CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

761037Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology/Toxicology Review

Date:	October 19, 2016
From:	Timothy J. McGovern, PhD, ODE Associate Director for Pharmacology and Toxicology, OND IO
BLA:	761037
Agency receipt date:	October 30, 2015
Drug:	KEVZARA (sarilumab) Solution for Subcutaneous Injection
Sponsor:	Sanofi-Aventis

Indication: Adult patients with moderately to severely active rheumatoid arthritis who have an inadequate response or intolerance to one or more disease modifying anti-rheumatic drugs

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

The primary pharmacology/toxicology reviewer and team leader concluded that the nonclinical data for KEVZARA (sarilumab) Solution for Subcutaneous Injection support approval for the indication listed above.

KEVZARA is a subcutaneous (SC) injection product containing the active pharmaceutical ingredient sarilumab at concentrations of 131.6 mg/mL or 175 mg/mL per pre-filled syringe; the deliverable volume is 1.14 mL for a total delivered dose of either 150 or 200 mg. Sarilumab is a recombinant humanized monoclonal antibody (IgG1) consisting of two disulfide-bonded human heavy chains. Sarilumab binds to both soluble and membrane-bound human interleukin-6 receptor alpha (IL-6Rα) to inhibit IL-6 mediated signaling. The recommended doses of sarilumab are either 150 or 200 mg administered once every 2 weeks. The Established Pharmacologic Class (EPC) for sarilumab is "interleukin-6 (IL-6) receptor antagonist". Another member of this class [ACTEMRA (tocilizumab)] was approved in 2010.

Pivotal nonclinical studies of sarilumab were conducted in Cynomolgus monkeys by intravenous and SC dosing since only monkeys were identified as a relevant species for sarilumab in a series of pharmacology studies. Fertility and juvenile toxicity studies were also conducted in mice with an analogous monoclonal antibody (REGN844). In toxicology studies up to 6 months duration, no target organs of toxicity were identified in monkeys at IV doses up to 50 mg/kg/week (6 months duration) or SC doses up to 100 mg/kg/week (3 months duration). The most common treatment-related findings included injection site inflammation and decreased neutrophils, fibrinogen and/or C-reactive protein; the latter findings are considered pharmacodynamics effects of the drug. Decreases in primary and secondary IgG responses to antigen (KLH) challenge were observed in a TDAR evaluation and were attributed to the immunosuppressive properties of sarilumab. The NOAEL of 50 mg/kg/week from the 6-month study provided a safety margin of > 80-fold compared to the recommended clinical doses based on systemic exposure comparisons.

Genetic toxicity studies were not applicable for this therapeutic biologic monoclonal antibody. The sponsor submitted a carcinogenicity risk assessment for sarilumab and the Executive Carcinogenicity Assessment Committee agreed with the Division that no additional studies were required to address the carcinogenic potential of sarilumab and that it was reasonable to manage risk for immune suppression mediated tumor initiation and/or tumor promotion by appropriate labeling. Dr. Salicru's review provides a detailed evaluation of the overall carcinogenic risk of sarilumab and the Division discussed labeling for section 13.1 with the Pharmacology/Toxicology Associate Directors.

Development and reproductive studies were conducted with sarilumab in monkeys and with an analogue molecule (REGN844) in mice. There were no observed effects on fertility in mice. In an expanded prenatal and postnatal development study in monkeys, no effects on evaluated parameters in neonates were observed. The NOAEL was the high dose of 50 mg/kg/week. Sarilumab was detected in the serum of neonates up to one month after birth, suggesting the drug had crossed the placenta.

Conclusion: I agree with the Division pharmacology/toxicology conclusion that this BLA can be approved from the pharmacology/toxicology perspective. The EPC for sarilumab is appropriate. I have reviewed and am in agreement with labeling revisions proposed by the Division; the labeling follows the Pregnancy and Lactation Labeling Rule (PLLR) format and is consistent with current labeling recommendations.

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/s/

TIMOTHY J MCGOVERN
10/19/2016

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION OF PRE-FILLED SYRINGE FOR SARILUMAB FORMULATION

Application number:	BLA 761037
Supporting document/s:	1 and 35
Applicant's letter date:	10/30/2015 and 08/15/2016
CDER stamp date:	10/30/2015 and 08/15/2016
Product:	KEVZARA® (Sarilumab; Anti-IL-6 Receptor Monoclonal Antibody) solution for subcutaneous injection
Indication:	Treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more disease-modifying anti-rheumatic drugs.
Applicant:	Sanofi-Aventis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Eleni Salicru, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Christine Ford, RPh

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Sanofi-Aventis submitted Biologics License Application (BLA) 761037 on October 30, 2015, for KEVZARA (sarilumab). The sarilumab drug product is provided ready for subcutaneous injection in a single-use pre-filled syringe (PFS) that is available in dosage forms of 150 mg and 200 mg. This review is a nonclinical safety evaluation of potential leachables for the sarilumab drug product PFS. The Applicant provided forced extraction and leachable studies of container closure system (CCS) components. The CCS for the sarilumab PFS formulation consists of a bulk PFS, finger flange, and plunger rod. The bulk PFS comes into direct contact with the drug product and consists of the syringe barrel, staked needle, soft needle shield, and elastomeric plunger stopper.

1.2 Brief Discussion of Nonclinical Findings

An evaluation of forced extraction studies of the CCS for the sarilumab drug product was used to guide the detection and identification of potential leachables. Leachable studies identified (b) (4) as compounds that leached into the drug product. The highest level of (b) (4) detected was in the 15 month 2-8°C sample at about (b) (4) ppm ((b) (4) mcg/mL), which corresponds to (b) (4) mcg/syringe. The Occupational Safety and Health Administration(OSHA) of the United States Department of Labor has set permissible exposure limits of (b) (4) at (b) (4) mg/m^3 total weight average (TWA) which corresponds to (b) (4) mg/day ((b) (4) mcg/day) when adjusting for inhalation intake ((b) (4) m^3/8-hour workday). This permissible exposure limit provides about a 3100-fold safety margin. No adjustment was made for the difference in route of exposure (inhalation vs. subcutaneous) in the safety qualification as they were assumed to be approximately equivalent. (b) (4) is considered qualified for safety based on the respective low estimated total daily intakes from the leachable studies. Leachable studies identified levels of (b) (4) from both the 15 month and 34 month sarilumab samples with concentrations up to (b) (4) ppm ((b) (4) mcg/mL), which corresponds to (b) (4) mcg/syringe. This was considered acceptable based upon the Product Quality Research Institute (PQRI) qualification threshold of 5 mcg/day for non-genotoxic and non-irritant chemicals.

There are no nonclinical safety concerns based on results from the leachables studies.

2 Drug Information

2.1 Drug

CAS Registry Number: 1189541-98-7

Trade Name: KEVZARA®

Generic Name: Sarilumab

Code Name: REGN88, SAR153191

Chemical Name: Human immunoglobulin G1 (IgG1) specific for human interleukin 6 receptor alpha (covalent tetramer consisting of 2 heavy and 2 light chains)

Immunoglobulin G1, anti-(human interleukin-6 receptor subunit alpha (IL-6Rα, membrane glycoprotein 80, CD126)); human monoclonal REGN88 gamma1 heavy chain (219-214′)-disulfide with human monoclonal REGN88 kappa light chain dimer (225-225″:228-228″)-bisdisulfide

Amino Acid Sequence:

Sarilumab Heavy Chain Amino Acid Sequence

EVQLVESGGG LVQPGRSLRL SCAASRFTFD DYAMHWVRQA PGKGLEWVSG ISWNSGRIGY[60]
ADSVKGRFTI SRDNAENSLF LQMNGLRAED TALYYCAKGR DSFDIWGQGT MVTVSSASTK[120]
GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG ALTSGVHTFP AVLQSSGLYS[180]
LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF[240]
CPPC of heavy chain
LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR[300]
VVSVLTVLHQ DWLNGKEYKC KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN[360]
QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD GSFFLYSKLT VDKSRWQQGN[420]
VFSCSVMHEA LHNHYTQKSL SLSPGK[446]

Sarilumab Light Chain Amino Acid Sequence

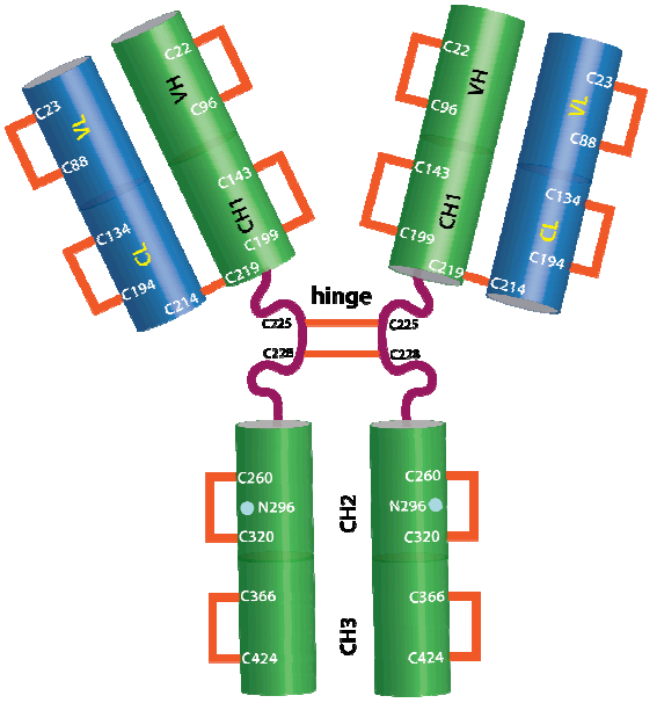
DIQMTQSPSS VSASVGDRVT ITCRASQGIS SWLAWYQQKP GKAPKLLIYG ASSLESGVPS[60]
RFSGSGSGTD FTLTISSLQP EDFASYYCQQ ANSFPYTFGQ GTKLEIKRTV AAPSVFIFPP[120]
SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT[180]
LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC[214]

Sarilumab antibody amino acid sequence with post-translational modifications denoted. The sequences of sarilumab heavy chain and light chain CDR regions are highlighted in blue. The cysteine residues (red) that have been confirmed to form the predicted disulfide bonds are connected by solid orange lines. The Fc N-linked glycosylation site at Asn[296] is highlighted in green. The heavy chain C-terminal Lys[446] (pink) is predominantly removed during protein expression.

Figure 1 Amino Acid Sequence of Sarilumab (Applicant's Figure)

Molecular Weight: 144 kDa

Structure:



Representation of the structure of sarilumab depicting the location of each of the intrachain and interchain disulfide bonds (orange). Heavy (green) and light (blue) chains are connected by interchain disulfide bonds; heavy chain dimerization is achieved through two heavy chain intermolecular disulfide bonds located within the hinge region. The Fc domain glycosylation site is also indicated (cyan).

Abbreviations: CH, constant region of heavy chain; CL, constant region of light chain; VH, variable region of heavy chain; VL, variable region of light chain

Figure 2 Schematic of Sarilumab Structure (Applicant's Figure)

Biochemical Description: Sarilumab is a recombinant human IgG1 monoclonal antibody consisting of two disulfide-bonded human heavy chains. Each heavy chain is covalently linked through a disulfide bond to a human kappa light chain. Sarilumab binds to both soluble and membrane-bound IL-6R.

Pharmacologic Class: IL-6R antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 100632: Sarilumab for indications of polyarticular juvenile idiopathic arthritis and rheumatoid arthritis (CDER/ODEII/DPARP)

(b) (4) (CDER/OAP/DTOP)

2.3 Drug Formulation

The sarilumab drug product is provided ready for subcutaneous injection in a single-use PFS that is available in dosage forms of 150 mg and 200 mg. Both dosage forms are formulated with (b) (4) histidine, (b) (4) arginine (b) (4) sucrose, polysorbate 20, and water for injection, as shown in **Table 1**.

Table 1 Qualitative Composition of Sarilumab Solution for Injection (Modified from Applicant's Table)

(b) (4)

2.4 Comments on Novel Excipients

No novel excipients are present in the drug product formulation.

2.5 Comments on Extractables and Leachables Studies with the CCS

The CCS for the sarilumab PFS formulation consists of a bulk PFS, finger flange, and plunger rod. The bulk PFS is considered the primary CCS for the sarilumab formulation, as it comes into direct contact with the drug product. The bulk PFS consists of the syringe barrel (1 mL long clear glass (b) (4) , staked needle (27 Gauge ½" (b) (4) , soft needle shield (elastomeric formulation), and elastomeric plunger

stopper [redacted]). **Figure 3** and **Figure 4** illustrate the PFS and its components.

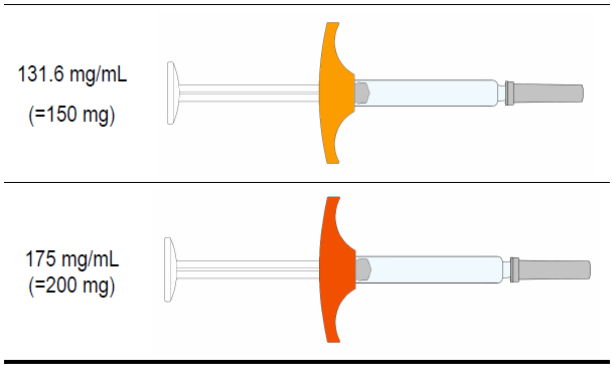


Figure 3 Illustration of PFS for Sarilumab Drug Product (Applicant's Figure)

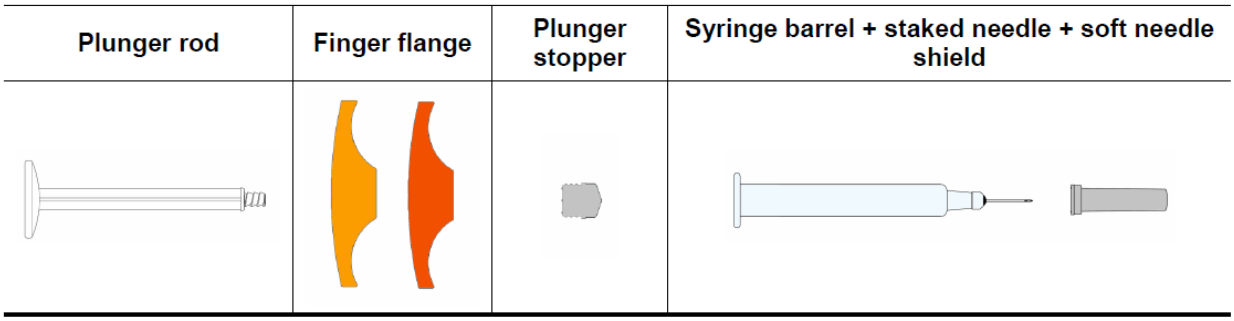


Figure 4 Illustration of PFS Components for Sarilumab Drug Product (Applicant's Figure)

This review is a nonclinical safety evaluation of potential leachables for the sarilumab PFS. Described in this section are the studies and the results; the nonclinical safety evaluation is included in the **Integrated Summary and Safety Evaluation** section.

Extractable Studies

An extractable evaluation was conducted on the CCS in order to identify possible leachables in the drug product. The glass component (syringe barrel) and rubber components (piston and needle shield) were exposed to exaggerated (stressed) conditions. The extraction conditions and analytical techniques used are shown in **Table 2**. As shown in **Table 2**, there were two pistons ([redacted]) and two needle shields ([redacted]) that were evaluated. Further, intact syringes containing either sarilumab [redacted] or placebo were exposed to extraction solvent in a 50°C incubator for 72 hours. The Applicant indicated that

extractables in intact syringes were considered more likely to become leachables than the component extracts.

The extracts were evaluated by inductively coupled plasma mass spectrometry (ICP-MS), direct injection gas chromatography mass spectrometry (GC-MS), headspace GC-MS (HS-GC-MS), and liquid chromatography with ultra violet detection mass spectrometry (LC-UV-MS) in order to determine metal compounds, semi-volatile organic compounds, volatile organic compounds, and non-volatile components, respectively. Results from the extractable studies informed the leachable studies, which are described below.

Table 2 Extraction Conditions and Analytical Techniques for Determination of Extractables (Applicant's Table)

Solvent System and Conditions		Component Evaluated (Yes/No)					Technique Used (Yes/No)			
		Barrel	Piston		Needle Shield	(b) (4)	LC-UV-MS CTP2685	GC-MS CTP3065	HS-GC-MS CTP3172	ICP-MS CTP2936
1M Sodium Chloride	121°C 1 Hr	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
pH 3 water		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Placebo		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
pH 9 water		Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
20% Ethanol	50°C 72 Hr	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Isopropanol		Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No
Hexane	Reflux 60°C 24 Hr	No*	Yes	Yes	Yes	Yes	No	Yes	No	No

* (b) (4) hexane reflux extraction is not required for this component

ICP-MS

ICP-MS analysis was used to determine potential metal compounds in the extracts from the syringe components exposed to stressed conditions. These extracts were compared with stressed and unstressed control samples. Elements that were present in the extracts from both the syringe components and the controls were not considered extractables. Any element that was significantly different than the control, or was not present in the control but was detected above the analytical evaluation threshold (AET) o (b) (4) mcg/mL, was evaluated as a potential leachable. Depending on the solvent system (pH 3, pH9, 1 M NaCl, or placebo), there were several elements that were detected above the AET of (b) (4) mcg/mL in either the barrel, needle shield ((b) (4) (b) (4) or piston ((b) (4)) extracts:

- (b) (4) was detected in the barrel ((b) (4) mcg/mL) and (b) (4) piston ((b) (4) mcg/mL) in pH 9 extracts.

- (b) (4) was detected in the (b) (4) needle shield in pH 3 extracts ((b) (4) mcg/mL), pH 9 extracts ((b) (4) mcg/mL), and 1 M sodium chloride (NaCl) extracts ((b) (4) mcg/mL).
- (b) (4) was detected in the barrel (b) (4) mcg/mL) and (b) (4) piston ((b) (4) mcg/mL) in pH 3 extracts; in the (b) (4) needle shield ((b) (4) mcg/mL) in pH 9 extracts; and in the (b) (4) needle shield ((b) (4) mcg/mL) and (b) (4) needle shield ((b) (4) mcg/mL) in 1 M NaCl extracts.
- (b) (4) was detected in the (b) (4) needle shield ((b) (4) mcg/mL), (b) (4) needle shield ((b) (4) mcg/mL), (b) (4) piston ((b) (4) mcg/mL), and (b) (4) piston ((b) (4) mcg/mL) in pH 3 extracts; in the (b) (4) needle shield ((b) (4) mcg/mL), (b) (4) needle shield ((b) (4) mcg/mL), and (b) (4) piston ((b) (4) mcg/mL) in pH 9 extracts; and in (b) (4) needle shield ((b) (4) mcg/mL) and (b) (4) piston ((b) (4) mcg/mL) in placebo extracts.
- (b) (4) was detected in the (b) (4) piston ((b) (4) mcg/mL) and (b) (4) piston ((b) (4) mcg/mL) in pH 3 extracts; and (b) (4) piston ((b) (4) mcg/mL) in placebo extracts.
- (b) (4) was detected in the (b) (4) needle shield ((b) (4) mcg/mL) and (b) (4) needle shield ((b) (4) mcg/mL) in pH 3 extracts; in the (b) (4) needle shield ((b) (4) mcg/mL) and (b) (4) needle shield ((b) (4) mcg/mL) in pH 9 extracts; in the (b) (4) needle shield ((b) (4) mcg/mL) in 1 M NaCl extracts; and in the (b) (4) needle shield ((b) (4) mcg/mL) and (b) (4) needle shield ((b) (4) mcg/mL) in placebo extracts.
- (b) (4) was detected in the barrel ((b) (4) mcg/mL) in 1 M NaCl extracts.

Intact syringes containing sarilumab placebo or (b) (4) were also evaluated under stressed conditions. Results from the extracts indicated that (b) (4) were present above the AET. For the placebo intact syringes, (b) (4) was detected at (b) (4) mcg/mL. For the (b) (4) intact syringes, (b) (4) were detected at (b) (4) mcg/mL, (b) (4) mcg/mL, and (b) (4) mcg/mL, respectively. The study report indicates that none of the three elements were detected at consistent concentrations between samples.

GC-MS

For direct injection GC-MS analysis for semi-volatile organic compounds, the total ion chromatograms (TIC) for each component extract solution under stressed conditions was compared to control samples. (b) (4) was used as a reference. (b) (4) was identified in the both the (b) (4) and (b) (4) rubber needle shield placebo extracts at about (b) (4) ppm, and also in the (b) (4) plunger placebo extract sample at about (b) (4) ppm. The Applicant indicated that this is not unexpected since (b) (4) is one of the main extract components in the organic stressed extracts. (b) (4) was identified in the (b) (4) rubber needle shield at (b) (4) ppm in the pH3 water extract, at (b) (4) ppm in the placebo sample, and at (b) (4) ppm to (b) (4) ppm in all the organic extracts (i.e., hexane, isopropanol, and 20% ethanol). (b) (4) was also identified in the (b) (4) rubber needle shield at (b) (4) ppm in the placebo extract, at (b) (4) ppm in the pH3 water samples, and at (b) (4) ppm in the organic extracts (i.e., 20% ethanol, isopropanol, and hexane).

(b) (4) was identified in the (b) (4) rubber needle shield in all of the aqueous, placebo, and organic extracts at about (b) (4) ppm. There were three unidentified peaks in the (b) (4) rubber needle shield extract. Peak 1 was in the pH3 water extract at about (b) (4) ppm. Peaks 2 and 3 were similar in the placebo extract and organic stressed extracts (i.e., isopropanol, hexane, and 20% ethanol) at about (b) (4) ppm and (b) (4) ppm, respectively. Of note, upon evaluation of the stressed and control intact (b) (4) and placebo syringes, (b) (4) (b) (4) and the unknown peaks were not identified. Lastly, upon evaluation of the (b) (4) plunger, an unidentified peak was found in the placebo extract at about (b) (4) ppm, in the hexane and 20% ethanol extracts at about (b) (4) ppm, and in the isopropanol extract at (b) (4) ppm. The peak was not in the intact (b) (4) or placebo stressed or control syringe samples.

HS-GC-MS

HS-GC-MS analysis was used to determine potential volatile organic compounds in the extracts from the syringe components, sarilumab (b) (4) intact syringes, and placebo intact syringes exposed to stressed conditions. These extracts were compared with stressed and unstressed control samples. (b) (4) were used as references. If the TIC for each extract was above the AET of (b) (4) mcg/mL and not present in the control samples further leachable evaluation was conducted.

Extracts from sarilumab (b) (4) and placebo intact syringes were not different from stressed and control samples. Also, no extractables were identified above the AET (b) (4) mcg/mL) in the extracts from the (b) (4) piston or (b) (4) needle shield. For the (b) (4) needle shield, (b) (4) was confirmed in all the aqueous extracts at about (b) (4) ppm to (b) (4) ppm. For the (b) (4) piston, (b) (4) was confirmed in the 1 M sodium chloride extract at about (b) (4) ppm. Further, one unidentified peak was present in the (b) (4) piston placebo extract at about (b) (4) ppm. This peak was not noted in other extract samples or in the sarilumab (b) (4) or placebo intact syringes. Two unidentified peaks were present in the glass barrel placebo extract at about (b) (4) ppm and (b) (4) ppm. Neither peak was detected in the other extract samples. Although the (b) (4) ppm peak was not detected in stressed and unstressed sarilumab (b) (4) and placebo intact syringes, a (b) (4) ppm peak was.

LC-UV-MS

LC-UV-MS analysis was used to determine potential non-volatile compounds in the extracts from the syringe components, sarilumab (b) (4) intact syringes, and placebo intact syringes exposed to stressed conditions. These extracts were compared with stressed and unstressed control samples. (b) (4) were used as external standards. Chromatogram results above the AET ((b) (4) mcg/mL), not present in the control, were evaluated. A peak was noted in the (b) (4) piston 20% extract at about (b) (4) ppb. Four peaks in the (b) (4) piston isopropanol sample were noted at estimated amounts ranging from (b) (4) ppm to (b) (4) ppm; one of the peaks was identified as (b) (4) The isopropanol samples of (b) (4) pistons showed four peaks as well, with estimated amounts ranging from (b) (4) ppb to (b) (4) ppm; as with the (b) (4) piston, one of the peaks was confirmed as (b) (4) (b) (4) was also confirmed in the (b) (4) (estimated

amounts of (b) (4) ppm in placebo extract and (b) (4) ppm in isopropanol extract) and (b) (4) (estimated amount of (b) (4) ppb in placebo extract) needle shields. No extractables were identified in the placebo syringe. In the (b) (4) syringe, one peak was noted above the AET in intact stressed syringes at levels ranging from (b) (4) ppb to (b) (4) ppb; this peak had also been noted in the (b) (4) and (b) (4) needle shields.

A summary of aqueous extractables detected above the AET, as summarized in the text above, is shown in **Table 3**.

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Table 3 Summary of Aqueous Extractables Detected Above the AET (Applicant's Table)

Report Page Number Reference	Compound	Identification criteria to be used for leachable monitoring	Detection Technique			
			ICP/MS	GC/MS	HS/GC/MS	LC/UV/MS
7-8	(b) (4)		X			
7-8			X			
7-8			X			
7-8			X			
7-8			X			
7-8			X			
11,28				X		
11				X		
11				X		
11				X		
11				X		
11				X		
12				X		
21					X	
21					X	
22					X	
22					X	
22					X	
29						X
32						X

*m/z = mass to charge ratio; RRT = Relative Retention Time

**RRT determined as needed to compare to peaks observed in leachable evaluation.

***m/z values in table are most abundant ion observed

Leachable Studies:
The leachable studies evaluated compounds that had leached from the CCS into the drug product. The extractable studies described above guided the leachable evaluation. Sarilumab (b) (4) stability samples stored at (b) (4) °C (recommended storage temperature) for 15 months (process validation samples) or 34 months (oldest available samples) were evaluated. The solutions were evaluated by ICP-MS, GC-MS, HS-GC-MS, and LC-UV-MS in order to determine metal compounds, semi-volatile organic compounds, volatile organic compounds, and non-volatile components, respectively. Any result above the AET ((b) (4) mcg/mL) was considered significant.

ICP-MS
ICP-MS was used to determine potential metal compounds as leachables. ICP-MS identified the presence of (b) (4) at levels above the AET in the 15 month sample at about (b) (4) ppm. (b) (4) was also detected in the 34 month sample but at levels below the AET. As a (b) (4) is used during manufacturing of the PFS, the study results support that (b) (4) is likely a leachable. (b) (4) were also detected above the AET, but were not considered by the Applicant as leachables due to their (b) (4) .

GC-MS
GC-MS analysis determined potential semi-volatile organic compounds as leachables by evaluating TIC. (b) (4) was used as a reference. Peaks above the AET were detected from both the 15 month and 34 month sarilumab samples with concentrations of (b) (4) ppm and (b) (4) ppm, respectively. The peak was confirmed to be (b) (4) an oxidation product of (b) (4). The mass spectrum and relative retention time of this peak matched an unknown peak identified in the extractable studies. In the extractable studies (aqueous and isopropanol extracts of the rubber piston and stressed and unstressed samples of the sarilumab (b) (4) and placebo intact syringes) this compound was detected at similar concentrations as in the leachable studies.

HS-GC-MS
HS-GC-MS analysis determined potential volatile organic compounds as leachables by evaluating TIC. There were no volatile organic compounds determined above the AET that were considered as leachables.

Of note, two peaks above the AET were identified by mass spectrum and retention time match as (b) (4). Both compounds were considered to be (b) (4) (b) (4) was measured in the leachable samples at about (b) (4) ppm to (b) (4) ppm. This amount was expected based on the amount present in (b) (4). (b) (4) was present in the leachable samples at about (b) (4) ppm to (b) (4) ppm.

LC-UV-MS
LC-UV-MS analysis determined potential non-volatile compounds as leachables by chromatogram. (b) (4) were used as external standards. Peaks above the AET were detected in both the 15 month and 34

month sarilumab samples with concentrations of (b) (4) ppm and (b) (4) ppm, respectively. The peak was confirmed to be (b) (4) an oxidation product of (b) (4). This compound was also identified by direct injection GC-MS. The mass spectrum and relative retention time of this peak matched an unknown peak identified in the extractable studies. In the extractable studies this peak was present in both the aqueous and organic extracts of the (b) (4) piston and also in stressed and unstressed samples of the sarilumab (b) (4) and placebo intact syringes.

3 Studies Submitted

3.1 Studies Reviewed

Title	eCTD Sequence Number	SDN	Module and Section
Extractable Evaluation of Prefilled Syringe Container Closure Components to be Used for Sarilumab (REGN88) Placebo and Drug Products (Report No.: REGN-150.10.164 [QUA-(b) (4) 2015-13433])	0000, 0034	1, 35	Module 3 Quality Section 3.2.P.2 (Pharmaceutical Development)
Leachable Evaluation of Pre-filled Syringes to be used with Sarilumab (REGN88) Drug Products (Report No.: REGN-150.10.173 [QUA-(b) (4)-2015-13435])	0000, 0034	1, 35	Module 3 Quality Section 3.2.P.2 (Pharmaceutical Development)
Identification and Toxicological Assessment of (b) (4) in Leachable and Extractable Samples (Report No.: REGN-150.5.480)	0000	1	Module 3 Quality Section 3.2.P.5.5 (Control of Drug Product, Characterization of Impurities)

Abbreviations: eCTD = electronic common technical document; SDN = supporting document number

11 Integrated Summary and Safety Evaluation

This review provides a safety assessment of potential leachables from the primary CCS for the sarilumab drug product, based on data submitted by the Applicant and other available information. The Applicant is seeking approval for the drug product at doses of 200 mg and 150 mg to be delivered once every two weeks via a subcutaneous injection from a single-dose PFS in a 1.14 mL volume. Of note, although the drug product is delivered once every two weeks, any comparisons made in this evaluation are based on acceptable levels per day. Adjustments for dosing intervals were not made in order to provide a conservative safety assessment. In general, for leachable evaluations, any nonmetal compound with expected patient exposure below the PQRI thresholds of 5 mcg/day (for compounds lacking genotoxic potential and irritant potential) and 1.5 mcg/day (for compounds with genotoxic potential) was considered qualified for safety regardless of the available nonclinical data.[1]

Leachable Evaluation:

[1]Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products. Product Quality Research Institute. August 2006.

Leachable ICP-MS studies identified [redacted] at levels above the AET of [redacted] mcg/mL (ppm). [redacted] were not considered to be leachables due to their [redacted]. On the other hand, since a [redacted] is used during manufacturing of the PFS, the study results support that [redacted] is likely a leachable. [redacted] is [redacted] used in numerous manufacturing processes.[2] [redacted] does not have an established permitted daily exposure (PDE) in the ICH Q3D guidance. It is classified by the National Institute for Occupational Safety and Health (NIOSH) Registry of Toxic Effects of Chemical Substances (RTECS) as having [redacted].[2] NIOSH has established occupational guidelines which recommend exposure limits of [redacted] on a TWA over an 8-hour workday as [redacted] mg/m^3 and a short term exposure limit (STEL) of [redacted] mg/m^3.[3] OSHA has set permissible exposure limits of [redacted] at [redacted] mg/m^3 TWA.[4] Using the more conservative [redacted] mg/m^3 exposure limit set by OSHA, and adjusting for inhalation intake ([redacted] m^3/8-hr workday), yields a level of [redacted] mg/day ([redacted] mcg/day).

In the sarilumab drug product leachable studies, [redacted] was detected in the 34 month sample but at levels below the AET. [redacted] levels in the 15 month 2-8°C sample were about [redacted] ppm ([redacted] mcg/mL). This level of [redacted] corresponds to [redacted] mcg/syringe. This amount is about 44000-fold less than the OSHA exposure limit of [redacted] mg/day ([redacted] mcg/day). Further, applying an adjustment factor of 14-fold to account for differences in exposure frequency and health status of workers compared to patients provides about a 3100-fold safety margin. No adjustment was made for the difference in route of exposure (inhalation vs. subcutaneous) in the safety qualification as they were assumed to be approximately equivalent. [redacted] is considered qualified for safety based on the respective low estimated total daily intakes from the leachable studies.

Leachable HS-GC-MS studies identified levels of [redacted] and [redacted] above the AET of [redacted] mcg/mL (ppm). Both compounds were considered to be [redacted] was measured in the leachable samples at about [redacted] ppm to [redacted] ppm. The Applicant indicated that this amount was expected based on the amount present in [redacted]. [redacted] was present in the leachable samples at about [redacted] ppm to [redacted] ppm. Both [redacted] are classified by [redacted] and are thus considered [redacted] with low toxic potential and of lower risk to human health.[5] [redacted] states that [redacted] mg per day or less (corresponding to [redacted] ppm) would be acceptable without justification. The levels of [redacted] identified from the leachable analysis are both well below this suggested amount.

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[2]U.S. National Library of Medicine ChemIDplus website search for [redacted] http://chem.sis.nlm.nih.gov/chemidplus/name/[redacted]

[3]NIOSH Pocket Guide to Chemical Hazards website: http://www.cdc.gov/niosh/npg/[redacted]

[4]OSHA Chemical Sampling Information for [redacted] Soluble Compound: https://www.osha.gov/dts/chemicalsampling/data/[redacted]

[5]Impurities: [redacted].

In addition an unknown compound was also identified by GC-MS and LC-MS as a potential leachable compound. This compound had been previously identified as an unknown semi-volatile organic compound by GC-MS and LC-MS in extractable studies of the PFS. In the extractable studies, the unknown compound was identified by retention time and mass spectra in samples of the (b) (4) piston.

After the unknown compound was detected in the leachable studies, an attempt was made to identify it. Comparison to mass spectra in the National Institute of Standards and Technology (NIST) database did not determine any potential candidates. Still, it was determined that the unknown compound shared several ions with mass to charge ratios that matched the fragmentation pattern of (b) (4) Thus, it was speculated that the unknown compound was somehow related to (b) (4) which is used in the manufacture of the (b) (4) pistons. Further, a peak from a sample of (b) (4) oxidized in the lab matched that of the unknown compound. After further experimental evaluations, the unknown compound was confirmed as (b) (4) which is an oxidation product of (b) (4). In theory the process of oxidation should makes (b) (4) less reactive and/or toxic than (b) (4) **Table 4** provides the chemistry information for (b) (4) and (b) (4)

Table 4 Chemistry Information for (b) (4) and (b) (4)

Compound:	(b) (4)
CASRN:	
Molecular Formula:	
Structure:	
Molecular Weight:	

[1]From ChemIDplus Toxnet Database
Abbreviations: CASRN = Chemical Abstracts Service Registry Number

Limited toxicity information is publically available for (b) (4) As such, the literature evaluation provided here pertains to the available information for its parent compound (b) (4) (b) (4) In the pharmaceutical industry,

(b) (4)

(b) (4) Relevant information from these evaluations are summarized below.

- (b) (4)
- **Irritation:** (b) (4) mg undiluted (b) (4) was found to be slightly irritating to the skin of rabbits after 24-hour semi-occlusive application on intact and abraded skin. (b) (4) was also slightly irritating to the eye of rabbits when tested in the Draize test.[12]
- **Repeat dose toxicity:** After 28-day exposure in rats at 25, 250, and 500 mg/kg/day (b) (4) the liver was the main target organ of toxicity at doses of 250 and 500 mg/kg/day. The NOAEL was (b) (4) mg/kg/day.[12] In long term feeding studies male rats were fed diets equivalent to about 7.5, 23, 75, 22, and 450 mg/kg/day (b) (4) for up to 76 weeks. In this study, the liver was identified as a target organ at doses greater than 75 mg/kg/day.[12]
- **Genetic toxicity:** (b) (4) was not mutagenic in the Ames assay at levels up to 10000 mcg/plate, with and without metabolic activation.[12] Overall, (b) (4) was not clastogenic in vitro (chromosome aberration assays, sister chromatid exchange assays, DNA damage and repair assays) or in vivo (bone marrow micronucleus assay with mice, dominant lethal assay with rats, heritable translocation assay with mice).[12]
- **Carcinogenicity:** In a bioassay for possible carcinogenicity, groups of 50 F344 rats/sex and 50 B6C3F1 mice/sex were fed either 3000 ppm or 6000 ppm of (b) (4) for either 105 weeks or for 107 or 108 weeks, respectively. The NTP concluded that under the study conditions, (b) (4) was not carcinogenic.[13]

(b) (4)

- **Developmental Toxicity:** Based on data from two oral teratogenicity tests in mice, there was no evidence that (b) (4) was teratogenic at repeat doses of up to 800 mg/kg/day during gestation days 7 and 13 or after a single dose of (b) (4) up to 1800 mg/kg.
- **Other Toxicities:** Numerous studies in mice have evaluated the possible effect of repeat dose high exposures of (b) (4) on lung toxicity and shown that lung damage may occur at doses of 450 mg/kg.

