kg and 30 mg/kg groups. The depletion of eosinophils continued through PPD28 (statistically significant, MEDI-563 was administered on PPD14 and PPD28). Peripheral blood eosinophil counts were 0/μL in 8 of 11 females in the 10 mg/kg group and 13 of 13 females in the 30 mg/kg group on PPDs 7, 14, and/or 28. Eosinophil counts began to increase gradually in most females in the 10 and 30 mg/kg dose groups by PPDs 91, 136, and 180 with eosinophil counts in a few females approaching the lowest control eosinophil counts by PPD91 and most counts were similar to the control by PPD180. However, Adult Females #2502, 2503, 2506, and 2509 in the 10 mg/kg group and #3508, 3509, and 3514 in the 30 mg/kg group had peripheral blood eosinophil counts that remained ≤10/μL through PPD180, indicating a lack of recovery. Decreased eosinophil counts are attributed to the pharmacological action of benralizumab.

Basophil counts were transiently increased for females in the 10 and 30 mg/kg group on PPD7 relative to the control group; however, no differences were evident at later time

points. Basophils were a known target of benralizumab; however, no changes of cell counts were evident.

At GD91, 4 of 19 females (Adult Females #3501, 3509, 3511, and 3516) in the 30 mg/kg group had mild decreases of neutrophil counts that were lower than counts the control females; however, the levels observed were within the expected range of variability for pregnant females at GD91, and the group mean did not attain statistical significance.

Table 51 Hematology parameters in adult female monkeys on GD20-22, GD91, PPD7, PPD14, PPD28, PPD91, PPD136, and PPD180±1

Parameters	Time	Control	10 mg/kg	30 mg/kg
Eosinophils cells/ μ L	GD 20-22	167.1	203.6	131.8
	GD 91	87.9	47.8*	11.3*
	PPD 7	95.5	13.7*	2.3*
	PPD 14	149.6	13.0*	0.7*
	PPD 28	203.5	21.9*	0.7*
	PPD 91	243.3	55.3*	20.8*
	PPD 136	154.2	73.2*	59.4*
	PPD 180±1	163.2	113.0	197.5
Basophils cells/ μ L	GD 20-22	49.1	60.6	47.1
	GD 91	50.1	44.1	44.8
	PPD 7	26.4	62.3*	108.0*
	PPD 14	28.0	35.5	34.4
	PPD 28	25.6	32.9	35.1
	PPD 91	27.8	31.2	39.9
	PPD 136	32.4	37.2	39.9
	PPD 180±1	39.6	40.1	36.6
WBCs $10^3/\mu$ L	GD 20-22	15.07	15.59	13.13
	GD 91	11.45	11.38	10.12
	PPD 7	9.74	14.18	14.19
	PPD 14	9.98	11.45	10.39
	PPD 28	8.54	10.43	9.60
	PPD 91	9.36	11.18	11.00
	PPD 136	8.93	10.33	11.14
	PPD 180±1	10.12	11.09	11.03
Neutrophils cells/ μ L	GD 20-22	8455.5	8542.6	6442.2
	GD 91	5001.9	5701.6	4299.9
	PPD 7	4565.9	7235.1	8097.0
	PPD 14	4637.9	5767.5	5059.9
	PPD 28	3349.8	4968.5	4167.6
	PPD 91	3585.1	5033.6	4436.3
	PPD 136	3109.1	4271.9	4766.6
	PPD 180±1	4131.2	5380.3	5052.8

Clinical chemistry: Blood samples for evaluation of serum chemistry parameters were only collected from adult females on days of confirmed abortion, stillbirth, or unscheduled necropsy. See other sections for clinical chemistry measurements.

Immunophenotyping: Blood samples for immunophenotyping of lymphocytes, monocytes, and NK cells were obtained from mothers on GD18-GD22, GD20-GD22, GD91, postpartum day (PPD7), PPD28, PPD180±1, and the day of unscheduled necropsy (as applicable). Cellular antigens and cell populations were quantified, using specific antibodies against the marker. Flow-Count™ fluorospheres were used for real time quantification of selected peripheral blood subsets.

There were no changes of B lymphocytes, Total T lymphocytes, T-helper lymphocytes, T-cytotoxic/suppressor lymphocytes, NK cells, or monocytes in mothers attributed to treatment with benralizumab at 10 or 30 mg/kg.

CD3-/CD14+ monocytes were transiently increased to ≥150% of the prestudy baseline high value in 8 of 11 monkeys from the 10 mg/kg group on PPD7. Samples from the remaining 3 of 14 monkeys from the 10 mg/kg group were not available for evaluation, because the monkeys had been removed from the study due to infant stillbirths. The cause of the increases was not evident; however, no change was evident in the 30 mg/kg group and the monocyte counts returned to baseline by PPD28. The changes in monocyte counts in the 10 mg/kg group were consistent with hematology data.

Serum Immunoglobulin: Blood samples for measurement of serum immunoglobulin M, G, A, and E (IgM, IgG, IgA, and IgE) levels were collected from adult females on GD20-22, GD91, PPD7, PPD14, PPD28, PPD91, and PPD180±1. IgM, IgG, IgA, and IgE were measured using ELISA methods.

There were no MEDI-563-related alterations in maternal serum IgM, IgG, IgA, or IgE during the gestation or postpartum periods.

Toxicokinetics: Time points for collection of blood samples for measurement of MEDI-563 and ADA are listed in Table 48. MEDI-563 was measured in Cynomolgus monkey serum samples using enzyme-linked immunosorbent assay (ELISA) method (MedImmune SOP CT-9615). The assay was an indirect capture colorimetric ELISA in which mouse anti-IL-5 receptor alpha antibody was coated onto a microtiter plate to capture soluble recombinant IL-5 receptor alpha. MEDI-563 binds to an epitope of the receptor separate from that which binds to the mouse anti-IL-5 receptor alpha antibody. Bound MEDI-563 was detected using an alkaline phosphatase labeled mouse monoclonal anti-human IgG Fc gamma specific antibody. BluePhos® was used as substrate for the colorimetric reaction. Color intensity at 650 nm was measured to determine the concentration of MEDI-563. The assay's lower limit of quantitation (LLOQ) was determined to be 0.066 µg/mL and the upper limit of quantitation (ULOQ) was determined to be 2.00 µg/mL.

C_{max} and AUC values for serum MEDI-563 increased in an approximate dose proportional manner after the 1st dose (GD 20-22) and 9th dose (GD 133). Accumulation ratios (i.e., 1.53 and 1.18) for doses of 10 and 30 mg/kg were relatively minimal when comparing exposures after the 9th dose relative to the 1st dose. Mean half-lives ranged from 7.92 to 10.8 days. Mean clearance ranged from 5 to 7.92 mL/day/kg.

Average serum concentrations of MEDI-563 measured prior to the 4th (GD63), 7th (GD105), and 9th (GD133) IV administrations of MEDI-563 were 50.6±21.7, 51.3±16.2, and 60.2±16.1 µg/mL for Group 2 adult females and 180±86.3, 201±89.8, and 183±81.0 µg/mL for Group 3 adult females, respectively. These observations indicated that steady-state had been achieved by the ninth dose and that exposure to MEDI-563 was maintained in adult females over the dosing period.

Figure 32 Toxicokinetic parameters for MEDI-563 in female adult monkeys after the 1st and 9th doses

Text Table 5: Summary of Mean Toxicokinetic Results of MEDI-563 in Adult Female Cynomolgus Monkeys

Group (Dose)	Dose No. (GD)	Toxicokinetic Parameters						
		T _{max} (d)	C _{max} (µg/mL)	AUC(0-14d) (µg·d/mL)	AUC _{inf} (µg·d/mL)	CL (mL/d/kg)	t _{1/2} (d)	AR
2 ^a (10 mg/kg)	1 st Dose (GD20-22)	0.00139 (0.00139-0.00139)	222±39.9	1120±316	1790±787	6.42±2.21	9.71±2.93	1.53 ± 0.254
	9 th Dose (GD133)	0.00139 (0.00139-0.00139)	273±45.4	1710±343	N/A	6.07±1.22	7.92±1.53	
3 ^b (30 mg/kg)	1 st Dose (GD20-22)	0.00139 (0.00139-0.00139)	763±124	4040±617	6930±3110	4.98±1.68	10.8±5.51	1.18 ± 0.226
	9 th Dose (GD133)	0.00139 (0.00139-3)	957±972	4760±1290	N/A	6.72±1.78	8.39±3.80	

TK Parameters and accumulation ratio (AR) were rounded to 3 significant figures after calculations were performed. Parameters are shown as mean ± SD, except for T_{max}, which is shown as median (range).

AUC(0-14d): area under the concentration-time curve from 0 to 14 days post dose

AR determined using AUC(0-14d), GD133/AUC(0-14d), and GD20-22.

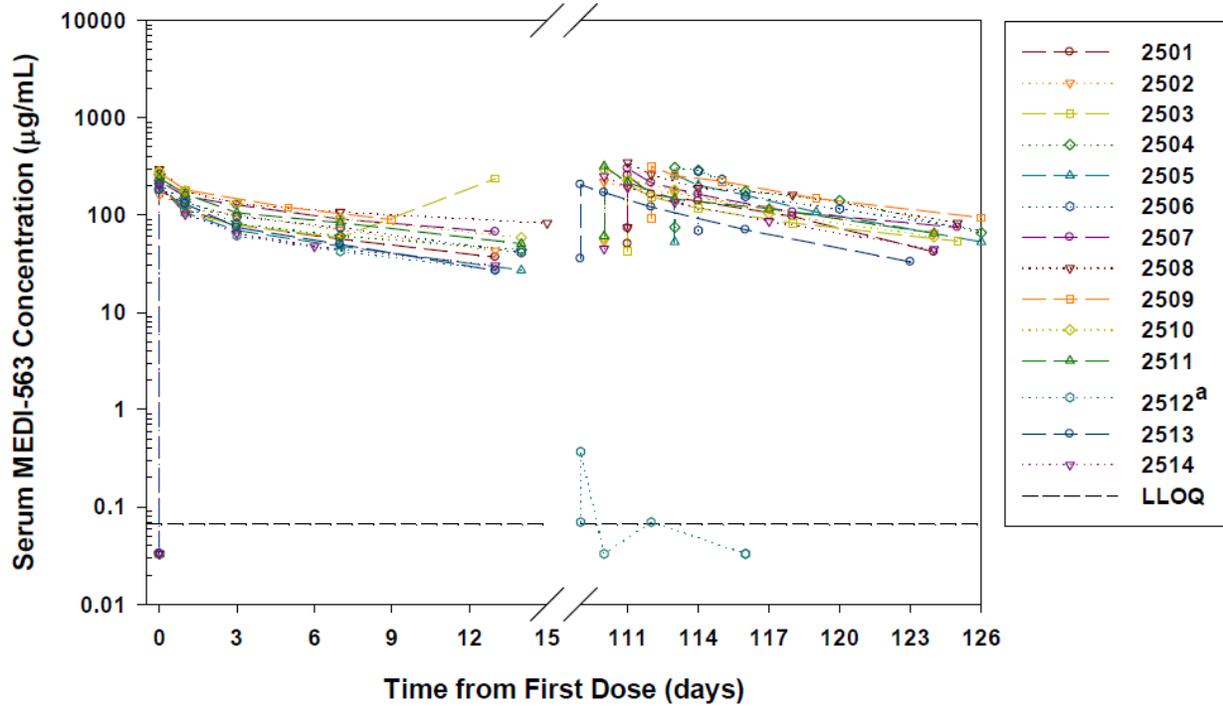
N/A - not applicable.

^a For 9th dose, data for Adult Female 2512 were excluded from summary statistics due to anti-drug antibodies formation, which affected the overall TK profile of the adult female.

^b For 9th dose, data for adult females (3501 and 3506) were excluded from summary statistics due to formation of Anti-drug antibodies, which affected the overall TK profile of the adult females.

(Excerpted from the Sponsor's submission)

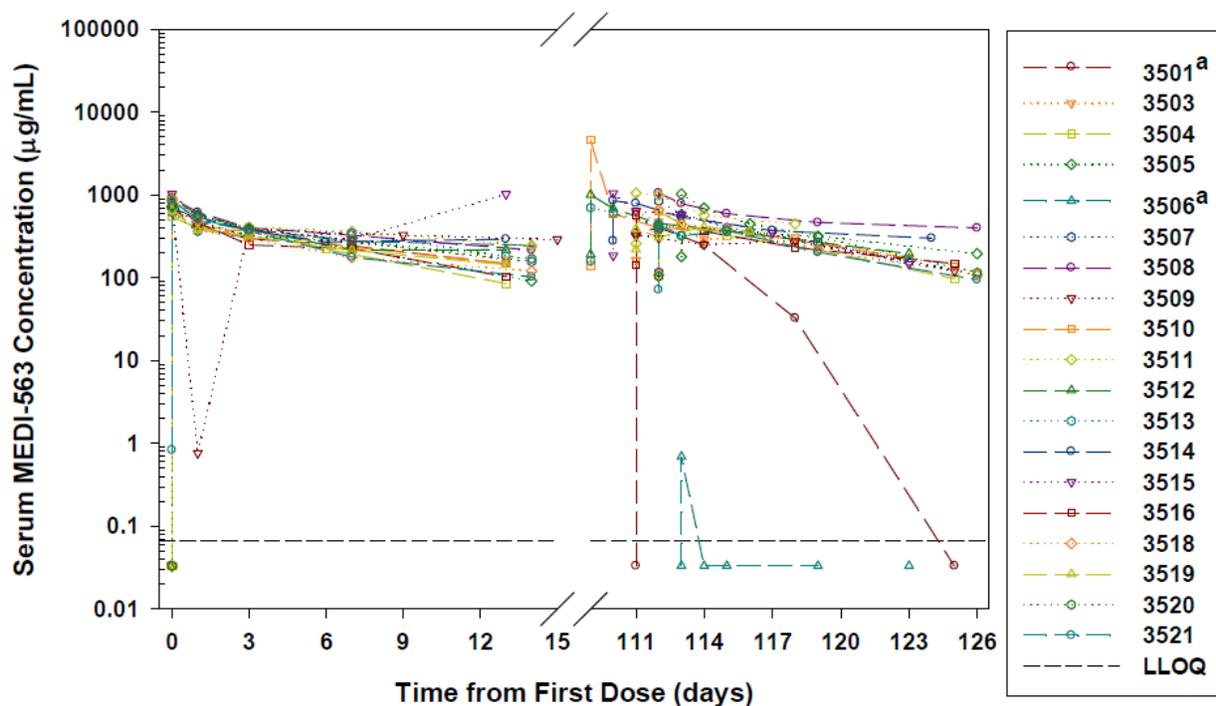
Figure 33 Individual Serum MEDI-563 Concentration-Time Profiles For Group 2 Adult Female Cynomolgus Monkeys following Bi-Weekly IV Administration of MEDI-563 at 30 mg/kg (First and Ninth Dose Profiles Only)



Note: ^aAdult Female was tested positive for Anti-Drug Antibodies prior the 9th Dose

(Excerpted from the Sponsor's submission)

Figure 34 Individual Serum MEDI-563 Concentration-Time Profiles For Group 3 Adult Female Cynomolgus Monkeys following Bi-Weekly IV Administration of MEDI-563 at 30 mg/kg (First and Ninth Dose Profiles Only)



Note: ^aAdult Female was tested positive for Anti-Drug Antibodies prior the 9th Dose

(Excerpted from the Sponsor's submission)

Anti-MEDI-563 antibodies were measured in Cynomolgus monkey serum samples using an enzyme-linked immunosorbent assay (ELISA) method (MedImmune SOP CT-9614). In the assay, anti-MEDI-563 antibodies present in the samples were captured by MEDI-563 coated on a microtiter plate. Bound anti-MEDI-563 antibodies were then detected using biotinylated-MEDI-563 and horseradish peroxidase (HRP)-labeled streptavidin. Tetramethylbenzidine was used as substrate for the colorimetric reaction and the plates were measured at 450nm. Optical density (OD) responses were proportional to the amount of anti-MEDI-563 antibodies.