Leachable GC-MS studies identified levels of (b) (4) from both the 15 month and 34 month sarilumab samples with concentrations of (b) (4) ppm and (b) (4) ppm, respectively. Similarly, leachable LC-UV-MS studies identified levels of (b) (4) in both the 15 month and 34 month sarilumab samples with concentrations of (b) (4) ppm and (b) (4) ppm, respectively. The highest level of detected (b) (4) (i.e., (b) (4) ppm or (b) (4) mcg/mL) corresponds to (b) (4) mcg/syringe. Since the literature for (b) (4) indicated that it lacks genotoxic potential, and the exposure of (b) (4) is below the PQRI threshold of 5 mcg/day, it is considered qualified for safety.

(b) (4) was employed during manufacturing to (b) (4). Of note, (b) (4) were identified as subvisible particles upon evaluation for particulate matter by microflow imaging (MFI). However, (b) (4) was not detected as an extractable or a leachable.

Overall, there appear to be no nonclinical safety concerns for the sarilumab drug product as described in the leachable studies for the PFS primary CCS.

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/s/

ELENI M SALICRU
09/22/2016

TIMOTHY W ROBISON
09/22/2016
I concur

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

PHARMACOLOGY/TOXICOLOGY BLA LABELING REVIEW AND EVALUATION

Application number:	BLA 761037
Supporting document/s:	1 and 13
Applicant's letter date:	10/30/2015 and 03/16/2016
CDER stamp date:	10/30/2015 and 03/16/2016
Product:	KEVZARA® (Sarilumab; Anti-IL-6 Receptor Monoclonal Antibody) Solution for Subcutaneous Injection
Indication:	Treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more disease-modifying anti-rheumatic drugs.
Applicant:	Sanofi-Aventis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Eleni Salicru, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Christine Ford, RPh

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761037 are owned by Sanofi-Aventis or are data for which Sanofi-Aventis has obtained a written right of reference.
Any information or data necessary for approval of BLA 761037 that Sanofi-Aventis does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug,

as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 761037.

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1 Executive Summary

1.1 Introduction

Sanofi-Aventis submitted Biologics License Application (BLA) 761037 on October 30, 2015, for KEVZARA (sarilumab). This is a review of the nonclinical sections of the Applicant's proposed labeling submitted on March 16, 2016 (supporting document number [SDN] 13). This review evaluated the Applicant's proposed prescribing information for Indications and Usage and Use in Specific Populations (both under Highlights of Prescribing Information), Section 8.1 (Pregnancy), Section 8.3 (Lactation), Section 11 (Description; first paragraph only), Section 12.1 (Mechanism of Action), and Section 13 (Nonclinical Toxicology). An integrated review and evaluation of the nonclinical pharmacology and toxicology studies to support the safety of sarilumab for approval was completed on August 24, 2016.

1.3 Recommendations

1.3.3 Labeling

Provided below are the recommended nonclinical changes to the sections of the Applicant's proposed prescribing information mentioned in the **Introduction**.

The underlined text is recommended for insertion and the ~~strikethrough~~ text is recommended for deletion.

HIGHLIGHTS OF PRESCRIBING INFORMATION
INDICATIONS AND USAGE
KEVZARA® is an interleukin-6 (IL-6) receptor antagonist indicated for treatment of (b) (4) adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more Disease-Modifying Anti-Rheumatic Drugs (DMARDs). (1.1)

USE IN SPECIFIC POPULATIONS

(b) (4)

- (b) (4) Discontinue drug or nursing taking into consideration importance of drug to mother. (8.2)

(b) (4)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to KEVZARA during pregnancy. Physicians are encouraged to register

patients and pregnant women are encouraged to register themselves by calling 1-8NN-NNN-NNNN.

Risk Summary

(b) (4) The limited human data with KEVZARA (b) (4) -in pregnant women are not sufficient to inform drug-associated risk for major birth defects and miscarriage. (b) (4) Monoclonal antibodies, such as sarilumab, are actively transported across the placenta (b) (4) -during the third trimester of pregnancy and may affect immune response in the in utero exposed infant *[see Clinical Considerations]*. From animal data, and consistent with the mechanism of action, levels of IgG, in response to antigen challenge, may be reduced in the fetus/infant of treated mothers *[see Clinical Considerations and Animal Data]*. In an animal reproduction study (b) (4), consisting of a combined embryo-fetal and pre- and postnatal development study with monkeys that received intravenous administration of sarilumab, there was no evidence of embryotoxicity or fetal malformations with exposures up to (b) (4) pproximately 84 times the (b) (4) maximum recommended human dose (MRHD) *[see Animal Data]*. The literature suggests that inhibition of IL-6 signaling may interfere with cervical ripening and dilatation and myometrial contractile activity leading to potential delays of parturition *[see Animal Data]*.

(b) (4) The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. (b) (4) All pregnancies, (b) (4) have a background (b) (4) risk of birth defect, loss or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is (b) (4) -2 to 4% (b) (4) and 15 to 20%, respectively (b) (4) ~~KEVZARA should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.~~

Clinical Considerations

Fetal/Neonatal Adverse Reactions

Monoclonal antibodies are increasingly transported across the placenta as pregnancy progresses, with the largest amount transferred during the third trimester. Risks and benefits should be considered prior to administering live or live-attenuated vaccines to infants exposed to KEVZARA in utero *[see Warnings and Precautions (5.6)]*. From the animal data, and consistent with the mechanism of action, levels of IgG, in response to antigen challenge, may be reduced in the fetus/infant of treated mothers *[see Animal Data]*.

Data

Animal Data

In (b) (4) a combined embryo-fetal and (b) (4) pre-/and postnatal development (b) (4) study, pregnant cynomolgus monkeys (b) (4) received sarilumab (b) (4) at intravenous (b) (4) doses of 0, 5, 15, or 50 mg/kg/week from confirmation of pregnancy at (b) (4) gestation day (GD) 20, throughout the period of organogenesis (up to approximately GD 50), and continuing to natural birth of infants at around GD 165. (b) (4) Maintenance of pregnancy was not affected at any doses. Sarilumab was not embryotoxic or teratogenic with exposures up to (approximately 84 times the (b) (4) MRHD (based on AUC with maternal intravenous (b) (4) doses (b) (4) up to 50 mg/kg/(b) (4) week (b) (4) Sarilumab had no effect on (b) (4) growth and development evaluated up to one month after birth. (b) (4) Sarilumab was detected in the serum of neonates up to one month after birth, suggesting that the antibody had crossed the placenta. (b) (4)

Following antigen challenge, decreased IgG titers attributed to the immunosuppressive action of sarilumab were evident in studies with older monkeys, with exposures up to approximately 80 times the MRHD (based on AUC with intravenous doses up to 50 mg/kg/week) and juvenile mice treated with an analogous antibody, which binds to murine IL-6Rα to inhibit IL-6 mediated signaling, at subcutaneous doses up to 200 mg/kg/week. These findings suggest the potential for decreased IgG titers, following antigen challenge, in infants of mothers treated with sarilumab.

Parturition is associated with significant increases of IL-6 in the cervix and myometrium. The literature suggests that inhibition of IL-6 signaling may interfere with cervical ripening and dilatation and myometrial contractile activity leading to potential delays of parturition. For mice deficient in IL-6 ($Il6^{-/-}$ null mice), parturition was delayed relative to wild-type ($Il6^{+/+}$) mice. Administration of recombinant IL-6 to $Il6^{-/-}$ null mice restored the normal timing of delivery.

8.2 Lactation

Risk Summary

(b) (4) No information is available (b) (4) on the presence of sarilumab in human milk, the effects of the drug on the breastfed infant, or the effects of the drug on milk production. Maternal IgG is present in human milk. If sarilumab is transferred into human milk, the effects of local exposure in the gastrointestinal tract and potential limited systemic exposure in the infant to sarilumab are unknown. The lack of clinical

data during lactation precludes clear determination of the risk of KEVZARA to an infant during lactation; therefore, the developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for KEVZARA and the potential adverse effects on the breastfed child from sarilumab or from the underlying maternal condition. (b) (4)

11 DESCRIPTION

(b) (4) Sarilumab is a recombinant (b) (4) monoclonal antibody of the IgG1 subclass ~~that binds IL-6 recepto~~ (b) (4) (b) (4)

Sarilumab is produced by recombinant DNA technology in Chinese Hamster Ovary cell suspension culture.

(b) (4)

(b) (4)

KEVZARA (sarilumab) injection (b) (4) for subcutaneous administration is supplied as a sterile, colorless to pale yellow, preservative-free (b) (4) solution of approximately pH 6.0 (b) (4) KEVZARA is supplied in a single-dose pre-filled syringe. Each syringe delivers 1.14 mL of solution containing ~~(~~200 mg or 150 mg~~)~~ of sarilumab, (b) (4) arginine (8.94 mg (b) (4)), histidine (3.71 mg (b) (4)), polysorbate 20 (2.28 mg (b) (4)), sucrose (57 mg (b) (4)) and Water for Injection, USP.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Sarilumab binds (b) (4) to both soluble and membrane-bound IL-6 receptors (sIL-6R~~α~~ and mIL-6R~~α~~), and has been shown to inhibit~~s~~ IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes, and fibroblasts. IL-6 has been shown to be involved in (b) (4) diverse (b) (4) physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation~~,~~ and differentiation~~,~~ (b) (4)

(b) (4)

IL-6 (b) (4) is also produced by synovial and endothelial cells leading to local production of IL-6 in (b) (4) joints (b) (4) affected by inflammatory processes such as rheumatoid arthritis. (b) (4)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term animal studies have been performed to establish the carcinogenicity potential of sarilumab. The literature supports that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. Available published evidence also supports that IL-6 signaling through the IL-6 receptor may be involved in pathways that lead to increased tumor growth, particularly at high levels of IL-6 exceeding normal physiological levels. The malignancy risk in humans from an antibody that disrupts signaling through the IL-6 receptor, such as sarilumab, is not clear. (b) (4)

Fertility (b) (4) and reproductive performance were unaffected in male and female mice (b) (4) treated with an analogous antibody, which binds to murine (b) (4) IL-6Rα (b) (4) to inhibit IL-6 mediated signaling, at subcutaneous doses of 10, 25, and 100 mg/kg twice per week.

11 Integrated Summary and Safety Evaluation

Presented below is the Applicant's proposed wording for the KEVZARA (sarilumab) product insert. The Applicant's proposed text is from their draft prescribing information dated March 16, 2016 (SDN 13). Also presented below are the nonclinical revisions to the Applicant's proposed labeling and a rationale for the proposed changes. The underlined text is recommended for insertion and the ~~strikethrough~~ text is recommended for deletion from the Applicant's proposed text.

The approved label for ACTEMRA (tocilizumab) was used for reference as it is the first approved IL-6 receptor antagonist, which is the same FDA established pharmacologic class (EPC) as sarilumab. Further, the product insert for ACTEMRA was undergoing Pregnancy and Lactation Labeling Rule (PLLR) conversion with consultation from the Division of Pediatric and Maternal Health (DPMH) and was referenced for language pertaining to Sections 8.1 and 8.2.

Applicant's Proposed Labeling for Indications and Usage and Use in Specific Populations in the Highlights of Prescribing Information Section:

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

KEVZARA® is an interleukin-6 (IL-6) receptor antagonist indicated for treatment of (b) (4) adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more Disease-Modifying Anti-Rheumatic Drugs (DMARDs). (1.1)

USE IN SPECIFIC POPULATIONS

(b) (4)

- (b) (4) Discontinue drug or nursing taking into consideration importance of drug to mother. (8.2)

(b) (4)

DPARP's Proposed Nonclinical Labeling for Indications and Usage and Use in Specific Populations in the Highlights of Prescribing Information Section:

HIGHLIGHTS OF PRESCRIBING INFORMATION

INDICATIONS AND USAGE

KEVZARA® is an interleukin-6 (IL-6) receptor antagonist indicated for treatment of (b) (4) adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more Disease-Modifying Anti-Rheumatic Drugs (DMARDs). (1.1)

USE IN SPECIFIC POPULATIONS

(b) (4)

- (b) (4) Discontinue drug or nursing taking into consideration importance of drug to mother. (8.2)

(b) (4)

Rationale for Changes in Indications and Usage and Use in Specific Populations in the Highlights of Prescribing Information Section:

General edits were made to remove unnecessary text.

Nonclinical reviewed the EPC class for sarilumab and no nonclinical changes were suggested. IL-6 receptor antagonist is recognized as an FDA EPC. The pharmacology studies conducted with sarilumab support the classification of sarilumab as an IL-6 receptor antagonist.

The first bullet statement under the Use in Specific Populations was removed based on discussions with the Clinical Team. (b) (4)

(b) (4)

Applicant's Proposed Labeling for Sections 8.1 and 8.2:

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to KEVZARA during pregnancy. Physicians are encouraged to register patients and pregnant women are encouraged to register themselves by calling 1-8NN-NNN-NNNN.

Risk Summary

(b) (4) with KEVZARA (b) (4) in pregnant women. (b) (4) monoclonal antibodies are transported across the placenta (b) (4) during the third trimester. In animal reproduction (b) (4) intravenous administration of sarilumab to (b) (4) pproximately 84 times the (b) (4) (b) (4) (b) (4) all pregnancies, (b) (4) have a background (b) (4) of (b) (4) 2 to 4% (b) (4) and 15 to 20% (b) (4). KEVZARA should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Animal Data

In (b) (4) pre-/postnatal development (b) (4) study, pregnant cynomolgus monkeys (b) (4) sarilumab (b) (4) intravenous (b) (4) doses of 5, 15, or 50 mg/kg/week from (b) (4) gestation to natural birth (b) (4) (approximately 84 times the (b) (4) based on AUC (b) (4) Sarilumab had no effect on (b) (4) evaluated up to one month after birth (b) (4) Sarilumab was detected in the serum of neonates up to one month. (b) (4)

8.2 Lactation

Risk Summary

(b) (4) no information (b) (4) the presence of sarilumab in human milk, the effects on the breastfed infant, or the effects on milk production. (b) (4)

DPARP's Proposed Nonclinical Labeling for Sections 8.1 and 8.2:
8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to KEVZARA during pregnancy. Physicians are encouraged to register patients and pregnant women are encouraged to register themselves by calling 1-8NN-NNN-NNNN.

Risk Summary

(b) (4) The limited human data with KEVZARA (b) (4) in pregnant women are not sufficient to inform drug-associated risk for major birth defects and miscarriage. (b) (4) Monoclonal antibodies, such as sarilumab, are actively transported across the placenta (b) (4) during the third trimester of pregnancy and may affect immune response in the in utero exposed infant *[see Clinical Considerations]*. From animal data, and consistent with the mechanism of action, levels of IgG, in response to antigen challenge, may be reduced in the fetus/infant of treated mothers *[see Clinical Considerations and Animal Data]*. In an animal reproduction study (b) (4), consisting of a combined embryo-fetal and pre- and postnatal development study with monkeys that received intravenous administration of sarilumab, there was no evidence of embryotoxicity or fetal malformations with exposures up to (b) (4) pproximately 84 times the (b) (4) maximum recommended human dose (MRHD) *[see Animal Data]*. The literature suggests that inhibition of IL-6 signaling may interfere with cervical ripening and dilatation and myometrial contractile activity leading to potential delays of parturition *[see Animal Data]*.

(b) (4) The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. (b) (4) All pregnancies,

(b) (4) have a background (b) (4) risk of birth defect, loss or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is (b) (4) 2 to 4% (b) (4) and 15 to 20%, respectively (b) (4) pregnancy loss. ~~KEVZARA should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.~~

Clinical Considerations

Fetal/Neonatal Adverse Reactions

Monoclonal antibodies are increasingly transported across the placenta as pregnancy progresses, with the largest amount transferred during the third trimester. Risks and benefits should be considered prior to administering live or live-attenuated vaccines to infants exposed to KEVZARA in utero *[see Warnings and Precautions (5.6)].* From the animal data, and consistent with the mechanism of action, levels of IgG, in response to antigen challenge, may be reduced in the fetus/infant of treated mothers *[see Animal Data].*

Data

Animal Data

In (b) (4) a combined embryo-fetal and (b) (4) pre-/and postnatal development (b) (4) study, pregnant cynomolgus monkeys (b) (4) received sarilumab (b) (4) at intravenous (b) (4) doses of 0, 5, 15, or 50 mg/kg/week from confirmation of pregnancy at ~~early~~ gestation day (GD) 20, throughout the period of organogenesis (up to approximately GD 50), and continuing to natural birth of infants at around GD 165. (b) (4) Maintenance of pregnancy was not affected at any doses. Sarilumab was not embryotoxic or teratogenic with exposures up to (approximately 84 times the (b) (4) MRHD (based on AUC with maternal intravenous (b) (4) doses (b) (4) up to 50 mg/kg/- (b) (4) (b) (4) Sarilumab had no effect on (b) (4) growth and development evaluated up to one month after birt (b) (4) Sarilumab was detected in the serum of neonates up to one month after birth, suggesting that the antibody had crossed the placenta. (b) (4)

Following antigen challenge, decreased IgG titers attributed to the immunosuppressive action of sarilumab were evident in studies with older monkeys, with exposures up to approximately 80 times the MRHD (based on AUC with intravenous doses up to 50 mg/kg/week) and juvenile mice treated with an analogous antibody, which binds to murine IL-6Rα to inhibit IL-6 mediated signaling, at subcutaneous doses up to 200

mg/kg/week. These findings suggest the potential for decreased IgG titers, following antigen challenge, in infants of mothers treated with sarilumab.

Parturition is associated with significant increases of IL-6 in the cervix and myometrium. The literature suggests that inhibition of IL-6 signaling may interfere with cervical ripening and dilatation and myometrial contractile activity leading to potential delays of parturition. For mice deficient in IL-6 ($Il6^{-/-}$ null mice), parturition was delayed relative to wild-type ($Il6^{+/+}$) mice. Administration of recombinant IL-6 to $Il6^{-/-}$ null mice restored the normal timing of delivery.

8.2 Lactation

Risk Summary

(b) (4) No information is available (b) (4) on the presence of sarilumab in human milk, the effects of the drug on the breastfed infant, or the effects of the drug on milk production. Maternal IgG is present in human milk. If sarilumab is transferred into human milk, the effects of local exposure in the gastrointestinal tract and potential limited systemic exposure in the infant to sarilumab are unknown. The lack of clinical data during lactation precludes clear determination of the risk of KEVZARA to an infant during lactation; therefore, the developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for KEVZARA and the potential adverse effects on the breastfed child from sarilumab or from the underlying maternal condition. (b) (4)

Rationale for Changes in Sections 8.1 and 8.2:
General edits were made to Sections 8.1 and 8.2 to add appropriate subheadings and to remove unnecessary text. DPMH was not consulted with regards to Sections 8.1 and 8.2 of this product insert. However, the product insert for ACTEMRA was undergoing PLLR conversion with DPMH consultation. As such, Sections 8.1 and 8.2 of the KEVZARA product label were modified to reflect general changes to the ACTEMRA product label that apply to both products (e.g., placental transfer of monoclonal antibodies, consideration of risks and benefits before administering live or live-attenuated vaccines to infants, estimated background risk of major birth defects and miscarriage, presence of maternal IgG in human milk and the unknown effects in the gastrointestinal tract if drug was transferred into human milk, etc.).

Additional language is proposed for the Risk Summary and Animal Data in Section 8.1 to highlight that IL-6 is involved in successful parturition and that blocking the IL-6 receptor by sarilumab may interfere with successful parturition in pregnant females taking sarilumab. The literature suggests that IL-6 is broadly expressed in the female reproductive tract and gestational tissues and has been shown to be involved in embryo implantation, placental development, and pregnancy tolerance.[1] IL-6 plays a role in the

[1]Prins JR, Gomez-Lopez N, Robertson SA. 2012. Interleukin-6 in pregnancy and gestational disorders.

local inflammation at the time of parturition to recruit leukocytes to the cervix and uterine wall, which induce cervix ripening and dilatation.[2,3,4] For mice deficient in IL-6 ($Il6^{-/-}$ null mice), parturition was delayed relative to wild-type ($Il6^{+/+}$) mice.[5]

For the animal data, more details were provided about the timing of sarilumab exposure in the ePPND study. Language was added to indicate that sarilumab in the serum of neonates up to one month after birth suggests placental transfer of the drug. Also, in the 6-month once weekly IV study of sarilumab in monkeys both primary and secondary IgG responses to KLH were slightly decreased with ≥5 mg/kg/week of the test article. In the juvenile toxicity study in mice with REGN844 (murine surrogate monoclonal antibody to sarilumab that binds mouse IL-6 receptor α), SC doses up to 200 mg/kg/week slightly decreased IgG responses after antigen challenge in males at all doses and at all the time points evaluated. As such, language was added that based on the effects of sarilumab on IgG titers in older monkeys following antigen challenge (with exposures up to approximately 80 times the MRHD [based on AUC with intravenous doses up to 50 mg/kg/week]) and juvenile mice treated with an analogous antibody which binds to murine IL-6 receptor α, there is the potential for decreased IgG titers, following antigen challenge, in infants of mothers treated with sarilumab.

Applicant's Proposed Labeling for Section11:

11 DESCRIPTION

(b) (4) is a human (b) (4) monoclonal antibody that binds (b) (4) IL-6 receptor (b) (4)

Sarilumab is produced by recombinant DNA technology in Chinese Hamster Ovary cell suspension culture.

(b) (4)

(b) (4)

J Reprod Immunol 95(1-2):1-14.

[2]Hassan SS, Romero R, Haddad R, et al. 2006. The transcriptome of the uterine cervix before and after spontaneous term parturition. Am J Obstet Gynecol 195(3):778-786.

[3]Törnblom SA, Klimaviciute A, Byström B, et al. 2005. Non-infected preterm parturition is related to increased concentrations of IL-6, IL-8 and MCP-1 in human cervix. Reprod Biol Endocrinol 3:39-49.

[4]Winkler M. 2003. Role of cytokines and other inflammatory mediators. BJOG 110(20):118-123.

[5]Robertson SA, Christiaens I, Dorian CL, et al. 2010. Interleukin-6 is an essential determinant of on-time parturition in the mouse. Endocrinology 151(8):3996-4006.

KEVZARA [redacted] for subcutaneous administration is supplied as a sterile, colorless to pale yellow, preservative-free liquid [redacted] of approximately pH 6.0. [redacted] is supplied in a single-dose pre-filled syringe. Each syringe delivers 1.14 mL (200 mg or 150 mg) of [redacted] arginine ([redacted]), histidine ([redacted]), polysorbate 20 [redacted]), sucrose ([redacted]) and Water for Injection, USP.

DPARP's Proposed Nonclinical Labeling for Section 11:
11 DESCRIPTION

[redacted] Sarilumab is a recombinant [redacted] monoclonal antibody of the IgG1 subclass ~~that binds~~ [redacted]

Sarilumab is produced by recombinant DNA technology in Chinese Hamster Ovary cell suspension culture.

[redacted]

[redacted]

KEVZARA (sarilumab) injection [redacted] for subcutaneous administration is supplied as a sterile, colorless to pale yellow, preservative-free [redacted] solution of approximately pH 6.0 [redacted] KEVZARA is supplied in a single-dose pre-filled syringe. Each syringe delivers 1.14 mL of solution containing ~~(~~200 mg or 150 mg~~)~~ of sarilumab, [redacted] arginine (8.94 mg [redacted]), histidine (3.71 mg [redacted]), polysorbate 20 (2.28 mg [redacted]), sucrose (57 mg [redacted]) and Water for Injection, USP.

Rationale for Changes in Section 11:
Revisions to the first paragraph of Section 11 were made by nonclinical to be consistent with the ACTEMRA label. [redacted] "sarilumab" was added to the first sentence because the first paragraph describes the drug substance. Information related to [redacted]

Edits in the remaining paragraphs of Section 11 were made by the Office of Biotechnology Products (OBP).

Applicant's Proposed Labeling for Section 12.1:
12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Sarilumab binds (b) (4) to both soluble and membrane-bound IL-6 receptors (sIL-6R (b) (4) and mIL-6R (b) (4), and inhibits IL-6-mediated signaling. IL-6 is a pleiotropic cytokine (b) (4) diverse (b) (4) such as proliferation, differentiation, (b) (4)

DPARP's Proposed Nonclinical Labeling for Section 12.1:
12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Sarilumab binds (b) (4) to both soluble and membrane-bound IL-6 receptors (sIL-6R (b) (4) and mIL-6R (b) (4)), and has been shown to inhibits IL-6-mediated signaling through these receptors. IL-6 is a pleiotropic pro-inflammatory cytokine produced by a variety of cell types including T- and B-cells, lymphocytes, monocytes, and fibroblasts. IL-6 has been shown to be involved in (b) (4) diverse (b) (4) physiological processes such as T-cell activation, induction of immunoglobulin secretion, initiation of hepatic acute phase protein synthesis, and stimulation of hematopoietic precursor cell proliferation, and differentiation, (b) (4)

IL-6 (b) (4) is also produced by synovial and endothelial cells leading to local production of IL-6 in (b) (4) joints (b) (4) affected by inflammatory processes such as rheumatoid arthritis. (b) (4)

Rationale for Changes in Section 12.1:
Revisions to Section 12.1 were made to be consistent with the ACTEMRA label, as both KEVZARA and ACTEMRA are IL-6 receptor antagonists with a similar mechanism of action. Also, any potential (b) (4) was removed. The last sentence was removed because (b) (4)

Applicant's Proposed Labeling for Section 13.1:
13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term animal studies have been performed to establish the carcinogenicity potential of sarilumab. (b) (4)

Fertility (b) (4) in male and female mice (b) (4) antibody (b) (4) IL-6Rα (b) (4)

DPARP's Proposed Nonclinical Labeling for Section 13.1:
13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term animal studies have been performed to establish the carcinogenicity potential of sarilumab. The literature supports that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. Available published evidence also supports that IL-6 signaling through the IL-6 receptor may be involved in pathways that lead to increased tumor growth, particularly at high levels of IL-6 exceeding normal physiological levels. The malignancy risk in humans from an antibody that disrupts signaling through the IL-6 receptor, such as sarilumab, is not clear. (b) (4)

Fertility (b) (4) and reproductive performance were unaffected in male and female mice (b) (4) treated with an analogous antibody, which binds to murine (b) (4) IL-6Rα (b) (4) to inhibit IL-6 mediated signaling, at subcutaneous doses of 10, 25, and 100 mg/kg twice per week.

Rationale for Changes in Section 13.1:
Based on species specificity, a rodent carcinogenicity study with sarilumab was not feasible. As such, the Applicant had previously indicated that any potential risk for malignancies would be managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. Thus, additional text is proposed for inclusion in the first paragraph of Section 13.1 to provide a balanced description of the published literature regarding the potential role of the IL-6/IL-6 receptor pathway in cancer. A request for an evaluation of the proposed labeling for Section 13.1 was made to the ECAC by email on July 22, 2016. In addition, a face-to-face meeting with the ECAC occurred on August 30, 2016 to discuss the proposed labeling. In consultation with the ECAC the text included above was agreed upon. Consideration was given to summarizing the current literature but being consistent with other sections in the product

insert where it is stated that the impact of treatment with KEVZARA on the development of malignancies in not known (Section 5.4).

The first paragraph of Section 13.1 was also revised to remove language about the fact that (b) (4)

The paragraph in Section 13.1 regarding fertility and reproductive performance has been updated to include the doses used in the SC fertility study in mice with REGN844. Further, the word (b) (4) was replaced with “analogous” when referring to the description of the REGN844 antibody.

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/s/

ELENI M SALICRU
09/02/2016

TIMOTHY W ROBISON
09/02/2016
I concur

Pharmacology and Toxicology Secondary Review for BLA 761037

TO: BLA 761037 (KEVZARA [sarilumab])

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

DATE: September 2, 2016

Sanofi-Aventis submitted an original Biologics License Application (BLA) on October 30, 2015 for KEVZARA (sarilumab). Sarilumab was a human immunoglobulin G1 (IgG1) monoclonal antibody (mAb) that binds to both soluble and membrane bound human Interleukin-6 receptor alpha (IL-6Rα) to inhibit IL-6 mediated signaling. KEVZARA was proposed for the treatment of adult patients with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response or intolerance to one or more disease-modifying anti-rheumatic drugs (DMARDs). The planned doses of KEVZARA were 150 or 200 mg q2 weeks by subcutaneous (SC) injection.

Dr. Salicru's reviews dated August 24, 2016 and September 2, 2016 evaluated the Sponsor's nonclinical safety assessment of sarilumab.

I concur with the recommendations of Dr. Salicru's reviews dated August 24, 2016 and September 2, 2016 that the nonclinical pharmacology and toxicology profile of sarilumab has been adequately studied and KEVZARA should be approved from the nonclinical perspective.

The Sponsor has a complete nonclinical development program for sarilumab.

Pharmacology: Sarilumab binds human and Cynomolgus monkey IL-6Rα with an equilibrium dissociation constant (K_d) of 54 pM and 123 pM, respectively, but no binding of sarilumab to the murine IL-6Rα was observed. This supported selection of the Cynomolgus monkey as the relevant species for nonclinical assessment of sarilumab.

Toxicology: The toxicity profile of sarilumab was evaluated in a number of GLP-compliant repeat-dose toxicology studies in Cynomolgus monkeys by the intravenous (IV) route of administration for up to 6 months in duration and by the SC route for 3 months duration. See Dr. Salicru's review for further details. The pivotal study in the review of the BLA was the 6-month study. In the 6-month toxicology study, Cynomolgus monkeys (6/sex/group) received sarilumab at IV doses of 0 (placebo control), 0.5, 5, 15, and 50 mg/kg/week. Four monkeys/sex/group were sacrificed after the 26-week dosing period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period. From the assessments of standard panels of hematology and coagulation parameters, test-article related effects were noted on white blood cell and neutrophil counts and fibrinogen levels. Neutrophil counts (absolute and percent) were decreased at week 25 in all male and female drug-treated groups in a non-dose related

manner. These decreases were generally reversible at the end of the recovery period. White blood cells were decreased in males treated with doses ≥5 mg/kg/week and in all female drug-treated groups, which were attributed to treatment-related decreases of neutrophil counts. Fibrinogen levels from Week 4 to the end of the dosing period were decreased in a non-dose related manner in males and females that received doses ≥5 mg/kg/week. From the assessment of a standard panel of clinical chemistry parameters, C-reactive protein (CRP) levels were decreased at Weeks 4, 12, and 25 for males that received doses ≥15 mg/kg/week. Decreases of CRP levels were observed at Weeks 4 and 12 in females that received doses ≥5 mg/kg/week. These decreases were reversible by the end of the recovery period. Changes of neutrophil counts and decreases of fibrinogen and CRP levels might be considered biomarkers of treatment with an anti-IL-6R mAb such as sarilumab. Increased IL-6 levels at Weeks 4, 12, and 25 in males and females that received doses ≥5 mg/kg/week were attributed to an inhibition of receptor-mediated clearance and had reversed by the end of the recovery period. No target organs of toxicity were identified with doses up to 50 mg/kg/week. From the TDAR assessment, there were small decreases in both the primary and secondary IgG responses to KLH challenge in males and females that received doses ≥5 mg/kg/week; this change was attributed to the immunosuppressive properties of sarilumab. The NOAEL was identified at the high dose of 50 mg/kg/week based upon no findings of dose-limiting toxicity or target organs of toxicity. Exposure at the NOAEL provided a safety margin >80 times the exposures at proposed clinical doses.

Carcinogenicity: At the EOP2 meeting during IND development, the Sponsor provided a carcinogenicity risk assessment in support of their position that a carcinogenicity study with sarilumab was not feasible or needed. The Division and the Executive Carcinogenicity Assessment Committee (ECAC) concurred. It was agreed that it was reasonable for the Sponsor to manage any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion by appropriate labeling, clinical monitoring, and post-marketing surveillance. The recommendation was made to the Sponsor that the product label for sarilumab should include a balanced description of the literature to address the carcinogenic potential of sarilumab in relationship to the carcinogenic risk to humans for the chronic use of sarilumab. In the current BLA submission, the Sponsor submitted an updated carcinogenicity risk assessment for sarilumab.

Dr. Salicru's review extensively evaluated the role of the IL-6/IL-6R pathway in carcinogenesis. The carcinogenic potential of the IL-6/IL-6R signaling pathway has been investigated in published scientific literature (Lu et al., 2006; Hideshima et al., 2007; Heikkila et al., 2008; Lippitz, 2013). The published literature suggests that IL-6/IL-6R signaling is tumor promoting. For example, IL-6 signaling by the IL-6R/gp130 complex and subsequent downstream activation of the JAK/STAT, MAPK, and PI3K/Akt pathways has been implicated in the tumorigenesis of multiple myeloma, ovarian cancer, lung cancer, bladder cancer, breast cancer, colon cancer, and prostate cancer (Bharti et al., 2016; Yao et al., 2013; Hideshima et al., 2001; Berishaj et al., 2007; Bharti et al., 2015). However, the literature also indicates that the IL-6 pathway may confer an anti-tumor role by supporting the adaptive immune response. In particular, IL-6 trans-

signaling may be instrumental in facilitating anti-tumor T cell responses. IL-6 can support T-cell anti-tumor effects either at the lymph node or at the tumor microenvironment. For instance, acute activation of the IL-6/IL-6R pathway can influence the trafficking of lymphocytes to lymph nodes in order to induce their activation, proliferation, and polarization towards phenotypes that oppose the immunosuppressive tumor microenvironment.

In consultation with the ECAC, the following statement was developed for use in Section 13.1 of the product label to provide a balanced description of the literature to address the carcinogenic potential of sarilumab in relationship to the carcinogenic risk to humans for the chronic use of sarilumab.

The literature supports that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. Available published evidence also supports that IL-6 signaling through the IL-6 receptor may be involved in pathways that lead to increased tumor growth, particularly at high levels of IL-6 exceeding normal physiological levels. The malignancy risk in humans from an antibody that disrupts signaling through the IL-6 receptor, such as sarilumab, is not clear.

Reproductive Toxicity:
Reproductive and developmental toxicology studies were conducted with sarilumab in monkeys and with REGN844 (a surrogate monoclonal antibody that binds murine IL-6Rα) in mice.

In a fertility study, male and female CD-1(ICR) mice received REGN844 twice per week by SC administration at 0 (placebo control), 10, 25, or 100 mg/kg/dose (total weekly doses of 20, 50, and 200 mg/kg, respectively). There were no test article-related effects on any of the fertility parameters examined in the study (e.g., mating/fertility index, number of corpora lutea, number of late resorption, number of dead fetuses, pre-implantation loss, or post-implantation loss).

In an enhanced prenatal and postnatal development (ePPND) study, pregnant Cynomolgus monkeys received sarilumab by IV infusion at doses of 0 (placebo control), 5, 15, or 50 mg/kg/week. Dosing began on gestation day (GD) 20 and continued through the end of gestation with either delivery around GD 160 to GD 165 or abortion/embryo-fetal death. Maternal animals and neonates were observed for 28-32 days after delivery. Evaluation of hematology and coagulation parameters in the maternal animals found decreases of mean white blood cell, lymphocyte, and neutrophil counts in all drug-treated groups with no relation to dose on GD 153 and/or lactation day (LD) 7. Fibrinogen levels were slightly decreased in all test article-treated groups (not dose related) on GD 153, LD 7, and/or LD 30. In neonates, no test article-related effects were noted on any of the parameters evaluated (birth examinations, clinical signs, body weight, functional and morphological development, hematology, coagulation, serum chemistry, immunophenotyping, and ADA). Sarilumab was detected in the serum of neonates up to one month after birth, suggesting that the antibody had

crossed the placenta. The Nonclinical Reviewer highlighted some concerns about the adequacy of the ePPND study (e.g., small numbers of neonates in each group at the time of scheduled necropsy, absence of immune function testing [e.g., TDAR] in the offspring during the postnatal phase, presence of drug in the circulation of the neonates at the time of necropsy, which may lead to uncharacterized effects beyond the one month of age in monkeys). The NOAEL for the ePPND study was identified as 50 mg/kg/week based upon no evidence of embryotoxicity or fetal malformations.

Parturition is associated with significant increases of IL-6 in the cervix and myometrium. The literature suggests that inhibition of IL-6 signaling may interfere with cervical ripening and dilatation and myometrial contractile activity leading to potential delays of parturition. For mice deficient in IL-6 ($Il6^{-/-}$ null mice), parturition was delayed relative to wild-type ($Il6^{+/+}$) mice. Administration of recombinant Il-6 to $Il6^{-/-}$ null mice restored the normal timing of delivery.

Labeling: Dr. Salicru's review dated September 2, 2016 evaluated and recommend changes as necessary to product labeling in Indications and Usage (Established Pharmacological Classification) and Use in Special Populations under Highlights of Prescribing Information, Section 8.1 (Pregnancy), Section (b) (4), Section 12.1 (Mechanism of Action), and Section 13.1 (Carcinogenesis, Mutagenesis, Impairment of Fertility). I concur with Dr. Salicru's recommendations for changes to the product label. See Dr. Salicru's reviews for additional details of changes to the product labeling.

When possible, consistency was maintained between the ACTEMRA® (tocilizumab) product and KEVZARA® (sarilumab) labels since both are IL-6 receptor antagonists.

Recommendation: From the nonclinical perspective, approval of the application is recommended.

There are no outstanding nonclinical issues.

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/s/

TIMOTHY W ROBISON
09/02/2016
From the nonclinical perspective, approval of the application is recommended.

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:	BLA 761037
Supporting document/s:	1 and 10
Applicant's letter date:	10/30/2015 and 02/26/2016
CDER stamp date:	10/30/2015 and 02/26/2016
Product:	KEVZARA® (Sarilumab; Anti-IL-6 Receptor Monoclonal Antibody) Solution for Subcutaneous Injection
Indication:	Treatment of adult patients with moderately to severely active rheumatoid arthritis who have had an inadequate response or intolerance to one or more disease-modifying anti-rheumatic drugs
Applicant:	Sanofi-Aventis
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Eleni Salicru, PhD
Supervisor/Team Leader:	Timothy Robison, PhD, DABT
Division Director:	Badrul Chowdhury, MD, PhD
Project Manager:	Christine Ford, RPh

Template Version: September 1, 2010

Disclaimer

Except as specifically identified, all data and information discussed below and necessary for approval of BLA 761037 are owned by Sanofi-Aventis or are data for which Sanofi-Aventis has obtained a written right of reference.
Any information or data necessary for approval of BLA 761037 that Sanofi-Aventis does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug,

as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of BLA 761037.

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1 Executive Summary

1.1 Introduction

Sanofi-Aventis submitted an original Biologics License Application (BLA) on October 30, 2015, for Kevzara (sarilumab) as a solution for subcutaneous (SC) injection. Sarilumab is a human immunoglobulin G1 (IgG1) monoclonal antibody that binds to both soluble and membrane bound human interleukin-6 receptor alpha (IL-6Rα) to inhibit IL-6 mediated signaling. Kevzara is proposed for the treatment of adult patients with moderately to severely active rheumatoid arthritis (RA) who have had an inadequate response or intolerance to one or more disease-modifying antirheumatic drugs (DMARDs). This review evaluates the nonclinical pharmacology and toxicology studies to support the safety of sarilumab for approval.

1.2 Brief Discussion of Nonclinical Findings

The nonclinical safety program for sarilumab was performed in cynomolgus monkeys, which were established to be the most pharmacologically relevant nonclinical species. As determined by surface plasmon resonance (SPR), sarilumab binds human IL-6R and cynomolgus monkey IL-6R with equilibrium dissociation constants (K_D) of 54.4 pM and 123 pM, respectively.

Results from a number of GLP-compliant repeat-dose toxicology studies in cynomolgus monkeys with sarilumab by intravenous (IV) administration for durations up to 6 months and by SC administration for 3 months did not identify any significant dose-limiting toxicity or target organs of toxicity at IV doses up to 50 mg/kg/week or SC doses up to 100 mg/kg/week (two weekly doses of 50 mg/kg). There were no deaths that were attributed to treatment with sarilumab. Microscopic findings were limited to effects at the injection site (minimal to moderate perivascular mixed inflammatory cell infiltrates). The most common effects related to treatment with sarilumab were decreased levels of neutrophils, fibrinogen, and/or C-reactive protein (CRP). These decreases were considered pharmacodynamic (PD) effects of inhibiting IL-6 signaling. In most cases, these effects were not dose-dependent and were generally reversible during the recovery period. The 6-month study revealed slight decreases in primary and secondary IgG responses following antigen challenge.

Based on species specificity, a rodent carcinogenicity study with sarilumab was not considered feasible. The Executive Carcinogenicity Assessment Committee (ECAC) concurred that a carcinogenicity study was not feasible. No nonclinical studies were required to evaluate the potential carcinogenicity of sarilumab. A review of the scientific literature related to the role of IL-6/IL-6R pathway in cancer was conducted. The literature indicates that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. Available published evidence also supports that IL-6 signaling through the IL-6R may be involved in pathways that lead to tumorigenesis. The malignancy risk in humans from an antibody that disrupts signaling through the IL-6R, such as sarilumab, is unknown.

The reproductive and developmental toxicity of sarilumab was evaluated in an enhanced pre- and postnatal development (ePPND) study in cynomolgus monkeys. Further, a murine surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα (i.e., REGN844) was developed and used for a fertility study in mice.

In the ePPND study with pregnant cynomolgus monkeys, sarilumab at IV doses up to 50 mg/kg/week, from the time that pregnancy was confirmed, throughout the period of organogenesis, and continuing to natural birth of the infants, produced no evidence of embryotoxicity or fetal malformations. However, there were concerns about the adequacy of the study based upon the small number of animals per group. Sarilumab was detected in the serum of neonates up to one month after birth suggesting that the antibody had crossed the placenta.

Fertility and reproduction were unaffected in male and female mice treated with REGN844 at SC doses up to 100 mg/kg twice per week.

REGN844 was used for a repeat-dose toxicity study in juvenile mice. In this study, treatment of mice (starting at 14 days of age) with REGN844 at SC doses up to 200 mg/kg/week resulted in immunosuppression based upon findings of slightly decreased IgG responses after antigen challenge in males at all doses and at all the time points evaluated. These findings were reversible at the end of the recovery period. Decreased IgG responses after antigen challenge were also evident in the 6-month monkey study.

1.3 Recommendations

1.3.1 Approvability

From the nonclinical perspective, BLA 761037 is recommended for approval.

There are no outstanding nonclinical issues.

1.3.2 Additional Non Clinical Recommendations

None.

1.3.3 Labeling

Nonclinical sections of the product label will be evaluated in a separate review.

2 Drug Information

2.1 Drug

CAS Registry Number: 1189541-98-7

Trade Name: KEVZARA®

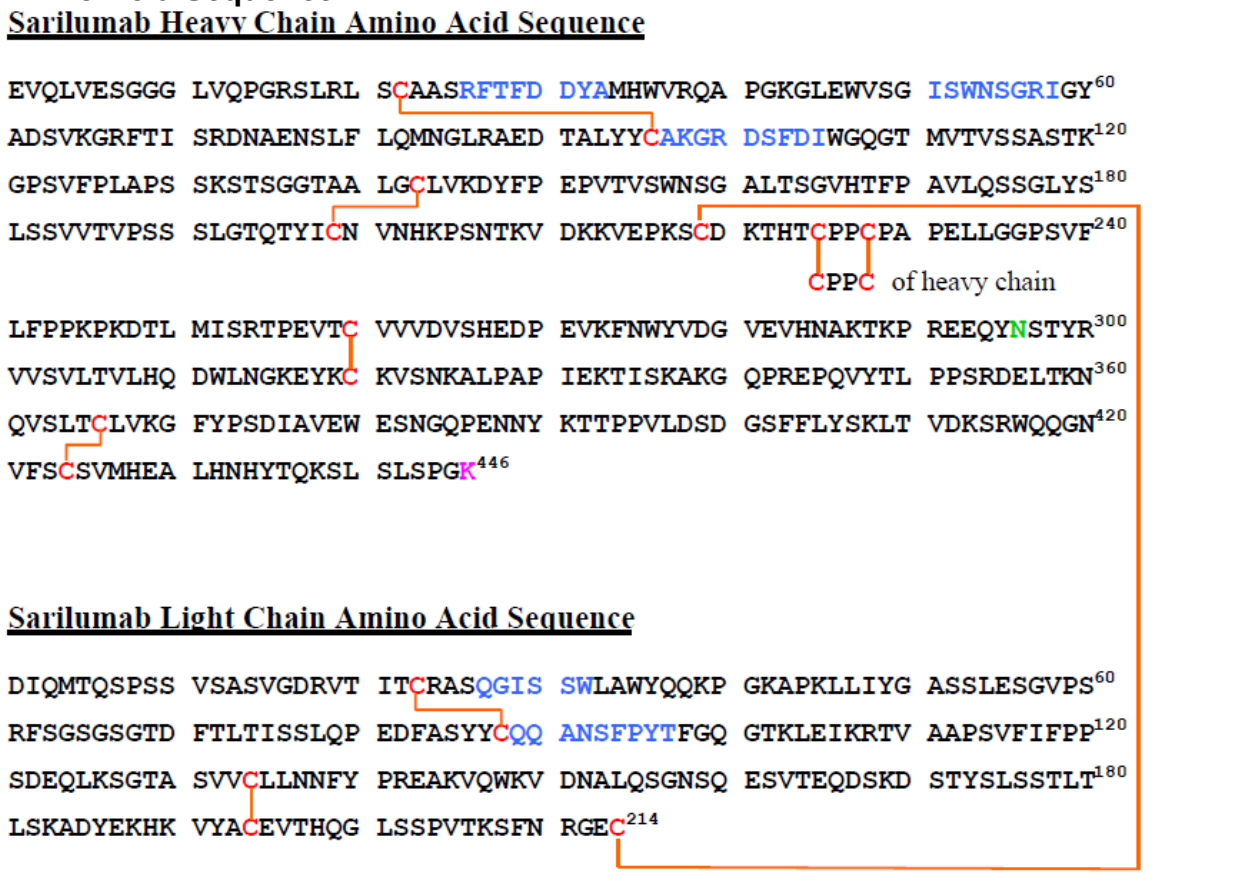
Generic Name: Sarilumab

Code Name: REGN88, SAR153191

Chemical Name: Human IgG1 specific for human IL-6Rα (covalent tetramer consisting of 2 heavy and 2 light chains)

Immunoglobulin G1, anti-(human interleukin-6 receptor subunit alpha (IL-6Rα, membrane glycoprotein 80, CD126)); human monoclonal REGN88 γ1 heavy chain (219-214′)-disulfide with human monoclonal REGN88 κ light chain dimer (225-225″:228-228″)-bisdisulfide

Amino Acid Sequence:

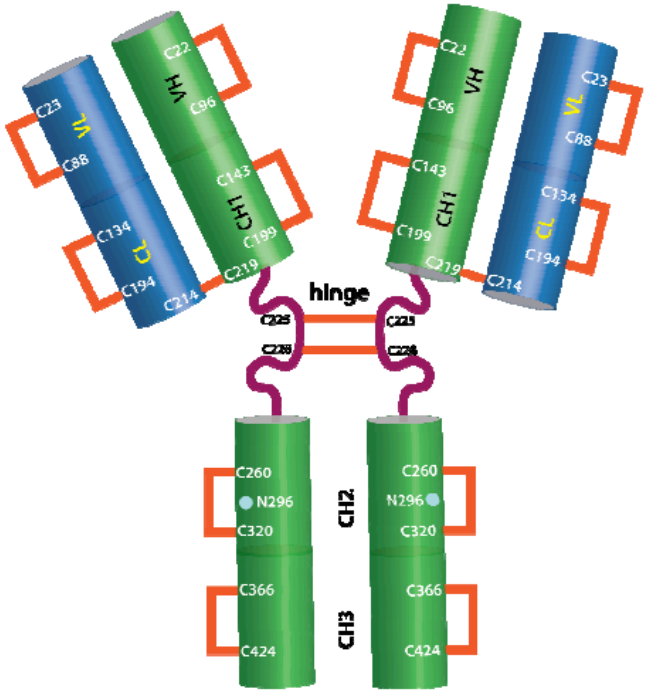


Sarilumab antibody amino acid sequence with post-translational modifications denoted. The sequences of sarilumab heavy chain and light chain CDR regions are highlighted in blue. The cysteine residues (red) that have been confirmed to form the predicted disulfide bonds are connected by solid orange lines. The Fc N-linked glycosylation site at Asn^{296} is highlighted in green. The heavy chain C-terminal Lys^{446} (pink) is predominantly removed during protein expression.

Figure 1 Amino Acid Sequence of Sarilumab (Applicant's Figure)

Molecular Weight: 144 kDa

Structure:



Representation of the structure of sarilumab depicting the location of each of the intrachain and interchain disulfide bonds (orange). Heavy (green) and light (blue) chains are connected by interchain disulfide bonds; heavy chain dimerization is achieved through two heavy chain intermolecular disulfide bonds located within the hinge region. The Fc domain glycosylation site is also indicated (cyan).

Abbreviations: CH, constant region of heavy chain; CL, constant region of light chain; VH, variable region of heavy chain; VL, variable region of light chain

Figure 2 Schematic of Sarilumab Structure (Applicant's Figure)

Biochemical Description: Sarilumab is a recombinant human IgG1 monoclonal antibody consisting of two disulfide-bonded human heavy chains. Each heavy chain is covalently linked through a disulfide bond to a human kappa light chain. Sarilumab binds to both soluble and membrane-bound IL-6R.

Pharmacologic Class: IL-6R antagonist

2.2 Relevant INDs, BLAs and DMFs

IND 100632: Sarilumab for indications of polyarticular juvenile idiopathic arthritis (pJIA) and RA (CDER/ODEII/DPARP)

(b) (4) (CDER/OAP/DTOP)

2.3 Drug Formulation

The sarilumab drug product is provided ready for SC injection in a single-use prefilled syringe that is available in dosage forms of 150 mg and 200 mg. Both dosage forms

are formulated with (b) (4) histidine, (b) (4) arginine (b) (4) sucrose, polysorbate 20, and water for injection, as shown in **Table 1**.

Table 1 Qualitative Composition of Sarilumab Solution for Injection (Modified from Applicant's Table)

(b) (4)

2.4 Comments on Novel Excipients

No novel excipients are present in the drug product formulation.

2.5 Comments on Impurities/Degradants of Concern

None. A nonclinical safety evaluation of extractables and leachables of the pre-filled syringes to be used with the sarilumab drug product will be conducted separately.

2.6 Proposed Clinical Population and Dosing Regimen

Kevzara is proposed for use in adult patients with moderately to severely active RA who have had an inadequate response or intolerance to one or more DMARDs. The planned dose of Kevzara is 150 or 200 mg once every two weeks by SC injection.

2.7 Regulatory Background

Provided below is a brief summary of the regulatory background related to the development program for sarilumab.

- The initial submission for IND 100632 by Regeneron was received on October 16, 2007. The proposed first in human (FIH) Phase 1 clinical study was deemed safe to proceed based on the 30-day safety review.

- The End of Phase 2 (EOP2) meeting with Sanofi-Aventis and Regeneron to discuss the planned Phase 3 clinical and nonclinical programs occurred on September 15, 2011 (see meeting minutes dated September 26, 2011). The Applicant's nonclinical questions, the Division's responses, and the meeting discussion are reproduced below. The EOP2 meeting related to Chemistry, Manufacturing, and Control (CMC) information occurred on October 26, 2011 (see meeting minutes dated November 14, 2011).

"Question 10: The general toxicity studies include a duration of sarilumab treatment in monkeys of up to 3 months SC and 6 months intravenously (IV). Does the Agency agree that the general toxicity program is sufficient to support the initial registration of sarilumab by SC route?

Division Response: Yes, we agree.

Question 11: Does the Agency agree that the 2 reproductive studies (fertility study in mice with the fully murine surrogate against mouse IL-6R and enhanced pre/postnatal developmental study in monkeys) plus the available data in the public domain on IL-6 inhibition (including a (b) (4) ***) are sufficient for the initial registration?***

Division Response: It appears that the (b) (4) *information that could not be used to support your application. We have some concerns about the adequacy of the enhanced pre/postnatal developmental study in monkeys using sarilumab. First, numbers of neonates in each group were relatively small. Second, immune function testing in the offspring during the postnatal phase was not conducted (e.g., TDAR). Last, sarilumab was not totally cleared from the circulation of F1 offspring at the time of necropsy (around postnatal day 30) and therefore the drug may have uncharacterized effects beyond the one month of age in monkeys. We are aware that sarilumab has been shown to be immunosuppressive in adult monkeys (e.g., decreased neutrophil counts, decreased effects in the TDAR assay in the 6-month study) as well as in adult humans. The investigator's brochure, informed consent, and potential product labeling will need to use existing knowledge on sarilumab and the IL-6 pathway to report the potential immunosuppressive effects of sarilumab in infants of mothers exposed to sarilumab.*

Sanofi Clarification Request: The Sponsor acknowledges the Agency's comments. To address the Agency's concerns, the Sponsor proposes to conduct a GLP subcutaneous pre- /postnatal toxicity study in mice using the murine surrogate against IL-6R, REGN844. This is the same molecule used in the fertility study in mice. The pre-/postnatal study would include TDAR evaluation and be of sufficient duration to clear the test article from the F1 offspring. This study

would be conducted for BLA submission. Upon completion of this mouse pre-postnatal study, the Sponsor would consider the Agency's concerns addressed and that the reproductive toxicity package would have appropriately evaluated the target for registration. Does the Agency agree that after completion of this pre-/postnatal toxicity study in mice that no additional reproductive toxicity studies are needed for registration?

Discussion: The Division reiterated concerns about the adequacy of the enhanced pre/postnatal developmental study in monkeys using sarilumab, but stated that additional developmental toxicity studies are not necessarily required if Sanofi agrees to incorporate information regarding the potential immunosuppressive effects of the product in infants in the Informed Consent (IC), the Investigator's Brochure (IB), and the potential product label as follows: "Infant monkeys were evaluated up to 1 month of age in the enhanced PPND study with monkeys and immune functional testing was not performed. Based on the findings from the 6-month toxicity study in adult monkeys and available adult human clinical data, sarilumab possesses the potential to cause immunosuppressive effects in the infants of mothers treated with sarilumab". Sanofi asked if it would be acceptable to include this information in the documents noted above and (b) (4) The Division noted that it is Sanofi's choice to take this approach. However, (b) (4) . Sanofi asked whether the statement can be (b) (4) It was stated that the agency is not recommending (b) (4) .

Question 12: Does the Agency agree that, given the available nonclinical data with sarilumab (including general toxicity and reproductive studies in animals) and available information on molecules with the same mechanism of action in terms of inhibition of IL-6R, (b) (4)

Division Response: No, we do not agree. (b) (4)

Sanofi Clarification Comment: Thank you for the response. Should it be decided that (b) (4)

Question 13: The Sponsor conducted a weight-of-evidence based carcinogenicity risk assessment based on results from toxicology studies with sarilumab and REGN844 (the fully-murine surrogate against mouse IL-6R), effects of sarilumab in antitumor pharmacology models, and a review of published literature following IL-6R activation and inhibition. Does the Agency agree that, given the available data with sarilumab and data in the literature on IL-6 inhibition, no additional in vitro and in vivo studies (e.g., in vitro cell-based assays, xenograft models, tumor promotion models, 2-year bioassays) with sarilumab are necessary for registration?

Division Response: Your carcinogenicity assessment position paper is being reviewed by DPARP. In order to complete the review of your carcinogenicity assessment position paper, we need the study report on characterization of the effects of sarilumab (REGN88) on STAT3 activation and tumor xenograft growth (Report Number REGN88-MX-11050-SR-01V1). Your proposal on the carcinogenicity assessment will be also discussed with the Executive

Carcinogenicity Assessment Committee at which time we will address whether further carcinogenicity assessment for sarilumab is needed. After this issue is discussed with the Executive Carcinogenicity Assessment Committee, we will respond to this question.

Sanofi Clarification Request: The Sponsor appreciates the review of the position paper by DPARP at this time. Please note that on 12 September 2011 the requested study report on the characterization of the effects of sarilumab (REGN88) on STAT3 activation and tumor xenograft growth (Report Number REGN88-MX-11050-SR-01V1) was submitted to the IND 100632 (Serial No. 0125).

As noted in the Agency's response, prior to responding to Question 13, the Division will discuss the sponsor's carcinogenicity assessment position paper with the Executive Carcinogenicity Assessment Committee. Can the Division provide feedback on the timeline when the sponsor can expect to receive a definitive response on whether an additional carcinogenicity assessment for sarilumab is required in support of registration?

Discussion: The Division clarified that discussion of a carcinogenicity assessment position paper with the Executive Carcinogenicity Assessment Committee is a routine occurrence, but that definitive timeline for a response cannot be given. The Division assured Sanofi that review and feedback will be accomplished in as timely manner as possible, given the resources available."

- It was communicated to the Applicant (see information request [IR] dated December 4, 2013) that based on the nonclinical evaluation of the Applicant's proposed carcinogenicity assessment, that no additional nonclinical studies were required to address the carcinogenic potential of sarilumab.

- A pre-BLA meeting occurred on October 22, 2014, with Sanofi-Aventis and Regeneron to discuss specific clinical, nonclinical, and regulatory questions related to the BLA submission for sarilumab (see meeting minutes dated November 21, 2014). It was agreed upon at the meeting that the sarilumab nonclinical program was sufficient to support submission and review of a BLA. It was also agreed upon that Drug Abuse Liability Assessment (DALA) studies and abuse liability assessment analyses would not need to be performed. The pre-BLA meeting related to the CMC information occurred on December 16, 2014 (see meeting minutes dated February 9, 2015).

- The BLA for sarilumab (150 and 200 mg pre-filled syringes) was submitted on October 30, 2015, and the application was filed on December 29, 2015, for standard review.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology Studies:

Study Title	Study Number	Reviewed
Determination of the Equilibrium Binding Constants for the Interaction of REGN844 with Mouse IL-6Rα	REGN844-MX-09073	G. Lee (IND 100632)

Study Title	Study Number	Reviewed
Inhibition of IL-6 Binding to Mouse IL-6Rα by REGN844	REGN844-MX-09074	E. Salicru (BLA 761037)
In vitro Inhibition of Mouse IL-6Rα Signaling by REGN844	REGN844-MX-09075	E. Salicru (BLA 761037)
In Vivo Pharmacodynamic Activity of IL-6Rα Blockade in Mice by REGN844	REGN844-MX-09076	G. Lee (IND 100632)
In Vivo Pharmacodynamic Activity of mIL-6Rα Blockade in a Murine Model of Rheumatoid Arthritis by REGN844 Antibody	REGN844-MX-14076	E. Salicru (BLA 761037)
Evaluation of Fc Effector Function for REGN88	REGN88-MX-07015	E. Salicru (BLA 761037)
Determination of Equilibrium Binding Constants for the Interaction of Human and Monkey IL-6 Receptor with REGN88	REGN88-MX-07016	G. Lee (IND 100632)
Characterization of the Effects of Sarilumab (REGN88) on STAT3 Activation and Tumor Xenograft Growth	REGN88-MX-11050	G. Lee (Version 1 IND 100632); E. Salicru (Version 2 BLA 761037)
Characterization of REGN88 Activity in a Cell Proliferation Assay	REGN88-MX-11077	G. Lee (IND 100632)
Cross-Reactivity of Sarilumab (REGN88) Against IL6Rα of Non-Human Primate and Non-Primate Species	REGN88-MX-11078	E. Salicru (BLA 761037)
In Vivo Pharmacodynamic Activity of IL-6Rα Blockade in Humanized Mice by Sarilumab/REGN88	REGN88-MX-13129	E. Salicru (BLA 761037)
In Vitro Studies Demonstrating that REGN88 Directly Blocks Binding of IL-6 to IL-6Rα	REGN88-MX-14072	E. Salicru (BLA 761037)

Toxicology:

Study Title	Study Number	Reviewed
SAR153191 (REGN88): Exploratory 4-Week Subcutaneous and Intravenous Toxicity Study in Mice with REGN844	DIV1267	G. Lee (IND 100632)
A 4-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 9-Week Recovery Period	REGN88-TX-06040 ((b) (4)-460003)	G. Lee (IND 100632)

Study Title	Study Number	Reviewed
A 13-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by an 8-Week Recovery Period	REGN88-TX-06037 ((b) (4)-460004)	G. Lee (IND 100632)
A 13-Week Subcutaneous Injection Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 12-Week Recovery Period	REGN88-TX-06038 ((b) (4)-460005)	G. Lee (IND 100632)
A 13-Week Bridging Subcutaneous Toxicity Study of REGN88 in Cynomolgus Monkeys	REGN88-TX-09053 ((b) (4)-460024)	G. Lee (IND 100632)
A 6-Month Once Weekly 30-Minute Intravenous Infusion Study of REGN88 in Cynomolgus Monkeys Followed by a 12-Week Recovery Period	REGN88-TX-08031 ((b) (4)-460012)	G. Lee (IND 100632)
SAR153191 (REGN88): Subcutaneous Fertility Study in Mice with REGN844	REGN844-TX-09048 (FER0480)	G. Lee (IND 100632)
Study of the Effects of REGN88 on Embryo-Fetal Development and Pre- and Post-Natal Development When Administered Weekly by Intravenous Infusion to Pregnant Cynomolgus Monkeys	REGN88-TX-08030 ((b) (4).223.33)	G. Lee (IND 100632)
SAR153191/REGN88: Exploratory Subcutaneous 4-Week Toxicity Study in the Juvenile Mouse with REGN844	REGN844-TX-12082 (JUP0016)	G. Lee (IND 100632)
REGN844: 9-Week Subcutaneous Toxicity Study in the Juvenile Mouse Followed by a 13-Week Recovery Period	REGN844-TX-13063 (JUV0030)	E. Salicru (BLA 761037)
Amended Carcinogenicity Risk Assessment		E. Salicru (BLA 761037)
Cross-Reactivity Study of Biotinylated REGN88 with Normal Human and Cynomolgus Monkey Tissues	REGN88-TX-06036 (IM1436)	G. Lee (IND 100632)

3.2 Studies Not Reviewed

Pharmacokinetic Studies:

Study Title	Study Number
Validation of a Bioanalytical Method for the Quantitative Measurement of REGN844 in Mouse Serum	REGN844-AV-09101-VA-01 V2

Study Title	Study Number
Validation of a Bioanalytical Method for the Quantitative Measurement of Total REGN88 in Monkey Serum	REGN88-AV-07003-VA-01 V3
Validation of a Bioanalytical Method for Semi-Quantitative Analysis of REGN88 Reactive Antibodies in Monkey Serum	REGN88-AV-07006-VA-01V2
Validation of a Bioanalytical Method for Detection of REGN88 Reactive Antibodies in Monkey Serum Using Electrochemiluminescence	REGN88-AV-09011-VA-01V2
Development of a Bioanalytical Method for the Quantitative Measurement of Free REGN88 in Rat Serum	REGN88-MX-14147-SR-OIV1
The Determination of the Pharmacokinetics of Anti-IL6Rα Antibody (REGN88) Following a Single Subcutaneous and Intravenous Administration at 5 mg/kg to Female Sprague-Dawley Rats	PK06006-88-SA_OJV2
The Determination of the Pharmacokinetics of Anti-IL6Rα Antibody (REGN88) Following a Single Subcutaneous and Intravenous Administration at 1 mg/kg to Sprague-Dawley Rats	PK07002-88-SA _ 01 V2
The Determination of the Pharmacokinetics, Bioavailability and Dose Ranges of Anti-IL6Rα Antibody (REGN88) Following a Single Subcutaneous and Intravenous Administration at 0.5 mg/kg, 1mg/kg and 3mg/kg to Female Sprague-Dawley Rats Respectively	PK07004-88-SA_OJV2
A Single Intravenous or Subcutaneous Dose Pharmacokinetics/Pharmacodynamics Study of REGN88, an Anti-IL6R Monoclonal Antibody, in Cynomolgus Monkeys	REGN88-PK-06041 ((b) (4).223.26)
The Pharmacokinetics and Bioavailability of REGN88 in Sprague-Dawley Rats: Comparison of P1 and P2 Process Lots	REGN88-PK-09060 ((b) (4) #PK09003)
A Single Dose Pharmacokinetics and Bioavailability Study of REGN88 Monoclonal Antibody in Cynomolgus Monkeys (GLP): a Process Comparability Study	REGN88-PK-09092 ((b) (4).223.39)

Toxicology:

Study Title	Study Number
Sarilumab: Supplemental Pharmacokinetic Analyses in Support of Pharmacokinetic and Toxicokinetic Studies in the Mouse and Monkey (Abbreviated Report)	REGN88-MX-14095-PK-Ol V2

Study Title	Study Number
A 4-Week Subcutaneous and Intravenous Tolerability Study of REGN88 in Cynomolgus Monkeys	IL6R-TX-06029 ([(b) (4)].223.24)

3.3 Previous Reviews Referenced

Pharmacology/Toxicology IND 100632 Review and Evaluation dated May 12, 2016.

4 Pharmacology

A number of pharmacology studies were submitted and reviewed under IND 100632; a brief synopsis of these studies is included below (see Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016 for more details). Also provided below is a more detailed review of pharmacology studies that were not previously reviewed under IND 100632 or that were submitted to BLA 761037.

4.1 Primary Pharmacology

The equilibrium binding constants for sarilumab binding to human and monkey IL-6R were determined by the SPR Biacore assay [Study No. REGN88-MX-07016; non-GLP]. The K_D (calculated equilibrium dissociation constant) was 54.4 pM for the human IL-6R and 123 pM for the monkey IL-6R. Sarilumab has a higher affinity for the human IL-6R than for the monkey IL-6R. The difference in K_D of sarilumab binding to human and monkey IL-6R was mostly due to the faster dissociation rate constant (K_d) for the monkey receptor (~ 2-fold).

The equilibrium binding constants for the interactions of three batches of REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) with mouse IL-6Rα monomer (mIL-6Rα-MMH) and mouse IL-6Rα dimer (mIL-6Rα-hFc) were measured using a SPR Biacore assay [Study No. REGN844-MX-09073; non-GLP]. The mean K_D for the three batches of REGN844 that were tested was 203 pM and 8.3 pM for binding to mIL-6Rα-MMH and mIL-6Rα-hFc, respectively.

The biological activity of IL-6 induced signal transduction of several lots of sarilumab was evaluated using two different cell lines (HepG2 and DS-1) [Study No. REGN88-MX-11077-SA-01V1; non-GLP]. In HepG2 cells (human hepatocytic cell line endogenously expressing IL-6Rα and gp130), sarilumab inhibited IL-6-mediated signal transducer and activator of transcription 3 (STAT3) activity in a luciferase assay with an IC_{50} of ~150 pM in the presence of a constant concentration of 50 pM IL-6; no increase in luciferase activity was detected in HepG2 cells when incubated with sarilumab in the absence of IL-6. IL-6 stimulated proliferation of DS-1 cells (human B-cell line) in a dose-dependent manner (EC_{50} of ~0.7pM). This proliferation was inhibited by sarilumab with an IC_{50} of ~140 pM in the presence of a concentration of 1.0 pM IL-6.

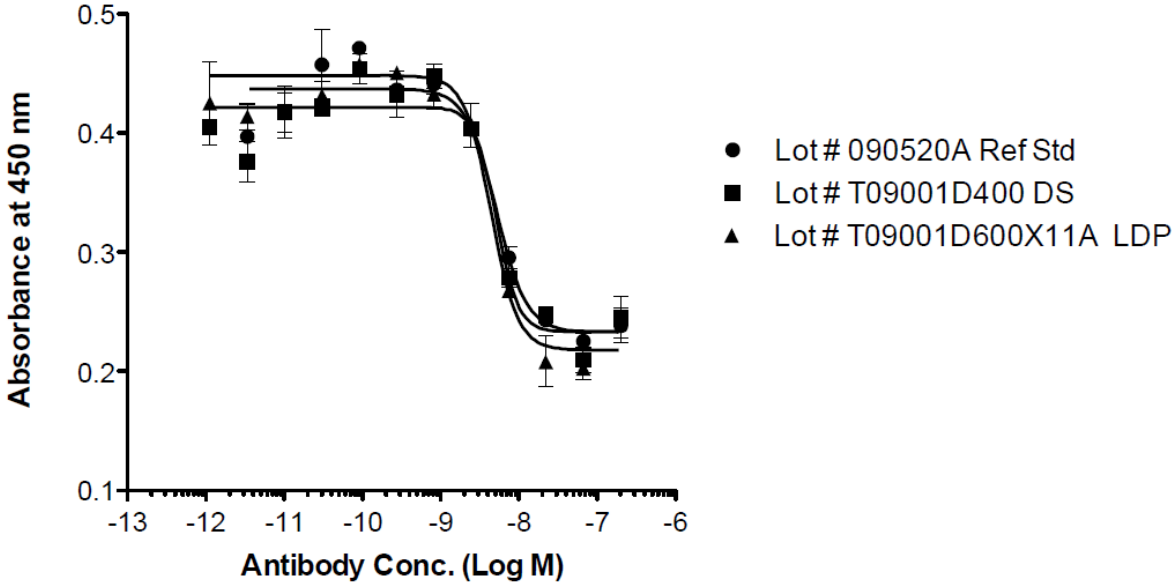
The in vivo PD activity of IL-6Rα blockade in mice by REGN844 was evaluated by challenging mice with turpentine after pretreatment with REGN844 [Study No. REGN844-MX-09076; non-GLP]. Pre-treatment with REGN844 (≥5 mg/kg) completely inhibited the turpentine-induced elevation of serum amyloid A (SAA) protein, an

indicator of the acute inflammatory response in mice. Further, IL-6 serum levels were increased after turpentine injection, and were elevated even further after dosing of >1.5 mg/kg REGN844.

Study Title: Inhibition of IL-6 Binding to Mouse IL-6Rα by REGN844 (Study No. REGN844-MX-09074-SR-01V2; non-GLP)

Methods: A competition Enzyme-Linked Immunosorbent Assay (ELISA) was used to evaluate the ability of REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) to block mouse IL-6Rα-hFc binding to plate-coated human IL-6. REGN844 (3-fold serial dilutions from 200 nM to 3 pM) was added to mouse IL-6Rα-hFc (10 nM fixed final concentration) and incubated for 1 hour prior to transferring to plates coated with human IL-6. Of note, the dose response binding curve for mouse IL-6Rα-hFc binding to plates coated with human IL-6 was determined by incubating mouse IL-6Rα-hFc (600 nM to 10 pM) with plate-coated human IL-6 in the absence of REGN844.

Results: Mouse IL-6Rα-hFc binding to plate-coated human IL-6 was found to be dose dependent with an EC_{50} of ~80 nM. The three lots of REGN844 that were tested (Lot Nos: 090520A Reference Standard; T09001D400 Drug Substance; and T09001D600X11A Labeled Drug Product) were found to inhibit binding of mouse IL-6Rα-hFc (10 nM) to plate-coated human IL-6 in a dose-dependent manner with IC_{50} values of 5 nM, 5 nM, and 4 nM, respectively (see **Figure 3**).