One female (#2512; [1/14, 7%]) in the 10 mg/kg group and two females (#3501 and 3506; [2/19, 11%]) in the 30 mg/kg group tested positive for ADA prior to the 9th dose (GD133). Findings of ADA correlated with decreases of serum MEDI-563 concentrations in these three females as can be seen in Figure 33 and Figure 34 above (serum concentration vs. time). Exposure to MEDI-563 was maintained in all other adult females where anti-MEDI-563 antibodies were not detected. Infants of #2512, #3501, and #3506 died on gestation days 140, 165, and 144, respectively.

Table 52 Immunogenicity results in adult female monkeys treated with MEDI-563**Table 5.1.4-1 Summary of Immunogenicity Results in Adult Female Cynomolgus Monkeys following Bi-Weekly IV Administration of MEDI-563**

Group (Dose)	Animal ID	Onset Day	Highest Titer Observed
2 (10 mg/kg)	2512	GD63	1310720
3 (30 mg/kg)	3501	GD133	1280
3 (30 mg/kg)	3506	GD105	81920

(Excerpted from the Sponsor's submission)

Immunogenicity results for three other adult females (#3508, 3515, and 3516 in the 30 mg/kg group) showed detectable ADA titers that would be considered immunopositive. However, the titers (1:10 or 1:20) were just above the assay LLOQ (<1:10) and were considered to be false positive results. Drug exposures in these females were unaffected.

Necropsy: The mothers were released from the study once their infant was euthanized on approximately Birth Day (BD) 199 (\pm 2 days).

Dosing Solution Analysis:

(b) (4)

(b) (4)

F1 Generation: Infants followed from birth through BD199±2**Table 53 Blood collection time points from infants for assessments of immunophenotyping, hematology parameters, immunoglobulins, benralizumab concentrations, anti-test article antibody titers, and anti-KLH IgM and IgG**

Birth Day	Time Points	Samples Collected ^a
BD7	At approximately the same time of day that the sample is collected from the adult female	TK, ATA
BD14	Prior to dosing the adult female	Flow, Ig
BD28	Prior to dosing the adult female	Hem, Flow, Ig, TK, ATA
BD91	At approximately the same time of day that the sample is collected from the adult female	Hem, Ig, TK, ATA
BD136	Not applicable	Hem
BD180 ± 1	Pre-KLH Challenge	Hem, Flow, Ig, TK, KLH-Ig, ATA
BD187 ± 1	7 days post-KLH Challenge	KLH-Ig
BD190 ± 1	10 days post-KLH Challenge	KLH-Ig
BD194 ± 1	14 days post-KLH Challenge	KLH-Ig
BD199 ± 2	Prior to necropsy, if applicable	Hem, Flow, Ig, TK, ATA
As applicable	Day of unscheduled necropsy	Hem, Flow, Ig, TK, ATA

^a ATA – anti-therapeutic antibody; Flow – flow cytometry; Hem – hematology; Ig – immunoglobulin; KLH-Ig - KLH antibodies (IgG and IgM); TK – toxicokinetic

If sufficient volume could not be collected, the priority for sample collection was: 1) Flow, 2) Hem, 3) KLH-Ig, 4) Ig, 5) TK, and 6) ATA.

(Excerpted from the Sponsor's submission)

Embryo/Fetal Measurements conducted using Ultrasound Monitoring: Ultrasounds were performed bi-weekly (i.e., approximately once every 2 weeks) during gestation for assessments of general condition, heart rate, and developmental landmarks of the embryo/fetus. The developmental landmarks included gestational sac (GS) dimensions and greatest length (GL) of the embryo/fetus. Prior to GD50, the GS was measured in 3 dimensions (cephalocaudal, ventrodorsal, and transverse) and a mean value obtained. GL of the embryo/fetus was determined by obtaining a sagittal scan through the midline and obtaining a maximum length from the top of the cranium to the base of the tail (crown/rump length). Measurements of humerus and femur length (obtainable after GD50), biparietal diameter, occipitofrontal diameter, head circumference, and abdominal circumference were obtained.

Fetal heart rates were generally unaffected on GDs 31-33, 45-47, 59-61, 73-75, 87-89, 101-103, 115-117, 129-131, 143-145, and 157-159. However, Fetus #3141 from Mother #3514 in the 30 mg/kg group had low heart rates on GDs 129-131, 143-145, and 157-159 of 104, 148, and 90 bpm, respectively, relative to mean control values ranging from 182.93 to 191.62 bpm. No differences in heart rate were evident for other fetuses in the 30 mg/kg group. Fetus #3141 survived to study termination (BD199).

Crown-rump lengths were unaffected in the 10 and 30 mg/kg groups on GDs 31-33, 45-47, 59-61, and 73-75 relative to the control. Gestational sac lengths in the 10 and 30 mg/kg groups were unaffected on GDs 31-33 and 45-47. Humerus and femur lengths, biparietal and occipitofrontal diameters, head and abdominal circumference were unaffected in the 10 and 30 mg/kg groups on GDs 59-61, 73-75, 87-89, 101-103, 115-117, 129-131, 143-145, and 157-159.

Table 54 Heart rates for fetuses on GDs 129-131, 143-145, and 157-159

Parameter:		Heart Rate (bpm)		
		GD129-GD131	GD143-GD145	GD157-GD159
GROUP 1	STATISTIC			
	Mean	185.77	183.17	190.22
	SD	17.13	26.14	16.32
	N	13	12	9
Statistical Sig				
GROUP 2	Mean	191.62	180.46	175.33
	SD	25.14	18.49	17.74
	N	13	13	9
	Statistical Sig			
GROUP 3	Mean	182.93	182.23	166.25
	SD	30.88	20.28	34.33
	N	14	13	8
	Statistical Sig			

Mortality: Fetuses were evaluated for mortality/morbidity by ultrasound evaluations. Neonates were observed daily for mortality/morbidity.

Overall, there were 13 embryonic or fetal losses on study (13 of 50 or 26.0%). Group loss incidences were 3 of 15 (20.0%) in the control group, 3 of 14 (21.4%) in the 10 mg/kg group, and 7 of 21 (33.3%) in the 30 mg/kg group.

Fetal losses occurring prior to approximately GD140 were considered abortions and those occurring on or after approximately GD140 were considered stillbirths, since infants born on GD140 or later would be expected to survive.

Embryonic losses during major organogenesis (prior to GD50) occurred for one mother (#1501) in the control group and one mother (#3502) in the 30 mg/kg group on GD46. Female #3502 was observed with triplets. Fetuses were determined to be non-viable by ultrasound.

There was 1 abortion that occurred shortly after major organogenesis (GD52) in the 30 mg/kg/dose group (Adult Female #3517). The female showed evidence of red vaginal discharge on GD51 and red vaginal discharge with red material inside and under the cage on GD52. The red material was considered evidence of fetal tissue.

There were 10 abortions/stillbirths that occurred during the third trimester (\geq GD100): 2 of 15 (13.3%) in the control group, 3 of 14 (21.4%) in the 10 mg/kg group, and 5 of 21 (23.8%) in the 30 mg/kg group. With one exception (control fetus lost on GD102), remaining losses occurred late in the third trimester of gestation at the time of expected or actual parturition (\geq GD138). One loss in the 10 mg/kg group and 2 losses in the 30

mg/kg group were most likely due to incidental complications associated with parturition (i.e., breech presentation); two mothers (#2512 and #3501) were ADA positive. If these losses were excluded, third trimester fetal losses in MEDI-563-dosed groups were similar to the control group (2 of 15 fetuses [13.3%] in the control group, 2 of 14 fetuses [14.3%] in the 10 mg/kg group, and 3 of 21 fetuses [14.3%] in the 30 mg/kg group. Incidences of third trimester loss were comparable to results from control pregnancies in 8 enhanced PPND studies of similar design at the Testing Facility (23 losses for 149 mothers [15.4%] in control groups; range 0% to 28.6%).

Table 55 Fetal Losses: Abortions and Stillbirths (Fetal #/Mother #)

Parameter	Control	10 mg/kg	30 mg/kg
Total # of Pregnant Females	15	14	21
Pregnancy Losses Total (M/F/U)	3 (1/0/2)	3 (1/2/0)	7 (3/2/2)
Overall Pregnancy Loss, %	20%	21.4%	33.3%
Abortions (Fetal Deaths <GD140)	2 (13.3%) GD46 (NA/1501) GD102 (1060/1506)	0	3 (14.3%) GD46 (NA/3502) GD52 (NA/3517) GD138 (3196/3519)
Fetal Stillbirths (Deaths ≥GD140)	1 (6.7%) GD162 (1031/1503)	3 (21.4% [2 of 14, 14.3% ^b]) GD140 (2126/2512) ^{a, c} GD160 (2106/2510) GD171 (2131/2513)	4 (19.0% [2 of 21, 9.5% ^b]) GD144 (3061/3506) ^c GD151 (3071/3507) ^a GD155 (3106/3510) GD165 (3011/3501) ^{a, c}

a. breech fetuses

b. Fetal losses if breech fetuses were excluded

c. Mothers were ADA positive.

Aborted fetuses (GD100-GD139) and stillborn fetuses (GD140 or older) underwent external, visceral, and heart evaluation. In general, these fetuses had no detectable developmental abnormalities, changes in external or visceral evaluations, body weight, or changes in measurements that were attributed to MEDI-563. The majority of the body and viscera for Fetus No. 1060 (control, aborted on GD102) was cannibalized by its mother; the evaluation of this aborted fetus was limited to the head. Placentas were only available for 2 aborted/stillborn fetuses (Fetus #2131 and #3061 in the 10 and 30 mg/kg groups, respectively) obtained during the C-section; both placentas were considered normal.

Table 56 Findings for Aborted/Stillborn Fetuses in the Control, 10 mg/kg, and 30 mg/kg groups occurring during the third trimester

Group	Infant #	Day	Findings
Control	#1031	GD162	The fetal evaluation performed for stillborn Fetus #1031 (Control, Adult Female #1503) was unremarkable.
Control	#1060	GD102	The majority of the body and viscera for Fetus No. 1060 (control,

Group	Infant #	Day	Findings
			aborted on GD102) was cannibalized by its mother; the evaluation of this aborted fetus was limited to the head.
10 mg/kg	#2106	GD160	The fetal evaluation performed for stillborn Fetus #2106 (10 mg/kg, Adult Female #2510) was unremarkable.
10 mg/kg	#2126	GD140	The tail of Fetus #2126 (10 mg/kg group, Adult Female #2512) was observed protruding from its mother's vaginal canal. This female had decreased red blood cell mass on GD140 (decreased red blood cell count, hemoglobin concentration, and hematocrit). During the unscheduled C-section on GD140, the tail and left foot of the non-viable fetus were in the birth canal. This was a breech fetus. The mother was ADA positive.
10 mg/kg	#2131	GD171 (/GD157)	Fetus #2131 (10 mg/kg/dose, Adult Female #2513) was non-viable at the ultrasound examination conducted on GD171. The stillborn fetus was obtained by C-section. On external and visceral evaluations, Fetus #2131 was found with the umbilical cord wrapped twice around its neck and was in an advanced state of autolysis with all tissues soft and pliable. It was last observed viable at the GD157 ultrasound. The heart in Fetus No. 2131 was noted to be diffusely soft and pale in color with 2 mm wall thickness in the left ventricle and small papillary muscles. This soft thin walled heart was considered likely due to advanced intrauterine autolysis. The amniotic fluid was cloudy for Fetus #2131 and a sample was collected for evaluation. The amniotic fluid sample was considered normal (some light growth of <i>acinetobacter baumannii</i> , <i>flavobacterium species</i> , and coagulase negative <i>Staphylococcus spp</i> was detected for Fetus #2131).
30 mg/kg	#3071	GD151	Fetus #3071 (30 mg/kg group, Adult Female #3507) was observed as breech during ultrasounds conducted on GD144 and/or GD150 ultrasounds. This fetus was likely delivered breech and the death was classified as a stillbirth.
30 mg/kg	#3011	GD165	Fetus #3011 (30 mg/kg group, Adult Female No. 3501) was observed as breech during ultrasounds conducted on GD144 and/or GD150 ultrasounds. The infant was stillborn on GD165 and had evidence of acute cephalic trauma with red discoloration of the brain and accumulation of red coagulated material (hemorrhage) over the brain. The findings were attributed to the birth process. The mother was ADA positive.
30 mg/kg	#3061	GD144 (/GD130)	Fetus #3061 (30 mg/kg group, Adult Female #3506) was non-viable at the ultrasound examination conducted on GD144. The stillborn fetus was obtained by C-section. Fetus #3061 was last observed as viable at the GD130 ultrasound. It had a low body weight compared to fetuses of similar gestational age. However, this fetus had extensive postmortem autolysis and had likely been dead for some time prior to C-section and potentially of a lesser gestational age with autolysis contributing to the observed reduced weight. The skullcap was markedly flexible with failure of fusion of calvarial symphyses and there was liquefaction of the brain, which has been reported in fetuses that died <i>in utero</i> , secondary to autolysis. Fetus #3061 had a small head (reduced biparietal diameter, reduced occipitofrontal diameter, and reduced horizontal head circumference). These findings may have affected the measurements of the head and crown rump or crown hip lengths. The heart of Fetus #3061 was considered abnormal because it was diffusely soft and grey in color, with 2 mm walls in both ventricles,

Group	Infant #	Day	Findings
			most likely related to postmortem intrauterine autolysis. The amniotic fluid was cloudy for Fetus #3061 and a sample was collected for evaluation. The amniotic fluid sample was considered normal (no bacterial growth was detected). The mother was ADA positive.
30 mg/kg	#3196	GD138	The fetal evaluation performed for aborted Fetus #3196 (30 mg/kg group, Adult Female #3519) was unremarkable.
30 mg/kg	#3106	GD155	The fetal evaluation performed for stillborn Fetus #3106 (30 mg/kg group, Adult Female #3510) was unremarkable.