REGN844 inhibits binding of mIL-6Rα-hFc (10 nM) to plate-coated hIL-6. Three lots of REGN844 were assayed and IC_{50} values were determined to be: 5 nM (Lot # 090520A - reference standard, filled circles), 5 nM (Lot # T09001D400 - drug substance, filled squares), and 4 nM (Lot # T09001D600X11A - labeled drug product lot, filled triangles) respectively, using a sigmoidal dose response model. Mouse IL-6Rα-hFc binding was detected using a HRP conjugated goat anti-hFc antibody and the plate-bound IL-6Rα-hFc was determined by the absorbance at 450 nm.

Figure 3 REGN844 Dose-Dependent Inhibition of Mouse IL-6Rα-hFc Binding to Human IL-6 (Applicant's Figure)

Study Title: In vivo Pharmacodynamic Activity of mIL-6Rα Blockade in a Murine Model of Rheumatoid Arthritis by REGN844 Antibody (Study No. REGN844-MX-14076; non-GLP)

Methods: A collagen induced arthritis (CIA) model of RA in DBA/1 mice was used to determine the effects of blocking mouse IL-6Rα with REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) on the development of joint inflammation (onset and degree) and bone erosion. Groups received SC injections of either a murine isotype control antibody (30 mg/kg REGN1299) or REGN844 (10 or 30 mg/kg) twice weekly for 5 weeks.

Results: Upon visual observation for onset of inflammation and swelling after collagen immunization, mice treated with the isotype control antibody REGN1299 had an increased incidence of collagen-induced inflammation beginning at about Week 4 (20% of mice) and seen through Day 58 (70% of mice) towards the end of the study. In comparison, mice treated with 10 mg/kg REGN844 had no observed inflammation or swelling, while 10% (1/10) of mice treated with 30 mg/kg REGN844 developed joint inflammation (limited to a single digit) at Week 7. Further, the degree of disease inflammation was high in the mice treated with the isotype control antibody REGN1299 (i.e., 7/10 mice had inflammation present in at least one limb, with a majority of those

animals [5/7] having inflammation in more than one limb by Day 61). In comparison, no mice treated with 10 mg/kg REGN844 and one mouse treated with 30 mg/kg REGN844 had disease inflammation. At the end of the study (Day 61) bone erosion for all four mouse limbs was assessed by Quantum FX micro CT scanning. For the mice treated with the REGN1299 isotype control, 50% of the mice (5/10) had bone erosion in at least one limb, with a majority of those animals (4/5) having erosion in more than one limb. In comparison, no animals treated with 10 mg/kg REGN844 and only one mouse treated with 30 mg/kg REGN844 had signs of bone erosion.

Study Title: In Vivo Pharmacodynamic Activity of IL-6Rα Blockade in Humanized Mice by Sarilumab/REGN88 (Study No. REGN88-MX-13129-SR-01V1; non-GLP)

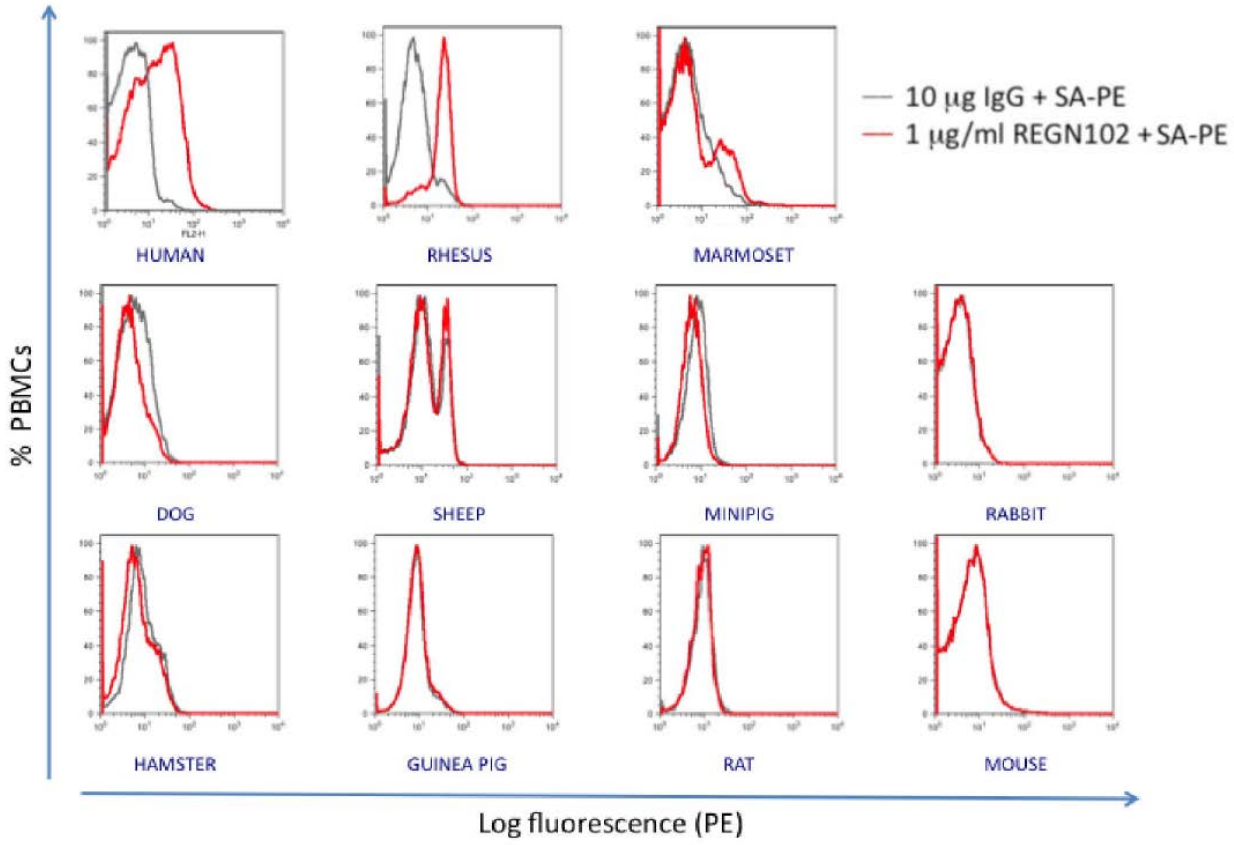
Methods: A turpentine model of acute inflammation in genetically engineered double-humanized IL-$6^{hu/hu}$IL-$6R\alpha^{hu/hu}$ mice was used to determine the PD effect of sarilumab (0.015 mg/kg to 15 mg/kg) on circulating SAA. Of note, the double-humanized mice were made using homologous recombination to substitute the endogenous mouse Il6 gene locus with the corresponding human IL-6 genomic sequences containing the exon region. Further, the mouse Il6ra gene encoding the extracellular domain of IL-6Rα was replaced with the corresponding human genomic DNA encoding the extracellular domain of human IL-6Rα. This resulted in a chimeric IL-6Rα with mouse-specific intracellular signaling but with an extracellular domain that could now be recognized by sarilumab and human IL-6.

Results: Administration of turpentine to the double-humanized mice increased serum SAA. If sarilumab was given to the mice 24 hours prior to the turpentine challenge, serum SAA levels were decreased in a dose-dependent manner, most notably at doses ≥1.5 mg/kg. In the same experiment, human IL-6 levels were measured and were only slightly increased after administration of turpentine. If sarilumab was given to the mice 24 hours before the administration of turpentine then IL-6 levels were increased in a dose-dependent manner at doses ≥1.5 mg/kg through inhibition of endogenous receptor-mediated clearance.

Study Title: Cross-Reactivity of Sarilumab (REGN88) Against IL6Rα of Non-Human Primate and Non-Primate Species (Study No. REGN88-MX-11078-SA-01V1; non-GLP)

Methods: Whole blood from human, rhesus monkey, marmoset, dog, sheep, mini pig, rabbit, hamster, guinea pig, rat, and mouse was used to evaluate the binding of sarilumab to cell surface IL-6Rα. In brief, after lysing red blood cells and fixing white blood cells, the white blood cells were incubated with 10 mcg/mL human polyclonal IgG to block endogenous Fc receptors. Subsequently, the white blood cells were incubated without (control) or with 1 mcg/mL REGN102-biotin antibody (mouse IgG2a with the same Fv fragment as sarilumab). Flow cytometry was used to evaluate the cross-reactivity of sarilumab binding to cell surface IL-6Rα in primary peripheral blood mononuclear cells (PBMC).

Results: As shown in **Figure 4**, binding of REGN102 in rhesus monkey PBMC was similar to that in humans. Binding was also evident in a small percentage of marmoset PBMCs. In contrast, no binding was evident in any of the other species evaluated.



Fluorescence intensity was measured in PBMC gated on forward side scatter. Gray line represents signal samples pre-incubated with excess human IgG and stained with streptavidin-PE, red line represents signal in samples incubated with REGN102-biotin (same Fv fragment as Sarilumab) and stained with streptavidin-PE.

Figure 4 Cross-Reactivity of Sarilumab Binding to Cell Surface IL-6Rα in Primary PBMC (Applicant's Figure)

Study Title: In Vitro Studies Demonstrating that REGN88 Directly Blocks Binding of IL-6 to IL-6Rα (Study No. REGN88-MX-14072-SR-01V1; non-GLP)

Methods: A SPR Biacore study was used to determine if REGN88 could directly block the binding of human IL-6 (200 nM) to sensor chip-captured human IL-6Rα saturated with either REGN88 or a non-blocking anti-IL-6Rα monoclonal antibody. An ELISA competition assay was used to measure the ability of REGN88 or an isotype control IgG1 antibody to directly block the binding of human IL-6Rα (100 pM) to human IL-6 immobilized on microtiter plates. A functional cell-based luciferase reporter assay was employed to determine the ability of REGN88 to block soluble human IL-6Rα trans-signaling in vitro by using a human cell line (i.e., human embryonic kidney 293 [HEK293]) that lacked IL-6Rα expression but that stably expressed human gp130 and a STAT3 response element linked to a luciferase reporter gene. This luciferase reporter

cell line was activated by the human IL-6 and soluble human IL-6Rα complex but not by human IL-6 alone.

Results: In the SPR Biacore assay, REGN88 completely blocked IL-6 from binding to sensor chip-captured human IL-6Rα. The average signal for bound IL-6 for the buffer alone surface was 117 RU. In comparison, when the sensor chip-captured human IL-6Rα was saturated with the non-blocking control monoclonal antibody or REGN88 the average signal was 131 RU or -38 RU, respectively.

In the ELISA competition assay, REGN88 blocked human IL-6Rα from binding to human IL-6 in a concentration-dependent manner with an IC_{50} value of 108 pM. In comparison, the isotype control IgG1 antibody did not block the binding between IL-6Rα and IL-6.

In the luciferase reporter assay, when HEK293 cell line overexpressing gp130 were stimulated with 1 nM of IL-6Rα and increasing concentrations of IL-6 an EC_{50} of 2.2 nM was observed. When REGN88 was preincubated with the 1 nM of IL-6Rα and then added to the cells in the presence of 12.5 nM IL-6, the activation of the cells was blocked with an IC_{50} of 860 pM. Increasing concentrations of IL-6 alone did not activate trans-signaling under the conditions tested.

Study Title: In vitro Inhibition of Mouse IL-6Rα Signaling by REGN844 (Study No. REGN844-MX-09075-SR-01V2; non-GLP)

Methods: An in vitro cell proliferation assay with a mouse B-cell hybridoma cell line (B9 cells) was used to determine the ability of REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) to inhibit IL-6 activity. The B9 cells were treated without (control) or with REGN844 at concentrations from 10 nM to 0.17 pM in the presence of either human or mouse IL-6 (0.5 pM). After 3 days, cell growth was assessed using WST-8 (a colorimetric indicator of cell number).

Results: A dose-dependent increase in B9 cell proliferation was seen with IL-6 resulting in an EC_{50} of approximately 0.2 pM for both human and mouse IL-6 ((b) (4) vendor; 1 of 3 vendors tested for mouse IL-6). In the presence of REGN844, proliferation of B9 cells was inhibited by both human and mouse ((b) (4) vendor) IL-6 (0.5 pM) with IC_{50} values of 60 pM and 110 pM, respectively.

Study Title: Evaluation of Fc Effector Function for REGN88 (Study No. REGN88-MX-07015-SR-01V2; non-GLP)

Methods: Antibody-dependent cell-mediated cytotoxicity (ADCC) was assessed using PBMC as effector cells. The target cells were two human lymphoma cell lines (KG-1 and XG-1) and a hepatocytic cell line (HepG2); flow cytometry analysis with biotinylated REGN88 or a commercial anti-IL-6R mouse monoclonal antibody labeled with phycoerythrin (PE) was used to determine the expression of IL-6R on these cell lines. For the ADCC assay, the target cells were incubated for 10 minutes with serial dilutions

of REGN88 (final concentrations ranged from 10 nM to 0.169 pM). The PBMC, which had previously been stimulated with IL-2 for 3 days, were then added to the target cells/REGN88. After 4 hours, cell lysis (cytotoxicity) was determined based on the amount of lactate dehydrogenase (LDH) enzyme in the solution. Rituxamab treatment of Daudi lymphoma cells as the target cells served as a positive control to show the activity of PBMC in the ADCC experiment.

Complement-dependent cytotoxicity (CDC) was evaluated also using the KG-1, XG-1, and HepG2 cell lines as target cells. The target cells were first treated with serial dilutions of REGN88 (50 nM to 0.845 pM). Subsequently, 5% normal human serum with complement components was added to the target cells. Cell survival was assessed using CellTiter-Blue. Rituxamab treatment of Daudi lymphoma cells as the target cells served as a positive control in the CDC experiment.

Experiments using SPR Biacore technology were performed to assess the in vitro binding of REGN88 to a panel of recombinant Fcγ receptor proteins and to purified C1q protein on a dextran-coated (CM5) chip surface using two surface format binding schemes. In the first surface format, rituximab, REGN88, and REGN88 bound to human IL-6R were immobilized by anti-$(Fab')_2$ chip and then varying concentrations of Fcγ and C1q were evaluated for binding. In the second format, Fcγ receptor proteins were immobilized by anti-His antibody and varying concentrations of rituximab, REGN88, and REGN88 bound to IL-6R were assessed for binding.

Results: Flow cytometry analysis of biotinylated REGN88 and a commercial PE-labeled anti-IL-6R mouse monoclonal antibody indicated that the highest expression of IL-6R was seen on KG-1 cells and then XG-1 cells (order of magnitude less than KG-1 cells). HepG2 cells showed very low expression of IL-6R.

In the ADCC assay, treatment of Daudi cells with rituximab and then the addition of the PBMC effector cells increased ADCC activity in a dose-dependent manner. In comparison, little or no ADCC activity was noted for KG-1, XG-1, or HepG2 cells that were treated with REGN88 and then exposed to PBMC.

In the CDC assay, treatment of Daudi cells with rituximab and 5% normal human serum complement increased the percent cytotoxicity in a dose-dependent manner. In comparison, KG-1, XG-1, or HepG2 cells that were treated with REGN88 and 5% normal human serum complement did not show any increase in cytotoxicity. Of note, KG-1 and XG-1 cells not treated with REGN88 had about 40% baseline cytotoxicity in the presence of 5% normal human serum complement compared to its absence. In contrast, HepG2 cells not treated with REGN88 had -80% baseline cytotoxicity in the presence of 5% normal human serum complement compared to its absence, indicating proliferation.

Under the conditions tested in the SPR Biacore binding study using the first surface format binding scheme, neither REGN88 nor rituximab exhibited binding with C1q at concentrations from 10 mcM to 78.1 nM. Similarly, when immobilized REGN88 was

bound to human IL-6R there was no evident interaction with C1q. Binding of surface-captured rituximab and REGN88 to FcγRI (high affinity receptor for Fc domain antibody binding) was noted with equilibrium dissociation constants of 0.4 nM and 0.5 nM, respectively. In comparison, immobilized REGN88 bound to human IL-6R had a dissociation constant of 0.4 nM. Binding of surface-captured REGN88 to low affinity recombinant human Fcγ receptors (i.e., FcγRIIa [R131], FcγRIIa [H131], FcγRIIb, FcγRIIIa [V176], FcγRIIIa [F176], and FcγRIIIb) exhibited dissociation constants from 0.7 to 3.1 mcM. Similar dissociation constants were noted for low affinity Fcγ receptors binding to immobilized REGN88 bound to human IL-6R (i.e., K_d = 0.9 to 4 mcM). In comparison, rituximab binding to the low affinity Fcγ receptors had equilibrium dissociations constants from 0.8 to 7.3 mcM.

Under the conditions tested in the SPR Biacore binding study with the second surface format binding scheme, binding of rituximab and REGN88 to immobilized FcγRI resulted in equilibrium dissociation constants of 3.4 nM and 5.9 nM, respectively. REGN88 bound to soluble human IL-6R-mFc fusion protein had an equilibrium dissociation constant of 4.1 nM. When the effect of clustering rituximab and REGN88 with an anti-human kappa $(Fab')_2$ clustering antibody was assessed, the equilibrium dissociation constants were calculated to be 2.8 nM and 6.3 nM, respectively. In comparison, clustering REGN88 bound to soluble human IL-6R-mFc fusion protein resulted in a K_d of about 4.8 nM. Binding of surface-captured low affinity Fcγ receptors to rituximab and REGN88 resulted in equilibrium binding constants from 0.6 mcM to 5.9 mcM and 2.5 mcM to 19.1 mcM, respectively. In comparison, when REGN88 was bound to soluble human IL-6R-mFc fusion protein, affinities to the immobilized low affinity Fcγ receptors were increased with equilibrium binding constants from 0.3 mcM to 12.8 mcM. When the effect of clustering rituximab and REGN88 with $(Fab')_2$ was assessed, the equilibrium dissociation constants ranged from 0.5 mcM to 5.9 mcM and 0.7 mcM to 7.6 mcM, respectively. In comparison, when REGN88 bound to soluble human IL-6R-mFc fusion protein was clustered with $(Fab')_2$, affinities to the immobilized low affinity Fcγ receptors had equilibrium binding constants from 0.4 mcM to 30 mcM. See **Table 2**.

Table 2 Summary of Equilibrium Dissociation Constants for REGN88 with Immobilized Fcγ Receptors (Applicant's Table)

	Rituximab	Rituximab + $(Fab')_2$	REGN88	REGN88 + $(Fab')_2$	REGN88 +hIL-6R-mFc	REGN88+hIL-6R-mFc +$(Fab')_2$	hIL-6R-mFc
Surface	K_D (M)	K_D (M)	K_D (M)	K_D (M)	K_D (M)	K_D (M)	K_D (M)
FcγR1	3.4×10^{-9}	2.8×10^{-9}	5.9×10^{-9}	6.3×10^{-9}	4.1×10^{-9}	4.8×10^{-9}	5.7×10^{-9}
FcγRIIa (R131)	2.3×10^{-6}	4.0×10^{-6}	7.6×10^{-6}	4.2×10^{-6}	2.1×10^{-6}	0.8×10^{-6}	0.5×10^{-6}
FcγRIIa (H131)	1.1×10^{-6}	1.0×10^{-6}	5.7×10^{-6}	3.2×10^{-6}	1.8×10^{-6}	1.5×10^{-6}	0.3×10^{-6}
FcγRIIb	5.9×10^{-6}	5.9×10^{-6}	19.1×10^{-6}	7.6×10^{-6}	12.8×10^{-6}	30.0×10^{-6}	0.3×10^{-6}
FcγRIIIa (V176)	0.6×10^{-6}	0.5×10^{-6}	2.5×10^{-6}	0.7×10^{-6}	0.3×10^{-6}	0.4×10^{-6}	0.4×10^{-6}
FcγRIIIa (F176)	2.6×10^{-6}	4.1×10^{-6}	8.2×10^{-6}	5.0×10^{-6}	2.9×10^{-6}	2.1×10^{-6}	1.0×10^{-6}
FcγRIIIb	5.2×10^{-6}	3.2×10^{-6}	13.3×10^{-6}	5.7×10^{-6}	9.9×10^{-6}	5.2×10^{-6}	ND[a]

Equilibrium dissociation constants obtained for the interaction of immobilized Fcγ receptors and REGN88 or comparator antibody. Fcγ receptors were immobilized *via* capture with an anti-Penta-His Tag polyclonal antibody.

[a] ND (Not Determined). An equilibrium dissociation constant could not be calculated due to a minimal level of detectable binding interaction.

4.2 Secondary Pharmacology

No secondary pharmacology studies were conducted.

4.3 Safety Pharmacology

No stand-alone safety pharmacology studies were conducted.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Brief Summary

Nonclinical pharmacokinetic (PK) studies were conducted with sarilumab in cynomolgus monkeys and rats. A summary of the mean PK parameters for total sarilumab in monkeys is shown in **Table 3**. In brief, following single IV (1 and 15 mg/kg) or SC doses (1, 5, and 15 mg/kg), AUC values increased greater than dose proportionally. Subcutaneous bioavailability was determined to be about 75%, based on AUC comparisons between the IV and SC doses at 1 and 15 mg/kg. The volume of distribution was low and consistent with the blood volume.

Table 3 Summary of Mean Pharmacokinetic Parameters for Total Sarilumab in Monkeys (Applicant's Table)

Study No. (Compliance)	Route	Dose (mg/kg)	Sex (n)	t_{max} (h)	C_{max} (µg/mL)	C_{max}/Dose (µg/mL per mg/kg)	$t_{½}$[a] (h)	$AUC_{0-\infty}$[b] (µg•h/mL)	$AUC_{0-\infty}$/Dose[b] (µg•h/mL per mg/kg)	V_{ss} (mL/kg)	MRT[c] (h)	CL[d] (mL/h/kg)	%F[e]
[REGN88-PK-06041] (GLP)	IV	1	M+F (3+3)	0.589	39.8	39.8	29.1	1660	1660	NC	45.9	0.627	NA
		15	M+F (3+3)	1.59	533	35.5	233 / 69.9	79200 / 79100	5280 / 5270		260 / 257	0.193 / 0.193	
	SC	1	M+F (3+3)	41	10.7	10.7	30.0	1280	1280	NA	79.6	0.833	77.4
		5	M+F (3+3)	72	57.9	11.6	113 / 35.6	13100 / 13100	2620 / 2620		158 / 156	0.401 / 0.402	NA
		15	M+F (3+3)	112	177	11.8	226 / 58.5	62400 / 62300	4160 / 4150		279 / 276	0.261 / 0.262	78.7
[REGN88-PK-09092] (GLP)	SC	5 (C1P2F2)[f]	M+F (5+5)	84.0	73.2	14.6	148 / 26.4	15800	3180	NA	146	0.320	NA
		5 (C1P2F3)[f]	M+F (5+5)	88.8	59.9[g]	12.0	166 /38.5	14400	2890		172	0.360	NA
		5 (C2P1F3)[f]	M+F (5+5)	74.4	50.9[g]	10.2	144 / 41.0	11400	2290		163	0.459	51.6
	IV	5 (C2P1F3)[f]	M+F (5+5)	0.813	244	48.8	134 / 35.4	22000	4410	44.8[h]	134	0.231	NA

Note: Values reported before and after the '/' represent values above and below target saturation, respectively.
[a] $t_{1/2}$ values presented were determined using user-defined λ_z values from report [REGN88-PK-06041-SA-01V1].
[b] Values rounded to 3 significant figures from original reports.
[c] Values are MRT_{INF} and MRT_{last} for studies [REGN88-PK-06041] and [REGN88-PK-09092], respectively.
[d] Values reported for the SC route are CL/F values.
[e] %F was calculated using AUC_{last} values (see [TS 2.6.5]).
[f] For details of cell lines and formulations (see [TS 2.6.5.3]).
[g] Values significantly lower ($p<0.05$) than those in other SC groups.
[h] Volume of distribution based on the terminal phase (V_z) was calculated by Phoenix WinNonlin for study [REGN88-PK-09092].
NA = Not applicable; NC = Not calculated.

No distribution, metabolism, or excretion studies with sarilumab were conducted.

5.2 Toxicokinetics

Toxicokinetics are discussed in **Section 6**.

6 General Toxicology

6.1 Single-Dose Toxicity

No single-dose toxicity studies were conducted.

6.2 Repeat-Dose Toxicity

GLP-compliant repeat-dose toxicology studies were conducted with sarilumab in pharmacologically relevant cynomolgus monkeys for durations of 4-weeks (IV), 13-weeks (IV and SC), and 6-months (IV). A non-GLP-compliant repeat-dose toxicology study was conducted in mice. Since mice were not a pharmacologically relevant species the study was conducted with REGN844, a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα. These repeat-dose toxicology studies were reviewed under IND 100632 and a brief summary is provided below. Refer to Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016, attached in the appendices, for additional details.

In addition, two repeat-dose toxicity studies were conducted in juvenile mice with REGN844 (non-GLP exploratory 4-week study and GLP 9-week study). The 4-week exploratory study was reviewed under IND 100632 (Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016). A detailed review of the 9-week toxicity study in the juvenile mouse is provided below.

Mouse

Study Title: SAR153191 (REGN88): Exploratory 4-Week Subcutaneous and Intravenous Toxicity Study in Mice with REGN844 (Study No. (b) (4) 1267; non-GLP)

REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) was given to Crl:CD1(SD) mice that were about 6 to 7 weeks of age (10/sex/group) twice per week by SC administration at 0 (placebo control), 5, 25, or 100 mg/kg/dose (10, 50, or 200 mg/kg/week, respectively) or one time per week by IV administration at 25 mg/kg/dose for 4 weeks. There were no mortalities in the study and no test article-related effects on any of the study parameters (clinical signs, body weights, hematology, clinical chemistry, organ weights, gross pathology, and histopathology). Systemic exposure (mean C_{max} and AUC values) increased with dose and there was no sex difference in exposure. Drug accumulation was observed over the 4-week dosing period.

Cynomolgus Monkey

Study Title: A 4-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 9-Week Recovery Period (Study No. REGN88-TX-06040 [(b) (4) -460003]; GLP)

Sarilumab was given to cynomolgus monkeys (5/sex/group) one time per week by IV infusion at 0 (placebo control), 5, 10, or 40 mg/kg/dose for 5 weeks. Three monkeys/sex/group were sacrificed one week after a 5-week main study period and the remaining 2 monkeys/sex/group were sacrificed after a 9-week recovery period. There were no mortalities in the study. There were no test article-related effects on clinical signs, body weight, ophthalmoscopy, electrocardiogram (ECG), blood pressure, body temperature, urinalysis, gross pathology, and histopathology.

Hematology and coagulation evaluation revealed a possible test article effect on neutrophils, lymphocytes, and fibrinogen and clinical chemistry evaluation revealed an effect on CRP levels. In Week 1, a decrease in mean neutrophil counts (percent and absolute) was noted for males in the low dose and high dose groups and for females in all dose groups. In Week 4, neutrophil counts (percent and absolute) were also decreased in low dose and high dose males and in high dose females. Percent lymphocytes were increased in low dose and high dose males and in females in all dose groups in Week 1. Mean levels of fibrinogen were decreased in all test article-treated males and females in Weeks 1 and 13. The effects noted on neutrophils, lymphocytes,

and fibrinogen were reversible after the recovery period. Clinical chemistry evaluation showed a decrease in CRP levels in all test article-treated females in Weeks 1 and 4.

An assessment of organ weights showed that mean thymus weights (absolute and relative to body weight) were decreased in males and females in all test article-treated groups but lacked a dose response. The decrease was reversible after the recovery period.

Toxicokinetic analysis indicated that mean total concentrations of REGN88 (sarilumab) increased proportionally between the three doses. Increases in trough levels were also noted and did not return to baseline before the next dose, suggesting drug accumulation. There did not appear to be any sex differences in toxicokinetics. ADA was detected in two monkeys in the low dose group and three monkeys in the mid dose group. In these animals, peak increases in sarilumab levels were similar to other animals but lower trough levels at 24 hours postdose suggested increased drug clearance.

Study Title: A 13-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by an 8-Week Recovery Period (Study No. REGN88-TX-06037 [(b) (4)-460004]; GLP)

Sarilumab was given to cynomolgus monkeys (6/sex/group) one time per week by IV infusion at 0 (placebo control), 1, 10, or 50 mg/kg/dose for 13 weeks. Four monkeys/sex/group were sacrificed after a 13-week main study period and the remaining 2 monkeys/sex/group were sacrificed after an 8-week recovery period. There were no test-article related mortalities in the study. Of note, one high dose male was found dead on Day 31 and the death was attributed to gavage error when giving nutritional supplementation. Also, one female in the mid dose recovery group died prior to sacrifice on Day 123 and the death was considered likely due to amoebiasis. There were no test article-related effects on body weight, ophthalmoscopy, ECG, blood pressure, body temperature, urinalysis, gross pathology, and histopathology.

With regard to clinical signs, male monkeys treated with all dose levels of sarilumab had slightly higher incidences of fecal findings during the main study period than control males. Female monkeys treated with all dose levels of sarilumab had an increased incidence of swollen urogenital area and this persisted during the recovery period in the mid dose and high dose groups.

Evaluation of hematology and coagulation parameters identified a decrease in mean neutrophil counts (absolute and percent) in males and females in all test article-treated groups in Weeks 3 and 13, compared to the control groups. A decrease in mean levels of fibrinogen was seen in males and females in the mid and high dose groups in Weeks 3 and 13. Clinical chemistry evaluation indicated that mean levels of CRP were decreased in males and females in all test article-treated groups in Week 0 and in the mid and high dose groups in Week 3. During Week 13, mean CRP levels were lower in females in all test article-treated groups.

An assessment of organ weights showed that mean thymus weights (absolute and relative to body weight) were increased in males and females in all test article-treated groups but lacked a dose response. There were also increased spleen weights (absolute and relative to body weight) in the males in the high dose group and females in all test article-treated groups compared to controls.

Toxicokinetic analysis indicated that systemic exposure (AUC_{all}) increased with dose. No sex differences were noted. Drug accumulation was seen between Week 1 and Week 13 for males and females in the mid and high groups but not in the low dose groups. No ADA was detected in the mid and high dose groups but 11/12 monkeys in the low dose group had ADA. The ADA in the low dose group appeared to correlate with decreased drug exposure at the end of the main study period due to increased clearance.

Study Title: A 13-Week Subcutaneous Injection Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 12-Week Recovery Period (Study No. REGN88-TX-06038 [(b) (4)-460005]; GLP)

Sarilumab was given to cynomolgus monkeys (6/sex/group) twice per week by SC injection at 0 (placebo control), 1, 5, 15, or 50 mg/kg/dose (2, 10, 30, or 100 mg/kg/week, respectively) for 13 weeks. Four monkeys/sex/group were sacrificed 1 week after a 13-week main study period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period. There were no mortalities in the study. There were no test article-related effects on clinical signs, body weight, ophthalmoscopy, ECG, blood pressure, body temperature, urinalysis, organ weight, and gross pathology.

Evaluation of hematology and coagulation parameters showed a test article-related effect on neutrophils and fibrinogen. Noted in Week 3 was a decrease in mean neutrophil counts in males in all test article-treated groups and in females in all test article-treated groups except for the 15 mg/kg/dose group. In Week 13, neutrophil counts were still decreased in all test article-treated male groups. In females, the decrease in neutrophils was noted in all test article-treated groups except for the 50 mg/kg/dose group. The test article-related decreases in neutrophils appeared to reverse by the end of the recovery period. Decreases in mean levels of fibrinogen were seen in males and females in ≥5 mg/kg/dose groups in Weeks 3 and 13. Evaluation of clinical chemistry parameters identified a decrease in mean CRP levels in males in ≥5 mg/kg/dose groups and in females in all test article-treated groups in Weeks 0 and 3.

Histopathological evaluation determined that the most notable test article-related finding was minimal to moderate perivascular mixed inflammatory cell infiltrates at the injection site, which was reversible at the end of the recovery period.

Toxicokinetic analysis indicated that mean serum concentrations of sarilumab increased with dose. No sex differences were noted. Drug accumulation was seen over the

course of the study for males and females in all dose groups except the low dose group. No ADA was detected in the 0, 15 or 50 mg/kg/dose groups but all animals in the 1 mg/kg/dose group and 4/12 animals in the 5 mg/kg/dose group had ADA. The ADA in the low dose group appeared to correlate with decreased drug exposure at the end of the main study period due to increased clearance.

Study Title: A 13-Week Bridging Subcutaneous Toxicity Study of REGN88 in Cynomolgus Monkeys (Study No. REGN88-TX-09053 [(b) (4)-460024]; GLP)

The objective of this study was to compare the potential toxicity of process 1 (P1) sarilumab to a newer process 3 (P3) sarilumab. Sarilumab was given to cynomolgus monkeys (4/sex/group) twice per week by SC injection at 0 (P3 placebo control), 5 (P3), 50 (P3), or 50 (P1) mg/kg/dose (10 [P3], 100 [P3], or 100 [P1] mg/kg/week, respectively) for 13 weeks. There were no mortalities in the study. There were no test article-related effects on clinical signs, body weight, ophthalmoscopy, heart rate, ECG, blood pressure, body temperature, clinical chemistry, urinalysis, organ weight, and gross pathology.

Evaluation of hematology and coagulation parameters identified a test article-related effect on neutrophils, white blood cells, and fibrinogen. Mean neutrophil counts were decreased in all test article-treated groups of both males and females in Week 4. Mean white blood cell numbers were decreased in all test-article treated male groups in Week 4, which was attributed to the decline of neutrophil counts. Mean levels of fibrinogen were decreased in males in the 50 (P3) and 50 (P1) mg/kg/dose groups and in females in all test article-treated groups in both Weeks 4 and 12. The clinical chemistry evaluation found that an effect on CRP could not be discerned because baseline levels (Week -3) of CRP in the test article-treated groups were lower than in the control groups for both males and females.

Histopathological evaluation determined that the most notable test article-related finding was minimal perivascular mononuclear infiltrate at the injection site. This finding was noted in all test article-treated males and in females in the 50 (P3) and 50 (P1) mg/kg/dose groups. Of note, 1/4 males in the 50 (P1) mg/kg/dose group had an adenoma in the adrenal cortex (benign neoplasm). Also, 1/4 females in the 5 (P3) mg/kg/dose group had moderate squamous metaplasia in the cervix. These preneoplastic and neoplastic findings were considered incidental.

Toxicokinetic evaluation indicated that the mean systemic exposure of sarilumab (mean serum concentration and $AUC_{0\text{-}24h}$) in the 5 (P3) and 50 (P3) mg/kg/dose groups increased dose proportionally. When comparing the 50 (P3) and the 50 (P1) mg/kg/dose groups, for males and females combined, $AUC_{0\text{-}24h}$ in the 50 (P3) mg/kg/dose group was about 16% less than in the 50 (P1) mg/kg/dose group over the course of the study (Week 0 to Week 11). No sex differences were noted. Drug accumulation was seen over the course of dosing (Week 0 to Week 11). ADAs were found in 1/4 males and 1/4 females in the 5 (P3) mg/kg/dose groups but not in either the 50 (P3) or the 50 (P1) mg/kg/dose groups.

Study Title: A 6-Month Once Weekly 30-Minute Intravenous Infusion Study of REGN88 in Cynomolgus Monkeys Followed by a 12-Week Recovery Period (Study No. REGN88-TX-08031 [(b) (4)-460012]; GLP)

Sarilumab was given to cynomolgus monkeys (6/sex/group) once per week by IV infusion at 0 (placebo control), 0.5, 5, 15, and 50 mg/kg/week for 26 doses. Four monkeys/sex/group were sacrificed after the 26-week main study period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period. There were no test article-related mortalities in the study. Of note, one male in the 0.5 mg/kg/week group was found dead on Day 159 and the death was attributed to accidental choking. Also, one male in the 15 mg/kg/week group was sacrificed early on Day 133 due to moderate typhlocolitis; the relationship to the test article was considered unclear. There were no test article-related effects on clinical signs, body weight, ophthalmoscopy, ECG, blood pressure, body temperature, urinalysis, gross pathology, and histopathology.

The hematology and coagulation evaluation identified test-article related effects on neutrophils, white blood cells, and fibrinogen. At Week 25, neutrophil counts (absolute and percent) were lower in all test article-treated groups for both males and females, although this decrease was not dose related. In most cases, the decrease seemed to be reversible at the end of the recovery period. At Week 25, white blood cells appeared to be decreased in males treated with ≥5 mg/kg/week of the test article and in females in all test article-treated groups, which was attributed to the decline of neutrophil counts. There was also a trend towards a non-dose-related decrease in fibrinogen levels from Week 4 to the end of the main study period in males and females in groups treated with ≥5 mg/kg/week of the test article.

The clinical chemistry evaluation found that CRP levels were decreased in Weeks 4, 12, and 25 for males treated with ≥15 mg/kg/week of the test article. In females, decreases in CRP levels were noted in Weeks 4 and 12 when treated with ≥5 mg/kg/week of the test article. These decreases were reversible by the end of the recovery period. Mean IL-6 levels were increased in male and female groups treated with ≥5 mg/kg/week of the test article at Weeks 4, 12, and 25. These increases were considered a PD effect and were reversed by the end of the recovery period.

An evaluation of organ weights revealed an increase in mean thymus weights (absolute and relative to body weight) in males in all test article-treated groups and in females treated with ≥5 mg/kg/week of the test article. These increases reversed by the end of the recovery period.

Evaluation of a T-cell dependent antibody response (TDAR) identified that there was a small decrease in both primary and secondary IgG responses to keyhole limpet hemocyanin (KLH) administration in males and females treated with ≥5 mg/kg/week of the test article.

Toxicokinetic evaluation determined that mean systemic exposure of sarilumab (AUCs and peak serum levels) increased with dose. No difference in exposure was noted between sexes. Drug accumulation was evident between the 1st and the 25th dose for test article doses ≥5 mg/kg in both males and females. All animals in the low dose group and 4/6 males and 1/6 females in the 5 mg/kg/week group had ADA. ADA appeared to decrease overall exposure by increasing drug clearance.

Juvenile Toxicity

A detailed review of the 9-week toxicity study in the juvenile mouse is provided below.

Study title: REGN844: 9-Week Subcutaneous Toxicity Study in the Juvenile Mouse Followed by a 13-Week Recovery Period

Study no.:	(b) (4) Study No.: 8297885 Sanofi-Aventis Reference No.: JUV0030 Regeneron Reference No.: REGN844-TX-13063
Study report location:	SDN 10; eCTD Sequence No. 0009
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 1, 2014 (animal arrival/allocation) April 8, 2014 (first treatment for toxicity and recovery animals)
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Drug: REGN844 (murine monoclonal antibody against mouse IL-6Rα); lot #: T09001D600X11A; % purity: 98.7

Key Study Findings

- Fourteen day old Crl:CD1(ICR) mice were treated once per week for 9 weeks with REGN844 at SC doses of 20, 60, or 200 mg/kg. A 13-week recovery period was included to assess reversibility of any test article-related findings.
- There were no test article-related deaths.
- There were no test article-related changes in hematology or clinical chemistry parameters in any of the dose groups. Of note, an assessment of CRP was not made.
- Microscopic findings of increased incidence and/or severity were noted in the femur + marrow, sternum + marrow, lymph nodes (axillary and mandibular), and injection site (neck). These findings were reversible at the end of the recovery period. A minimal increase in hemopoiesis was noted in the femur + marrow of high dose males and females. Females in the mid dose group also had a minimal increase in hemopoiesis in the femur + marrow and sternum + marrow. Lymphoid hyperplasia was noted with increased incidence in the axillary lymph

nodes of mid dose and high dose males and females in all dose groups. Lymphoid hyperplasia was also noted in the mandibular lymph nodes of high dose males and females but at a lesser incidence than in the axillary lymph nodes. At the injection site the main findings were inflammation of the subcutis and dermis. High dose males and females in all test article-treated groups had minimal to slight subcutis inflammation. High dose males and females in the mid dose group had minimal to slight inflammation of the dermis.

- The main test article-related effect was observed in the TDAR assessment as a decrease in the IgG responses to KLH challenge in males at all doses and at all the time points evaluated. These findings were attributed to the immunosuppressive properties of REGN844. Decreased IgG responses to antigen challenge were reversible at the end of the recovery period. A decrease in the IgM response to KLH was only noted 6 days after KLH challenge in males in all test article-treated groups.
- Total serum IgG concentrations were increased at mid (60 mg/kg/week) and high doses (200 mg/kg/week) of the test article for males and females, which might be attributed to administration of exogenous IgG (i.e., REGN844). IgM concentrations were only increased for females in the high dose group (200 mg/kg/week). These findings were reversible by the end of the recovery period.
- C_{max} and $AUC_{0-\tau}$ were generally dose proportional between groups. T_{max} was 24 hours for the low dose group and 72 hours for both the mid and high dose groups. There did not appear to be any sex differences in REGN844 toxicokinetic parameters.

Methods

Doses:	See **Table 4**
Frequency of dosing:	One time/week on postnatal days (PND) 14, 21, 28, 35, 42, 49, 56, 63, and 70 (recovery animals were dosed on PND 70 and then given a 13-week recovery period prior to necropsy)
Dose volume:	10 mL/kg
Route of administration:	SC injection (neck)
Formulation/Vehicle:	REGN844 placebo product (10 mM histidine, 0.13% w/v polysorbate 20, 6% sucrose, pH 6.0)
Species/Strain:	Mouse/Crl:CD1(ICR)
Number/Sex/Group:	See **Table 4**
Age:	PND 14 at initiation of dosing
Satellite groups:	TDAR and TDAR recovery subgroups, Immunotoxicity subgroup, Toxicokinetic subgroup (See **Table 4)**
Study design:	See **Table 4**
Deviation from study protocol:	The protocol deviations were not considered to have affected the study results or interpretation. Of note, for PND 37 toxicity group mice each of the first 6 animals per group were sampled for hematology and clinical chemistry evaluations and were then killed and discarded without tissues being retained or immunophenotyping by flow cytometry. Because this procedure was done to each group, it was not considered by the Study Director to have affected the study results or interpretation. The Reviewer concurs.

Table 4 Study Design for 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Group	Description	Dose level (mg/kg/ week)	Number of Animals in Group			
			Toxicity (Subgroup 1)		Tox Recovery (Subgroup 1)	
			Male	Female	Male	Female
1	Control	0	18	18	6	6
2	Low	20	18	18	6	6
3	Intermediate	60	18	18	6	6
4	High	200	18	18	6	6
			TDAR (Subgroup 2)		TDAR Recovery (Subgroup 2)	
			Male	Female	Male	Female
1	Control	0	12	12	12	12
2	Low	20	12	12	12	12
3	Intermediate	60	12	12	12	12
4	High	200	12	12	12	12
			Immunotoxicity (Subgroup 3)		Toxicokinetics[a] (Subgroup 4)	
			Male	Female	Male	Female
1	Control	0	12	12	6	6
2	Low	20	12	12	21	21
3	Intermediate	60	12	12	21	21
4	High	200	12	12	21	21

a for toxicokinetic investigations only; no other experimental observation data from these animals were reported

Observations and Results

Mortality

All animals were checked twice daily (AM and PM). There were no deaths during the study in any of the main toxicity groups. In the toxicokinetic groups, three deaths occurred as follows: male #140 and female #542 in the low dose group and male #218 in the mid dose group. Necropsy evaluations were not conducted on these animals, but the deaths were not considered related to drug treatment since deaths were not seen in the main toxicity animals and there were no evident clinically observable abnormalities.

In the immunotoxicity groups there were two animals that were sent to necropsy during Week 10. One animal had a protruding eye (male #54 in the control group) and the other animal had eye damage (male #117 in the low dose group), which were both confirmed at the time of necropsy.

Clinical Signs

A detailed clinical exam was performed on all animals every week. In addition, all toxicity and recovery animals were observed 1 to 4 hours after dosing during the dosing phase and all recovery animals were observed weekly during the recovery phase.

During the dosing phase, minimal to moderate sores/lesions were observed primarily on the back and neck (injection site) of test article-treated animals in the high dose group. Of note, one male in the low dose group also had moderate sores/lesions on the neck. These findings were reversible during the recovery period.

Body Weights

All animals were individually weighed during the predose phase (Day -1), once weekly from Day 1 (before dose), and before necropsy. There were no test article-related effects on body weight or body weight gain for toxicity group animals during the dosing phase of the study.

Feed Consumption

Food consumption was determined at least once weekly from PND 21 for each cage of toxicity and recovery animals and calculated as g/animals/day. There were no test article-related effects on food consumption during the dosing or recovery phases.

Ophthalmoscopy

A mydriatic agent was administered into the eyes and ophthalmic exams were conducted on toxicity and recovery animals in the control and high dose groups in Week 8 of the dosing phase and at the end of the 13-week recovery phase. There were no test article-related changes noted by ophthalmic examination during the dosing or recovery phases.

Hematology

Blood samples for hematology evaluations were obtained via cardiac puncture, right before exsanguination, from 6 animals/sex/group on PND 37 (interim euthanized animals; see above for deviations from study protocol) and from 3 animals/sex/group on PND 78 and PND 79 (end of dosing phase). During the recovery phase, blood samples were obtained on PND 160 (end of recovery phase) from the retro-orbital sinus (under isoflurane anesthesia) from 3 animals/sex/group in the control and high dose groups. There were no effects on hematology parameters during the dosing or recovery phases that were considered test article-related.

An evaluation of coagulation parameters was not made.

Clinical Chemistry

Blood samples for clinical chemistry evaluations were obtained via cardiac puncture, right before exsanguination, from 6 animals/sex/group on PND 37 (interim euthanized animals; see above for deviations from study protocol) and from 3 animals/sex/group on PND 78 and PND 79 (end of dosing phase). During the recovery phase, blood samples were obtained on PND 160 (end of recovery phase) from the retro-orbital sinus (under isoflurane anesthesia) from 3 animals/sex/group in the control and high dose groups. There were no test article-related effects on clinical chemistry parameters during the dosing or recovery phases.

An assessment for CRP was not made.

Urinalysis

Not evaluated.

Gross Pathology

At the time of scheduled necropsy, juvenile animals were given isoflurane anesthesia followed by exsanguination and then a complete macroscopic evaluation was conducted. On PND 37, the first 6 animals/sex/group were euthanized and discarded without further examination (see above for deviations from study protocol). The remaining animals per sex/group were necropsied 3 days following completion of the dosing period (PND 78 and PND 79) and also following completion of the 13-week recovery period (start of Week 22; PND 161).

Macroscopic findings were noted with increased incidence at the injection site (neck) of high dose animals compared to control group animals and included fur loss (1/12 males), masses (1/12 females), and sores (3/12 males and 1/12 females). Histopathological findings at the injection site were also noted.

Large axillary lymph nodes were noted in high dose males (2/12) and females (1/12) compared to control male and female groups (1/12 and 0/12, respectively). Females in the high dose group also had red areas on their axillary lymph nodes (2/12) compared to the control group (0/12). Histopathological findings in the axillary lymph nodes were also noted.

Table 5 Macroscopic Findings from 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period

Tissue/Observation	Males				Females			
	REGN844 (mg/kg/week)				REGN844 (mg/kg/week)			
	0	20	60	200	0	20	60	200
SC, Neck n=	12	12	12	12	12	12	12	12
Fur loss	0	0	0	1	0	0	0	0
Mass	0	0	0	0	0	0	0	1
Sore	0	0	0	3	0	0	0	1
Lymph Node, Axillary n=	12	12	12	12	12	12	12	12
Large	1	0	0	2	0	0	0	1
Red	1	0	1	0	0	1	1	2
Lymph Node, Mandibular n=	12	12	12	12	12	12	12	12
Large	0	0	0	1	0	0	1	0
Red focus	0	0	0	0	0	0	0	1
Lymph Node, Mesenteric n=	12	12	12	12	12	12	12	12
Red	0	0	0	0	0	0	0	1
Liver n=	12	12	12	12	12	12	12	12
Large	0	1	2	4	0	0	0	0
Pale	0	0	0	0	0	0	1	0

Tissue/Observation	Males				Females			
	REGN844 (mg/kg/week)				REGN844 (mg/kg/week)			
	0	20	60	200	0	20	60	200
Lung n=	12	12	12	12	12	12	12	12
Dark	0	0	1	3	0	2	0	0
Red area	0	0	0	0	0	0	1	0
Ovary n=	NA	NA	NA	NA	12	12	12	12
Cyst	NA	NA	NA	NA	0	0	2	1
Seminal Vesicle n=	12	12	12	12	NA	NA	NA	NA
Small	0	0	0	1	NA	NA	NA	NA
Skin/Subcutis n=	12	12	12	12	12	12	12	12
Sore	0	0	0	1	0	0	0	0
Spleen n=	12	12	12	12	12	12	12	12
Large	0	0	1	2	0	0	0	1
Thick	0	1	0	0	0	0	0	0
Uterus n=	NA	NA	NA	NA	12	12	12	12
Distension	NA	NA	NA	NA	0	1	1	2

Abbreviations: n = number of animals examined; NA = not applicable; SC = subcutaneous

Organ Weights

The following organs were collected and weighed at the time of scheduled necropsy (paired organs were weighed together): brain, heart, kidneys, liver, lungs with main stem bronchi and bronchioles, ovary with oviduct, prostate with seminal vesicles, spleen, testis and epididymis, and thymus. Adrenal glands were not weighed; there were no findings for the adrenal glands in the 4-week dose range finding study with mice or in studies with monkeys. There were no test article-related effects on organ weights during the dosing or recovery phases.

Histopathology

Adequate Battery

An adequate battery of tissues and organs were examined. The following tissues were examined microscopically for animals in the control and high dose groups: adrenals; aorta; axillary lymph node of SC site; brain; cecum; colon; esophagus; dosing sites; duodenum; eyes; epididymis; femur with bone marrow; gall bladder; GALT/Peyers patch; gross lesions; Harderian gland; heart; ileum; jejunum; joint, femorotibial; kidney; lacrimal gland; larynx; liver; lungs with main stem bronchi and bronchioles; lymph node, mandibular; lymph node, mesenteric; mammary gland; mandibular salivary gland; muscle, quadriceps; nerve, optic; nerve, sciatic; ovary with oviduct; pancreas; parotid salivary gland; pituitary; prostate; rectum; seminal vesicle; skeletal muscle, diaphragm; skin and subcutis; spinal cord, cervical; spleen; sternum with bone marrow; stomach (glandular and non-glandular); sublingual salivary gland; testis; thymus; thyroid with parathyroid; tongue; trachea; ureter; urinary bladder; uterus with cervix; vagina.

For animals in the low and mid dose groups only the injection site, axillary lymph node, femoral bone marrow, sternal bone marrow, and macroscopic observations were examined microscopically.

Peer Review
The Applicant performed an independent peer review.

Histological Findings
Test article-related histopathology findings were noted in the femur + marrow, sternum + marrow, lymph nodes (axillary and mandibular), and injection site (neck). These findings were reversible at the end of the recovery period. A minimal increase in hemopoiesis was noted in the femur + marrow (3/12 males and 4/12 females) and the sternum + marrow (3/12 males and 4/12 females) of high dose males and females. Females in the mid dose group also had a minimal increase in hemopoiesis in the femur + marrow (2/12) and sternum + marrow (2/12). Findings of hemopoiesis were considered a compensatory response and not adverse.

Lymphoid hyperplasia was noted with increased incidence in the axillary lymph nodes of males (mid dose: 6/12; high dose: 8/12) and females (low dose: 7/12; mid dose 8/12; and high dose: 9/12) compared to control groups (4/12 and 2/12, respectively). Lymphoid hyperplasia was also noted in the mandibular lymph nodes of high dose males and females but at a lesser incidence than in the axillary lymph nodes (2/12 males and 1/12 females).

At the injection site the main findings were inflammation of the subcutis and dermis. High dose males and females in all test article-treated groups had minimal to slight subcutis inflammation. High dose males and females in the mid dose group had minimal to slight inflammation of the dermis.

Table 6 Histopathology Findings from 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period

Tissues/Observations	Males				Females			
	REGN844 (mg/kg/week)				REGN844 (mg/kg/week)			
	0	20	60	200	0	20	60	200
Femur + Marrow n=	12	12	12	12	12	12	12	12
Increased hemopoiesis	0	0	0	3	0	0	2	4
…minimal	0	0	0	3	0	0	2	4
Sternum + Marrow n=	12	12	12	12	12	12	12	12
Increased hemopoiesis	0	0	0	3	0	0	2	4
…minimal	0	0	0	3	0	0	2	4
Lymph Node, Axillary n=	11	12	12	12	12	12	12	12
Lymphoid hyperplasia	4	4	6	8	2	7	8	9
…minimal	4	4	6	7	2	7	8	9
…slight	0	0	0	1	0	0	0	0

Tissues/Observations	Males				Females			
	REGN844 (mg/kg/week)				REGN844 (mg/kg/week)			
	0	20	60	200	0	20	60	200
Pigment	0	0	1	0	0	0	1	3
…minimal	0	0	1	0	0	0	1	3
Inflammation	0	0	0	0	0	0	0	2
…minimal	0	0	0	0	0	0	0	1
…slight	0	0	0	0	0	0	0	1
Lymph Node, Mandibular n=	12	0	0	12	12	0	1	12
Lymphoid hyperplasia	0	0	0	2	0	0	1	1
…minimal	0	0	0	1	0	0	0	1
…slight	0	0	0	1	0	0	0	0
…moderate	0	0	0	0	0	0	1	0
SC, Neck n=	12	12	12	12	12	12	12	12
Inflammation, subcutis	1	0	1	3	1	2	5	11
…minimal	1	0	1	2	1	2	4	9
…slight	0	0	0	1	0	0	1	2
Granuloma	2	2	2	2	4	4	7	2
…minimal	2	2	2	2	4	4	7	2
Inflammation, dermis	1	1	1	3	1	1	3	1
…minimal	1	1	0	1	1	1	3	1
…slight	0	0	1	0	0	0	0	0
…moderate	0	0	0	2	0	0	0	0
Inflammation, muscle	0	0	0	0	0	0	2	0
…minimal	0	0	0	0	0	0	2	0
Abscess	0	0	0	0	0	0	0	1
…moderate	0	0	0	0	0	0	0	1
Liver n=	12	1	2	12	12	0	1	12
Agonal congestion/hemorrhage	0	1	2	4	0	0	0	0
Focal necrosis	0	0	0	0	0	0	0	1
…minimal	0	0	0	0	0	0	0	1
Hemopoiesis	0	0	0	1	0	0	0	0
…minimal	0	0	0	1	0	0	0	0
Stomach n=	12	1	0	12	12	0	1	12
Cystic glands	0	0	0	3	2	0	0	2
…minimal	0	0	0	3	2	0	0	2
Tongue n=	12	0	0	12	12	0	0	12
Granuloma	0	0	0	2	0	0	0	1
…minimal	0	0	0	2	0	0	0	1

Abbreviations: n = number examined; SC = subcutaneous

Special Evaluation

TDAR

KLH (300 mcg/kg) was given by IV administration to all animals in the TDAR subgroup during the dosing phase on Day 29 (PND 42) and Day 36 (PND 49) and during the recovery phase on Recovery Day 70 (PND 140) and Recovery Day 77 (PND 147). Blood samples were taken from animals in the dosing phase on Days 35, 42, and 50 (PND 48, 55, and 63, respectively) and from animals in the recovery phase on Recovery Days 76, 83, and 91 (PND 146, 153, and 161, respectively). Subsequently, blood samples were processed and serum was assayed by ELISA for Anti-KLH IgM and IgG antibodies.

On Day 35 (PND 48), 6 days after initial KLH administration, IgM response to KLH in males was reduced in all test article-treated groups. At all other blood sampling time points (both during the dosing and recovery periods) there were no apparent differences between the KLH responses in test article-treated groups versus the control group. Of note, there was large intra animal variability in the IgM response to KLH in the control and test article-treated groups. See **Table 7**.

Table 7 IgM Response to KLH in Males during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

KLHM

Group/ Subgroup/ Sex	Phase	Dosing.			Recovery.		
	Day	35	42	50	76	83	91
1/2/M	Mean	521	1262	799	406	1723	771
	SD	488.6	705.2	582.5	69.9	1033.9	443.3
	N	6	6	12	6	6	12
2/2/M	Mean	199*	1303	445	414	2710	1207
	SD	69.6	767.8	333.4	65.0	2180.9	687.2
	N	6	6	12	6	6	12
3/2/M	Mean	280	1528	573	<528	1407	<829
	SD	125.6	696.1	478.3	454.4	754.7	563.8
	N	6	6	12	6	6	12
4/2/M	Mean	218*	1858	505	483	1577	2396
	SD	84.8	2324.9	384.2	362.9	2712.2	2881.5
	N	6	5	10	6	6	12
	Statistics	AT	A	A	X5	A	X5

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
T = Rank-transformed data

X5 = Not analysed (values above/below the limit of quantitation)

On Day 50 (PND 63), 14 days after the second KLH administration, IgM responses to KLH in females were statistically reduced in the mid dose group. Of note, there was large intra animal variability in the IgM response to KLH in the control and test article-treated groups. Overall, there did not appear to be an effect of test article on the IgM response in females. See **Table 8**.

Table 8 IgM Response to KLH in Females during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

KLHM

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

Group/ Subgroup/ Sex	Phase	Dosing.			Recovery.		
	Day	35	42	50	76	83	91
1/2/F	Mean	335	1828	1322	436	3181	1430
	SD	94.5	343.4	504.7	154.7	1818.5	767.6
	N	6	6	12	6	5	12
2/2/F	Mean	274	2004	899	<447	2421	1066
	SD	78.5	1745.5	563.0	305.6	1042.6	715.3
	N	6	6	12	6	5	12
3/2/F	Mean	283	1896	692*	<860	<842	1115
	SD	83.5	2171.7	554.2	929.6	933.0	690.5
	N	6	6	12	6	6	12
4/2/F	Mean	322	2468	1146	<220	<1168	1061
	SD	115.8	984.8	555.4	122.2	972.0	677.4
	N	6	6	11	6	6	12
	Statistics	A	A	A	X5	X5	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's
X5 = Not analysed (values above/below the limit of quantitation)

The IgG responses to KLH challenge in males appeared decreased in all the test article-treated groups compared to the control group at all the time points, but particularly on dosing Day 50 (PND 63) when the IgG decrease was statistically significant for the low and high dose groups compared to the corresponding control group. On Recovery Day 83 (PND 153) the control IgG response was elevated compared to Recovery Day 76 (PND 146). Comparably, the IgG responses in the test article-treated groups on Recovery Day 83 (PND 153) were decreased. On Recovery Day 91 (PND 161) the control IgG response was still elevated but there was no relative difference between the test article-treated groups and the control group. See **Table 9**. Decreased IgG responses to antigen challenge were attributed to the immunosuppressive properties of REGN844.

Table 9 IgG Response to KLH in Males during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

KLHG

Group/ Subgroup/ Sex	Phase	Dosing.			Recovery.		
	Day	35	42	50	76	83	91
1/2/M	Mean	596	11276	27594	381	16593	19471
	SD	554.8	10037.1	29533.6	121.3	17635.7	15945.4
	N	6	6	12	6	6	12
2/2/M	Mean	<194	5569	8233*	382	9093	28395
	SD	62.9	4101.1	7885.8	115.8	8961.1	19121.0
	N	6	6	12	6	6	12
3/2/M	Mean	460	5800	12014	<427	6752	16306
	SD	369.2	3898.5	13422.5	429.1	7973.6	14076.4
	N	6	6	12	6	6	12
4/2/M	Mean	295	3672	8947*	<398	2640	19793
	SD	66.3	4314.9	12212.1	269.5	3593.2	16730.0
	N	6	5	10	6	6	12
	Statistics	X5	AT	AT	X5	AT	A

* P<=0.05
** P<=0.01
*** P<=0.001
X5 = Not analysed (values above/below the limit of quantitation)
A = ANOVA and Dunnett's
T = Rank-transformed data

The IgG responses to KLH challenge in females appeared comparable between all the test article-treated groups and the control group at all the time points during the dosing phase. On Recovery Day 83 (PND 153) the control IgG response was elevated compared to Recovery Day 76 (PND 146). Comparably, the IgG responses in the mid and high dose groups were decreased on Recovery Day 83 (PND 153). On Recovery Day 91 (PND 161) the control IgG response was even more elevated but there was no relative difference between the test article-treated groups and the control group. See **Table 10**.

Table 10 IgG Response to KLH in Females during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

KLHG

Group/ Subgroup/ Sex	Phase	Dosing.			Recovery.		
	Day	35	42	50	76	83	91
1/2/F	Mean	367	9519	51041	693	14430	37447
	SD	105.1	3548.1	27674.7	511.1	14285.3	18892.5
	N	6	6	12	6	5	12
2/2/F	Mean	326	11282	35152	<350	22346	32139
	SD	60.4	9270.3	29196.4	222.1	10913.3	21377.5
	N	6	6	12	6	5	12
3/2/F	Mean	339	11722	29250	<828	3946	27836
	SD	103.7	17415.1	50651.7	900.4	4167.8	20433.8
	N	6	6	12	6	6	12
4/2/F	Mean	329	14581	41194	<226	<6878	33287
	SD	131.8	13469.6	20154.5	93.4	4692.3	19810.7
	N	6	6	11	6	6	12
	Statistics	A	A	A	X5	X5	A

A = ANOVA and Dunnett's
X5 = Not analysed (values above/below the limit of quantitation)

Immunophenotyping in Blood, Spleen, and Mesenteric Lymph Node

Blood samples were collected from animals in the immunotoxicity subgroup on PND 74 (Recovery Day 4) and PND 161 (Recovery Day 91). Further, mesenteric lymph node and spleen samples from toxicity group animals were taken on PND 78 (Day 65 at end of dosing phase) and PND 162 (Day 92 at end of recovery phase). Blood and tissue samples were then processed for immunophenotyping of T cells, B cells, natural killer (NK) cells, and monocytes by flow cytometry.

Flow cytometry immunophenotyping of blood samples indicated that cell counts in the male and female control groups were generally comparable between PND 74 (Recovery Day 4) and PND 161 (Recovery Day 91). There were instances where cell counts either increased or decreased statistically in the test article-treated groups compared to the respective control groups on either PND 74 or PND 161. The following statistical changes in cell counts were noted in males: CD3+/CD4+ cells were decreased in the low dose group on PND 74; CD3+/CD8+ cells were decreased in the low dose, mid dose, and high dose groups on PND 161; CD19+ cells were increased in the low dose group on PND 74; and NK cells were increased in the low dose group on PND 161. The following statistical changes in cell counts were noted in females: CD3+, CD3+/CD4+, and CD3+/CD8+ cells were all decreased in the low dose group on PND 74; CD19+ cells were increased in the high dose group on PND 74; and NK cells were decreased in the mid dose group on PND 74. In most instances, a biological relevance of these test article effects is unlikely due to a lack of a dose response. Of note, CD14+ cells appeared to be unaffected by the test article in both males and females.

Flow cytometry immunophenotyping data of splenocytes from spleen samples and mesenteric lymph node cells from mesenteric lymph node were difficult to interpret due

to the large variation between the animals in each group. As such, it was difficult to determine whether the following statistical changes in cell counts seen in males and females were due to treatment with the test article: CD3+/CD4+ splenocytes were decreased in mid dose and high dose males on PND 78; NK splenocytes were increased in high dose females on PND 162; CD14+ splenocytes were increased in mid dose and high dose females on PND 162; and CD19+ and CD14+ mesenteric lymph node cells were decreased in low dose and mid dose males on PND 162.

Serum IgM and IgG Concentrations

Blood samples taken from the immunotoxicity subgroup animals on PND 74 (Recovery Day 4) and on PND 161 (Recovery Day 91) were processed and the serum was assayed by ELISA for total IgM and IgG concentrations. Total serum IgM levels for males were not affected by test article treatment at either Recovery Days 4 and 91 (PND 74 or PND 161, respectively). In contrast, total serum IgG levels in mid dose and high dose males were increased on PND 74, which might be attributed to administration of exogenous IgG (i.e., REGN844). See **Table 11**.

Table 11 Serum IgM and IgG Concentrations in Males during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

Group/ Subgroup/ Sex	Phase	IGM g/L Recovery.		IGG g/L Recovery.	
	Day	4	91	4	91
1/3/M	Mean	0.17	0.23	0.80	2.34
	SD	0.067	0.085	0.442	2.284
	N	6	6	6	6
2/3/M	Mean	0.17	0.24	1.06	3.15
	SD	0.043	0.089	0.294	1.518
	N	6	6	6	6
3/3/M	Mean	0.15	0.14	2.11***	1.68
	SD	0.030	0.034	0.245	0.632
	N	6	6	6	6
4/3/M	Mean	0.21	0.23	4.14***	2.29
	SD	0.032	0.094	0.466	1.590
	N	6	6	6	6
	Statistics	A	A	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's

Total serum IgM levels for females in the high dose group were increased on PND 74. Further, total serum IgG levels in mid dose and high dose females were also increased on PND 74. See **Table 12**.

Table 12 Serum IgM and IgG Concentrations in Females during the 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Test Article		Control	REGN844		
Group		1	2	3	4
Dose level (mg/kg/week)		0	20	60	200

Group/ Subgroup/ Sex	Phase	IGM g/L Recovery.		IGG g/L Recovery.	
	Day	4	91	4	91
1/3/F	Mean	0.17	0.37	1.01	1.68
	SD	0.087	0.135	0.463	0.620
	N	6	6	6	6
2/3/F	Mean	0.18	0.30	1.02	1.45
	SD	0.045	0.132	0.326	0.808
	N	6	6	6	6
3/3/F	Mean	0.22	0.26	2.01**	2.22
	SD	0.077	0.129	0.291	1.091
	N	6	6	6	6
4/3/F	Mean	0.35*	0.34	3.95***	2.03
	SD	0.137	0.239	0.554	0.891
	N	6	6	6	6
	Statistics	A	A	A	A

* P<=0.05
** P<=0.01
*** P<=0.001
A = ANOVA and Dunnett's

Toxicokinetics

Blood samples for toxicokinetic evaluations were collected from the orbital sinus according to the schedule shown in **Table 13**. In brief, blood was drawn from 3 toxicokinetic animals/sex from all groups (control and drug treated) at predose on PND 49. On PND 70, blood was drawn from 3 toxicokinetic animals/sex from drug-treated groups (not the control group) at predose and following the 9th dose at 24 (included control group), 48, 72, 96 and 168 hours. Samples were also collected from 3 toxicokinetic animals/sex from all groups (control and drug-treated) at the recovery necropsy. Of note, an assessment for ADA was not conducted.

Table 13 Schedule for Toxicokinetic Blood Sampling for 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Group and Sex	Animals bled at the following time points (hours post dose):							
	PND 49: Pre-dose	PND 70: Pre-dose	24	48	72	96	168	End of Recovery++
1M	61-63		64-66					64-66
2M	127-129	130-132	133-135	136,138+	139-141	142-144	145-147	130-132
3M	208-210	211-212#	214-216	217-219	220-222	223-225	226-228	211-213
4M	289-291	292-294	295-297	298-300	301-303	304-306	307-309	292-294
1F	461-463		464-466					464-466
2F	527-529	530-532	533-535	536-538	539-541	542-544	545-547	530-532
3F	608-610	611-613	614-616	617-619	620-622	623-625	626-628	611-613
4F	689-691	692-694	695-697	698-700	701-703	704-706	707-709	692-694

no sample obtained for animal number 213
+ no sample obtained for animal number 137
++ blood samples were taken under terminal anaesthesia

As shown in **Table 14**, after the 9th weekly SC dose, C_{max} and AUC ($AUC_{0-\tau}$) were generally dose proportional between groups. T_{max} was 24 hours for the low dose group and 72 hours for both the mid and high dose groups. There did not appear to be any sex differences in REGN844 toxicokinetic parameters.

Table 14 Toxicokinetic Parameters of Total REGN844 for 9-Week Subcutaneous Toxicity Study with REGN844 in the Juvenile Mouse Followed by a 13-Week Recovery Period (Applicant's Table)

Parameter	Units	Dose	REGN844 20 mg/kg	REGN844 60 mg/kg	REGN844 200 mg/kg
C_{max}	µg/mL	9	296	1040	2670
$AUC_{0-\tau}$	h•µg/mL	9	40700	156000	381000
t_{max}	h	9	24	72	72
C_{trough}	µg/mL	5	82.8	293	1380
C_{trough}	µg/mL	8	149	591	2130
C_{trough}	µg/mL	9	188	854	1940

$AUC_{0-\tau}$ = Area under the drug concentration-time curve during a dosing interval; C_{max} = Maximum observed concentration during a dosing interval; C_{trough} = Trough drug concentration at the end of dosing interval (at 168 h following a dose); t_{max} = Time to maximum concentration

Dosing Solution Analysis

The formulated doses were between 95.9% to 105.9%, 94.9% to 101.4%, and 95.0% to 105.8% for the low, mid, and high concentration formulations, respectively, and were considered acceptable for dosing (i.e., formulated doses were within acceptance criteria

of 90-110% with a %CV ≤10%). REGN844 was not detected in the control samples analyzed.

7 Genetic Toxicology

No genetic toxicology studies were conducted based on ICH S6 (Guidance for Industry S6 Preclinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals).

8 Carcinogenicity

At the time of the EOP2 meeting for IND 100632, September 15, 2011, the Applicant provided a carcinogenicity risk assessment in support of their position not to conduct additional nonclinical studies to evaluate the carcinogenic potential of sarilumab. The Applicant's assessment (as described in the Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016) included the following:

- Evidence from the literature that inhibition of IL-6 leads to inhibition of activation of phosphorylated STAT 3 (p-STAT3). The assumption is that a decrease in p-STAT3 activation decreases the potential for tumor growth, differentiation, and proliferation of different tumor cell types. Further, inhibition of p-STAT3 prevents the expression and activity of immunosuppressive factors which would then allow for increased immunity against tumors.

- In vitro and/or in vivo studies with sarilumab using prostate (Du145) and lung (NCI-H1650) tumor cell lines showed inhibition of p-STAT3 activation and inhibition of tumor growth in tumor xenograft animal models. In the Du145 prostate tumor cell line, inhibition of p-STAT3 activation and tumor growth by sarilumab resulted in induction of cleaved caspase-3 suggesting a role for apoptosis in the anti-tumor effects of sarilumab.

- Evidence in the literature of the role of IL-6 in tumor immunity and the relationship between IL-6Rα inhibition and tumor promotion was outweighed by evidence that the lack of IL-6 inhibition promotes tumor formation. However, because sarilumab is expected to be immune suppressive, any potential risks for malignancies in patients are better managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches than any additional nonclinical studies [ICH S6(R1)].

- Observed tumors in the monkey and mouse studies using sarilumab and REGN844, respectively, were not considered test article-related.

- During the BLA review for tocilizumab (IL-6R antagonist) an animal carcinogenicity assessment was not required. The Applicant affirmed that since both sarilumab and tocilizumab have the same target and mechanism of action, there should be no difference with respect to their carcinogenic potential.

Reviewer's note: For the tocilizumab review, a carcinogenicity study was not considered feasible in CD-1 mice due to immunogenicity issues with the surrogate antibody. The potential role of IL-6 signaling in cancer was not considered.

Based on the nonclinical evaluation of the Applicant's proposed carcinogenicity assessment, the nonclinical reviewer and the ECAC agreed that no additional nonclinical studies were required to address the carcinogenic potential of sarilumab (see Pharmacology/Toxicology IND 100632 Review and Evaluation dated May 12, 2016). They also agreed that it was reasonable for the Applicant to manage any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. The recommendation was made to the Applicant that the product label for sarilumab should include a balanced description of the literature to address the carcinogenic potential of sarilumab in relationship to the carcinogenic risk to humans for the chronic use of sarilumab (see IR dated December 4, 2013).