Infant deaths occurring after natural delivery are listed in Table 57. The overall incidence of neonatal deaths was 4 of 37 [10.8%] for all groups with 2 [2/12, 16.7%] occurring in the control group and 2 [2/14, 14.3%] occurring in the 30 mg/kg group. The historical control incidence of infant death for macaques ranges from approximately 10% to 12%. In 3 of the 4 cases of infant death (2 infants in the control group and 1 in the 30 mg/kg group), the cause was attributed to lack of maternal care, which has been characterized as a background finding in primigravid Cynomolgus monkeys.

Table 57 Infant deaths occurring after natural delivery

Group	Infant #	Day of death	Findings
Control	#1071	GD166	Infant #1071, born on GD166, was rejected by its mother beginning on the day of birth. The infant was hand-reared (bottle-fed) and thriving, as evidenced by increase in body weight from BD1 to BD3. However, due to logistical challenges of hand-rearing an infant, the infant was euthanized on BD3. Grossly, this infant had dark red discoloration of the parenchyma of the liver that correlated with vascular congestion histologically (likely postmortem). The heart had a right ventricular wall thickness of 3 mm and a left ventricular wall of 4 mm; the right ventricular wall may have been thickened. Histologically, the lung of this animal had moderate hyaline membranes and deposition of amphophilic material within alveoli, which suggested some degree of respiratory difficulty and distress in this infant. There was minimal lymphoid depletion of the thymic cortex.
Control	#1136	GD139	Infant #1136 was born prematurely at GD139. It had decreased activity on BD1 and was small in size. It weighed only 185.4 g at birth and 189.7 g on BD3; it was small compared to other infants in all measurements. Its mother rejected the infant beginning on BD3. Similar to Infant No. 1071, due to logistical challenges of hand-rearing an infant, the infant was euthanized on BD3. There were no gross findings in this infant. Infant # 1136 was immature in its skeletal maturation because there was no ossification of the tarsal or carpal bones. This infant had a small body size, with decreased measurements in all dimensions. The early gestational day of birth (premature, GD139) likely accounted for the small size of the infant and immature skeletal ossification. Histologically this infant had diffuse lymphoid depletion in the spleen, thymus, and lymph nodes, and deposition of amphophilic material and squamous epithelium in the alveoli of the lungs, and depletion of mature and band neutrophils in the bone marrow with an increase in proportion of myeloid precursors. The lymphoid depletion and lung changes suggested possible stress and some degree of respiratory difficulty in this infant, which would be consistent with premature birth.

Control	#1046	BD71	Infant #1046 was born on GD172, but was euthanized on BD71 following lack of ability to continue independently on study after its mother (Adult Female #1504) became moribund and was euthanized on PPD67. This infant was not included in the tabulation of infant losses for the study, due to the maternal-related circumstances of its death. For several days after death of the mother, attempts were made to continue raising Infant # 1046 by co-housing her with a colony juvenile monkey (female, approximately 1 year old), but on BD71, the infant was observed to be very weak and have blood in the perianal area. The infant was moved to an incubator and put under veterinary observation, but remained non-responsive and had nystagmus and bloody feces, leading to the decision to euthanize the infant. Macroscopic findings at necropsy included abnormal content (red) in the cecum and colon, along with red foci of the mucosa of the cecum, and green foci of the cranial and middle lung lobes (no histologic correlate for the latter). Histologic findings were secondary to the general illness of the infant (congestion of the lungs, lymphoid depletion of the thymus, and decreased size/number of germinal centers in the inguinal lymph node).
30 mg/kg	#3056	BD3	Infant #3056 (born on GD171) was found dead on BD3. It was observed with mild scleral hemorrhage and mild gingival hemorrhage during the BD1 physical examination. At the BD1 muscle tonus examination, the resting knee and resting hip had little or no flexion/abduction at the joint; although the observations for the elicited state were similar to other infants at the same age. It was lethargic on BD2. Grossly, this infant had multifocal brown and white discolored areas in the stomach that correlated to minimal hemorrhage in the lamina propria and edema in the submucosa, with minimal erosions. Histologically this infant had diffuse lymphoid depletion in the spleen and lymph nodes, and deposition of amphophilic material and squamous epithelium in the alveoli of the lungs. Findings suggested stress and some degree of respiratory difficulty in this infant. The heart evaluation was normal. The number and morphology of the skeletal bones were within normal limits for infant monkeys.
30 mg/kg	#3036	BD14	The mother of Infant # 3036 (Adult Female # 3503, 30 mg/kg/dose) died on PPD10 due to complications related to the retained placenta. Subsequently, Infant # 3036 was cross-fostered to a lactating colony female (Adult Female #3603), but the infant was found dead on BD14. The physical examination and muscle tonus evaluation on BD1, the neurobehavioral evaluations on BD3 and BD7, and infant measurements were within the range of normal variability for neonatal monkeys. At the external evaluation on BD14, this infant had decreased body mass, dehydration, and sunken eyes consistent with failure to thrive. Grossly, this infant had a small thymus that correlated with moderate diffuse lymphoid depletion. Histologically there was diffuse moderate lymphoid depletion in the spleen, thymus, and lymph nodes. The lungs of this infant also had minimal intra-alveolar hemorrhage and increased alveolar macrophages, which was considered to be associated with moribundity. The heart evaluation was normal. The number and morphology of the skeletal bones were within normal limits for infant monkeys.

Incidences of aborted, stillborn fetuses, and fetal/neonatal survival appeared to be unaffected by treatment with benralizumab. Incidences of stillborn fetuses were higher in drug-treated group; however, no dose-response relationship was evident.

Clinical signs: Infants were observed at least twice daily (a.m. and p.m.), starting from the day of birth (BD1) until the final measurements were collected on the day of euthanasia (BD199 ± 2 days) for changes in general appearance and behavior.

There were no treatment-related clinical signs for infants. Within 4 to 8 hours after birth, respiration rates and heart rates of infants from the 10 and 30 mg/kg groups were unaffected relative to the control group.

Body weights: Body weights of infants were collected on BD1, BD3, BD7, BD14, BD28, BD56, BD84, BD112, BD140, BD168, BD196, and on the day of necropsy.

Body weight gain and absolute body weights were unaffected for infants from mother treated with doses of 10 or 30 mg/kg.

Table 58 Infant body weights (grams) from BD1 to BD 196

Parameter	Control	10 mg/kg	30 mg/kg
BD1	332.63	331.28	337.81
BD28	421.06	424.33	412.21
BD56	531.40	548.72	543.78
BD84	691.51	676.02	688.61
BD112	825.74	820.80	814.90
BD140	934.80	920.24	955.92
BD168	1063.21	1041.88	1085.64
BD196	1170.81	1153.22	1195.08
BD196, % of Control	100.0%	98.5%	102.1%
▲ BD196 – BD 1	838.2 g	821.9 g	857.3 g
BW Gain, % of BD1	252%	248.1%	253.8%
BW Gain, % of Control	100.0%	98.4%	100.7%

Feed consumption: Food consumption was not recorded for infants. During the latter stages of the study, the total food consumption recorded was likely to include some food biscuits consumed by the infant, but it was attributed to the adult female.

External Assessment and Morphometric Measures of Infants: An external assessment of the infant for shape and symmetry of the head and body and collection of the following measurements were performed on BD1, BD28, BD84, BD168, and BD199 ± 2 days at necropsy: crown-rump length, femur length (both), feet length, horizontal head circumference, biparietal diameter, occipitofrontal diameter, chest circumference, and anogenital distance. The infants were not sedated for these procedures (except on BD28 in conjunction with radiograph collection) unless measurements could not be obtained on alert infants. At necropsy (collection at BD199 ± 2 days), infants were euthanized for these procedures.

Overall external evaluations as well as evaluations of the hand digits, feet digits, and external genitalia were normal on BD1, BD28, BD84, BD168, and BD199. Head and limbs of infants were judged to be symmetrical on BD1, BD28, BD84, BD168, and BD199.

Crown rump length, chest circumference, femur length (right and left) foot length (right and left), biparietal diameter, occipitofrontal diameter, horizontal head circumference, anogenital distance (males and females) were unaffected on BD1, BD28, BD84, BD168, and BD199.

External, visceral, and heart evaluations of infants in the control and drug-treated groups on BD199 were normal.

Neurological assessment of Infants: Neonatal neurobehavioral assessments consisted of neonatal muscle tone evaluated on BD1 (within 4-8 hours after birth) and a neonatal neurobehavioral test battery conducted on BD3, BD7, and BD14.

No differences of neonatal muscle tonus were evident between control and drug-treated groups on BD1.

The neurobehavioral test battery conducted on BD3, BD7, and BD14 did not detect any differences between control and drug-treated groups on BD3, BD7, and BD14.

Skeletal Evaluation (Infants): On BD28, when the infant was separated from the mother for other study-related procedures, the infant was sedated and skeletal radiographs were taken. For any early infant deaths/euthanasia, radiographs were taken on the day of necropsy. The skeletal evaluations included the skull (cranial base, cranial vault and midface), forelimb (humerus, radius/ulna, carpals, metacarpals, and digits) hindlimb (femur, tibia/fibula, tarsals, metatarsals, and digits), sternum (sternbrae), vertebrae (cervical, thoracic, lumbar and sacral and coccygeal), pectoral girdle (clavicles, scapulae, and ribs), and pelvic girdle (pubis, ilium, and ischium).

Overall skeletal evaluations were normal for infants in the control, 10 mg/kg, and 30 mg/kg groups with the exception of infant #3091 in the 30 mg/kg group.

Infant #3091 in the 30 mg/kg group had an abnormal lumbar vertebra where the right side of the 4th lumbar vertebra was malformed (wedge-shaped) resulting in scoliosis and deviation of the spinal column to the left. Scoliosis has been reported as an occasional incidental finding in this colony of cynomolgus macaques. Olson *et al.* (Laboratory Animal Science 41: 380-381, 1991) reported that a lateral curvature of the thoracic spine was noted in one 9-year old adult male cynomolgus macaque during routine examination of a group of eight male cynomolgus macaques. Plain radiographs disclosed marked rotation and deformity of the thoracic spine as well as lateral curvature. The greatest deformity was present at T7-T8; however, there were no missing or fractured vertebrae, and there were no exostoses or mineralization of supporting ligaments, as might occur with trauma or ankylosing spondylitis. The preliminary diagnosis was scoliosis of undetermined etiology. Peterson *et al.* (Journal of Medical Primatology 26: 267-275, 1997) reported on the spontaneous incidence of congenital defects in the Rhesus and Cynomolgus macaque colonies at the California Regional Primate Research Center over a 14-year period from 1983 to 1996. The

calculated malformation rates were 0.9% (40/4390) and 0.3% (3/965) for Rhesus and Cynomolgus monkeys, respectively. Most of the observed malformations in both species affected the musculoskeletal and the cardiovascular systems, while a smaller number of defects were observed in the gastrointestinal, urogenital, endocrine, and central nervous systems. One male Rhesus monkey infant at term was reported to have a congenital scoliosis of the cervical/lumbar region of the spine (Lordosis, scoliosis, arthrogryposis). The finding of scoliosis in one monkey from the 30 mg/kg group appears to be most likely a spontaneous finding, unrelated to treatment with benralizumab. Embryo-fetal development of IL-5-deficient mice has been reported to be generally unaffected relative to wild-type mice; no skeletal malformations have been reported (Immunity 4: 15-24, 1996 and a personal communication (b) (4)

Ophthalmologic Examinations (Infants): An ophthalmologic examination was conducted on each infant near the end of postnatal weeks 4, 8, 13, and 26, when possible, when the mother and infant were separated for other procedures.

No treatment-related ophthalmic findings were evident during postnatal weeks 4, 8, 13, and 26.

Hematology (Infants): Blood samples for measurement of hematology parameters were collected on BD28, BD91, BD136, BD180, and BD199 ± 2.

Infants exposed *in utero* to MEDI-563 in the 10 and 30 mg/kg groups had decreased peripheral blood eosinophil counts: 2 of 7 infants and 6 of 10 infants on BD28, 8 of 9 infants and 9 of 10 infants on BD91, and 3 of 8 infants and 6 of 12 infants on BD136, respectively. Peripheral blood eosinophil counts had risen in most infants by BD180 and by BD199 (± 2 days) to counts comparable to controls for all infants except Infant # 3141 in the 30 mg/kg group. Eosinophil counts in infant #3141 remained at 0/μL, which correlated with the bone marrow eosinophil evaluation for this animal.

Table 59 Hematology parameters in infants on BD28, BD91, BD136, BD180, and BD199 ± 2

Parameters	Time	Control	10 mg/kg	30 mg/kg
Eosinophils cells/μL	BD28	111.8	105.4	36.0
	BD91	125.2	25.2	17.9
	BD136	97.9	100.6	69.8
	BD180	130.1	94.5	118.3
	BD199±2	295.9	294.9	269.4
WBCs 10 ³ /μL	BD28	8.30	9.36	8.67
	BD91	10.62	12.31	12.84
	BD136	10.93	13.89	14.49
	BD180	14.40	14.38	14.74
	BD199±2	13.61	15.44	15.67
Neutrophils cells/μL	BD28	2319.1	2493.6	3296.1
	BD91	2331.3	3145.2	3096.8
	BD136	2374.4	3032.0	3255.2

	BD180	4287.4	4090.1	4288.2
	BD199±2	3193.6	5271.3	4167.8

Bone marrow: For infants (scheduled and unscheduled sacrifices) and adult females (unscheduled), 2 bone marrow smears were collected from the seventh rib (or femur if the infant necropsied was less than 1 month in age or if it could not be obtained from the seventh rib). Bone marrow smears were not collected from infants or adult females found dead or that were not able to be necropsied immediately following euthanasia. For the infants (scheduled euthanasia), bone marrow cytologic preparations were evaluated and myeloid:erythroid ratio (M:E) were determined and quantified for each of these infants. The smears were evaluated for morphologic or maturation abnormalities.