In the current BLA submission, the Applicant submitted an amended carcinogenicity risk assessment for sarilumab. The changes were made to provide clarity and/or to add/update information to the original amendment, but did not alter the Applicant's previous assessment. The most notable addition was the inclusion of an appendix with historical control incidence of adrenal cortical adenomas and hyperplasia from several contract research organizations (see **Table 16**) and adrenal neoplastic findings and hyperplasia reported in the literature (see **Table 17**). The amendment also included historical control data for tumor and pre-neoplastic (hyperplasia) findings in the adrenal gland of cynomolgus monkeys from the conducting laboratories (as requested in the nonclinical IR to the Applicant dated July 24, 2013).

As shown in **Table 15**, in general repeat-dose toxicity studies (5-week IV, 13-week IV, 13-week SC [two studies], and 6-month IV), 54 monkeys were treated with placebo and 186 monkeys were treated with sarilumab at doses from 0.5 to 100 mg/kg/week. Further, in an IV ePPND study, 12 monkeys were treated with placebo and 36 monkeys were treated with sarilumab at doses from 5 to 50 mg/kg/week. No tumors were observed in the 6-month monkey study with doses of sarilumab up to 50 mg/kg/day. Mice were treated with a surrogate to sarilumab (REGN844) in both a general toxicity study and a fertility study. In a 4-week exploratory toxicity study, 20 mice received placebo and 80 mice received REGN844 at doses from 10 to 200 mg/kg/week. In the fertility study, 48 mice received placebo and 144 mice received REGN844 from 20 to 200 mg/kg/week.

Table 15 Total Animals/Dose in Toxicity Studies with Sarilumab (Monkeys) and REGN844 (Mice) (Applicant's Table)

Sarilumab Toxicity Study Types	Dose (mg/kg/week)													
	0	0.5	1	2	5	10	15	20	25	30	40	50	100	200
Total Monkeys in General Toxicity Studies	54	12	12	12	22	42	12			12	10	24	28	
Total Monkeys in ePPND Study	12				12		12					12		
Total Mice in General Toxicity Study [b]	20					20			20			20		20
Total Mice in Fertility Study	48							48				48		48

a = monkeys were administered sarilumab and mice were administered REGN844

b = excluding mice for toxicokinetic determinations

As indicated in the Pharmacology/Toxicology IND 100632 Review and Evaluation dated May 12, 2016, there were 3 monkeys and 1 mouse from the above mentioned studies with tumors. One male monkey (#4703) in the 100 mg/kg/week high dose group of the 13-week SC bridging study had an adrenal cortical adenoma. One female monkey (#2528) in the 10 mg/kg/week mid dose group of the 5-week IV study had cervical papilloma. Further one female monkey in the 10 mg/kg/week low dose group (#4716) of the 13-week SC bridging study had squamous metaplasia in the cervix and uterus. One female mouse (#183) in the high dose group of the SC fertility study also had squamous metaplasia of the cervix. These findings were considered by the Applicant and the nonclinical Reviewer as unrelated to the test article based on historical control data and the isolated incidences of these findings and lack of dose-response relationships.

In the toxicity studies, hyperplasia was noted in various organs of monkeys treated with sarilumab and mice treated with REGN844. These findings occurred in both control and test article treated animals and were therefore not considered test article related. See **Table 16** for the incidence of neoplastic and nonneoplastic proliferative findings in the adrenal gland cortex of cynomolgus monkeys from the historical control database of four contract research laboratories and **Table 17** for the incidence of spontaneous neoplastic and hyperplastic findings in the adrenal gland of macaque monkeys from the literature.

Table 16 Contract Research Organizations Historical Control Databases of Incidence of Neoplastic and Nonneoplastic Proliferative Findings in the Adrenal Gland Cortex of Cynomolgus Monkeys (Applicant's Table)

Source	Date range for of data	Sex	Number of Cortical Adenoma Reported	Number of Cortical/Zona Fasciculata Hyperplasia Reported	Number of tissues/animals examined [a]
(b) (4)	2006-2011	M	0	1	182
		F	0	0	191
	2005-2013	M	0	4	1219
		F	1	4	1221
	1999-2010 [c]	M	1	0	401
		F	3	0	406
	2004-2010	Both	0	36	320
	2005-2012	M	0	1	128
		F	0	0	127
	2005-2012	M	0	28	1349
		F	1	19	1179

a = Some databases referred to number of tissues examined while other databases referred to the number of animals
b = US only and went back to 2005 for (b) (4) site
c = Available historical control database extract (b) (4) Database for Mauritius-origin monkeys is for 1999-2009 and for Asian-origin monkeys is for 2000-2010.
d = The incidence of adenoma, hyperplasia, and number of animals was derived using the values for the cynomolgus monkeys from Indonesia (adrenal left & right), Cambodia (adrenal, adrenal left & right), and China (adrenal, adrenal bilateral, adrenal left & right). For those monkey where hyperplasia was recorded in the right and left adrenal gland separately, it could not be ascertained how many monkeys may have had bilateral changes, and therefore, may have been counted twice.

Table 17 Incidence of Spontaneous Neoplastic and Hyperplastic Findings in the Adrenal Gland of Macaque Monkeys from the Literature (Applicant's Table)

Source	Year	Macaque Species	Number of adrenal neoplastic findings reported	Number of Hyperplasia Reported	Comment
Kaspareit, et al. (1; Appendix 7)	2013	cynomolgus	7 cortical adenoma (4M/3F) [a,b] 1 hemangioma (F)	Not in scope of poster	Covers spontaneous tumors from studies conducted at Covance in Münster, Germany from 1992-2012 Number of monkeys not noted.
Lowenstine, LJ (2)	1986	cynomolgus	1 cortical carcinoma (M) 1 adrenal adenoma (M)	Not in scope of paper	363 nonhuman primates in survey covering 63 species
		macaque	8 cortical adenoma 5 pheochromocytoma		Article did not specify the species of macaque for findings
McClure, et al. (3)	1980	rhesus	3 cortical adenoma (2M/F) 1 pheochromocytoma (M)	Not in scope of paper	Covers multiple nonhuman primate species from approx. 1968-1980 and 2,176 necropsies
Squire, et al. (4)	1978	cynomolgus	1 cortical carcinoma (M) 1 medullary pheochromocytoma (F)	Not in scope of chapter	Total number of monkeys not noted in chapter
		rhesus	1 hemangioma (M) 1 cortical carcinoma (M) 1 medullary pheochromocytoma (F) 1 fibroma (M)		
		macaque	1 adenoma		No additional details on species given in reference

Source	Year	Macaque Species	Number of adrenal neoplastic findings reported	Number of Hyperplasia Reported	Comment
Ito, et al. (5)	1992	cynomolgus	None reported	Nodular hyperplasia of zona fascicularis cells (10M / 3F)	221 monkeys/sex evaluated from 1979-1990 in authors laboratories
Sato, et al. (6)	2012	cynomolgus	None reported	Nodular hyperplasia of cortical cells (eosinophilic / vacuolated) (no incidence described)	332 males and 328 females from 1998-2011 in authors laboratories

a = Personal communication with J. Kaspareit regarding poster presented at the 12th FELASA SECAL Congress, Barcelona, Spain, 10–13 June 2013. Spontaneous adenomas were found in 1 control monkey and 3 treated monkeys for males, and 1 control monkey and 2 treated monkeys for females.

b = Includes 4 adenomas originally noted in Kaspareit et al paper from Experimental Tox Path, 2007; 59: 163-169 that was referenced in the sarilumab Carcinogenicity Risk Assessment document

IL-6 has been shown to have both pro-inflammatory and anti-inflammatory properties.[1] Dysregulation of the IL-6/IL-6R pathway has been implicated in various inflammatory diseases (e.g., RA and systemic juvenile idiopathic arthritis) and cancers (e.g., multiple myeloma, lymphoma, melanoma, ovarian cancer, and prostate cancer).[2,3,4,5] Although the literature generally supports that the IL-6/IL-6R pathway is involved in tumorigenesis, the literature also supports potential anti-tumor properties as well.

Role of IL-6/IL-6R Pathway in Tumor Progression

A relationship between inflammation and cancer is believed to exist.[6] Cancer cells and cancer stem cells have been shown to overexpress and secrete IL-6 into the tumor microenvironment.[7,8,9] Acting as a pro-inflammatory cytokine at the microenvironment, IL-6 may create conditions that promote cancer growth.[10] For instance, IL-6 has been

[1]Scheller J, Chalaris A, Schmidt-Arras D, et al. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 1813(5):878-888.
[2]Hirano T, Matsuda T, Turner M, et al. 1988. Excessive production of interleukin 6/B cell stimulatory factor-2 in rheumatoid arthritis. Eur J Immunol 18(11):1797-1801.
[3]Madhok R, Crilly A, Watson J, et al. 1993. Serum interleukin 6 levels in rheumatoid arthritis: correlations with clinical and laboratory indices of disease activity. Ann Rheum Dis 52(3):232-234.
[4]Isobe A, Sawada K, Kinose Y, et al. 2015. Interleukin 6 receptor is an independent prognostic factor and a potential therapeutic target of ovarian cancer. PLoS One 10(2):1-20.
[5]Adler HL, McCurdy MA, Kattan MW, et al. 1999. Elevated levels of circulating interleukin-6 and transforming growth factor-β1 in patients with metastatic prostatic carcinoma. J Urol 161(1):182-187.
[6]Rakoff-Nahoum S. 2006. Why cancer and inflammation? Yale J Biol Med 79(3-4):123-30.
[7]Cohen S, Bruchim I, Graiver D, et al. 2013. Platinum-resistance in ovarian cancer cells is mediated by IL-6 secretion via the increased expression of its target cIAP-2. J Mol Med 91(3):357-368.
[8]Nilsson MB, Langley RR, Fidler IJ. 2005. Interleukin-6, secreted by human ovarian carcinoma cells, is a potent proangiogenic cytokine. Cancer Res 65(23):10794-10800.
[9]Middleton K, Jones J, Lwin Z, et al. 2014. Interleukin-6: an angiogenic target in solid tumours. Crit Rev Oncol Hematol 89(1):129-139.
[10]Lu H, Ouyang W, Huang C. 2006. Inflammation, a key event in cancer development. Mol Cancer Res 4(4):221-233.

shown to be a growth factor for the survival of multiple myeloma cells and thus anti-IL-6 antibodies have been studied in clinical trials for the treatment of multiple myeloma.[11] High serum levels of IL-6 have also been correlated with poorer outcome in cancer patients with various types of cancers (e.g., gastric, pancreatic, melanoma, breast, colorectal, myeloma, and lung).[12,13]

IL-6 signaling through the IL-6R/gp130 complex and subsequent downstream activation of the Janus kinase (JAK)/STAT, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase (PI3K)/Akt pathways has been implicated in the tumorigenesis of multiple myeloma, ovarian cancer, lung cancer, bladder cancer, breast cancer, colon cancer, and prostate cancer.[14,15,16,17,18] Potential mechanisms of IL-6 in tumorigenesis include transformation, growth, proliferation, and/or drug resistance of the cancer.[19] In particular, IL-6/IL-6R activation of STAT3 (JAK/STAT pathway) has been shown to induce genes that prevent apoptosis (e.g., Bcl-2, Bcl-xL, survivin) and promote cell proliferation (e.g., c-myc, CycD1).[20] In human breast cancer cells survivin transcription is increased when STAT3 is bound to the promoter but blocked when STAT3 is inhibited, which induces apoptosis.[21] By affecting the expression of genes associated with cell death and cell survival/proliferation, STAT3 has been directly implicated in the initiation, promotion, and progression of various tumor types (e.g., pancreatic, colorectal, lung, gastric and breast).[22] For instance, p-STAT3 (i.e., activated STAT3) has been implicated in lung adenocarcinomas. In cell lines derived from lung

[11] Hideshima T, Mitsiades C, Tonon G, et al. 2007. Under-standing multiple myeloma pathogenesis in the bone marrow to identify new therapeutic targets. Nat Rev Cancer 7(8):585-598.
[12] Heikkila K, Ebrahim S, Lawlor DA. 2008. Systematic review of the association between circulating interleukin-6 (IL-6) and cancer. Eur J Cancer 44(7):937-945.
[13] Lippitz BE. 2013. Cytokine patterns in patients with cancer: a systematic review. Lancet Oncol 14(6):218-228.
[14] Bharti R, Dey G, and Mandal M. 2016. Cancer development, chemoresistance, epithelial to mesenchymal transition and stem cells: A snapshot of IL-6 mediated involvement. Cancer Lett 375(1):51-61.
[15] Yao X, Huang J, Zhong H, et al. 2013. Targeting interleukin-6 in inflammatory autoimmune diseases and cancers. Pharmacol Ther 141(2):125-139.
[16] Hideshima T, Nakamura N, Chauhan D, et al. 2001. Biologic sequelae of interleukin-6 induced PI3-K/Akt signaling in multiple myeloma. Oncogene 20(42): 5991–6000.
[17] Berishaj M, Gao SP, Ahmed S, et al. 2007. Stat3 is tyrosine-phosphorylated through the interleukin-6/glycoprotein 130/Janus kinase pathway in breast cancer. Breast Cancer Res 9(3):R32.
[18] Bharti R, Dey G, Ojha PK, et al. 2015. Diacerein-mediated inhibition of IL-6/IL-6R signaling induces apoptotic effects on breast cancer. Oncogene Nov 30 Epub ahead of print.
[19] Guo Y, Xu F, Lu T, et al. 2012. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev 38(7):904-910.
[20] Li N, Grivennikov SI, and Karin M. 2011. The unholy trinity: inflammation, cytokines, and STAT3 shape the cancer microenvironment. Cancer Cell 19(4):429-431.
[21] Gritsko T, Williams A, Turkson J, et al. 2006. Persistent activation of stat3 signaling induces survivin gene expression and confers resistance to apoptosis in human breast cancer cells. Clin Cancer Res 12(1):11-19.
[22] Rosell R, Bertran-Alamillo J, Molina MA, et al. 2009. IL-6/gp130/STAT3 signaling axis in cancer and the presence of in-frame gp130 somatic deletions in inflammatory hepatocellular tumors. Future Oncology 5(3):305-308.

cancer, agents that blocked IL-6 signaling through the IL-6R/gp130 pathway could decrease p-STAT3 levels and inhibit lung tumor formation.[23]

A non-GLP-compliant study was conducted to characterize the effects of sarilumab on STAT3 activation and tumor xenograft growth. The study was reviewed under IND 100632 but since that the time the Applicant amended the study report to add experimental results not previously reported. This amended study report was submitted to the BLA and is reviewed here.

Study Title: Characterization of the Effects of Sarilumab (REGN88) on STAT3 Activation and Tumor Xenograft Growth (Study No. REGN88-MX-11050-SR-01V2; non-GLP)

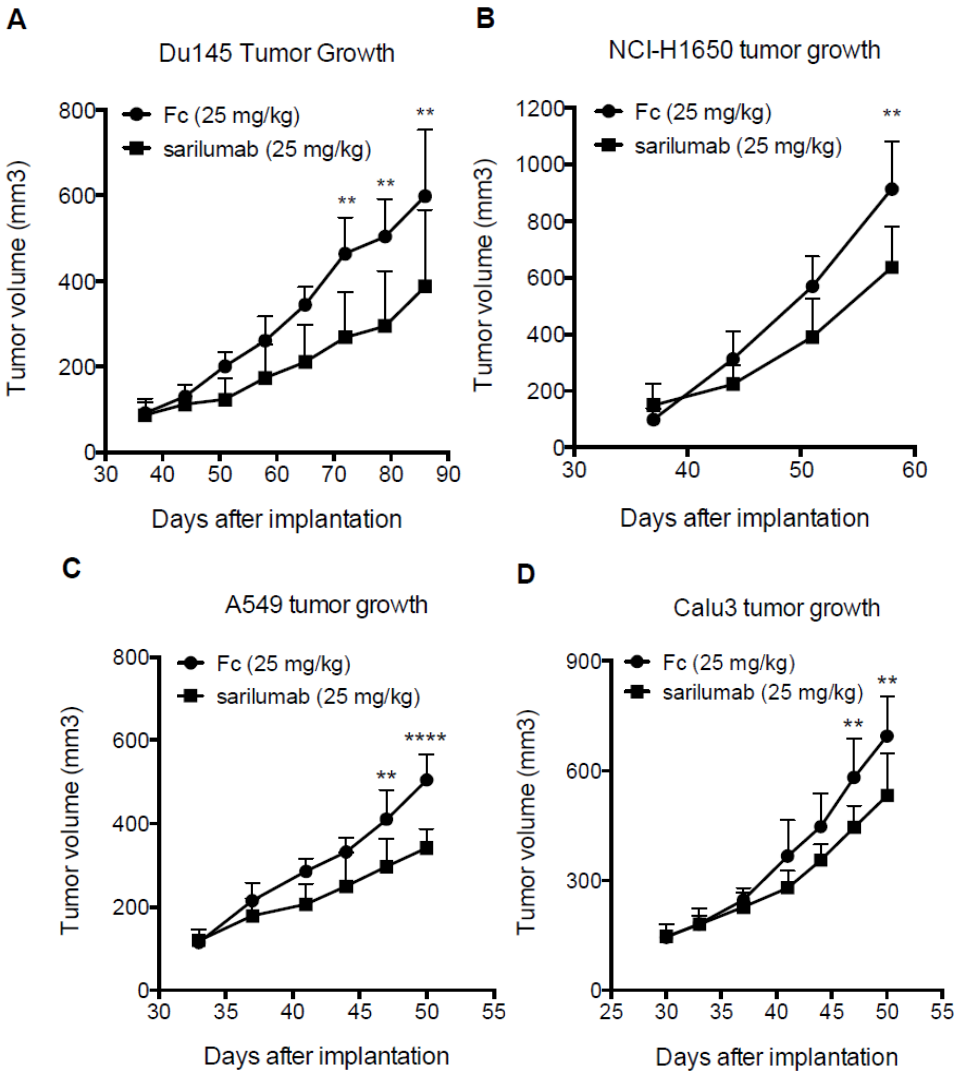
Methods: The effects of REGN88 on tumor growth were evaluated by subcutaneously implanting human prostate carcinoma (Du145 cell line) or human lung carcinoma (NCI-H1650, A549, or Calu3 cell lines) tumors into the right hind flank of C.B.-17 Severe Combined Immunodeficiency (SCID) mice (lack T and B cells) and monitoring for tumor growth. When the tumor xenografts were about 100 mm^3 (Du145), 125 mm^3 (NCI-H1650), 120 mm^3 (A549) and 150 mm^3 (Calu3) in size, the mice were treated with 25 mg/kg hFc (control) or 25 mg/kg REGN88 once per week over 49 days for Du145 and 21 days for NCH-H1650, and twice per week over 18 days for A549 and 21 days for Calu3 and then were sacrificed. Tumor size was measured regularly throughout the study treatment.

Further, dissected Du145 tumor sections were prepared and histologically examined for detection of cleaved caspase-3 (activated during apoptosis), Ki67 (cellular marker for cell proliferation), and platelet endothelial cell adhesion molecule 1 (PECAM-1; marker for degree of tumor angiogenesis).

Western blot analysis of p-STAT3 was performed on two prostate cancer cell lines (Du145 and PC3) and four lung cancer lines (NCI-H1975, NCI-H1650, A549, and Calu3) that were cultured and treated with hFc or REGN88, with or without IL-6. Western blot analysis for p-STAT3 was also performed on Du145 tumor xenografts from tumor bearing mice treated with a single dose of 25 mg/kg hFc control or sarilumab for 48 hours.

Results:
Over the course of treatment, the growth of Du145, NCI-H1650, A549, and Calu3 xenografts in mice treated with 25 mg/kg REGN88 was reduced by about 41%, 40%, 43%, and 30%, respectively, compared to mice treated with hFc control (see **Figure 5**).

[23]Gao SP, Mark KG, Leslie K, et al. 2007. Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest 117(12):3846-3856.



Mice bearing established Du145 **(Fig. 1A)**, NCI-H1650 **(Fig. 1B)**, A549 (**Fig. 1C**) and Calu3 (**Fig. 1D)** tumors (n = 5 mice per treatment group except for Calu3, n=6) were treated with hFc control protein or sarilumab (REGN88) at 25 mg/kg, once per week for Du145 and NCI-H1650 tumors, or twice per week for A549 and Calu3 tumors. The line graphs depict the average tumor volume ± SD over the course of treatment. Individual tumor weight and mouse body weight data are shown in Tables 3-10. Tumor sizes were analyzed using Two-way ANOVA with Bonferroni's multiple comparison test (** $p<0.01$, **** $p<0.0001$).

Figure 5 REGN88 Inhibits Growth of Du145, NCI-H1650, A549, and Calu3 Xenografts (Applicant's Figure)

Immunohistochemistry analysis of Du145 tumor xenografts that had been treated with REGN88 showed an increase in staining for cleaved caspase-3 compared to control hFc. Ki67 and PECAM-1 levels did not appear to be affected by REGN88 treatment.

Western blot analysis indicated that 5/6 cell lines (i.e., Du145, NCI-H1975, NCI-H1650, A549, and Calu3) expressed basal levels of p-STAT3, indicating basal STAT3 activation; basal STAT3 activation was not evident in the PC3 prostate cancer cell line.

Of the 5/6 cell lines with basal p-STAT3, sarilumab reduced p-STAT3 in all cases except for in the H1975 cell line. Further, in the Du145, NCI-H1650, A549, and Calu3 cell lines, the addition of IL-6 increased p-STAT3 above basal levels; this effect was blocked by sarilumab. Further, western blot analysis of Du145 xenografts from mice treated with a single dose of 25 mg/kg sarilumab for 48 hours showed a decrease in the expression of p-STAT3.

The Applicant's study data, taken together with the information from the public literature, imply that a drug, such as sarilumab, which blocks IL-6 signaling through the IL-6R, might be capable of diminishing tumorigenesis.

Role of IL-6/IL-6R Pathway in Tumor Suppression

Although the literature generally supports that IL-6/IL-6R signaling is tumor promoting, available data suggest that the IL-6 pathway may also have an anti-tumor role by supporting the adaptive immune response.[24] In particular, IL-6 trans-signaling may be instrumental in facilitating anti-tumor T cell responses. IL-6 can support T-cell anti-tumor effects either at the lymph node or at the tumor microenvironment. For instance, acute activation of the IL-6/IL-6R pathway can influence the trafficking of lymphocytes to lymph nodes in order to induce their activation, proliferation, and polarization towards phenotypes that oppose the immunosuppressive tumor microenvironment.[25] IL-6 also supports T cell proliferation after T cell receptor stimulation and protects T cells from apoptosis by inducing Bcl-2 and Bcl-xL (anti-apoptotic) and cFos and JunB (proto-oncogenic).[26,27,28]

The IL-6/IL-6R trans-signaling pathway has recently been shown to shift CD4+T cells away from the T regulatory (T_{reg}) toward the Th17 phenotype (i.e., a shift from an immune suppressed to an immune activated state that supports anti-tumor immunity). In a murine model of aggressive melanoma, IL-6 was required for reducing T_{reg}-mediated immune suppression and for effective priming of CD8+ effector T cells in the tumor-draining lymph node.[29] In a different study, IL-6 trans-signaling was found to augment lymphocyte trafficking to lymph nodes by affecting circulating lymphocytes and high endothelial venules (HEV) to boost immune surveillance.[24,25]

Further, acute activation of IL-6 trans-signaling was found to support the adhesive properties of the tumor endothelium. Studies in tumor-bearing mice showed STAT3

[24]Fisher DT, Chen Q, Skitzki JJ, et al. 2011. IL-6 trans-signaling licenses mouse and human tumor microvascular gateways for trafficking of cytotoxic T cells. J Clin Invest 121(10):3846-3859.
[25]Fisher DT, Appenheimer MM, and Evans SS. 2014. The Two Faces of IL-6 in the Tumor Microenvironment. Semin Immunol 26(1): 38-47.
[26]Naugler WE, Karin M. 2008. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 14(3):109-119.
[27]Dejean AS, Beisner DR, Ch'en IL, et al. 2009. Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. Nat Immunol 10(5):504-513.
[28]Rose-John S. 2012. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 8(9):1237-1247.
[29]Sharma MD, Hou DY, Baban B, et al. 2010. Reprogrammed foxp3(+) regulatory T cells provide essential help to support cross-presentation and CD8(+) T cell priming in naive mice. Immunity 33(6):942-954.

activation in tumor-associated endothelial cells after systemic administration of IL-6. This process occurred at different steps in the adhesion cascade to strengthen the contact between circulating cytotoxic T cells (i.e., CD8+ effector T cells) and the tumor endothelium.[24,25]

In a nonimmunogenic tumor system, B16 melanoma cells transfected with the human IL-6 complementary DNA had slower tumor growth in vivo. The developing tumors had a noticeable stromal matrix, infiltration of inflammatory cells, fewer mitotic figures, and fewer blood vessels. These findings appeared to be related to a greater adhesion of the IL-6-transfected melanoma cells to stromal matrix proteins and a less prominent vascular response (intradermal angiogenesis assay).[30] The authors proposed that these findings were due to an enhancement of cytotoxic T-cell function by IL-6 against the tumors.

IL-6R Knockout Mice[31]

A targeted null knockout for IL-6R ($Il6ra^{tm1.2Jcbr}$ mutation) was generated in C57BL/6 mice. In these mice, there was a decreased incidence of liver tumors after exposure to diethylnitrosamine (chemical which induces hepatocellular carcinoma). Mice fed standard chow and given diethylnitrosamine had increased hepatocyte apoptosis, decreased hepatocyte proliferation, and consequently fewer and smaller hepatocellular carcinomas compared to wild-type mice. In contrast, mice fed a high fat diet and treated with diethylnitrosamine displayed normal liver apoptosis and hepatocellular carcinoma development.

Reviewer's Conclusions

As described above, published data exists to indicate that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. However, the literature generally supports that IL-6 signaling through the IL-6R may be involved in pathways that lead to tumorigenesis (transformation, growth, proliferation, and/or drug resistance of the cancer). Further, IL-6R knockout mice have a phenotype associated with decreased tumor incidence in response to treatment with various chemical carcinogens as compared to wild-type mice.

Still, the nonclinical review for IND 100632 pointed out that the potential carcinogenic role of IL-6 in the endocrine glands and female reproductive tract could not be dismissed, since IL-6 is made in the endocrine glands (e.g., adrenal cortex) and elevated IL-6 levels have been described in diethylstilbestrol (DES)-induced vaginal metaplasia in animal studies. Also, since sarilumab is considered to be immunosuppressive there is an overall safety concern for malignancies that should be taken into account. Based on species specificity, a rodent carcinogenicity study with sarilumab is not feasible. As such, the Applicant indicated that any potential risk for

[30]Sun WH, Kreisle RA, Phillips AW, et al. 1992. In vivo and in vitro characteristics of interleukin 6-transfected B16 melanoma cells. Cancer Res 52(19):5412-5415.

[31]Gruber S, Straub BK, Ackermann PJ, et al. 2013. Obesity Promotes Liver Carcinogenesis via Mcl-1 Stabilization Independent of IL-6Rα Signaling. Cell Reports 4(4):669-680.

malignancies would be managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. Based on the available information, this approach was reasonable from the nonclinical perspective.

Overall, the malignancy risk in humans from an antibody that disrupts signaling through the IL-6R, such as sarilumab, is unknown.

9 Reproductive and Developmental Toxicology

Reproductive and developmental toxicology studies were conducted with sarilumab in monkeys or with REGN844 (a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) in mice. These studies were reviewed under IND 100632 (Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016) and a brief summary is provided below.

9.1 Fertility and Early Embryonic Development

The Applicant conducted a fertility study in mice with REGN844 (a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα). This study was reviewed under IND 100632 (see Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016, for additional details) and a brief summary is provided below.

Study Title: SAR153191 (REGN88): Subcutaneous Fertility Study in Mice with REGN844 (Study No. REGN844-TX-09048 [FER0480]; GLP)

REGN844 (surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) was given to CD-1(ICR) mice (24/sex/group) twice per week by SC administration at 0 (placebo control), 10, 25, or 100 mg/kg/dose (20, 50, or 200 mg/kg/week, respectively). Males were treated for 4 weeks before cohabitation, during cohabitation (up to 14 days maximum), and until necropsy. Females were treated 2 weeks before cohabitation, during cohabitation (up to 14 days maximum), and until Gestation Day (GD) 7 (GD 0 was considered the day that evidence of mating was observed).

There were three deaths in the study (one male in the control group on Day 36, one male in the high dose group on Day 17, and one female in the low dose group on Day 14). The causes of death were not determined but were not considered test article-related as one death was in the control group and the death in the high dose group occurred early in the study. Toxicokinetic evaluation indicated that mean serum concentrations of REGN844 increased with dose in males (dose proportional) and females (greater than dose proportional).

There were no test article-related effects on clinical signs, body weights, food consumption, organ weights, gross pathology, histopathology, or fertility parameters examined in the study (e.g., mating/fertility index, number of corpora lutea, number of late resorption, number of dead fetuses, preimplantation loss, postimplantation loss). The estrous cycle was not evaluated in the study.

9.2 Prenatal and Postnatal Development

The Applicant conducted an ePPND study in monkeys. This study was reviewed under IND 100632 (see Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016, for additional details) and a brief summary is provided below.

Study Title: Study of the Effects of REGN88 on Embryo-Fetal Development and Pre- and Post-Natal Development When Administered Weekly by Intravenous Infusion to Pregnant Cynomolgus Monkeys (Study No. REGN88-TX-08030 [(b) (4).223.33]; GLP)

Sarilumab was given to pregnant cynomolgus monkeys (12/sex/group) one time per week by IV infusion at 0 (placebo control), 5, 15, or 50 mg/kg/week. Dosing began on GD 20 (GD 0 was considered to be the middle of the 3 or 5 day mating season) and continued through the end of gestation with either delivery around GD 160 to GD 165 or abortion/embryo-fetal death. Maternal animals and neonates were observed for 28-32 days after delivery.

Maternal Animals

There were no mortalities in maternal animals that were attributed to the test article. Also, there were no test article-related effects on clinical signs, body weight, clinical chemistry, immunophenotyping, and hormone concentrations (17-β estradiol, progesterone, and prolactin).

Evaluation of hematology and coagulation parameters in maternal animals identified decreases of mean lymphocyte, neutrophil, and white blood cell counts in all test article-treated groups (not dose related) on GD 153 and/or lactation day (LD) 7. Further, fibrinogen levels were slightly decreased in all test article-treated groups (not dose related) on GD 153, LD 7, and/or LD 30. Sarilumab exposure ($AUC_{0\text{-}168h}$) appeared to increase proportionally with dose and slight accumulation was noted over the course of the dosing period. ADA was detected in three low dose and one mid dose animal before dosing with sarilumab on GD 20.

Neonate Animals

In the neonates, there were no test article-related effects on any of the parameters evaluated (birth examinations, clinical signs, body weight, functional and morphological development, hematology, coagulation, serum chemistry, immunophenotyping and ADA) over the limited examination period up to 30 days after birth. Of note, decreases in mean lymphocyte counts and white blood cells were seen in the high dose group on DB (day after birth) 30, but due to large within group variability the significance of these effects could not be determined. Sarilumab concentrations were increased with dose.

The nonclinical reviewer highlighted some concerns about the adequacy of the ePPND study (e.g., small numbers of neonates in each group at the time of scheduled necropsy, absence of immune function testing [e.g., TDAR] in the offspring during the postnatal phase, presence of drug in the circulation of the neonates at the time of

necropsy, which may lead to uncharacterized effects beyond the one month of age in monkeys).

10 Special Toxicology Studies

A GLP-compliant tissue cross-reactivity study of biotinylated sarilumab with normal human and cynomolgus monkey tissue was conducted. This study was reviewed under IND 100632 and a brief summary is provided below. Refer to the Pharmacology/Toxicology IND Review and Evaluation dated May 12, 2016, for additional details.

Study Title: Cross-Reactivity Study of Biotinylated REGN88 with Normal Human and Cynomolgus Monkey Tissues (Study No. REGN88-TX-06036 [IM1436]; GLP)

The positive tissue staining for biotinylated sarilumab between human and monkey tissues appeared similar with some exceptions. In human tissues, positive staining for biotinylated sarilumab was detected in the cytoplasm and/or cytoplasmic granules of various sites but not at any membrane sites. Similar findings were noted in monkey tissues with the exception that positive staining was noted in mammary gland epithelium from one monkey donor. Cytoplasmic and cytoplasmic glandular staining was considered of little toxicological concern.

11 Integrated Summary and Safety Evaluation

Sanofi-Aventis submitted an original BLA on October 30, 2015, for Kevzara (sarilumab). Sarilumab is a human IgG1 monoclonal antibody that binds to both soluble and membrane IL-6Rα to inhibit signaling by IL-6. Kevzara is proposed for the treatment of adult patients with moderately to severely active RA who have had an inadequate response or intolerance to one or more DMARDs. The planned dose of Kevzara is 150 or 200 mg once every two weeks by SC injection.

IL-6 is widely known as a pleiotropic cytokine that has both pro-inflammatory and anti-inflammatory effects.[1] Many different cell types including T-cells, B-cells, monocytes, keratinocytes, and endothelial cells produce IL-6.[32] IL-6 plays an important role in infection and trauma and also mediates fever and the acute phase response. As depicted in **Figure 6**,[33] IL-6 regulates its cellular activities by binding to either the membrane or soluble form of the IL-6R to elicit either the classical or trans-signaling pathway, respectively. In both pathways IL-6 binding to the IL-6R results in the dimerization of gp130, which is expressed on the surface of a number of cell types. Upon formation of the IL-6/IL-6R/gp130 complex, downstream phosphorylation and activation of the JAK/STAT, MAPK, or PI3K/Akt pathways can occur to initiate gene expression and subsequent cellular events.

[32] Mihara M, Hashizume M, Yoshida H, et al. 2012. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. Clin Sci (Lond) 122(4):143-159.
[33] Jones SA, Horiuchi S, Topley N, et al. 2001. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J 15(1):43-58.

IL-6 and the IL-6R have been linked to inflammatory joint disorders such as RA.[34] For instance, soluble IL-6Rα levels are increased in RA[35] and IL-6 levels are increased in the serum and synovial fluid of RA patients.[36] As such, there are potential clinical applications in the treatment of RA with drug products (such as sarilumab) that block the IL-6/IL-6R pathway. To date, tocilizumab is the only approved drug product that antagonizes the IL-6R to block IL-6/IL-6R signaling.

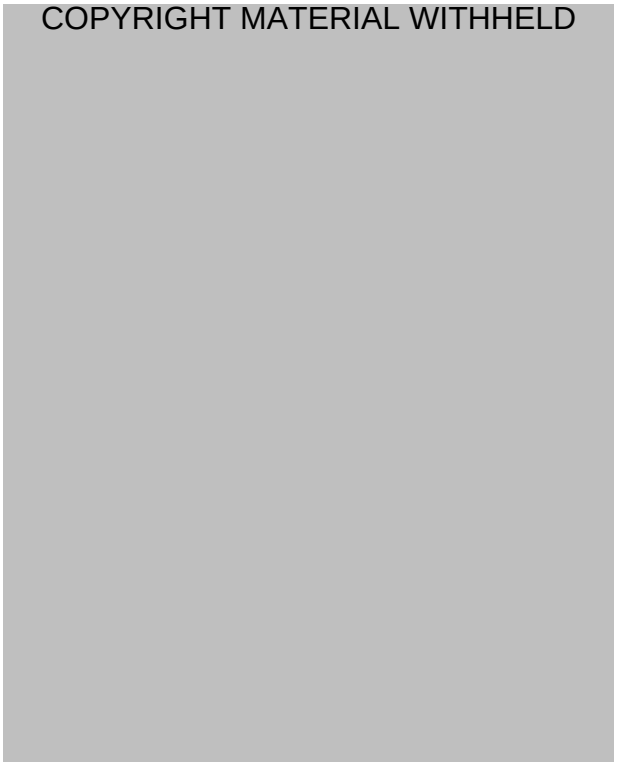


Figure 6 Schematic of IL-6 Signaling through the Membrane IL-6 Receptor and Soluble IL-6 Receptor (Figure 1 from FASEB J 15(1):43-58.)[37]

Sarilumab is a recombinant human IgG1 monoclonal antibody specific for human IL-6Rα and binds to both soluble and membrane-bound forms of the receptor. Sarilumab has a molecular weight estimated to be 144 kDa and is a covalent tetramer consisting of two heavy and two light chains. Each heavy chain is covalently linked through a disulfide bond to a human kappa light chain.