Brush preparations for bone marrow cytology were stained and evaluated from infants necropsied on BD199 (± 2 days). A 200 cell count was completed on one smear from each animal. Smears were scanned at 200x and 600x (oil immersion) magnification to assess overall cellularity, stain quality, number of megakaryocytes, and to locate a suitable area for cell counting. Cell counting and cellular morphologic evaluation was performed at 1000x (oil immersion) magnification. Myeloid, erythroid, lymphoid and eosinophilic cell types were counted. Other cells types were observed but not identified unless in excessive numbers or displayed abnormal morphology. Megakaryocytes often were not encountered during the 1000x cell count; the number of megakaryocytes on the smears were evaluated at lower magnification and not reported unless abnormal in morphology or number.

As noted above, infants exposed *in utero* to MEDI-563 at 10 or 30 mg/kg had decreased peripheral blood eosinophil counts on Birth Day (BD) 28, BD91, and BD136. The incidence and severity of the eosinophil depletion was dose-dependent. Eosinophil counts recovered to counts comparable to controls by BD180 in all infants except in one infant #3141 in the 30 mg/kg group, which remained depleted through BD199±2 (peripheral eosinophil count of 0/μL on BD91, 136, 180, and 199) correlating with cytological evidence of depleted bone marrow eosinophils at BD199 (± 2 days) for this animal.

Table 60 Individual bone marrow evaluations for the control, 10 mg/kg, and 30 mg/kg groups on BD199 ±2

Animal Number	Birth Day	M:E ¹	% Eosinophils ²	% Lymphocytes ³	Comments
Group 1: 0 mg/kg IV					
1021	199	1.7	5.6	39.0	NA
1056	199	1.7	9.4	57.5	NA
1086	199	1.5	5.0	76.5	Minimal lymphoid hypercellularity
1096	199	0.9	4.2	49.5	NA
1106	199	1.1	18.9	31.0	NA
1111	199	1.7	9.6	81.5	Minimal lymphoid hypercellularity
1126	199	1.5	8.4	58.0	NA
1141	199	NA	NA	NA	UTE ⁴
1151	199	1.1	11.3	27.5	NA
Average		1.4	9.0	52.6	
Group 2: 10 mg/kg IV					
2011	199	1.5	11.7	62.0	NA
2021	199	1.5	9.2	54.5	NA
2031	199	1.2	13.0	69.0	NA
2046	199	1.3	10.6	64.5	NA
2051	199	1.7	9.6	54.0	NA
2061	199	2.1	8.1	79.0	Minimal lymphoid hypercellularity
2076	199	1.4	11.3	49.0	NA
2081	199	1.6	9.8	33.5	NA
2091	199	2.0	8.2	131.5	Mild lymphoid hypercellularity
2116	199	1.0	13.1	107.5	Mild lymphoid hypercellularity
2141	199	1.7	15.7	88.0	Minimal lymphoid hypercellularity
Average		1.5	10.9	72.0	
¹ M:E = myeloid:erythroid ratio. Values represent the myeloid component of the ratio with the erythroid component = 1.0 (for example, M:E of 1.7:1.0 is presented as 1.7) ² Indicates the percentage of eosinophils per myeloid cells counted ³ Indicates the percentage of lymphocytes per 200 myeloid cells counted ⁴ Cells could not be distinguished, cells too compressed to identify NA = Not applicable; UTE = Unable to evaluate					

Animal Number	Birth Day	M:E ¹	% Eosinophils ²	% Lymphocytes ³	Comments
Group 3: 30 mg/kg IV					
3046	199	1.2	11.7	83.0	Minimal lymphoid hypercellularity
3081	199	1.0	18.6	63.0	NA
3091	199	1.4	11.9	70.5	Minimal lymphoid hypercellularity
3116	199	1.2	13.0	42.0	NA
3126	199	1.2	8.1	55.0	NA
3131	199	1.2	12.0	61.5	NA
3141	199	1.1	0.0	58.5	No eosinophils observed
3151	199	1.2	8.3	39.0	NA
3161	199	1.0	7.8	75.0	Minimal lymphoid hypercellularity
3181	199	0.8	8.8	40.5	NA
3206	199	1.0	8.9	55.0	NA
3211	199	1.6	11.4	63.5	NA
Average		1.2	10.0	58.9	

¹M:E = myeloid:erythroid ratio. Values represent the myeloid component of the ratio with the erythroid component = 1.0 (for example, M:E of 1.7:1.0 is presented as 1.7)

²Indicates the percentage of eosinophils per myeloid cells counted

³Indicates the percentage of lymphocytes per 200 myeloid cells counted

NA = Not applicable

Keyhole Limpet Hemocyanin (KLH) Challenge (Infants): To evaluate T-cell dependent antibody response (TDAR), all infants were immunized on BD 180 ± 1 with keyhole limpet hemocyanin (KLH) injected intramuscularly (IM) in the thigh. Blood samples were collected on BDs 180 (pre-challenge), 187 (Day 7 post-challenge), 190 (Day 10 post-challenge), and 194 (Day 14 post-challenge). Anti-KLH IgM and IgG antibodies were measured using a validated center point titer (CPT) ELISA method.

There were no benralizumab-related changes observed in anti-KLH IgM and anti-KLH IgG titer values in infant monkeys from the 10 and 30 mg/kg groups as compared to the control group.

Serum Immunoglobulin: Blood samples for measurement of serum immunoglobulin M, G, A, and E (IgM, IgG, IgA, and IgE) levels were collected from infants on BD14, BD28, BD91, BD180 ± 1, and BD199 ± 2. IgM, IgG, IgA, and IgE were measured using ELISA methods.

Infants exposed *in utero* to MEDI-563 in the 10 and 30 mg/kg groups had measurable levels of serum IgM and IgG comparable to control infants at all time points. Serum IgA values were below the quantitation limit in control and drug-treated infants on BDs 14 and 28; however, values were comparable between control and drug-treated infants on BDs 91, 180 ± 1, and 199 ± 2. Serum IgE values in infants were below the quantitation

limit at all time points evaluated with the exception of sporadic measurable values within all groups including control at either BD 180 \pm 1 or BD 199 \pm 2.

Immunophenotyping: Blood samples for immunophenotyping of lymphocytes, monocytes, and NK cells were obtained from infants on BD14, BD28, BD180 \pm 1, BD199 \pm 2, and the day of unscheduled necropsy (as applicable). Cellular antigens and cell populations were quantified, using specific antibodies against the marker. Flow-Count™ fluorospheres were used for real time quantification of selected peripheral blood subsets.

There were no changes of B lymphocytes, Total T lymphocytes, T-helper lymphocytes, T-cytotoxic/suppressor lymphocytes, NK cells, or monocytes in infants attributed to *in utero* exposure to benralizumab in the 10 or 30 mg/kg groups. It was noted that NK cell counts were decreased in infants from the 30 mg/kg group relative to the control group; however, statistical significance was not achieved

Table 61 NK (CD3-/CD16+) cell counts in Infants on BDs 14, 28, 180, and 199

Parameter: Absolute CD3-/CD16+ (NK Cells) per μ L

		BD14	BD28	BD180	BD199 \pm 2
GROUP 1	STATISTIC				
	Mean	248.20	163.30	192.57	263.78
	SD	291.48	70.10	109.22	215.63
	N	10	10	7	9
	Statistical Sig				
2	Mean	223.20	154.71	192.73	172.30
	SD	135.64	89.86	197.36	135.39
	N	10	7	11	10
	Statistical Sig				
3	Mean	168.00	95.36	135.09	126.75
	SD	128.14	54.99	154.98	84.50
	N	8	11	11	12
	Statistical Sig				

* ANOVA with Dunnett's/Dunn's ($p \leq 0.05$)

Groups with $n < 3$ were excluded from statistical analysis

Table 62 NK (CD3-/CD159+) cell counts in infants on BDs 14, 28, 180, and 199Parameter: Absolute CD3-/CD159+ (NK Cells) per μL

		BD14	BD28	BD180	BD199±2
GROUP 1	STATISTIC				
	Mean	297.30	196.70	239.86	305.33
	SD	326.35	75.48	123.51	274.05
	N	10	10	7	9
	Statistical Sig				
GROUP 2	Mean	350.90	233.86	281.73	211.10
	SD	208.54	127.06	284.97	173.37
	N	10	7	11	10
	Statistical Sig				
GROUP 3	Mean	290.50	155.73	150.45	186.67
	SD	223.54	91.15	136.99	104.12
	N	8	11	11	12
	Statistical Sig				

* ANOVA with Dunnett's/Dunn's ($p \leq 0.05$)Groups with $n < 3$ were excluded from statistical analysis**Table 63 Monocyte cell counts in infants on BDs 14, 28, 180, and 199**Parameter: Absolute CD3-/CD14+ (Monocytes) per μL

		BD14	BD28	BD180	BD199±2
GROUP 1	STATISTIC				
	Mean	590.80	495.10	333.57	323.89
	SD	415.93	260.94	119.36	197.37
	N	10	10	7	9
	Statistical Sig				
GROUP 2	Mean	688.50	423.29	311.09	374.60
	SD	232.95	254.48	99.33	138.79
	N	10	7	11	10
	Statistical Sig				
GROUP 3	Mean	495.63	324.45	272.00	246.50
	SD	161.28	128.53	109.22	103.94
	N	8	11	11	12
	Statistical Sig				

* ANOVA with Dunnett's/Dunn's ($p \leq 0.05$)Groups with $n < 3$ were excluded from statistical analysis

Organ weights: For infants (scheduled and unscheduled euthanasia) euthanized, the following organs (when present) were weighed before fixation. Paired organs were weighed together unless gross abnormalities were present, in which case they were weighed separately.

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

Absolute and relative spleen weights were increased for infants in the 30 mg/kg group. Absolute and relative thymus weights were increased for infants in the 10 and 30 mg/kg groups. There were no correlating histopathological findings in the spleen and thymus. Thyroid and ovarian weights were decreased for infants in the 30 mg/kg group. The thyroid gland and ovaries were not evaluated by histopathological examination.

Table 64 Infant organ weights on BD199 ± 2

Organ	Control	10 mg/kg	30 mg/kg
Spleen g	1.7016	1.7267	2.2139 (130%)
Spleen g/g BW	0.0015	0.0015	0.0015 (120%)
Spleen g/g BrW	0.0250	0.0257	0.0322 (128.8%)
Thymus g	3.7819	4.3182 (114%)	5.1337 (135.7%)
Thymus g/g BW	0.0032	0.0037 (115.6%)	0.0042 (131.3%)
Thymus g/g BrW	0.0555	0.0642 (115.7%)	0.0743 (133.9%)
Thyroid g	0.1807	0.2061	0.1458 (80.6%)
Thyroid g/g BW	0.0002	0.0002	0.0001 (50%)
Thyroid g/g BrW	0.0026	0.0031	0.0021
Ovaries g	0.0832	0.1130	0.0573 (68.9%)
Ovaries g/g BW	0.0001	0.0001	0.0001
Ovaries g/g BrW	0.0013	0.0017	0.0009 (69.2%)

BW = body weight; BrW = brain weight

Histopathology: For infants (scheduled and unscheduled euthanasia and found dead) and adult females (unscheduled euthanasia and found dead), the following tissues and organs (or portions of), when present, were collected and the tissues were preserved in

10% neutral-buffered formalin (NBF) (except for the testes, which were initially preserved in Bouin's fixative and transferred to 10% NBF).

Table 65 Tissue collection list from the ePPND study with monkeys

Tissues Collected	
Cardiovascular	Urogenital
Aorta	Kidneys
Heart	Urinary Bladder
Digestive	Testes
Salivary Gland (mandibular)	Epididymides
Tongue	Prostate
Esophagus	Seminal Vesicles
Stomach	Ovaries
Small Intestine	Uterus
Duodenum	Cervix
Jejunum	Vagina
Ileum	Endocrine
Large Intestine	Adrenals
Cecum	Pituitary
Colon	Thyroid/Parathyroids ^a
Rectum	Skin/Musculoskeletal
Pancreas	Skin/Mammary Gland
Liver	Bone (femoral head)
Gallbladder	Bone (7th rib)
Respiratory	Skeletal Muscle (psoas and diaphragm)
Trachea	Nervous/Special Sense
Lung	Eyes with Optic Nerve
Lymphoid/Hematopoietic	Sciatic Nerve
Bone Marrow (sternum and femur)	Brain
Thymus	Spinal Cord (thoracic)
Spleen	Other
Lymph Nodes	Animal Number Tattoo
Inguinal	Gross Lesions
Mandibular	Injection Site(s) ^b
Mesenteric	

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

^b Adult female injection site(s)

For infants (scheduled and unscheduled euthanasia and found dead) and adult females (unscheduled euthanasia and found dead), the lymphoid tissues (thymus, spleen, and lymph nodes [inguinal, mandibular, and mesenteric]), heart, gross lesions, and lung tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined by a Study Pathologist certified by the American College for Veterinary Pathologists (ACVP). Additionally, for infants euthanized (scheduled), bone marrow histopathology sections were evaluated. When clinical history or clinical pathology indicate that additional tissue(s) should also be evaluated to determine cause of death, histopathology for that tissue(s) may have also been conducted, after approval of the Study Director, Study Pathologist, and Sponsor.

The histopathological examination was limited to the lymphoid tissues (thymus, spleen, and lymph nodes [inguinal, mandibular, and mesenteric]), heart, gross lesions, and lung tissues. In addition, bone marrow smears were evaluated as discussed above.

Histopathological findings were similar in infants from the control, 10 mg/kg, and 30 mg/kg groups. No findings appear to be attributable to benralizumab.

Table 66 Gross pathological and histopathological findings for infants in the control, 10 mg/kg, and 30 mg/kg groups that were sacrificed on BD199±2

Group	Infant #	Gross Pathological Findings	Histopathological Findings
Control	1021	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
Control	1111	None	LUNG: hemorrhage, multifocal, minimal, alveolar space LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal LYMPH NODE, MESENTERIC: increased size / number germinal centers, mild
Control	1141	None	-
Control	1151	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
Control	1056	LUNG: all lobes, discoloration, dark, mottled, red	HEART: infiltrate, mononuclear cell, multifocal, minimal, interstitium, myocardium LUNG: congestion, multifocal, mild, alveolus LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal SPLEEN: aggregate follicles, multifocal
Control	1086	ABDOMEN: Hernia: umbilical, with abdominal fat protrusion but no gastrointestinal involvement	THYMUS: cyst, multifocal No Correlating Lesion: Tissue collected consists of skin and underlying ABDOMEN, hernia (Gross) normal adipose tissue
Control	1096	None	LYMPH NODE, MANDIBULAR: increased size/number; mild; germinal center
Control	1106	None	LUNG: infiltrate, mononuclear cell, multifocal, minimal, parenchyma
Control	1126	None	LUNG: infiltrate, mononuclear cell, multifocal, minimal, parenchyma LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
10 mg/kg	2011	KIDNEY: cortex, area/foci, pale, tan, right, single (TGL) LYMPH NODE, MESENTERIC: Enlargement	KIDNEY: ectopic tissue, adrenal cortex, focal, subcapsular LUNG: infiltrate, mononuclear cell, multifocal, minimal, parenchyma LYMPH NODE, MESENTERIC: increased size / number germinal centers, minimal
10 mg/kg	2021	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, mild
10 mg/kg	2031	None	LUNG: infiltrate, mononuclear cell, multifocal,

Group	Infant #	Gross Pathological Findings	Histopathological Findings
			minimal, parenchyma LYMPH NODE, INGUINAL: extramedullary hematopoiesis, mild
10 mg/kg	2051	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
10 mg/kg	2061	None	LUNG: infiltrate, mononuclear cell, multifocal, minimal, parenchyma LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
10 mg/kg	2081	None	LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
10 mg/kg	2091	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
10 mg/kg	2141	None	LUNG: infiltrate, mononuclear cell, multifocal, minimal, parenchyma LYMPH NODE, INGUINAL: increased size/number, mild, germinal center
10 mg/kg	2046	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
10 mg/kg	2076	None	None
10 mg/kg	2116	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
30 mg/kg	3081	None	LYMPH NODE, MANDIBULAR: increased size/number, mild, germinal center
30 mg/kg	3091	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal, increased size/number, mild, germinal center LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal LYMPH NODE, MESENTERIC: increased size / number germinal centers, minimal
30 mg/kg	3131	None	None
30 mg/kg	3141	THYROID/PARATHYROID: not found, right, only a single large thyroid gland was present THYROID/PARATHYROID; Enlargement; left	LUNG: infiltrate, mononuclear cell, multifocal, mild, parenchyma LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
30 mg/kg	3151	None	LUNG: hemorrhage, multifocal, minimal, alveolar space infiltrate, mononuclear cell, multifocal, minimal, parenchyma; increased size / number germinal centers, mild, bronchus-associated lymphoid tissue LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
30 mg/kg	3161	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, mild

Group	Infant #	Gross Pathological Findings	Histopathological Findings
30 mg/kg	3181	None	HEART: infiltrate, mixed cell, focal, minimal, interstitium, myocardium LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal
30 mg/kg	3211	None	None
30 mg/kg	3046	None	LUNG: proteinaceous fluid, multifocal, moderate, alveolar space, likely related to the euthanasia process THYMUS: cyst, multifocal
30 mg/kg	3116	None	LYMPH NODE, INGUINAL: extramedullary hematopoiesis, minimal LYMPH NODE, MANDIBULAR: extramedullary hematopoiesis, minimal
30 mg/kg	3126	None	None
30 mg/kg	3206	None	LUNG: proteinaceous fluid, multifocal, mild, alveolar space, likely related to the euthanasia process; infiltrate, mononuclear cell, multifocal, minimal, parenchyma

Toxicokinetics: Blood samples for measurement of MEDI-563 and ADA were collected on BDs 7, 28, 81, and 180.

Exposure of infants to MEDI-563 was demonstrated by detecting MEDI-563 in the serum of infants. Average MEDI-563 concentration levels in 10 mg/kg group infants were 37.3 ± 14.2 , 14.5 ± 5.72 , and 0.749 ± 0.938 $\mu\text{g/mL}$ on BD7, BD28, and BD91, respectively. Average MEDI-563 concentration levels in 30 mg/kg group infants were 109 ± 35.4 , 53.4 ± 29.1 , and 2.10 ± 1.59 $\mu\text{g/mL}$ on BD7, BD28, and BD91, respectively. The table below (Table 67) shows serum concentrations of MEDI-563 in adult females and infants at the same time points; MEDI-563 levels observed in infants were consistent with placental transfer from the maternal circulation into the fetal circulation. MEDI-563 concentrations in infants on BD180 were below the limit of quantitation except for 1 infant in the 30 mg/kg group.

All infants tested negative for anti-MEDI-563 antibodies at all time points.

Table 67 Mean post-partum and birth day MEDI-563 concentrations for adult female and infants

Group (Dose)	PPD7 / BD7		PPD28 / BD28		PPD91 / BD91		PPD180 / BD180	
	Adult	Infant	Adult	Infant	Adult	Infant	Adult	Infant
2 (10 mg/kg)	56.3 ± 29.7 (n=11)	37.3 ± 14.2 (n=10)	64.1 ± 23.2 (n=11)	14.5 ± 5.72 (n=9)	7.20 ± 4.30 (n=11)	0.749 ± 0.938 (n=7)	0.448 ± 0.383 (n=4)	BLQ (n=0)
3 (30 mg/kg)	164 ± 102 (n=13)	109 ± 35.4 (n=12)	195 ± 84.9 (n=12)	53.4 ± 29.1 (n=11)	20.3 ± 14.2 (n=12)	2.10 ± 1.59 (n=10)	1.28 ± 1.05 (n=7)	0.130 (n=1)

PPD - Postpartum Day; BD - Birth Day; BLQ – Below the assay limit of quantitation

Note: PPD28 trough samples for adult females were taken after an additional postpartum dose on PPD14.

Concentrations for all infants on BD199 were BLQ.

Values are presented as mean ± SD (n)

(Excerpted from the Sponsor's submission)

10 Special Toxicology Studies

Study title: Cross-Reactivity Study of F-BIW-8405 with Normal Human and Cynomolgus Monkey Tissues.

Key study findings:

- Cross reactivity of F-BIW-8405 with normal human and Cynomolgus monkey tissues was assessed at concentrations of 1 and 10 µg/mL. Cross reactivity was observed in several tissues from humans and monkeys.
- The test article stained intravascular protein in a rheostatic pattern (i.e., distribution of blood components within tissues reflective of the pattern of blood flow or pattern of post-mortem blood settling or pooling in tissues [hypostatic congestion]). These findings were attributed to the presence of solubilized IL-5 receptor. Circulating eosinophils may have released the IL-5 receptor into the tissue vasculature. Although, this pattern of tissue cross reactivity was greater in humans than monkeys, it was not considered a safety issue.
- Cross reactivity was observed with mononuclear cells in the red pulp cords of Billroth from the spleens of humans and a monkey.
- Cross reactivity was observed with striated skeletal myocytes (peripheral cytoplasm >> cross-striations [filaments]) for both humans and monkey. A large mAb would not be expected to cross the cell membrane in vivo.

- Cross reactivity with striated cardiac myocytes (peripheral cytoplasm >> cross-striations [filaments]) was also observed for monkeys. A large mAb would not be expected to cross the cell membrane in vivo.
- The results of the tissue cross reactivity study raised no safety concerns. Staining of intravascular protein appeared to be consistent with binding to the IL-5 receptor. Staining of the cytoplasm of skeletal and cardiac muscle was not considered biologically relevant as a large mAb would not cross the cell membrane in an in vivo setting.

Study no. (b) (4) Study Numbers IM1231 and IM1232

Volume #, and page #: Volume 18, Pages 5074-5150 and 5151-5204

Conducting laboratory and location: (b) (4)

Date of study initiation: December 13, 2005 for both studies

GLP compliance: Yes

QA reports: yes (X) no ()

Drug, lot #, and % purity: Test article F-BIW-8405, supplied by the Sponsor as a 1.4 mg/mL solution, Lot No. PR051005D, (b) (4) No. A7329.

Methods

Study design: The objective of this study was to evaluate potential cross-reactivity of the test article with cryosections of normal human and Cynomolgus monkey tissues. The test article, BIW-8405, is a humanized monoclonal antibody directed against human IL-5 receptor alpha chain that was FITC-labeled (b) (4) and designated F-BIW-8405. The antibody was supplied by the Sponsor as a 1.4 mg/mL solution in PBS + 0.03% thimerisol.

In order to detect binding, F-BIW-8405 was applied to cryosections of normal human and Cynomolgus monkey tissues at two concentrations (1 µg/mL and 10 µg/mL). In preliminary staining runs, the lower concentration (1 µg/mL) of FITC-conjugated BIW-8405 (F-BIW-8405) was judged to be ideal on the basis of staining of positive control cryosections of IL-5R-transfected TF1 cells. The higher concentration was selected as 10 µg/mL as excessive nonspecific staining was not evident at that concentration (10 µg/mL). These preliminary studies also indicated that 20 µg/mL of test article was too high to use for human tissue cross-reactivity testing, given the amplification of the staining system used [tertiary indirect immunoperoxidase (FITC-conjugated test article and/or FITC-conjugated negative control antibody or assay control followed by rabbit anti-FITC, followed by goat anti-rabbit (EnVision Rb + peroxidase polymer)] and the excessive nonspecific staining observed at 20 µg/mL of FITC-negative control antibody and/or test article in human tissues.

Cryosections of IL-5R-transfected TF1 cells (TF1/IL-5R) served as positive control material, while cryosections of untransfected TF1 (wild type) cells (TF1/wild) served as negative control material. All slides were read by the Study Pathologist to identify the tissue or cell type stained and intensity of staining (graded \pm [equivocal], 1+ [weak], 2+ [moderate], 3+ [strong], 4+ [intense], or Neg [negative]).

Study design for human tissues: Tissues were obtained at autopsy or biopsy from humans. Generally, tissues from at least three donors were available. Fresh unfixed tissue samples were placed in molds and frozen on dry ice in OCT embedding medium. Tissues were sectioned at approximately 5 μ m and fixed for 1 minute in cold (2-8°C) acetone. Cryosections were prepared on the day of staining.

As a tissue control, rabbit anti-human β_2 -microglobulin was reacted with cryosections of all human tissues (except blood smears). The β_2 -microglobulin antigen is a minor Class I determinant found on most tissues that is strongly expressed on endothelium. Its demonstration is indicative of expression of cell surface proteins by the normal tissues.

Study design of monkey tissues: Tissues were obtained at necropsy from Cynomolgus monkeys. Generally, tissues from only one donor were available. Tissues were prepared in a similar manner as described for humans.

Results:

Human Tissues: Using an indirect staining method, the test article, F-BIW-8405, specifically stained the positive control material (cryosections of IL-5R-transfected TF1 cells (TF1/IL-5R)). Neither the test article, F-BIW-8405, nor the negative control antibody, F-hlgG1, specifically reacted with negative control tissues (cryosections of untransfected TF1 (wildtype) cells (TF1/wild)). Further, the negative control antibody F-hlgG1 did not react with the positive control cryosections of TF1/IL-5R.

Positive cross reactivity was observed with intravascular protein from adrenal, blood leukocytes, bone marrow, brain-cerebrum (cortex), brain-cerebellum, mammary gland, colon, esophagus, small intestine, stomach, heart, kidney, liver, lung, lymph node, ovary, fallopian tube (oviduct), pancreas, parathyroid, peripheral nerve, pituitary, placenta, salivary gland, skin, spinal cord, spleen, skeletal muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, uterus-body (endometrium), and uterus-cervix. In general, the test article stained intravascular protein in a rheostatic pattern (i.e., distribution of blood components within tissue reflective of pattern of blood flow or pattern of post-mortem blood settling or pooling in tissues consistent with hypostatic congestion). For example, in the adrenals, the pattern was principally staining of capsular and extra-adrenal adipose vessels with less staining of parenchymal vessels, probably reflective of how the blood pooled post-mortem in that tissue. In brain, test article staining of intravascular protein was observed in a rheostatic pattern in meningeal vessels with less staining of parenchymal vessels. In spleen, test article staining predominated in red pulp sinusoids (i.e., cords of Billroth).

Positive cross reactivity was observed with mononuclear cells in the red pulp cords of Billroth in all three spleen donors examined. Test article F-BIW-8405 staining of mononuclear cells was evident in the peripheral cytoplasm and probably at the membrane of these mononuclear cells; however, distinction between true membrane staining and intravascular protein evident on the (vascular) luminal side of the mononuclear cell was very difficult.

F-BIW-8405 stained striated skeletal myocytes in striated skeletal muscle in all three donors examined (peripheral cytoplasm >> cross-striations [filaments]). Test article F-BIW-8405 staining was observed as very fine granular, interrupted lines at the periphery of striated (skeletal) myocytes; however, distinction between true membrane staining and peripheral cytoplasm was difficult. In a few myocytes, extension of test article F-BIW-8405 staining into more centrally oriented (intracytoplasmic) myocyte striations was observed. The staining pattern was more consistent with staining of peripheral cytoplasm rather than membrane.

Test article F-BIW-8405 staining was not observed at the membrane or in the peripheral cytoplasm of striated cardiac myocytes from the three human heart samples evaluated.

Staining of β_2 -microglobulin in tissues indicated that tissues were suitable for inclusion in the cross-reactivity study.

Monkey Tissues: It is noted that generally only one donor animal was used per tissue, which is considered insufficient, although the study is judged to be minimally relevant.

Results with positive and negative controls were similar to that described above.

For bone marrow, F-BIW-8405 stained the membrane and cytoplasm of occasional clusters of myeloid progenitors consistent with reported expression of IL5-R by human eosinophils. Other myeloid progenitors (probable neutrophil series) and erythroid progenitors were negative for test article staining.

Positive cross reactivity was observed with intravascular protein from adrenal, bone marrow (probable eosinophil progenitors, membrane, cytoplasm), brain-cerebellum, eye, colon, esophagus, small intestine, stomach, heart (intravascular/intrachamber protein), kidney, liver, lymph node, pancreas, pituitary, placenta, prostate, salivary gland, spleen, skeletal muscle, thymus, thyroid, ureter, and uterus-body (endometrium). In general, the test article stained intravascular protein in a rheostatic pattern (i.e., distribution of blood components within tissue reflective of pattern of blood flow or pattern of post-mortem blood settling or pooling in tissues consistent with hypostatic congestion).

F-BIW-8405 also stained mononuclear cells in red pulp cords of Billroth in Cynomolgus monkey spleen. Test article F-BIW-8405 staining of mononuclear cells was evident in the peripheral cytoplasm and probably at the membrane of these mononuclear cells;

however, distinction between true membrane staining and intravascular protein evident on the (vascular) luminal side of the mononuclear cell was very difficult.

Positive cross reactivity was observed with striated (cardiac) myocytes (peripheral cytoplasm >> cross-striations [filaments]) and striated (skeletal) myocytes (peripheral cytoplasm >> cross-striations [filaments]).

F-BIW-8405 stained striated skeletal myocytes in striated skeletal muscle in the Cynomolgus monkey tissue examined (peripheral cytoplasm >> cross-striations [filaments]). Test article F-BIW-8405 staining was observed as very fine granular, interrupted lines at the periphery of striated (skeletal) myocytes; however, distinction between true membrane staining and peripheral cytoplasm was difficult. In a few myocytes, extension of test article F-BIW-8405 staining into more centrally oriented (intracytoplasmic) myocyte striations was observed.