The sarilumab drug product is provided ready for SC injection in a single-use prefilled syringe that is available in dosage forms of 150 mg and 200 mg. Both dosage forms

[34]Firestein GS. 2005. Immunologic mechanisms in the pathogenesis of rheumatoid arthritis. J Clin Rheumatol 11:S39-S44.
[35]Lipsky PE. 2006. Interleukin-6 and rheumatic diseases. Arthritis Res Ther 8 (Suppl 2):S4.
[36]Srirangan S and Choy EH. 2010. The role of interleukin 6 in the pathophysiology of rheumatoid arthritis. Ther Adv Musculoskel Dis 2(5):247-256.
[37]Jones SA, Horiuchi S, Topley N, et al. 2001. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. FASEB J 15(1):43-58.

are formulated with (b) (4) histidine, (b) (4) arginine (b) (4) sucrose, polysorbate 20, and water for injection.

The nonclinical safety program for sarilumab included pharmacology, PK, general toxicology, carcinogenicity assessment, reproductive and developmental toxicology, and special toxicology studies. An integrated summary of this information is provided below.

Pharmacology

In vitro SPR Biacore studies showed binding of sarilumab to human and cynomolgus monkey IL-6Rα (K_D values = 54 pM and 123 pM, respectively) but not to mouse IL-6Rα. Cross reactivity studies showed that the REGN102-biotin antibody (mouse IgG2a with the same Fv fragment as sarilumab) bound to IL-6Rα on human and rhesus monkey PBMC, as determined by flow cytometry, but not to IL-6Rα of any other species tested (dog, sheep, mini-pig, rabbit, hamster, guinea pig, rat or mouse). Signaling pathways mediated by IL-6 are evolutionarily conserved, but the sequence homology of IL6Rα subunit varies across species (homology to human at the protein level being 96%, 98%, 79%, 54% and 53% for cynomolgus monkey, rhesus monkey, pig, rat and mouse, respectively). The monkey was considered the most appropriate species for nonclinical evaluations with sarilumab.

In vitro SPR Biacore and ELISA completion assays (IC_{50} = 108 pM) demonstrated that sarilumab directly blocked the binding of IL-6 to human IL-6Rα. In a functional cell-based luciferase reporter assay, sarilumab blocked soluble human IL-6Rα trans-signaling in a HEK293 cell line overexpressing gp130, but not expressing IL-6Rα (IC_{50} = 860 pM). In two in vitro functional bioassays sarilumab blocked IL-6-mediated STAT luciferase activity in HepG2 cells and IL-6 stimulated proliferation in DS-1 cells.

The in vitro Fc effector function activity of sarilumab was evaluated in ADCC and CDC assays. In the ADCC assay, little activity was noted for cells treated with sarilumab and then exposed to PBMC. In the CDC assay, there was no increase in cytotoxicity for cells treated with sarilumab and normal human serum complement.

The in vivo PD activity of blocking IL-6Rα with sarilumab were performed in double humanized (IL-$^{6hu/hu}$ IL-6Rα$^{hu/hu}$) mice expressing human IL-6 and the ectodomain of human IL-6Rα. This resulted in a chimeric IL-6Rα with mouse-specific intracellular signaling but with an extracellular domain that could now be recognized by sarilumab and human IL-6. Administration of turpentine to the double-humanized mice increased serum SAA. If sarilumab was given to the mice 24 hours prior to the turpentine challenge, serum SAA levels were decreased in a dose-dependent manner, most notably at doses ≥1.5 mg/kg. In the same experiment, human IL-6 levels were measured and were only slightly increased after administration of turpentine. If sarilumab was given to the mice 24 hours before the administration of turpentine then IL-6 levels were increased in a dose-dependent manner at doses ≥1.5 mg/kg through inhibition of endogenous receptor-mediated clearance.

Additional studies in mice were performed using a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα (i.e., REGN844). The in vivo PD activity of blocking mouse IL-6Rα with REGN844 was evaluated in a murine CIA of RA. Mice treated with 10 mg/kg REGN844 had no observed inflammation or swelling, while 10% (1/10) of mice treated with 30 mg/kg REGN844 developed joint inflammation (limited to a single digit) at Week 7. Further, no animals treated with 10 mg/kg REGN844 and only one mouse treated with 30 mg/kg REGN844 had signs of bone erosion.

Safety Pharmacology

No stand-alone safety pharmacology studies were conducted. Of note, no test article-related effects were evident in behavior (clinical observation) or cardiovascular parameters (e.g., ECG evaluations, blood pressure, body temperature) in any of the repeat dose toxicology studies in monkeys. Respiratory safety pharmacology parameters were not assessed.

Pharmacokinetics/ADME/Toxicokinetics

Designated PK studies were conducted with sarilumab in cynomolgus monkeys and rats. After single IV (1 and 15 mg/kg) or SC doses (1, 5, and 15 mg/kg), AUC values in monkeys increased greater than dose proportionally. Subcutaneous bioavailability was determined to be about 75%, based on AUC comparisons between the IV and SC doses at 1 and 15 mg/kg. The mean $t_{1/2}$ of sarilumab in monkeys was 30 to 226 hours (1.25 to 9.4 days) for single SC doses from 1 to 15 mg/kg. The volume of distribution was generally consistent with the blood volume. See **Table 18** for a comparison of single dose sarilumab PK parameters between monkeys and humans.

In repeat dose studies of sarilumab in monkeys, exposure increased in a dose-proportional manner across a range of doses. No sex differences in exposure were noted. Sarilumab accumulation was observed after repeat dosing. Detectable levels of ADA were generally observed in the low and/or mid dose groups and appeared to correlate with increased drug clearance.

There were no distribution, metabolism, or excretion studies conducted with sarilumab.

Table 18 Comparison of PK Parameters of Sarilumab between Monkeys and Humans

Study	Dose	C_{max} (mg/L)	AUC (mg*day/L)	t_{max} (days)	$t_{1/2}$ (days)	Vd (L)
SC Single Dose Monkey Study (REGN88-PK-06041)	1 5 15 (mg/kg)	10.7 57.9 177	53.3 546/546 2600/2596	1.7 3.0 4.7	1.25 4.7/1.5 9.4/2.5	NA
SC Single Dose Study in Healthy Volunteers (TDU11373)	100 (mg)	7.77	45.0	2.50	2.23	NA

Study	Dose	C_{max} (mg/L)	AUC (mg*day/L)	t_{max} (days)	$t_{1/2}$ (days)	Vd (L)
SC Studies in RA Patients (PK from Section 12 of product label)	150 200 (mg)	20.0 35.6	202 395	2-4	2-4	7.3

Abbreviations: AUC = area under the curve; C_{max} = maximum serum concentration; NA = not applicable; PK = pharmacokinetics; RA = rheumatoid arthritis; SC = subcutaneous; $t_{1/2}$ = terminal half-life; t_{max} = maximum time to C_{max}

General Toxicology

The safety of sarilumab was evaluated in a number of GLP-compliant repeat-dose toxicology studies in cynomolgus monkeys with IV administration for durations up to 6 months (4-weeks, 13-weeks, and 6-months) and with SC administration for 3 months (two 13-week studies). Further, a non-GLP-compliant repeat-dose toxicology study was conducted in mice for 4-weeks with IV and SC doses of REGN844, a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα (no notable test article-related findings were seen in this study). In addition, two repeat-dose toxicity studies were conducted in juvenile mice with REGN844 (non-GLP exploratory 4-week study and GLP 9-week study).

Repeat-Dose Monkey Studies

For the purposes of this integrated summary, common findings seen among all five monkey studies are briefly summarized below. Since similar findings were noted in all the monkey studies, only the 6-month study is discussed in detail as it included some evaluations not made in the shorter term studies.

Overall, no significant dose-limiting toxicity or target organs of toxicity were observed in any of the monkey studies with sarilumab administered at IV doses up to 50 mg/kg/week or SC doses up 100 mg/kg/week (two weekly doses of 50 mg/kg). There were no deaths that were attributed to treatment with sarilumab. Microscopic findings were limited to effects at the injection site (minimal to moderate perivascular mixed inflammatory cell infiltrates). The most common effects related to treatment with sarilumab were decreased levels of neutrophils, fibrinogen, and/or CRP. These decreases were considered PD effects of inhibiting IL-6 signaling. In most cases, these effects were not dose-dependent and were generally reversible during the recovery period. ADA were detected at the low and/or mid dose groups in a majority of animals and in most cases appeared related to decreased drug exposure due to increased drug clearance. Evaluations unique to the 6-month study included the measurement of serum IL-6 levels, immunophenotyping of lymphocytes, and TDAR. Serum IL-6 concentrations were noticeably increased in all sarilumab treated groups but returned to baseline levels during the recovery period. Elevations of IL-6 might be attributed to inhibition of endogenous receptor-mediated clearance. Immunophenotyping of lymphocyte subpopulations did not identify any treatment-related effects; however, levels of variability were high, which might have obscured any drug-related effects. The TDAR assessment measured slight but statistically significant decreases in primary and secondary IgG responses to antigen (KLH) challenge.

In the 6-month toxicology study with a 12-week recovery, cynomolgus monkeys (6/sex/group) received IV doses of sarilumab at 0 (placebo control), 0.5, 5, 15, and 50 mg/kg/week for 26 doses. Four monkeys/sex/group were sacrificed after the 26-week main study period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period. There were two deaths in the study (one male in the 0.5 mg/kg group and one male in the 15 mg/kg group), but the deaths were not attributed to the test article. There were no test article-related effects on clinical signs, body weight, ophthalmoscopy, ECG, blood pressure, body temperature, urinalysis, gross pathology, and histopathology.

From the assessments of standard panels of hematology and coagulation parameters, test-article related effects were noted on neutrophils, white blood cells, and fibrinogen. At Week 25, neutrophil counts (absolute and percent) were decreased in all test article-treated groups for both males and females, in a non-dose related manner. These decreases were generally reversible at the end of the recovery period. At Week 25, white blood cells were decreased in males treated with ≥5 mg/kg/week of the test article and in females in all test article-treated groups, which were attributed to treatment-related decreases of neutrophil counts. From Week 4 to the end of the main study period, males and females in the ≥5 mg/kg/week groups trended towards a decrease in fibrinogen levels (non-dose related manner).

From the assessment of a standard panel of clinical chemistry parameters, CRP levels were decreased in Weeks 4, 12, and 25 for males treated with ≥15 mg/kg/week of sarilumab. In females, decreases in CRP levels were noted in Weeks 4 and 12 when treated with ≥5 mg/kg/week of sarilumab. These decreases were reversible by the end of the recovery period. Mean IL-6 levels were increased in male and female groups treated with ≥5 mg/kg/week of sarilumab at Weeks 4, 12, and 25. These increases were considered a PD effect (inhibition of receptor-mediated clearance) and were reversed by the end of the recovery period.

An evaluation of organ weights revealed an increase in mean thymus weights (absolute and relative to body weight) in males in all test article-treated groups and in females treated with ≥5 mg/kg/week of the test article. These increases reversed by the end of the recovery period.

From the TDAR assessment, there were small decreases in both the primary and secondary IgG responses to KLH challenge in males and females treated with ≥5 mg/kg/week of the test article.

Toxicokinetic evaluation found that mean systemic exposure of sarilumab (AUCs and peak serum levels) increased with dose. No difference in exposure was noted between sexes. Drug accumulation was evident between the 1st and the 25th dose for test article doses ≥5 mg/kg in both males and females. All animals in the low dose group and 4/6 males and 1/6 females in the 5 mg/kg/week group had ADA. ADA appeared to decrease overall exposure by increasing drug clearance.

The no observed adverse effect level (NOAEL) was identified as 50 mg/kg/week based upon no dose-limiting treatment-related histopathological findings in any organs or tissues. Mean systemic exposure at 50 mg/kg/week was 381040 mcg*hr/mL (15877 mg*day/L). This exposure provides safety margins more than 80 times the exposure at the proposed clinical doses (see **Table 19**).

Table 19 Safety Margins for Proposed Clinical Doses Based on AUC from 6-Month Repeat-Dose Toxicology Study in Monkeys

	Monkey NOAEL	Monkey AUC[1]	Safety Margin for Clinical Dose of 200 mg Q2W[2]	Safety Margin for Clinical Dose of 150 mg Q2W[3]
6-Month Repeat Dose Toxicology Study in Monkeys	50 mg/kg/week	31754 mg*day/L	80	157

Abbreviations: AUC = area under the curve; NOAEL = no observed adverse effect level; Q2W = every 2 weeks

Notes:

[1]Dosing in 6-month monkey study was once weekly by intravenous injection. Combined (males + females) mean weekly $AUC_{0\text{-}168\ hr}$ estimate at end of study = 381040 mcg*hr/mL (15877 mg*day/L). Monkey AUC was multiplied by 2 to account for Q2W dosing in humans.

[2]Steady State $AUC_{0\text{-}14\ days}$ for 200 mg Q2W in humans = 395 mg*day/L (9480 mcg*hr/mL) (from Applicant's Summary of Clinical Pharmacology for population PK RA Study POH0428)

[3]Steady State $AUC_{0\text{-}14\ days}$ for 150 mg Q2W in humans = 202 mg*day/L (4848 mcg*hr/mL) (from Applicant's Summary of Clinical Pharmacology for population PK RA Study POH0428)

Juvenile Toxicology Studies

Juvenile Crl:CD1(ICR) mice (14 days old at the start of dosing) were treated once per week for 9 weeks with REGN844 at SC doses of 20, 60, or 200 mg/kg. A 13-week recovery period was included to assess reversibility of any test article-related findings. There were no test article-related deaths. Clinical observations were limited to the injection site and included minimal to moderate sores/lesions on the back and/or neck of animals in the high dose group (200 mg/kg/week). These findings were reversible during the recovery period.

There were no test article-related changes in hematology or clinical chemistry parameters in any of the dose groups. Of note, an assessment of CRP was not made.

Microscopic findings of increased incidence and/or severity were noted in the femur + marrow, sternum + marrow, lymph nodes (axillary and mandibular), and injection site (neck). These findings were reversible at the end of the recovery period. A minimal increase in hemopoiesis was noted in the femur + marrow of high dose males and females. Females in the mid dose group also had a minimal increase in hemopoiesis in the femur + marrow and sternum + marrow. Findings of hemopoiesis were considered a compensatory response and not adverse. Lymphoid hyperplasia was noted with increased incidence in the axillary lymph nodes of mid dose and high dose males and females in all dose groups. Lymphoid hyperplasia was also noted in the mandibular lymph nodes of high dose males and females but at a lesser incidence than in the axillary lymph nodes. At the injection site the main findings were inflammation of the

subcutis and dermis. High dose males and females in all test article-treated groups had minimal to slight subcutis inflammation. High dose males and females in the mid dose group had minimal to slight inflammation of the dermis.

The effects of REGN844 on the immune system of juvenile mice were evaluated by a TDAR assay. The main test article-related effect observed after a TDAR assessment was a decrease in the IgG responses to KLH challenge in males at all doses and at all the time points evaluated. These findings were reversible at the end of the recovery period. A decrease in the IgM response to KLH was only noted 6 days after KLH administration in males in all test article-treated groups.

Total serum IgG concentrations were increased at mid (60 mg/kg/week) and high doses (200 mg/kg/week) of the test article for males and females, which might be attributed to administration of exogenous IgG (i.e., REGN844). IgM concentrations were only increased for females in the high dose group (200 mg/kg/week). These findings were reversible by the end of the recovery period.

Toxicokinetics of REGN844 (i.e., C_{max} and $AUC_{0-\tau}$) were generally dose proportional between groups after repeat SC doses for 9 weeks. T_{max} was 24 hours for the low dose group and 72 hours for both the mid and high dose groups. There did not appear to be any sex differences in REGN844 toxicokinetic parameters.

Genetic Toxicology

Genetic toxicology studies with sarilumab were not conducted. Based on ICH S6, genetic toxicology studies are not applicable to biotechnology-derived pharmaceuticals and are not necessary. As a monoclonal antibody sarilumab is not expected to interact directly with DNA or other chromosomal material.

Carcinogenicity

At the EOP2 meeting for IND 100632 the Applicant provided a carcinogenicity risk assessment in support of their position not to conduct additional nonclinical studies to evaluate the carcinogenic potential of sarilumab. Based on the nonclinical evaluation of the Applicant's proposed carcinogenicity assessment, the nonclinical reviewer (Grace Lee, PhD) and the ECAC agreed that no additional nonclinical studies were required to address the carcinogenic potential of sarilumab (see Pharmacology/Toxicology IND 100632 Review and Evaluation dated May 12, 2016). It was also agreed that it was reasonable for the Applicant to manage any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. The recommendation was made to the Applicant that the product label for sarilumab should include a balanced description of the literature to address the carcinogenic potential of sarilumab in relationship to the carcinogenic risk to humans for the chronic use of sarilumab (see IR for IND 100632 dated December 4, 2013).

In the current BLA submission, the Applicant submitted an amended carcinogenicity risk assessment for sarilumab. The changes were made to provide clarity and/or to

add/update information to the original amendment, but did not alter the Applicant's previous assessment. The most notable addition was the inclusion of an appendix with historical control incidence of adrenal cortical adenomas and hyperplasia from several contract research organizations and adrenal neoplastic findings and hyperplasia reported in the literature. The amendment also included historical control data for tumor and pre-neoplastic (hyperplasia) findings in the adrenal gland of cynomolgus monkeys from the conducting laboratories (as requested in the nonclinical IR to the Applicant dated July 24, 2013).

IL-6 has been shown to have both pro-inflammatory and anti-inflammatory properties, but in the context of RA is considered pro-inflammatory.[1] As an inflammatory cytokine, IL-6 signaling through the IL-6R/gp130 complex and subsequent downstream activation of the JAK/STAT, MAPK, and PI3K/Akt pathways has been implicated in tumorigenesis.[14,15,16,17,18] In particular, STAT3 activation (JAK/STAT pathway) has been shown to induce genes that prevent apoptosis (e.g., Bcl-xL) and promote cell proliferation (e.g., c-myc, CycD1).[20] By affecting the expression of such genes, STAT3 has been implicated in the initiation, promotion, and progression of various tumor types (e.g., pancreatic, colorectal, lung, gastric and breast).[22]

The Applicant studied the effects of sarilumab on tumor xenograft growth in immunocompromised mice and STAT3 activation. Over the course of treatment, the growth of Du145 (prostate carcinoma), NCI-H1650 (human lung carcinoma), A549 (lung cancer cell line), and Calu3 (lung cancer cell line) xenografts in mice treated with 25 mg/kg REGN88 were reduced by about 41%, 40%, 43%, and 30%, respectively, compared to mice treated with control. Western blot analysis indicated that 5 of the 6 cell lines evaluated expressed basal levels of p-STAT3, indicating basal STAT3 activation. Sarilumab was able to reduce basal p-STAT3 and also reduced p-STAT3 levels that were induced by the addition of IL-6.

The Applicant's study data, taken together with the pro-tumorigenic information for the IL-6/IL-6R pathway from the public literature, imply that a drug, such as sarilumab, which blocks IL-6 signaling through the IL-6R, might be capable of diminishing tumorigenesis.

Although the literature generally supports that IL-6 signaling through the IL-6R is tumor promoting, available data also supports that the IL-6 pathway may have an anti-tumor role by supporting the adaptive immune response at the tumor microenvironment.[24] For instance, acute activation of the IL-6/IL-6R pathway can influence the trafficking of lymphocytes to lymph nodes in order to induce their activation, proliferation, and polarization towards phenotypes that oppose the immunosuppressive tumor microenvironment.[25]

A targeted null knockout for IL-6R ($Il6ra^{tm1.2Jcbr}$ mutation) was generated in C57BL/6 mice. In these mice, there was a decreased incidence of liver tumors after exposure to diethylnitrosamine (chemical which induces hepatocellular carcinoma). Mice fed standard chow and given diethylnitrosamine had increased hepatocyte apoptosis,

decreased hepatocyte proliferation, and consequently fewer and smaller hepatocellular carcinomas compared to wild-type mice. In contrast, mice fed a high fat diet and treated with diethylnitrosamine displayed normal liver apoptosis and hepatocellular carcinoma development.

The nonclinical review for IND 100632 pointed out that the potential carcinogenic role of IL-6 in the endocrine glands and female reproductive tract could not be dismissed, since IL-6 is made in the endocrine glands (e.g., adrenal cortex) and elevated IL-6 levels have been described in diethylstilbestrol (DES)-induced vaginal metaplasia in animal studies. Also, since sarilumab is considered to be immunosuppressive there is an overall safety concern for malignancies that should be taken into account. Based on species specificity, a rodent carcinogenicity study with sarilumab was not considered feasible. As such, the Applicant indicated that any potential risk for malignancies would be managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches.

As described above, published data exists to indicate that the IL-6 pathway can mediate anti-tumor responses by promoting increased immune cell surveillance of the tumor microenvironment. However, the literature generally supports that IL-6 signaling through the IL-6R may be involved in pathways that lead to tumorigenesis. Further, IL-6R knockout mice have a phenotype associated with decreased tumor incidence in response to treatment with various chemical carcinogens as compared to wild-type mice. Overall, the malignancy risk in humans from an antibody that disrupts signaling through the IL-6R, such as sarilumab, is unknown.

Reproductive and Developmental Toxicology

Reproductive and developmental toxicology studies were conducted with sarilumab in monkeys and with REGN844 (a surrogate monoclonal antibody to sarilumab that binds mouse IL-6Rα) in mice. Overall, sarilumab did not appear to adversely affect the reproductive system.

In a fertility study, CD-1(ICR) mice (24/sex/group) were given REGN844 twice per week by SC administration at 0 (placebo control), 10, 25, or 100 mg/kg/dose (total weekly doses of 20, 50, and 200 mg/kg, respectively). Males were treated for 4 weeks before cohabitation, during cohabitation (up to 14 days maximum), and until necropsy. Females were treated 2 weeks before cohabitation, during cohabitation (up to 14 days maximum), and until GD 7 (GD 0 was considered the day that evidence of mating was observed). There were no test article-related effects on any of the fertility parameters examined in the study (e.g., mating/fertility index, number of corpora lutea, number of late resorption, number of dead fetuses, preimplantation loss, or postimplantation loss). The estrous cycle was not evaluated in the study.

In an ePPND study, sarilumab was given to pregnant cynomolgus monkeys (12/sex/group) one time per week by IV infusion at 0 (placebo control), 5, 15, or 50 mg/kg/week. Dosing began on GD 20 (GD 0 was considered to be the middle of the 3 or 5 day mating season) and continued through the end of gestation with either delivery

around GD 160 to GD 165 or abortion/embryo-fetal death. Maternal animals and neonates were observed for 28-32 days after delivery.

There were no mortalities in maternal animals that were attributed to the test article. Hematology and coagulation evaluation in the maternal animals showed decreases in mean lymphocyte, neutrophil, and white blood cell counts in all test article-treated groups (not dose related) on GD 153 and/or LD 7. Further, fibrinogen levels were slightly decreased in all test article-treated groups (not dose related) on GD 153, LD 7, and/or LD 30. Sarilumab exposure ($AUC_{0\text{-}168h}$) appeared to increase proportionally with dose and slight accumulation was noted over the course of the dosing period. ADA was detected in three low dose and one mid dose animal before dosing with sarilumab on GD 20.

In the neonates, no test article-related effects were noted on any of the parameters evaluated (birth examinations, clinical signs, body weight, functional and morphological development, hematology, coagulation, serum chemistry, immunophenotyping and ADA). Sarilumab concentrations were increased with dose.

The nonclinical reviewer highlighted some concerns about the adequacy of the ePPND study (e.g., small numbers of neonates in each group at the time of scheduled necropsy, absence of immune function testing [e.g., TDAR] in the offspring during the postnatal phase, presence of drug in the circulation of the neonates at the time of necropsy, which may lead to uncharacterized effects beyond the one month of age in monkeys). The nonclinical reviewer indicated that the potential effects of sarilumab on the immune system of infants from mothers exposed to sarilumab during pregnancy should be addressed by product labeling.

The NOAEL for the ePPND study was identified as 50 mg/kg/week based upon no evidence of embryotoxicity or fetal malformations. Mean systemic exposure at 50 mg/kg/week was 396455 mcg*hr/mL (16519 mg*day/L). This exposure provides safety margins more than 84 times the exposure at the proposed clinical doses (see **Table 20**).

Table 20 Safety Margins for Proposed Clinical Doses Based on AUC from ePPND Study in Monkeys

	Monkey NOAEL	Monkey AUC[1]	Safety Margin for Clinical Dose of 200 mg Q2W[2]	Safety Margin for Clinical Dose of 150 mg Q2W[3]
ePPND Study in Monkeys	50 mg/kg/week	33038 mg*day/L	84	164

Abbreviations: AUC = area under the curve; NOAEL = no observed adverse effect level; Q2W = every 2 weeks

Notes:

[1]Dosing in ePPND study was once weekly by intravenous injection. Combined (males + females) mean weekly $AUC_{0\text{-}168\ hr}$ estimate at end of study = 396455 mcg*hr/mL (16519 mg*day/L). Monkey AUC was multiplied by 2 to account for Q2W dosing in humans.

[2]Steady State $AUC_{0\text{-}14\ days}$ for 200 mg Q2W in humans = 395 mg*day/L (9480 mcg*hr/mL) (from Applicant's Summary of Clinical Pharmacology for population PK RA Study POH0428)

[3]Steady State $AUC_{0\text{-}14\ days}$ for 150 mg Q2W in humans = 202 mg*day/L (4848 mcg*hr/mL) (from Applicant's Summary of Clinical Pharmacology for population PK RA Study POH0428)

Special Toxicology Studies

In a GLP-compliant tissue cross-reactivity study of biotinylated REGN88 with normal human and cynomolgus monkey tissues, the positive tissue staining for biotinylated sarilumab between human and monkey tissues appeared similar with some exceptions. In human tissues, positive staining for biotinylated sarilumab was detected in the cytoplasm and/or cytoplasmic granules of various sites but not at any membrane sites. Similar findings were noted in monkey tissues with the exception that positive staining was noted in mammary gland epithelium from one monkey donor. Cytoplasmic and cytoplasmic glandular staining was considered of little toxicological concern.

Recommendation

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

Labeling

Nonclinical sections of the product label will be evaluated in a separate review.

12 Appendix/Attachments

Appendix I: Pharmacology/Toxicology IND 100632 Review and Evaluation dated May 12, 2016.

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

PHARMACOLOGY/TOXICOLOGY IND REVIEW AND EVALUATION

Application number:	100,632
Sequence Number:	2
Supporting document/s:	4, 10, 27, 35, 67, 119, 124, 130, 270, 281, 319, 331
Sponsor's letter date:	10/16/2007, 7/8/2008, 12/18/2009, 5/28/2010, 12/13/2010, 7/28/2011, 8/16/2011, 9/12/2011, 2/11/2013, 3/15/2013, 8/14/2013, 10/4/2013
CDER stamp date:	10/16/2007, 7/8/2008, 12/18/2009, 5/28/2010, 12/13/2010, 7/28/2011, 8/16/2011, 9/12/2011, 2/11/2013, 3/15/2013, 8/14/2013, 10/4/2013
Product:	SAR153191 (anti-IL6Rα) formerly REGN88
Indication:	Treatment of Rheumatoid Arthritis (RA)
Sponsor:	Sanofi-Aventis U.S., Inc. 55 Corporate Drive Bridgewater, NJ 08807
Review Division:	Division of Pulmonary, Allergy, and Rheumatology Products
Reviewer:	Grace S. Lee, Ph.D., D.A.B.T.
Supervisor/Team Leader:	Marcie L. Wood, Ph.D.
Division Director:	Badrul Chowdhury, M.D., Ph.D.
Project Manager:	Christine Chung, R.Ph.

Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Sarilumab (SAR153191 or REGN88) is a fully human monoclonal antibody IgG1, which is directed against the alpha subunit of the IL-6 receptor complex (IL-6Rα). Sarilumab is being developed for the treatment of patients with rheumatoid arthritis (RA), (b) (4) Several clinical studies (Phase 1 and 2) have been conducted in healthy volunteers, RA patients, or AS patients. Currently, sarilumab, formulated for subcutaneous (SC) injection, is in Phase 3 clinical development for the treatment of RA.

1.2 Brief Discussion of Nonclinical Findings

Sarilumab binds human and cynomolgus monkey IL-6Rα with an equilibrium dissociation constant (Kd) of 54 pM and 123 pM, respectively, but no binding of sarilumab to the murine IL-6Rα was observed. This supported selection of the Cynomolgus monkey as the relevant species for nonclinical assessment of sarilumab.

Five repeat-dose studies were conducted in monkeys using sarilumab (one 5-week IV, one 13-week IV, two 13-week SC, and one 6-month IV). Exposure to REGN88 increased with dose. There was no test article-related mortality in any of these studies. The test article-related microscopic findings were limited to injection site findings in a few studies. The most consistent findings in these studies were decreased levels of neutrophil and fibrinogen in males and females in test article-dosed groups during the dosing period. These findings were not generally dose-dependent, and the findings tended to be reversible during the recovery period. In addition, decreased levels of CRP were observed during the first several weeks of the dosing period in most of the monkey studies. In addition, there were markedly increased levels of IL-6 in all test article-dosed groups and slightly, but statistically significant (not dose-dependent) decreases in primary and secondary IgG responses to KLH administration in the TDAR assays in males and females at ≥5 mg/kg/week in the 6-month study. Overall, the findings in the general toxicity studies indicate that sarilumab is immunosuppressive in monkeys.

The sponsor submitted a carcinogenic risk assessment associated with sarilumab. Based on the weight of evidence for the available *in vitro* and *in vivo* data related to inhibition of IL-6 in tumor promotion in the literature, and the anti-tumor effects specifically observed with sarilumab in a tumor xenograft assay, the sponsor proposed that no additional nonclinical studies (e.g., 2-year carcinogenicity study in mice) are needed to evaluate the carcinogenic potential of sarilumab in patients. In agreement with the ECAC, no additional nonclinical studies are needed to address the carcinogenic potential of sarilumab. Any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion are better managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. The carcinogenic risk to humans for the chronic use of sarilumab in the product label should include a balanced description of the literature information available to address the carcinogenic potential of sarilumab.

The reproductive and developmental toxicity of sarilumab was evaluated in a fertility and early embryonic developmental toxicity study in mice using REGN844 and an enhanced pre- and postnatal developmental (PPND) toxicity study in cynomolgus monkeys using REGN88. In the fertility study, there were no test article-related effects on parental toxicity parameters or any reproductive and fertility parameters examined in the study, including number of corpora lutea, implantation sites, resorption, and viable fetuses. However, estrous cycle observation was not included in the study. In the enhanced PPND study, groups of 12 pregnant monkeys were intravenously administered REGN88 once weekly from gestation Day (GD) 20 through natural delivery (approximately GD 165), with subsequent maternal and offspring monitoring to approximately 28 days after delivery. There was no test article-related mortality. In maternal animals, there were decreased mean counts of neutrophils and decreased levels of fibrinogen in all test article-dosed groups. Decreases in neutrophil counts and fibrinogen levels were also consistently observed in general toxicity studies as described above. In F1 neonates, there were no apparent test article-related effects on parameters examined in the study. Exposure to REGN88 increased with dose in maternal and F1 animals.

There were some concerns about the adequacy of the ePPND study. First, numbers of neonates in each group at scheduled necropsy were relatively small (3 males and 3 females in the control group; 1 male and 6 females at LD; 2 males and 3 females at MD; 2 males and 2 females at HD). Second, immune function testing in the offspring during the postnatal phase was not conducted (e.g., TDAR), although REGN88 has been shown to be immunosuppressive in adult monkeys (e.g., decreased neutrophil counts in almost all general toxicity studies, decreased effects in the TDAR assay in the 6-month study) as well as in adult humans. Lastly, REGN88 was not totally cleared from the circulation of F1 offspring at the time of necropsy (around postnatal day 30), and thus the drug may have uncharacterized effects beyond one month of age in monkeys. These concerns were communicated to the sponsor at the EOP2 meeting. The Division did not request additional developmental toxicity studies, but requested that these deficiencies be described in the investigator's brochure (IB), informed consent (IC), and potential product labeling.

2 Drug Information

2.1 Drug

CAS Registry Number: Not available (NA)

Generic Name: Sarilumab

Code Name: REGN88, SAR153191

Chemical Name: NA

Molecular Formula/Molecular Weight: 144 kDa

Structure or Biochemical Description:
REGN88 is a covalent heterotetramer consisting of two, disulfide linked human heavy

chains, each covalently linked through disulfide bonds to a fully human kappa light chain. The antibody has a single N-linked glycosylation site in each heavy chain, located within the constant region in the Fc portion of the molecule. The REGN88 heavy chain has an IgG1 isotype constant region (a allotype). The variable domains of the heavy and light chains combine to form the IL-6Rα binding site within the antibody.

APPEARS THIS WAY ON ORIGINAL

REGN88 Heavy Chain

EVQLVESGGG LVQPGRSLRL SCAASRFTFD DYAMHWVRQA PGKGLEWVSG ISWNSGRIGY ADSVKGRFTI SRDNAENSLF[80]

LQMNGLRAED TALYYCAKGR DSFDIWGQGT MVTVSSASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG[160]

ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV DKKVEPKSCD KTHTCPPCPA PELLGGPSVF[240]

Heavy Chain

LFPPKPKDTL MISRTPEVTC VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTVLHQ DWLNGKEYKC[320]

KVSNKALPAP IEKTISKAKG QPREPQVYTL PPSRDELTKN QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTPPVLDSD[400]

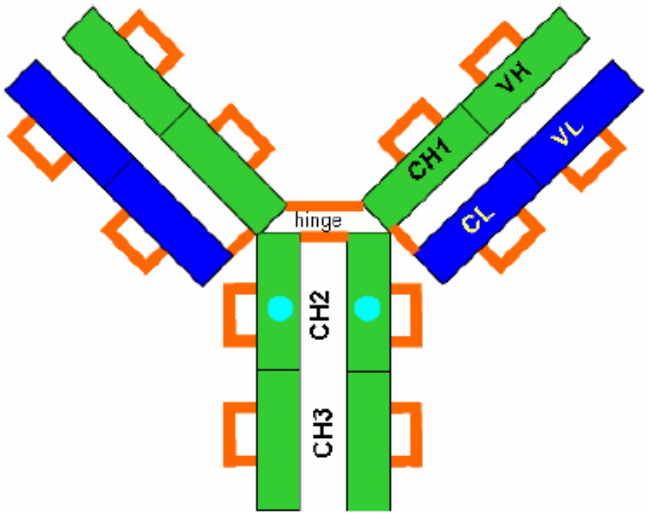
GSFFLYSKLT VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK[446]

REGN88 Light Chain

DIQMTQSPSS VSASVGDRVT ITCRASQGIS SWLAWYQQKP GKAPKLLIYG ASSLESGVPS RFSGSGSGTD FTLTISSLQP[80]

EDFASYYCQQ ANSFPYTFGQ GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ[160]

ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC[214]



Top: REGN88 antibody sequences with the post-translational modifications highlighted. The cysteines that form disulfide bonds are connected by solid lines. The Fc glycosylation site Asn[296] is marked in cyan. The C-terminal Lys446 of heavy chain is usually removed in vivo during production.