F-BIW-8405 stained striated cardiac myocytes from the Cynomolgus monkey tissue examined (peripheral cytoplasm >> cross-striations [filaments]). The test article F-BIW-8405 staining was observed as very fine granular, interrupted lines at the periphery of striated (cardiac) myocytes; however, distinction between true membrane staining and peripheral cytoplasm was difficult. In a few myocytes, extension of test article F-BIW-8405 staining into more centrally oriented (intracytoplasmic) myocyte striations was observed.

Staining of intravascular protein in human and monkey tissues: Cross reactivity was observed in several tissues from humans and monkeys. The test article stained intravascular protein in a rheostatic pattern (i.e., distribution of blood components within tissues reflective of the pattern of blood flow or pattern of post-mortem blood settling or pooling in tissues [hypostatic congestion]). These findings were attributed to the presence of solubilized IL-5 receptor (Journal of Immunology 169: 6459-6466, 2002; Growth Factors 19: 145-152, 2001; Antisense and Nucleic Acid Drug Development 10: 347-357, 2000). Circulating eosinophils may have released the IL-5 receptor into the tissue vasculature (Science 282 (no. 5392): 1279-1280, 1998; Journal of Immunology 169: 6459-6466, 2002; Growth Factors 19: 145-152, 2001; Antisense and Nucleic Acid Drug Development 10: 347-357, 2000). Although, this pattern of tissue cross reactivity was greater in humans than monkeys, it was not considered a safety issue at this time. Cross reactivity was observed with mononuclear cells (red pulp cords of Billroth, membrane, cytoplasm) in the spleen for both humans and monkeys. Cross reactivity was observed with striated (skeletal) myocytes (peripheral cytoplasm >> cross-striations [filaments]) for both humans and monkeys. Positive cross reactivity with striated (cardiac) myocytes (peripheral cytoplasm >> cross-striations [filaments]) was also observed for monkeys.

Table 68 Results of tissue cross reactivity studies with human and monkey tissues

	A	B	C	D	E	F
1	Tissue	Human Studies			Monkey Studies	
2		10 µg/mL	1 µg/mL	N	10 µg/mL	1 µg/mL
3	IL-5R-transfected TF-1 cells	3-4+	3-4+		3-4+	3-4+
4	Untransfected TF-1 cells	Neg	Neg		Neg	Neg
5	Adrenal-Intravascular protein	3+ (occas)	2-3+ (rare to occas)	3 of 3/3 of 3	3+ (occas)	2-3+ (occas)
6	Blood leukocytes-Eosinophils	Neg	Neg	3 of 3/3 of 3	-	-
7	Blood leukocytes-Intravascular Protein	1-2+ (freq)	1+ (occas to freq)	3 of 3/1 of 3	-	-
8	Bone marrow-Intravascular Protein	3-4+ (freq)	3+ (freq)	3 of 3/3 of 3	Neg	Neg
9	Bone marrow/eosinophils				3+ (occas)	2-3+ (rare)
10	Brain/cerebrum (cortex)-Intravascular Protein	2-3+ (rare to occas)	1-2+ (rare)	3 of 3/3 of 3	Neg	Neg
11	Brain/cerebellum-Intravascular Protein	1-2+ (rare to occas)	1+ (rare)	3of 3/1 of 3	2-3+ (rare to occas)	1+ (rare)
12	Breast/Mammary Gland-Intravascular Protein	2-3+ (very rare to freq)	1-2+ (rare)	2 of 2/1 of 2	Neg	Neg
13	Eye-Intravascular Protein	2-3+ (very rare)	Neg	1 of 1/0 of 1	1-2+ (rate to occas)	Neg
14	GIT/Colon-Intravascular Protein	2-4+ (occas to freq)	2-3+ (rare to occas)	3 of 3/1 of 3	Neg	Neg
15	GIT/Esophagus-Intravascular Protein	2-3+(rare to freq)	2+ (very rare)	3 of 3/3 of 3	2-4+ (rare to freq)	2-3+ (rare to occas)
16	GIT/Small Intestine-Intravascular Protein	2-3+ (rare to occas)	2+ (rare to occas)	3 of 3/3 of 3	3+ (occas)	2-3+ (rare to occas)
17	GIT/Stomach-Intravascular Protein	1-4+ (rare to freq)	1-3+ (occas to freq)	3 of 3/2 of 3	2-3+ (occas)	Neg
18	Heart-Intravascular Protein	1-3+ (rare)	Neg	3 of 3/0 of 3	2-3+ (occas to freq)	1-2+ (rare)
19	Heart-striated (cardiac) myocytes (peripheral cytop)	Neg	Neg		2-3+ (freq)	Neg
20	Kidney-Intravascular Protein	2+ (occas to freq)	1-2+ (rare)	3 of 3/2 of 3	1-2+ (rare)	1-2+ (rare)
21	Liver-Intravascular Protein	2-3+ (rare to freq)	1-2+ (rare)	3 of 3/3 of 3	2-3+ (occas)	1-2+ (rare)
22	Lung-Intravascular Protein	2-3+ (occas to freq)	1-2+ (rare to occas)	3 of 3/3 of 3	Neg	Neg
23	LN-Intravascular Protein	2-3+ (rare to occas)	1-2+ (rare)	2 of 3/2 of 3	2+ (rare)	1-2+ (rare)
24	Ovary-Intravascular Protein	2-3+ (rare to occas)	1-2+ (rare)	3 of 3/1 of 3	Neg	Neg
25	Fallopian tube (oviduct)-Intravascular Protein	2-3+ (occas to freq)	2+ (rare)	3 of 3/3 of 3	Neg	Neg
26	Pancreas-Intravascular Protein	3-4+ (occas to freq)	1-3+ (rare to occas)	3 of 4/3 of 4	2-3+ (Occas)	2+ (rare to occas)
27	Parathyroid-Intravascular Protein	2-3+ (occas)	2+ (rare to occas)	1 of 6/1 of 6	Neg	Neg
28	Peripheral nerve-Intravascular Protein	2-4+ (rare to freq)	2-4+ (rare to occas)	3 of 4/2 of 4	Neg	Neg
29	Pituitary-Intravascular Protein	2-3+ (occas to freq)	1-2+ (rare to occas)	3 of 3/2 of 3	3+ (occas)	2-3+ (rare to occas)
30	Placenta-Intravascular Protein	2-4+ (rare to freq)	1-3+ (rare to freq)	3 of 3/3 of 3	2-3+ (occas to freq)	2-3+ (occas to freq)
31	Prostate	Neg	Neg	3 of 3/3 of 3	1-2+ (rare)	Neg
32	Salivary Gland-Intravascular Protein	2+ (rare to occas)	1+ (rare)	2 of 3/1 of 3	2-3+ (occas)	1-2+ (rare)
33	Skin-Intravascular Protein	1-2+ (rare)	Neg	2 of 3/0 of 3	Neg	Neg
34	Spinal cord-Intravascular Protein	2-4+ (occas to freq)	1-3+ (rare to freq)	3 of 3/3 of 3	Neg	Neg
35	Spleen-Intravascular Protein	1-3+ (occas to freq)	1-2+ (rare)	3 of 3/1 of 3	2-4+ (freq)	2-3+ (freq)
36	Spleen-Mononuclear cells	1-3+ (rare-freq)	1-2+ (rare)	3 of 3/1 of 3	2-3+ (occas to freq)	2+ (occas to freq)
37	Striated (skeletal) muscle-Intravascular Protein	2-3+ (occas)	1-2+ (rare)	3 of 3/3 of 3	2-3+ (occas)	Neg
38	Striated (skeletal) muscle-striated (skeletal) myocy	2-3+ (freq)	1-2+ (rare)	3 of 3/2 of 3	2-3+ (freq)	Neg
39	Testis-Intravascular Protein	2-4+ (rare to freq)	2-3+ (rare to occas)	3 of 3/2 of 3	Neg	Neg
40	Thymus-Intravascular Protein	2-4+ (occas to freq)	1-4+ (rare to freq)	3 of 4/3 of 4	3+ (occas)	2-3+ (rare to occas)
41	Thyroid-Intramuscular Protein	2-3+(rare to occas)	1-2+ (rare)	3 of 3/2 of 3	2-3+ (occas)	2+ (rare)
42	Tonsil-Intramuscular Protein	2-3+ (occas to freq)	2+ (rare to occas)	3 of 4/2 of 4	Neg	Neg
43	Ureter-Intravascular Protein	2-3+ (rare to freq)	2-3+ (occas to freq)	3 of 3/1 of 3	3+ (occas)	2-3+ (rare to occas)
44	Urinary bladder-Intravascular Protein	2-4+ (rare to freq)	2-3+ (rare to occas)	3 of 6/3 of 6	Neg	Neg
45	Uterus-body (endometrium)-Intravascular Protein	2-3+ (occas)	2+ (occas)	2 of 3/1 of 3	2-3+ (occas)	1-2+ (rare to occas)
46	Uterus-cervix-Intravascular Protein	2-3+ (occas)	1-2+ (rare)	2 of 3/2 of 3	Neg	Neg

11 Integrated Summary and Safety Evaluation

Benralizumab is a humanized, afucosylated, immunoglobulin (Ig)G1 κ monoclonal antibody (mAb) that targets the alpha subunit of the human interleukin-5 receptor (IL-5R α), which is expressed on eosinophils and basophils.

In Vitro Pharmacology: Consistent with the expression of IL-5R α on human eosinophils and basophils, benralizumab exclusively stained peripheral blood eosinophils and basophils from healthy subjects. Eosinophils expressed an approximate 3-fold higher level of IL-5R α compared to basophils as quantified based on median fluorescence intensity. Further, benralizumab identified a small but specific fraction (approximately 0.9%) of bone marrow mononuclear cells (BMMNCs) that most likely represented the eosinophil/basophil lineage precursors. Eosinophils, basophils, and BMMNCs were potential targets for the action of benralizumab.

Binding of benralizumab to human and Cynomolgus monkey eosinophils and soluble human and Cynomolgus monkey IL-5R α was studied by using flow cytometry and BIAcore methods, respectively. EC₅₀ values of parental fucosylated anti-IL-5R mAb binding to eosinophils from humans and Cynomolgus monkeys were 26 and 40 pM, respectively. K_D values for binding of benralizumab to human and Cynomolgus monkey IL-5R α were 11 and 42 pM, respectively. Reduced binding (kinetic rate/binding constant) was observed with the benralizumab F(ab) fragment against human and monkey IL-5R α with K_D values of 1260 and 20500 pM, respectively.

Murine IL-5R α protein shares 68% homology with human IL-5R α . Binding of benralizumab to murine IL-5R α was characterized by flow cytometry using human embryonic kidney (HEK) 293F cells that expressed full length human or murine IL-5R α on the cell surface. There was no detectable binding of benralizumab to murine IL-5R α -expressing cells. Benralizumab displayed binding to human IL-5R α -expressing cells as expected. Benralizumab did not cross-react with murine IL-5R α .

The lack of binding of benralizumab to murine IL-5R α was used to assist in the identification of the human IL-5R α receptor epitope recognized by benralizumab. Extracellular human IL-5R α domains 1 (D1), 2 (D2), and 3 (D3) were replaced with corresponding murine IL-5R α domain sequences to create knockout variants or the reverse to create knock-in variants using transfected HEK293 F cells. The expression levels of all variants were monitored with anti-human or anti-mouse IL-5R α polyclonal antibodies using flow cytometry. Benralizumab bound only to constructs containing the human IL-5R α domain 1. Alignment of human, Cynomolgus monkey, and murine IL-5R α D1 amino acid sequences identified differences between the receptors and these areas were targeted to further characterize the binding epitope. Only swap mutants encoding human segment B were recognized by benralizumab; thus, identifying the region containing the binding epitope. Further refinement of the epitope was performed by swapping amino acids in segment B not conserved between the human and murine sequences. Substituting amino acids N40, N42, Q46, D56, and E58 in the human IL-5R α segment B with the corresponding murine residues had no effect on benralizumab binding to human IL-5R α . However, a single amino acid change to isoleucine at position

61 (I61) was sufficient to confer the benralizumab binding to murine IL-5R α . Conversely, benralizumab binding to human IL-5R α was obliterated by replacing I61 with the murine lysine residue at position 61 (K61), ultimately identifying amino acid I61 from the human IL-5R α as the critical residue binding. Segment B (amino acids 40-61) in D1 (including I61) was found to be 100% conserved between humans and Cynomolgus monkeys, thus explaining the cross reactivity of benralizumab with Cynomolgus monkey IL-5R α .

Both benralizumab (fucose negative) and the fucosylated (fucose positive) parent anti-IL-5R α mAb inhibited IL-5-induced proliferation of CTLL-2 cells transfected with recombinant human IL-5R with identical potencies (IC₅₀ = 0.3 nM). The inhibitory potency on IL-5-induced cell growth was 300-fold lower relative to its potency for inducing ADCC activity on eosinophils and basophils.

Benralizumab is an afucosylated (fucose negative) IgG1 κ mAb that targets IL-5R α . The absence of the monosaccharide, fucose, on the oligosaccharide core of a human IgG1 has previously been shown to result in an increased binding affinity to human Fc γ RIIIa and subsequently increased ADCC. ADCC-induced apoptosis of human eosinophils was compared between afucosylated (fucose negative) benralizumab and fucosylated (fucose-positive) benralizumab. Fucose negative benralizumab was expressed in the FUT8 gene deficient CHO cell line and fucose-positive benralizumab was expressed in the normal CHO cell line. To induce ADCC, purified PBMCs (1 x 10⁶ cells/well), as effector cells, and eosinophils (4 x 10⁴ cells/well), as target cells, were incubated with either fucose negative benralizumab or fucose-positive benralizumab at final concentrations of 0.01, 0.1, and 1 μ g/mL in the presence of IL-5 (1 ng/mL) for 20 hr. After the incubation period, cells were stained with FITC-labeled Annexin V. Annexin V is known to bind to apoptotic cells. Based upon bindings of Annexin V, fucose negative benralizumab induced apoptosis in the human eosinophils while fucose-positive benralizumab, having lower ADCC activity, exhibited much less apoptosis under the same conditions.

Benralizumab (fucose negative) was assessed for binding to Fc γ Rs and ADCC activity as compared to the fucosylated parental anti-IL-5R α mAb. Examinations of the binding affinity of benralizumab (fucose negative) to soluble human Fc γ R domains by surface plasmon resonance found that binding to human Fc γ RIIIa was increased 6-fold compared with the fucosylated (fucose positive) parental anti-IL-5R α mAb; however, binding was similar for all other Fc γ Rs. Comparable results were obtained with monkey Fc γ RIIIa.

The potency of benralizumab (fucose negative) to mediate eosinophil and basophil apoptosis by ADCC *in vitro* was examined and compared to its fucosylated (fucose positive) parental α IL-5R α mAb. ADCC assays were performed with autologous NK cells, as effector cells, and bone marrow mononuclear cell (BMMNCs; approximately 1% IL-5R α 1 cells), as target cells. For ADCC assays with peripheral blood-derived eosinophils or basophils, 10⁴ NK cells and 10⁴ eosinophils or basophils were co-incubated in 96-well plates in the presence of serial dilutions of benralizumab (fucose negative) or parent α IL-5R α mAb (fucose negative). Annexin V Alexa 647 was added.

Cells were analyzed on a flow cytometer and the percentage of Annexin V-positive eosinophils/basophils was measured. ADCC was determined by gating of Annexin V-positive target cells. Supernatants were collected at the end of ADCC assays to measure eosinophil cationic protein (ECP) and eosinophil-derived neurotoxin (EDN) levels by using an ELISA. Total ECP and EDN levels were determined by means of eosinophil lysis with 1% Triton X-100 (100% degranulation), and a mixture of the cytokines, RANTES (13 nmol/L), eotaxin (12 nmol/L), and IL-33 (6 nmol/L) was used as a positive control.

In the presence, but not absence, of autologous NK effector cells, benralizumab (fucose negative) induced eosinophil and basophil apoptosis, as assessed by means of Annexin V staining, with EC_{50} values of 0.9 and 0.5 pM, respectively. However, when the fucosylated (fucose positive) parental α IL-5R α mAb was used at concentrations 1000 times higher than the benralizumab EC_{50} (in the presence of NK effector cells), it did not induce target cell apoptosis above background levels, although its binding affinity for IL-5R α and its potency to inhibit IL-5-induced cell proliferation were indistinguishable from those of benralizumab (fucose negative). Benralizumab (fucose negative) also depleted human IL-5R α 1 BMMNCs when co-cultured with NK effector cells, whereas an irrelevant afucosylated (fucose negative) isotype control mAb was ineffective.

In contrast to stimulation with a mixture of cytokines (RANTES, eotaxin, and IL-33), eosinophil apoptosis induced by benralizumab was not associated with release of EDN or ECP, indicating a lack of significant eosinophil degranulation, no cellular necrosis, and reduced concerns that these toxic agents might be released in the *in vivo* setting.

The data suggested an enhanced benralizumab ADCC potency to deplete IL-5R α -expressing eosinophils, basophils, and BMMNCs in vitro resulted from its fucose deficiency.

Benralizumab and KM8400 used at concentrations more than 1000-fold greater than the EC_{50} for ADCC did not demonstrate complement-dependent cytotoxicity (CDC) with eosinophils purified from three different human donors. The lack of CDC activity of benralizumab with eosinophils was attributed to the low expression of IL-5R α on primary human eosinophils, which varies from 200 to 1000 copies/cell.

Collectively, benralizumab can deplete eosinophils and basophils through apoptosis induced by ADCC. The enhanced ADCC is attributed to the absence of the monosaccharide, fucose, on the oligosaccharide core of benralizumab, which results in an increased binding affinity to human Fc γ R1IIa and subsequently increased ADCC.

In Vivo Pharmacology: An IL-5-induced monkey eosinophilia model was established and the efficacy of benralizumab was evaluated in this model. A single administration of benralizumab was given by the intravenous route into these animals at doses of 0.01 or 0.3 mg/kg on day 10 after induction of IL-5-induced eosinophilia. The number of eosinophils in the peripheral blood of the monkeys that received IL-5 was dramatically increased. Eosinophil counts in animals administered a single dose of 0.3 mg/kg

benralizumab were significantly lower than in the controls. In 2 of 3 animals that received a dose of 0.01 mg/kg, eosinophil counts were also significantly lower than in the controls. Eosinophil counts were decreased in a dose-related manner. Benralizumab displayed pharmacological activity in monkeys indicating that it was a relevant species for toxicological testing.

A monkey model actively immunized (sensitized) to *Ascaris suum* antigen was developed and employed to evaluate the anti-asthmatic activity of benralizumab. To assess the effects of benralizumab on airway hyperreactivity (AHR) and bronchoalveolar lavage (BAL), monkeys received benralizumab by the intravenous route at a dose of 1 mg/kg at 24 hr prior to antigen challenge during week 24. During subsequent weeks following the single dose of benralizumab, AHR and BAL were evaluated. Increased eosinophil counts were observed in the BAL following *Ascaris* antigen challenge. A single intravenous dose of benralizumab at 1 mg/kg administered to monkeys during week 24 significantly decreased BAL eosinophil counts. The effect of benralizumab on eosinophil counts persisted over a 14-week observation period after dosing. As the benralizumab concentration in plasma decreased and became undetectable in all monkeys, the infiltration of eosinophils was observed again after antigen challenge in 3 monkeys (#1, 7, and 15). However, the infiltration of eosinophils remained suppressed 23 weeks after the benralizumab administration in two animals.

Development of airway hyperreactivity was evaluated by measuring reactivity to methacholine (MCh) aerosol inhalation by anesthetized animals before and 24 hr after inhalation of antigen during week 14. PC150, the concentration of inhaled MCh that caused a 50% increase in respiratory system resistance, was used as the indicator for evaluation. Development of AHR was expressed as the decrease in MCh PC150 after antigen inhalation (PostPC150/PrePC150). Only 2 (#7 and 8) of 5 immunized animals showed the development of AHR upon Ag challenge. AHR was evaluated on the two animals that tested positive for antigen reactivity. Tests performed at 1 day postdose and at weeks 3, 8, 14, and 23 postdose found that benralizumab suppressed the development of AHR in 1 of the 2 animals over a 23-week observation period. The value of Post PC150/Pre PC150 of monkeys #7 and #8 decreased to 55% and 39% upon Ag inhalation. These AHR values were almost as the same as those of the monkeys in the naturally immunized *Ascaris* model. Benralizumab completely suppressed the AHR value of monkey #7 at least for 8 weeks (until day 61). In monkey #8, the effect of benralizumab on the AHR suppression was detected 2 days after its administration. However, the significant AHR suppression was not detected thereafter except in week 8.

Safety Pharmacology: No standalone safety pharmacology studies were conducted with benralizumab; however, assessments of effects on CNS, cardiovascular, and respiratory function were incorporated into general toxicology studies. There was no evidence of any adverse effects of benralizumab on CNS, cardiovascular, or respiratory functions.

ADME: Pharmacokinetic parameters for benralizumab in monkeys and humans are listed in the table below. Half-lives of benralizumab in monkeys and humans were similar. Systemic clearance of benralizumab appeared to be higher in humans relative to monkeys. The volume of distribution in monkeys at low doses (0.1 mg/kg) was comparable to that in humans; however, at higher doses in monkeys (1, 10, and 30 mg/kg), the volume of distribution was greater. The volume of distribution at lower doses was generally consistent with the blood volume, although at higher doses, the volume of distribution more closely matched the extracellular fluid volume. The T_{max} following SC administration in monkeys was approximately 3 days. The SC bioavailability in monkeys was approximately 60%.

Table 69 Pharmacokinetic parameters for benralizumab in monkeys and humans

Species	T _{1/2} Days	Clearance mL/kg/day	V _z mL/kg
Cynomolgus monkeys ¹	7.83 to 20.4	3.6 to 8.37	50.52 to 177.12
Humans	15	4.16 (0.291 L/day)	45.7 (Central V _d) 35.7 (Peripheral V _d)

1. IV doses of 0.1, 1, 10, and 30 mg/kg

General Toxicology/Effects on Fertility: In a 39-week toxicology study, Cynomolgus monkeys (6/sex/group) received benralizumab (MEDI-563) at doses of 0 (IV/SC), 10 (IV), 25 (IV), and 30 (SC) mg/kg once every 2 weeks for a total of 20 doses. Selected animals (3/sex/group) were allowed a 12-week recovery period. Males and females were sexually mature adults to allow for fertility assessments.

Systemic drug exposure was highest in the 25 mg/kg IV group. Exposure for the 30 mg/kg SC group was intermediate between the 10 and 25 mg/kg IV groups.

One male in the 25 mg/kg IV group was euthanized on day 47 due to a right wrist anomaly; this finding was judged to have no relationship to treatment.

Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count and indicators of circulating erythrocyte mass that appeared to be reversible. Female 3501 was not dosed on day 57 due to these adverse clinical signs. After dosing resumed on day 71, platelet count decreased on day 87 and then gradually increased to baseline levels on day 267. Indicators of circulating erythrocyte mass were only minimally affected on day 87 and were greater than prestudy by day 183. The effects on platelets and erythrocytes (abnormal morphology) suggest a MEDI-563-related immune-mediated process (e.g., potential postdose reaction) that was considered adverse. It had fully resolved by day 267 despite continued dosing.

Eosinophil counts were decreased for males and females in the 10 and 25 mg/kg IV groups and 30 mg/kg SC group, which could be attributed to the pharmacological action of MEDI-563. The decreases were most pronounced for males in the 25 mg/kg IV groups. Eosinophil counts were consistently decreased by Day 63 and a few animals demonstrated an effect as early as Day 3. Lower peripheral blood eosinophil counts correlated with decreased eosinophils in the bone marrow.

Treatment-related bone marrow effects consisted of lower percentages of eosinophils for monkeys that received 10 or 25 mg/kg IV or 30 mg/kg SC. Decreased eosinophil counts was an expected pharmacologic effect of the test article.

Male and female fertility parameters were unaffected with doses up to 25 mg/kg IV.

Histopathological findings were judged to be spontaneous in nature and unrelated to treatment.

NOAELs were identified as the 10 mg/kg IV and 30 mg/kg SC based upon findings for Female 3501 in the 25 mg/kg IV dose group had a transient test article-related event after the fourth dose on day 43 that included adverse clinical signs of bruising/reddened areas around the eyes, on the face, chest and lower abdomen (petechiae and ecchymosis) and decrease in platelet count and indicators of circulating erythrocyte mass that appeared to be reversible. Systemic exposures for the 10 mg/kg IV group (2320 µg·day/mL) and 30 mg/kg SC group (4110 µg·day/mL) were significantly lower than the 25 mg/kg IV group (6100 µg·day/mL).

Table 70 Exposure margins on a mg/m² basis for the proposed clinical doses of 30 q4 weeks for the first 3 doses and 30 mg q4 weeks or q8 weeks thereafter, respectively

39-week IV/SC toxicology study with monkeys	Exposure margins for the clinical dose of 30 mg (0.5 mg/kg)
10 mg/kg – IV (NOAEL)	20
25 mg/kg – IV* (Dose-limiting toxicity)	50
30 mg/kg – SC (NOAEL)	60

* Systemic exposures for the 10 mg/kg IV group (2320 µg·day/mL) and 30 mg/kg SC group (4110 µg·day/mL) were significantly lower than the 25 mg/kg IV group (6100 µg·day/mL).

Table 71 Estimated exposure margins on an AUC basis for the proposed clinical doses of 30 mg q4 weeks for the first 3 doses and 30 mg q4 weeks or q8 weeks thereafter, respectively

39-week IV/SC toxicology study with monkeys	AUC _{0-2w} µg·day/mL	AUC _{0-8w} µg·day/mL	Exposure margins for a clinical dose of 30 mg
			Exposure margins for the clinical exposure at 30 mg q8 weeks AUC _{tau (q8 weeks), ss} = 60.7 µg·day/mL ^b
10 mg/kg – IV (NOAEL)	2320	9280	153
25 mg/kg – IV ^a (Dose-limiting toxicity)	6100	24400	402
30 mg/kg – SC (NOAEL)	4110	16440	270.8

a. Systemic exposures for the 10 mg/kg IV group (2320 µg·day/mL) and 30 mg/kg SC group (4110 µg·day/mL) were significantly lower than the 25 mg/kg IV group (6100 µg·day/mL).

b. From Dr. Yunzhao Ren (email dated June 23, 2017): The typical CL of a subject weighting 70kg with negative ADA is 0.291 L/day and the bioavailability is 58.9%. Therefore, the AUC_{tau} for a 30 mg Q8W dosing regimen at steady state is 60.7 µg·day/mL (or 60.7 mg·day/L).

Reproductive Toxicity: Pregnant Cynomolgus monkeys received benralizumab (MEDI-563) by bolus IV injection at doses of 0, 10, or 30 mg/kg on GD20-GD22 (dependent on pregnancy determination), on GD35, and once every 14 days thereafter through gestation. Further, MEDI-563 was administered to female adult monkeys on postpartum days 14 and 28. A maximum of 14 doses were administered.

There was no evidence of maternal toxicity with IV doses of benralizumab at 10 or 30 mg/kg. Complete or near-complete depletion of peripheral blood eosinophils was observed at GD91 in most adult females in the 10 mg/kg group and all females in the 30 mg/kg group. The depletion of eosinophils continued through PPD 28. Eosinophil counts began to increase gradually in most females in the 10 and 30 mg/kg dose groups by PPDs 91, 136, and 180 with eosinophil counts in a few females approaching the lowest control eosinophil counts by PPD 91 and most counts were similar to the control by PPD180. A few females in the 10 and 30 mg/kg groups had peripheral blood eosinophil counts that remained ≤10/µL through PPD180, indicating a lack of recovery. Decreased eosinophil counts were attributed to the pharmacological action of the benralizumab.

Incidences of aborted, stillborn fetuses, and fetal/neonatal survival appeared to be unaffected by treatment with benralizumab. Incidences of stillborn fetuses were higher in drug-treated group; however, no dose-response relationship was evident.

Infants exposed *in utero* to benralizumab in the 10 and 30 mg/kg groups had decreased peripheral blood eosinophil counts. Peripheral blood eosinophil counts had risen in most infants by BD180 and BD199 (± 2 days) to counts comparable to controls except infant

3141 in the 30 mg/kg group. Peripheral blood eosinophil counts in infant #3141 remained at 0/ μ L, which correlated with the bone marrow eosinophil evaluation for this animal.

Growth and neurological development of infants were unaffected by benralizumab. External, visceral, and heart evaluations of infants in the control and drug-treated groups on BD199 were normal.

Skeletal evaluations were generally normal. However, infant # 3091 in the 30 mg/kg group had an abnormal lumbar vertebra where the right side of the 4th lumbar vertebra was malformed (wedge-shaped) resulting in scoliosis and deviation of the spinal column to the left; this finding was judged to be not related to benralizumab.

There were no benralizumab-related changes observed in anti-KLH IgM and anti-KLH IgG titer values in infants from the 10 and 30 mg/kg groups as compared to the control group.

Infants exposed *in utero* to benralizumab in the 10 and 30 mg/kg groups had measurable levels of serum IgM and IgG comparable to control infants at all time points.

There were no changes of B lymphocytes, Total T lymphocytes, T-helper lymphocytes, T-cytotoxic/suppressor lymphocytes, NK cells, or monocytes in infants attributed to *in utero* exposure to benralizumab in the 10 or 30 mg/kg groups.

Exposure of infants to benralizumab was demonstrated by detecting benralizumab in the serum of infants. Benralizumab levels observed in infants were consistent with placental transfer from the maternal circulation into the fetal circulation. Benralizumab concentrations in infants on BD180 were below the limit of quantitation. Benralizumab was not teratogenic in monkeys.

Table 72 Estimated exposure margins on an AUC basis for the proposed clinical doses of 30 mg q4 weeks for the first 3 doses and 30 mg q4 weeks or q8 weeks thereafter, respectively

ePPND study with monkeys	Dose	AUC _{0-2w} μ g·day/mL	AUC _{0-8w} μ g·day/mL	Exposure margins for the clinical exposure at 30 mg q8 weeks AUC _{tau (q8 weeks), ss} = 60.7 μ g·day/mL
10 mg/kg – SC	1 st	1120	4480	73.8
	9 th	1710	6840	112.6
30 mg/kg – SC (NOAEL)	1 st	4040	16160	266.2
	9 th	4760	19040	313.7

Carcinogenic Potential:

The Sponsor's rationale for not conducting a carcinogenicity study with benralizumab was discussed with Executive Carcinogenicity Assessment Committee by email on January 30 and 31, 2013. It was agreed that a carcinogenicity study was not required for benralizumab. The key points of the discussion included:

1. To date, there is no evidence for proliferative or pre-neoplastic effects of benralizumab in any GLP-compliant repeat-dose IV or SC toxicologic study in Cynomolgus monkeys.
2. Since benralizumab does not bind to murine IL-5R α , direct assessment of carcinogenic risk of benralizumab in a classic 2-year rodent bioassay was not appropriate.
3. A murine surrogate is not required.
4. IL-5- and IL-5R α -deficient mouse models do not appear to be relevant for human risk assessment.

Based on the lack of suitable rodent models for assessment of carcinogenic risk of benralizumab, the Sponsor plans to use results from the completed repeat-dose toxicologic studies in Cynomolgus monkeys to provide nonclinical risk assessment regarding carcinogenic potential of benralizumab following its chronic administration.

The role of eosinophils in tumor development is unclear.

- 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 MCA-induced tumor incidence and growth were significantly attenuated in IL-5 transgenic mice of both the BALB/c strain and C57BL/6 background. Histological examination revealed that the protective effect of IL-5 was associated with significantly increased 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 2.5×10^5 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 wildtype or IL-5^{-/-} mice (Journal of Immunology 160: 345-350, 1998).

- 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 (DMBA)-induced hamster oral cancer model was utilized. Eosinophils were determined to progressively infiltrate into this model, and when ablated with 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 TGF α . Transgenic mice overexpressing TGF α 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.

Section 13.1 of the product label should include available information from the published scientific literature regarding the potential roles of IL-5 and eosinophils in cancer.

IL-5 Knockout Mice:

Findings with benralizumab were compared to the 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 helminth, *Mesocostoides 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 Review:

Labeling recommendations were provided for Indications and Usage (under Highlights of Prescribing Information), Section 8.1, Section 8.3, Section 12.1, and Section 13. Additions were denoted as underlined text. Deletions were denoted as ~~text~~.

Sponsor's Labeling:

----- **INDICATIONS AND USAGE** -----

TRADENAME is an interleukin-5 receptor alpha-directed cytolytic monoclonal antibody indicated ^{(b) (4)} add-on maintenance treatment ^{(b) (4)} patients with severe asthma aged ^{(b) (4)} years and older, with an eosinophilic phenotype.

Reviewer's Evaluation: The established pharmacological classification (EPC) was discussed with Drs. Jennifer Swisher (OPQ Reviewer), Sofia Chaudhry (Medical Officer), and Paul Brown (PharmTox Associate Director) as well as the CDER PTCC Nonclinical Biologics Subcommittee and it was agreed the Sponsor has sufficient data to support the proposed EPC of interleukin-5 receptor alpha-directed cytolytic monoclonal antibody.

Recommended labeling: No changes

Sponsor's Labeling:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

The data on pregnancy exposure from the clinical trials are insufficient to inform on drug-associated risk. Monoclonal antibodies such as benralizumab are transported across the placenta ^{(b) (4)}; therefore, potential ^{(b) (4)} a fetus ^{(b) (4)} likely to be greater during the ^{(b) (4)} third trimester of pregnancy. In a prenatal and postnatal development study conducted in cynomolgus monkeys, there was no evidence of fetal harm with IV administration of benralizumab throughout pregnancy at doses that produced exposures up to approximately ^{(b) (4)} times the exposure at the maximum recommended human dose (MRHD) of 30 mg SC [see Data]. ^{(b) (4)}

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

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 in the neonate.

Data

Animal Data

In a prenatal and postnatal development study, pregnant cynomolgus monkeys received benralizumab from beginning on GD20 to GD22 (dependent on pregnancy determination), on GD35, once every 14 days thereafter through gestation and 1-month postpartum (maximum 14 doses) at doses that produced exposures up to approximately (b) (4) times that achieved with the MRHD (on an AUC basis with maternal IV doses up to 30 mg/kg once every 2 weeks). Benralizumab did not elicit adverse effects on fetal or neonatal growth (including immune function) up to 6.5 months after birth.

Reviewer's Evaluation: Section 8.1 was modified to comply with current CDER labeling practices for PLLR conversions.

In the ePPND study, eosinophils counts were depleted in both mothers and infants. Recovery of eosinophil counts after discontinuation of drug exposure was slow. In a small number of mothers and infants, depletion of eosinophils was not reversible over the 6.5 month observation period.

Recommended labeling:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

The data on pregnancy exposure from the clinical trials are insufficient to inform on drug-associated risk. Monoclonal antibodies such as benralizumab are transported across the placenta (b) (4)

uring the third trimester of pregnancy; therefore, potential effects on a fetus are likely to be greater during the third trimester of pregnancy. In a prenatal and postnatal development study conducted in cynomolgus monkeys, there was no evidence of fetal harm with IV administration of benralizumab throughout pregnancy at

doses that produced exposures up to approximately (b) (4) times the exposure at the maximum recommended human dose (MRHD) of 30 mg SC [see Data] (b) (4)

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

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 in the neonate.

Data

Animal Data

In a prenatal and postnatal development study, pregnant cynomolgus monkeys received benralizumab from beginning on GD20 to GD22 (dependent on pregnancy determination), on GD35, once every 14 days thereafter through out the gestation period and 1-month postpartum (maximum 14 doses) at doses that produced exposures up to approximately 31 (b) (4) times that achieved with the MRHD (on an AUC basis with maternal IV doses up to 30 mg/kg once every 2 weeks). Benralizumab did not elicit adverse effects on fetal or neonatal growth (including immune function) up to 6.5 months after birth. There was no evidence of treatment-related external, visceral, or skeletal malformations. Benralizumab was not teratogenic in cynomolgus monkeys. Benralizumab crossed the placenta in cynomolgus monkeys. Benralizumab concentrations were approximately equal in mothers and infants on postpartum day 7, but were lower in infants at later time points. Eosinophil counts were suppressed in infant monkeys with gradual recovery by 6 months postpartum; however, recovery of eosinophil counts was not observed for one infant monkey during this period.

Sponsor's Labeling:

8.2 Lactation

Risk Summary

There is no information regarding the presence of benralizumab in human or animal milk, (b) (4)

Reviewer's Evaluation: Section 8.2 was modified to comply with current CDER labeling practices for PLLR conversions.

Recommended labeling:**8.2 Lactation****Risk Summary**

There is no information regarding the presence of benralizumab in human (b) (4). However, benralizumab is a humanized monoclonal antibody (IgG1/k-class), and immunoglobulin G (IgG) is present in human milk in small amounts. If benralizumab is transferred into human milk, the effects of local exposure in the gastrointestinal tract and potential limited systemic exposure in the infant to benralizumab are unknown. The developmental and health benefits of breast-feeding should be considered along with the mother's clinical need for benralizumab and any potential adverse effects on the breast-fed (b) (4) from benralizumab, or from the underlying maternal condition. (b) (4)

Sponsor's Labeling:**12 CLINICAL PHARMACOLOGY****12.1 Mechanism of Action**

Benralizumab is (b) (4) humanized afucosylated, monoclonal antibody (IgG1, kappa) (b) (4) binds to the alpha subunit of the human interleukin-5 receptor (IL-5R α) (b) (4). The IL-5 receptor is (b) (4) expressed on the surface of eosinophils and basophils. The absence of fucose in the Fc domain of benralizumab (b) (4) (45.5 nM) (b) (4) Fc γ RIII receptors on immune effectors cells such as natural killer (NK) cells leading to apoptosis of eosinophils and basophils through (b) (4) antibody-dependent cell-mediated cytotoxicity (ADCC).

(b) (4) inflammation is an important component in the pathogenesis of asthma. (b) (4)

Reviewer's Evaluation: Benralizumab is an afucosylated IgG1k mAb that targets IL-5R α . The absence of the monosaccharide, fucose, on the oligosaccharide core of a human IgG1 has previously been shown to result in an increased binding affinity to human Fc γ RIIIa and subsequently increased ADCC.

Binding of benralizumab to human eosinophils and soluble IL-5R α was studied by using flow cytometry and BIAcore methods, respectively. EC₅₀ values of parental fucosylated

anti-IL-5R mAb binding to eosinophils from humans was 26 pM. The K_D value for binding of benralizumab to human IL-5R α was 11 pM.

Benralizumab was assessed for binding to Fc γ R α s and ADCC activity as compared to the fucosylated parental anti-IL-5R α mAb. Examinations of the binding affinity of benralizumab to soluble human Fc γ R domains by surface plasmon resonance found that binding to human Fc γ RIIIa (K_D = 45.5 nM) was increased 6-fold compared with the fucosylated parental anti-IL-5R α mAb; however, binding was similar for all other Fc γ R α s.

The potency of benralizumab to mediate eosinophil and basophil apoptosis by ADCC *in vitro* was examined. In the presence, but not absence, of autologous NK effector cells, benralizumab induced eosinophil and basophil apoptosis, as assessed by means of Annexin V staining, with EC₅₀ values of 0.9 and 0.5 pM, respectively. However, when the fucosylated parental α IL-5R α mAb was used at concentrations 1000 times higher than the benralizumab EC₅₀ (in the presence of NK effector cells), it did not induce target cell apoptosis above background levels, although its binding affinity for IL-5R α and its potency to inhibit IL-5-induced cell proliferation were indistinguishable from those of benralizumab.

Potentially promotional wording/language was deleted from the first paragraph.

The second paragraph was modified to be essentially identical to labeling with approved IL-5 antagonist monoclonal antibodies, mepolizumab (NUCALA) and reslizumab (CINQAIR).

Recommended labeling:

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Benralizumab is a (b) (4) humanized afucosylated, monoclonal antibody (IgG1, kappa) (b) (4) that binds to the alpha subunit of the human interleukin-5 receptor (IL-5R α) with (b) (4) ~~(a dissociation constant of 11 (b) (4) pM)~~ (b) (4). The IL-5 receptor is (b) (4) expressed on the surface of eosinophils and basophils. In an *in vitro* setting, (b) (4) the absence of fucose in the Fc domain of benralizumab (b) (4) facilitate (b) (4) binding (45.5 nM) to (b) (4) Fc γ RIII receptors on immune effector cells, such as natural killer (NK) cells, leading to apoptosis of eosinophils and basophils through (b) (4) antibody-dependent cell-mediated cytotoxicity (ADCC).

Inflammation is an important component in the pathogenesis of asthma. Multiple cell types (e.g., mast cells, eosinophils, neutrophils, macrophages, lymphocytes) and mediators (e.g., histamine, eicosanoids, leukotrienes, cytokines) are involved in inflammation. Benralizumab, by binding to the IL-5R α chain, reduces (b) (4) eosinophils;

however, the mechanism of benralizumab action in asthma has not been definitively established.

Sponsor's Labeling:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of benralizumab.

Male and female fertility were unaffected based upon no adverse histopathological findings in the reproductive organs from cynomolgus monkeys treated with benralizumab for 9 months at IV doses up to 25 mg/kg or at SC doses of up to 30 mg/kg once every 2 weeks (approximately (b) (4) times the MRHD on an AUC basis, (b) (4)

Reviewer's Evaluation: The labeling in the first paragraph was modified to reflect available information from the published scientific literature regarding the potential roles of IL-5 and eosinophils in cancer. It was noted that this information was equivocal as the potential roles of IL-5 and eosinophils in cancer were not clear. The recommended labeling is essentially identical to labeling with approved IL-5 antagonist monoclonal antibodies, mepolizumab (NUCALA) and reslizumab (CINQAIR).

Recommended labeling:

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term animal studies have not been performed to evaluate the carcinogenic potential of benralizumab. Published literature using animal 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 that (b) (4) to (b) (4) IL-5R α (b) (4) such as benralizumab is unknown.

Male and female fertility were unaffected based upon no adverse histopathological findings in the reproductive organs from cynomolgus monkeys treated with benralizumab for 9 months at IV doses up to 25 mg/kg or at SC doses of up to 30 mg/kg once every 2 weeks (approximately 400 (b) (4) and 270 (b) (4) times the MRHD on an AUC basis; (b) (4)

12 Appendix/Attachments

Pharmacology and Toxicology reviews of nonclinical study reports under IND 100237 were reproduced in the present review rather than appending them.

APPEARS THIS WAY ON ORIGINAL

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
07/10/2017

CAROL M GALVIS
07/10/2017
I concur.

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR BLA

BLA Number: 761070

Applicant: MedImmune

Stamp Date: Nov. 16, 2016

Drug Name: Benralizumab

BLA Type: New

On initial overview of the 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 (b)(1) and (b)(2) 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).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		

**PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR
BLA**

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m ² or comparative serum/plasma levels) and in accordance with 201.57?	X		
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)	X		
11	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.
12	If the applicant is entirely or in part supporting the safety of their product by relying on nonclinical information for which they do not have the right to the underlying data (i.e., a 505(b)(2) application referring to a previous finding of the agency and/or literature), have they provided a scientific bridge or rationale to support that reliance? If so, what type of bridge or rationale was provided (e.g., nonclinical, clinical PK, other)?			Not applicable. The Sponsor owns the all of the data (351(a) application).

**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

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
01/09/2017