Bottom: Schematic of the structure of REGN88 with 16 disulfide bonds (orange). Heavy chain (green) and the light chain (blue) contain intermolecular disulfide bonds and dimerization is achieved through two heavy chain intermolecular disulfide bonds. Fc domain glycosylation site is indicated (cyan).

Figure 1 Amino Acid Sequence and Schematic Representation of REGN88 [taken directly from the Sponsor's submission (3.2.S.1.2)]

Pharmacologic Class: A monoclonal antibody (mAb) that binds to the alpha subunit of the IL-6 receptor complex (IL-6Rα) blocking the binding of the receptor to IL-6.

2.2 Relevant INDs, NDAs, BLAs and DMFs

(b) (4); DTOP

2.3 Drug Formulation

The REGN88 drug product is supplied as a (b) (4) subcutaneous administration. (b) (4)

Table 1 Nominal composition of REGN88 (b) (4) formulation [taken directly from the Sponsor's submission (3.2.P.1]]

(b) (4)

USP = United States Pharmacopeia; NF = National Formulary; Ph. Eur. = European Pharmacopeia; JP = Japanese Pharmacopeia;
NA = not applicable

Table 2 Nominal composition of REGN88 (b) (4) formulation [taken directly from the Sponsor's submission (3.2.P.1]]

(b) (4)

USP = United States Pharmacopeia; NF = National Formulary; Ph. Eur. = European Pharmacopeia; JP = Japanese Pharmacopeia; NA = not applicable

During clinical development, in order to optimize the manufacturing process, (b) (4)

In addition, a new formulation (F3) (b) (4) was developed to (b) (4) DP than is possible using the formulation (F2) currently in use in Phase 2. (b) (4) tested in the Phase 2 dose ranging studies in a volume compatible with the use of a pre-filled syringe (b) (4) that is planned for commercialization. The compositions of the F2 and F3 formulations are presented in Table 3.

Table 3 Comparison of F2 and F3 formulations [taken directly from the meeting package submitted under SDN 77, pp.11]

Components	Phase 2 Formulation (F2)	Phase 3 Formulation (F3)
Protein concentration	50, 75, 100 mg/mL	87.7, 131.6, 175.0 mg/mL
(b) (4) Histidine	(b) (4)	(b) (4) mM
Arginine (b) (4)		mM
Polysorbate 20		0.2%
Sucrose		5%
pH		6.0

Table 4 Summary of SAR153191 drug products [taken directly from the meeting package submitted under SDN 77, pp.11]

Drug product	Clinical phase use	Antibody-producing cell line	Excipients
(b) (4) F2	Phase 2 DP	(b) (4)	Formulation 2 [a]
F3	Proposed Phase 3 DP		Formulation 3 [b]
F3	Back-up Phase 3 DP		Formulation 3 [b]

[a] (b) (4)

[b] (b) (4) mM histidine, 0.2% polysorbate 20, (b) (4) mM arginine, and 5% sucrose

2.4 Comments on Novel Excipients

None

Arginine as an excipient can be found in approved intravenous drugs, but not in approved subcutaneous drugs. In the 13-week bridging subcutaneous study in monkeys, the control monkeys were dosed with the Phase 3 Formulation Placebo and there were no adverse findings.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Protocol

Not applicable for this PharmTox review

2.7 Previous Clinical Experience

A number of Phase 1, 2, and 3 studies with sarilumab have been completed, and there are ongoing Phase 2 and Phase 3 studies with RA patients (b) (4).

2.8 Regulatory Background

A preIND meeting was held on August 2, 2007.
The initial IND was submitted in October 2007.
A Type C meeting was held on February 23, 2011, to obtain the Division's feedback on the proposed Phase 3 development program for treatment of RA.
A Type B EOP2 meeting was held on September 15, 2011.
A preBLA meeting was held on October 22, 2014.

3 Studies Submitted

3.1 Studies Reviewed

Determination of the Equilibrium Binding Constants for the Interaction of Human and Monkey IL-6 Receptor with REGN88 [Study No. REGN844-MX-07016], submitted under SDN 4.

Determination of the equilibrium Binding Constants for the Interactions of REGN844 with Mouse IL-6Rα [Study No. REGN844-MX-09073], submitted under SDN 281.

Characterization of REGN88 Activity in a Cell Proliferation Assay [Study No. REGN88-MX-11077-SA-01V1], submitted under SDN 270.

SAR153191 (REGN88): Exploratory 4-week subcutaneous and intravenous toxicity study in mice with REGN844 [Study No. DIV1267], submitted under SDN 67.

A 4-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 9-Week Recovery Period [Study No. REGN88-TX-06040], submitted under SDN 4.

A 13-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by an 8-Week Recovery Period [Study No. REGN88-TX-06037], submitted under SDN 27.

A 13-Week Subcutaneous Injection Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 12-Week Recovery Period [Study No. REGN88-TX-06038], submitted under SDN 10.

A 13-Week Bridging Subcutaneous Study of REGN88 in Cynomolgus Monkeys [Study No. REGN88-TX-09053], submitted under SDN 119.

A 6-Month Once Weekly 30-Minute Intravenous Injection Study of REGN88 in Cynomolgus Monkeys Followed by a 12-Week Recovery Period [Study No. REGN88-TX-08031], submitted under SDN 35.

SAR153191 (REGN88): Subcutaneous Fertility Study in Mice with REGN844 [Study No. REGN844-TX-09048], submitted under SDN 119.

Study of the Effects of REGN88 on Embryo-Fetal Development and Pre- and Post-Natal Development When Administered Weekly by Intravenous Infusion to Pregnant Cynomolgus Monkey [Study No. REGN88-TX-08030], submitted under SDN 119.

SAR153191/REGN88: Exploratory Subcutaneous 4-Week Toxicity Study in the Juvenile Mouse with REGN844 [Study No. JUP0016], submitted under SDN 319.

Cross-Reactivity Study of Biotinylated REGN88 with Normal Human and Cynomolgus Monkey Tissues [Study Report: IM1436], submitted under SDN 4.

Characterization of the Effects of Sarilumab (REGN88) on STAT3 Activation and Tumor Xenograft Growth [Study Report: REGN88-MX-11050-SR-01V1], submitted under SDN 130.

3.2 Studies Not Reviewed

Selective study reports (as listed above) were reviewed, the remaining study reports will be reviewed at a later time point.

3.3 Previous Reviews Referenced

IND 100,632 Preliminary 30-day PharmTox Reviewed dated August 19, 2009 by Dr. Z. Alex Xu

4 Pharmacology

4.1 Primary Pharmacology

Title: Determination of the Equilibrium Binding Constants for the Interaction of Human and Monkey IL-6 Receptor with REGN88 [Study No. REGN844-MX-07016; non-GLP]

Method: Kinetic parameters for REGN88 binding to human and monkey IL-6 receptor (IL-6R) were measured using a BiaCore assay.

Result: Summary of binding results is shown in Table 5. The value of calculated equilibrium dissociation constant (K_D, k_{off}/k_{on}) was 54.4 pM for the human IL-6R and 123 pM for the monkey IL-6R. The data showed that that REGN88 has a higher affinity for the human IL-6R than for the monkey IL-6R, and this difference in K_D between human and monkey receptor binding to REGN88 is reflected for the most part in the faster K_d (~ 2-fold) for the monkey receptor.

Table 5 Summary of Binding Parameters for the Interaction of REGN88 with human and monkey IL-6 receptor [taken directly from the study report, pp. 9]

REGN88 Lot#	Ligand	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	Rmax (RU)	$t_{1/2}$
K07005M509	human IL6 receptor	8.52E+05	4.63E-05	5.44E-11	55	4.2 hr
K07005M509	monkey IL6 receptor	7.28E+05	8.93E-05	1.23E-10	53	2.2 hr

Title: Determination of the Equilibrium Binding Constants for the Interactions of REGN844 with Mouse IL-6Rα [Study No. REGN844-MX-09073; non-GLP]

Method: Kinetic parameters for the interaction of three batches of REGN844 (a mouse surrogate mAb that binds mouse IL-6Rα) with mouse IL-6Rα monomer (mIL-6Rα-MMH) and mouse IL-6Rα dimer (mIL-6Rα-hFc) were measured using a BiaCore assay.

Result: A summary of binding results is shown in Table 6. The mean value of calculated equilibrium dissociation constant (K_D) for three batches was 203 pM for binding to mIL-6Rα-MMH and 8.3 pM for binding to mIL-6Rα-hFc.

Table 6 Summary of Binding Parameters for the Interaction of REGN844 to mIL-6Rα-MMH or mIL-6Rα-hFc at pH 7.4 and 25°C [taken directly from the study report, pp.10]

REGN844 Lot # Tested	Antigen Tested	k_a ($M^{-1}s^{-1}$)	k_d (s^{-1})	K_D (M)	$T_{1/2}$ (hours)
090520A (Reference Standard)	mIL-6Rα-MMH	5.98E+05	1.26E-04	2.11E-10	1.5
	mIL-6Rα-hFc	1.06E+06	8.62E-06	8.13E-12	22.3
T09001D400 (Drug Substance)	mIL-6Rα-MMH	6.43E+05	1.32E-04	2.05E-10	1.5
	mIL-6Rα-hFc	1.25E+06	9.35E-06	7.50E-12	20.6
T09001D600X11A (Labeled Drug Product)	mIL-6Rα-MMH	6.52E+05	1.26E-04	1.93E-10	1.5
	mIL-6Rα-hFc	1.10E+06	1.03E-05	9.38E-12	18.6

Title: Characterization of REGN88 Activity in a Cell Proliferation Assay [Study No. REGN88-MX-11077-SA-01V1; non-GLP]

Method: The biological activity of several lots of REGN88 was tested in assays using 2 different cell types (HepG2 and DS-1 cells). Each assay is based on IL-6-induced signal transduction and measures subsequent activity. In the first assay, a human hepatocytic cell line (HepG2) endogenously expressing IL-6Rα and gp130 was transiently transfected with a Stat3-luciferase reporter plasmid (with 5 copies of the STAT3 response element in *cis* orientation with the luciferase gene). REGN88 diluted in OptiMEM® /FCS (100 nM to 1.7 pM final concentration) was added to the transfected cells followed by IL-6 at a constant concentration of 50 pM. For the dose response curve, IL-6 concentrations ranging from 15 nM to 2.5 pM were used. Negative control wells contained no IL-6. In the second assay, REGN88 was tested for its ability to block IL-6-induced proliferation of the DS-1 cell line. The DS-1 cell line is a human B lymphocyte cell line, and these cells proliferate in response to exogenous hIL-6. Using WST-8 or AlamarBlue (colorimetric and fluorescent indicators of cell number, respectively), a sensitive IL-6-dependent assay was developed. REGN88 diluted in RPMI/FCS was added to the DS-1 cells at concentrations ranging from 25 nM to 0.4 pM, including control wells without antibody, followed by the addition of IL-6 to a final concentration of 1pM. For the dose response curve, IL-6 concentrations ranging from 100 pM to 20 fM (final concentrations) were added to the wells. Negative control wells contained no IL-6.

Results: Luciferase activity in HepG2 cells stimulated by IL-6 with an EC_{50} of 30 pM and REGN88 inhibited IL-6-mediated luciferase activity with an IC_{50} of ~150 pM in the presence of a constant concentration of 50 pM IL-6 (Figure 2). The report states that no increased luciferase activity was detected in the cells when incubated with REGN88 in the absence of IL-6.

Similarly, IL-6 stimulated proliferation of DS-1 cells in a dose-dependent manner (EC_{50} of ~0.7pM), and REGN88 inhibited the IL-6-mediated proliferation with an IC_{50} of ~140 pM in the presence of a concentration of 1.0 pM IL-6 (Figure 3).

REGN88 from several lots had similar relative potencies in both assays (Table 7). The relative potencies of the REGN88 lots ranged from 64 to 124% of reference standard in the HepG2 luciferase assay. In the DS-1 assay, the potencies ranged from 92 to 141% of reference standard using AlamarBlue and from 80 to 143% using WST-8.

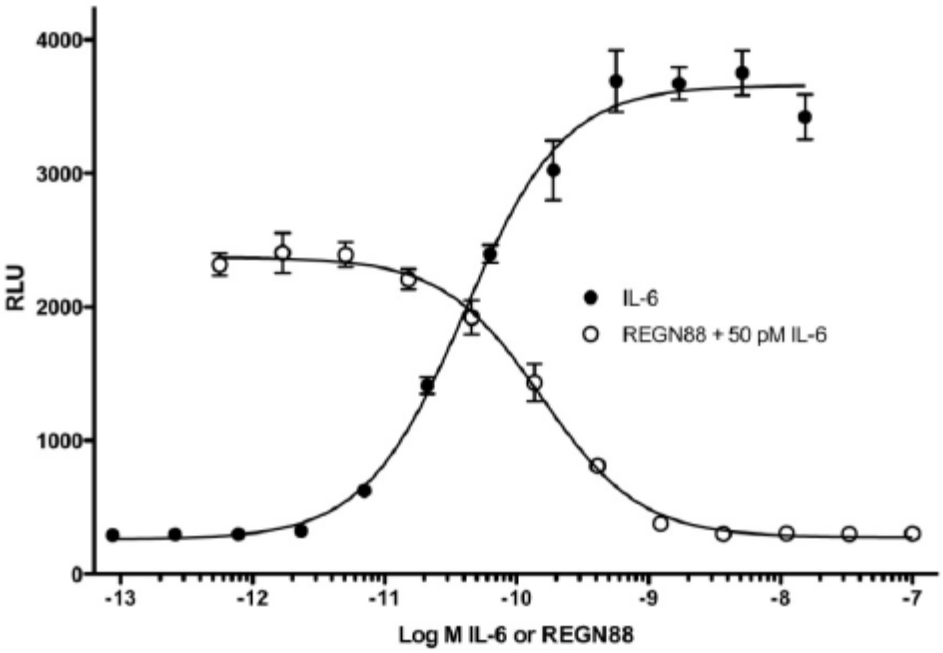


Figure 2 The inhibitory effect of REGN88 on IL-6 signaling in HepG2 cells [taken directly from the study report, pp.10]

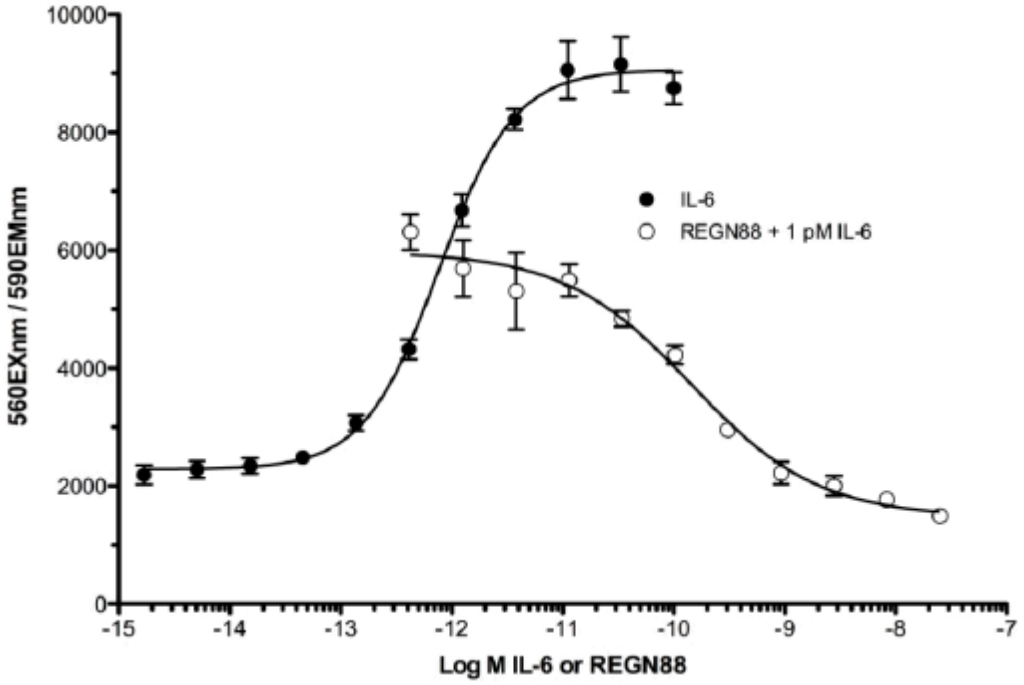


Figure 3 The inhibitory effect of REGN88 on IL-6-stimulated DS-1 cell proliferation [taken directly from the study report, pp.11]

Table 7 Potency of several lots of REGN88 in HepG2 and DS-1 assays [taken directly from the study report, pp. 12]

REGN88 Sample Lot #	HepG2 Transient Luciferase Assay		DS-1 Proliferation Assay AlamarBlue Detection		DS-1 Proliferation Assay WST-8 Detection	
	% Relative Potency	Number Of Replicates	% Relative Potency	Number Of Replicates	% Relative Potency	Number Of Replicates
K07005M500	77%	1	94%	1	NP	
K09004M419	120%	3	110%	5	NP	
K09005M419	117%	2	141%	2	NP	
K09003M410	79%	2	92%	2	123%	3
K08001D600A11A	115%	3	NP		107%	1
K08001M800A11	124%	3	NP		114%	2
K08002M509	64%	2	NP		126%	4
K08003M509	80%	2	NP		132%	4
K08004M509	66%	2	NP		97%	4
K08003D600A12A	NP[a]		NP		143%	1
K08003D600A11A	NP[a]		NP		80%	1
K08005M509	NP[a]		NP		93%	4

Table 2 summarizes relative potency data obtained using the initial potency assay (HepG2 Luciferase assay) and the current potency assay (DS-1 proliferation assay). AlamarBlue and WST-8 were evaluated as detection agents during development of the assay. The current validated release assay uses CellTiter Blue detection (analogous to AlamarBlue detection).
REGN88 Reference Standard = K06002D600X11A
% Relative Potency = IC_{50} Reference Standard / IC_{50} Test Article
NP: Not performed
[a] No longer being used as a release assay.

Title: *In vivo* Pharmacodynamics Activity of IL-6Rα Blockade in Mice by REGN844 [Study No. REGN844-MX-09076; non-GLP]

Method: To evaluate *in vivo* pharmacodynamic effect of blocking IL-6 activity by targeting IL-6Rα with REGN844, mice were challenged with a single subcutaneous (SC) injection of turpentine. In this study, female mice (age of 8 to 12 weeks) were pre-treated with REGN844 at concentrations ranging from 0.015 to 15 mg/kg for CD1 mice and 5.0 or 25 mg/kg for C57BL/6 mice as shown in Tables 8-9. Mice in the control groups were pretreated with an isotype-matched mouse mAb (REGN654). Mice were subsequently challenged with a SC injection of distilled turpentine or sterile PBS into the right hindlimb. Animals were euthanized at 20 hours following the challenge, and serum was obtained from terminal bleed samples. Serum amyloid A (SAA) protein and mouse IL-6 levels in serum were quantitated by ELISA using commercially available kits.

Table 8 Experimental design using CD1 mice [taken directly from the study report, pp.7]

No.	N=	Antibody	Dose	Challenge
1	3	Isotype Control	15 mg/kg	PBS
2	3	Isotype Control	15 mg/kg	Turpentine
3	3	REGN844	15 mg/kg	PBS
4	3	REGN844	0.015 mg/kg	Turpentine
5	3	REGN844	0.05 mg/kg	Turpentine
6	3	REGN844	0.15 mg/kg	Turpentine
7	3	REGN844	0.5 mg/kg	Turpentine
8	3	REGN844	1.5 mg/kg	Turpentine
9	3	REGN844	5.0 mg/kg	Turpentine
10	3	REGN844	15 mg/kg	Turpentine

Table 9 Experimental design using C57BL/6 mice [taken directly from the study report, pp.7]

No.	N=	Antibody	Dose	Challenge
1	3	Isotype Control	25 mg/kg	PBS
2	3	Isotype Control	25 mg/kg	Turpentine
3	3	REGN844	25 mg/kg	PBS
4	3	REGN844	5.0 mg/kg	Turpentine
5	3	REGN844	25 mg/kg	Turpentine

Results: Pre-treatment with REGN844 in either CD1 or C57BL/6 mice inhibited the turpentine-induced elevation of serum amyloid A (SAA) protein, an indicator of the acute inflammatory response (Figure 4). Complete inhibition of turpentine-induced SAA production was observed at ≥5 mg/kg of REGN844, whereas minimal or no effect was observed at doses below 0.5 mg/kg. An approximately 70% reduction of SAA level was observed using 1.5 mg/kg. In addition, serum levels of mouse IL-6 were elevated following turpentine injection (Figure 5), and these levels were further increased following dosing of REGN844 above 1.5 mg/kg.

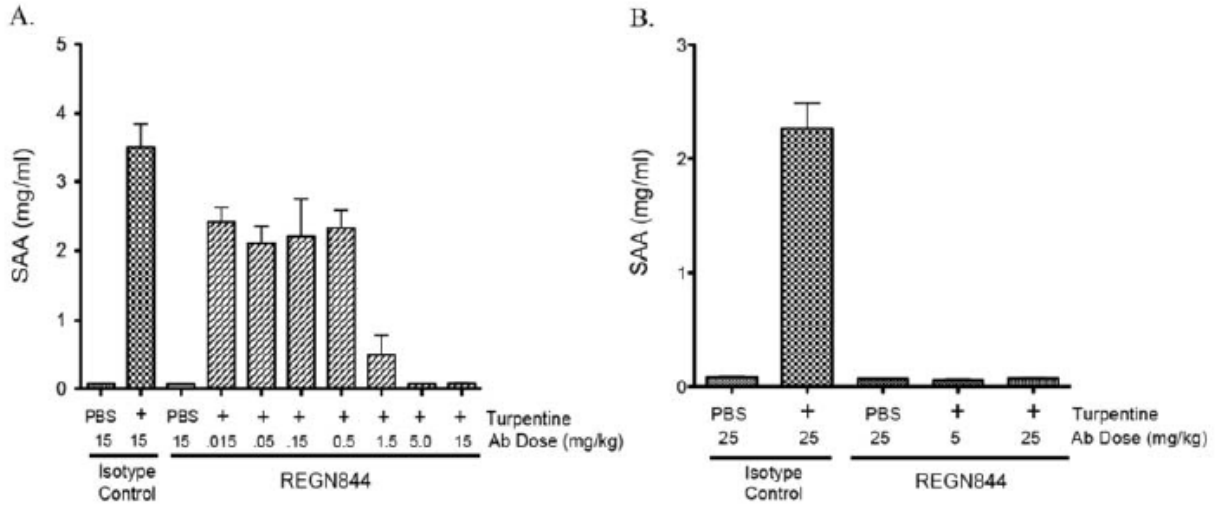


Figure 4 Dose-dependent reduction of serum amyloid A (SAA) by REGN844 in (A) wild type CD1 mice and (B) C57BL/6 mice [taken directly from the study report, pp. 8]

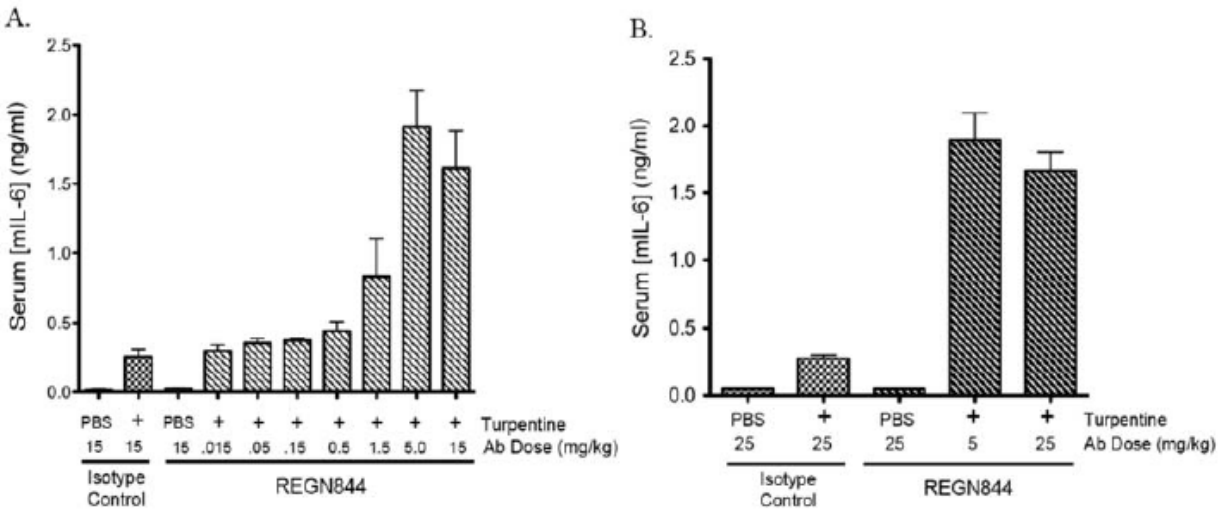


Figure 5 Elevated serum mIL-6 levels by REGN844 in the turpentine-induced inflammation model; (A) wild type CD1 mice and (B) C57BL/6 mice [taken directly from the study report, pp. 9]

6 General Toxicology

6.2 Repeat-Dose Toxicity

Study title: SAR153191 (REGN88): Exploratory 4-week subcutaneous and intravenous toxicity study in mice with REGN844

Study no.:	DIV1267
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 15, 2009
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	REGN844 (a mouse surrogate antibody to REGN88); Batch Lot # T09001D400; 98.2% purity by SEC-HPLC REGN844 labeled drug product; T09001D600X11A; 98.3% purity by SEC-HPLC

Key Study Findings

- Groups of 10 mice/sex/group were administered REGN844 (a mouse surrogate antibody to REGN88) at twice weekly subcutaneous doses of 0 (placebo control), 5, 25, or 100 mg/kg/dose or once weekly intravenous dose of 25 mg/kg/dose for 4 weeks.
- There was no mortality in the study. There were no test article-related effects on any parameters evaluated in the study.
- Systemic exposure (mean C_{max} and AUC values) increased with dose. There was no gender difference. Accumulation was observed over the 4-week dosing period.

Methods

Doses:	0 (Placebo; Group 1), 5 (Group 2), 25 (Group 3), and 100 (Group 4) mg/kg/dose; 25 mg/kg/week (Group 5); Placebo is an aqueous buffered solution, pH 6.0, containing 6% sucrose, 0.13% polysorbate 20, and 10 mM histidine
Frequency of dosing:	Twice weekly for Groups 1-4; once weekly for 4 weeks for Group 5
Route of administration:	Subcutaneous for Groups 2-4; Intravenous bolus for Group 5
Dose volume:	5 mL/kg
Formulation/Vehicle:	Sterile Water for Injection, U.S.P. was used for [redacted] (b) (4) placebo product; [redacted] (b) (4) REGN88 Placebo was used for Group 1 and as a diluent for REGN844 stock solution to make dose formulations for Groups 2-5.
Species/Strain:	Crl:CD1(SD) mice
Number/Sex/Group:	10 for the main study group
Age:	Approximately 6-7 weeks old at the initiation of dosing
Weight:	27.0-37.1 g for males and 20.0-29.3 g for females at the initiation of dosing
Satellite groups:	Toxicokinetic animals (12/sex for the control group; 24/sex/group for Groups 2-4; 27/sex for Group 5
Unique study design:	None
Deviation from study protocol:	No deviations were stated in the study report.

Observations and Results

Mortality

Viability check was performed twice daily.

All animals survived to the scheduled primary or recovery necropsies.

Clinical Signs

Clinical examinations were performed daily (at least twice on the dosing days; once on the nondosing days).

There were no test article-related clinical observations.

Body Weights

Body weight was measured weekly, and only body weights of main study animals were reported.

There were no test article-related effects on mean body weight or body weight gains.

Feed Consumption

Food consumption was measured twice weekly.

The study report states that meaningful comparisons of food consumption were not possible due to excessive food spillage in all groups including controls.

Hematology

Blood samples for hematology evaluation were collected from main study animals on Day 29. Food was withheld for at least 2 hours prior to blood collections. The first 5 mice/sex/group were used for hematology evaluation. The following hematology parameters were evaluated.

Hematology parameters [taken directly from the study report pp.158]:

Parameters evaluated	Units
White blood cell count	10^9/L
Red blood cell count	10^{12}/L
Hemoglobin	g/L
Hematocrit	%
Red cell distribution width	%
Mean corpuscular volume	fL
Mean corpuscular hemoglobin	pg
Mean corpuscular hemoglobin concentration	g/L
Platelets	10^9/L
Neutrophils	10^9/L
Eosinophils	10^9/L
Basophils	10^9/L
Lymphocytes	10^9/L
Monocytes	10^9/L
Large unstained cells	10^9/L
Reticulocytes	%
Reticulocytes (absolute)	10^{12}/L

There were no test article-related effects.

The mean level of eosinophils was markedly higher in males (+150% relative to the male control group) and modestly higher in females (+31% relative to the female control group) in Group 4. However, individual values were highly variable across the groups, and thus, the toxicological significance of this finding is unknown.

There were decreased mean reticulocytes, and increased mean levels of white blood cells, neutrophils, and monocytes in males in Group 4 compared to the values in the control

group. However, these changes were due to one male (Animal No. 034). Similarly, increased mean levels of white blood cells and lymphocytes were observed in females in Group 4 compared to the control group. Again, these changes were due to one female (Animal No. 081). Therefore, these findings were not considered test article-related.

Clinical Chemistry

Blood samples for clinical chemistry evaluation were collected from main study animals on Day 29. Food was withheld for at least 2 hours prior to blood collections. The last 5 mice/sex/group were used for clinical chemistry evaluation. The following serum chemistry parameters were evaluated. In addition, C-reactive protein was also evaluated.

Clinical chemistry parameters [taken directly from the study report pp.159]:

Parameters evaluated	Units
Aspartate aminotransferase	U/L
Alanine aminotransferase	U/L
Alkaline phosphatase	U/L
Gamma-glutamyl transferase	U/L
Glucose	mmol/L
Urea nitrogen	mmol/L
Creatinine	µmol/L
Total bilirubin	µmol/L
Total protein	g/L
Albumin	g/L
Globulin	g/L
Albumin/Globulin ratio	-
Total cholesterol	mmol/L
Triglycerides	mmol/L
Phosphorus	mmol/L
Calcium	mmol/L
Sodium	mmol/L
Potassium	mmol/L
Chloride	mmol/L

There were no test article-changes in clinical parameters.

Mean total bilirubin levels were higher in the males in Groups 1 and 2 and females in Groups 3 and 5, but these higher values were due to a single animal with a very high value in each of these groups. Thus, the changes in the test article-dosed groups were considered incidental.

Urinalysis

Urinalysis not performed.

Bone Marrow Smears

Smears were prepared for all main study mice that were euthanized, but not evaluated.

Gross Pathology

Necropsy was performed, and macroscopic observations were recorded for all main study animals found dead or euthanized during the study and for all animals surviving to scheduled necropsy.

The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted) [taken directly from the study report, pp.162]. Proximal to injection site tissue was only collected from animals in Group 5 (specified in Study Plan Amendment No. 1).

Tissue collection	Organ weights	Microscopic examination
Tissues with macroscopic observations (including masses)		X
Adrenal gland (2)	X	X
Brain (3 levels)	X	X
Epididymis (2)[a]	X	X
Heart	X	X
Gallbladder		X
Intestine: duodenum		X
Intestine: jejunum		X
Injection site(s) – SC and IV		X
Injection site; proximal (proximal to injection site) – SC and IV		X
Kidney (2)	X	X
Liver (3 lobes)	X	X
Lung (with bronchus)	X	X
Ovary (2)[b]	X	X
Spleen	X	X
Stomach: glandular		X
Stomach: nonglandular		X
Testis (2)[a]	X	X
Thymus	X	X
Uterus (body and horns)		X
Uterus: cervix		X
Vagina		X

[a] Fixed in Davidson's fixative

[b] Weighed with oviduct

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies as listed above. Paired organs were weighed together.

The increased mean adrenal gland weights (absolute and relative to body weight) were observed in males at ≥25 mg/kg/dose, but not in females (Table 10). There were no macroscopic or microscopic correlates to this weight change.

Table 10 Changes in organ weights from the 4-week IV mouse study

Dose Group / Organ	Male					Female				
	0	5 (SC)	25 (SC)	100 (SC)	25 (IV)	0	5 (SC)	25 (SC)	100 (SC)	25 (IV)
Adrenal Glands (g)	0.00665	0.00714 (+7%)	0.01007 (+51%)	0.01496 ** (+125%)	0.01098 * (+65%)	0.01398	0.0113 (-19%)	0.01253 (-10%)	0.01511 (+8%)	0.01481 (+6%)
AG/BW	0.01863	0.01976 (+6%)	0.02743 (+47%)	0.04007 ** (+115%)	0.03006 * (+61%)	0.04943	0.04125 (-17%)	0.04372 (-12%)	0.05568 (-13%)	0.04842 (-2%)

* P < 0.05; ** P < 0.01

Histopathology

Histopathologic examination was performed on all tissues listed above from all mice in Groups 1, 4, and 5, and subcutaneous injection sites from all mice in Groups 1 to 4 after the tissues were stained with hematoxylin and eosin. All macroscopic findings were evaluated by light microscopy.

Macroscopic observations were correlated with respective microscopic findings. To aid in the evaluation of intranuclear inclusions, formalin-fixed tissue kidney samples from Animal No. 33 were processed and embedded in plastic resin and examined by transmission electron microscopy (TEM).

Adequate Battery: A limited number of tissues was histopathologically examined, however, as this was a non-GLP exploratory study, the histology performed in this study was adequate for the purpose of this study.

Peer Review: The histopathology peer review was performed by a Sanofi-Aventis pathologist.

Histological Findings:
Histopathologic findings, including any occurrence of hyperplasia, are shown in Table 11. A REGN844-related increase in the incidence and severity of mixed inflammatory cell infiltrates was observed in subcuticular tissue at the injection site in both genders at ≥25 mg/kg/dose administered subcutaneously.

Table 11 Histological findings from the 4-week mouse study

Dose group / Tissues	Male					Female				
	G1	G2	G3	G4	G5	G1	G2	G3	G4	G5
Adrenal cortex										
Hyperplasia: subcapsular cell type A, focal	0/10	-	-	2/10	0/10	2/10	0/10	0/10	2/10	0/10
min	0	-	-	2	0	2	0	0	2	0
Kidney										
Hyperplasia: renal tubule, simple	5/10	-	-	0/10	0/10	2/10	-	-	2/10	0/10
min	5	-	-	0	0	2	-	-	2	0
Degeneration/ regeneration: renal tubule	0/10	-	-	2/10	0/10	0/10	-	-	0/10	0/10
min	0	-	-	2	0	0	-	-	0	0
Injection site										
Infiltrate: subcutis	0/10	0/10	3/10	2/10	0/10	2/10	2/10	4/10	5/10	1/10
min	0	0	3	2	0	2	2	3	1	1
mild	0	0	0	0	0	0	0	1	4	0

"-" represents "not examined"

Toxicokinetics

Serum samples (terminal samples) were collected from 3 TK animals per time point as follows: for control animals, serum was collected predose on Day 1, Day 25 (immediately prior to last injection), and on Day 29 (prior to necropsy); for Groups 2-5, serum was collected predose, then 24 and 72 hours postdose on Day 1 and last dose (Day 25 [SC; Groups 2-4] or Day 22 [IV; Group 5]) and on Day 29 prior to necropsy. Additional samples were collected for Group 5 animals immediately prior to the second dose (Day 8).

The study report does not provide information about the analysis of anti-drug antibody formation.

REGN844 concentrations in mouse serum were measured using a non-validated ELISA, which is capable of capturing REGN844. The lower limit of quantitation (LLOQ) of REGN844 was 1.56 ng/mL in the assay and 78 ng/mL in neat mouse serum.

REGN844 was not detected in any serum sample from control animals or in any predose (0h on Day1) samples from animals in REGN844-dosed groups. Systemic exposure (mean

C_{max} and AUC values) increased with dose (Table 12). There was no gender difference. Accumulation was observed over the 4-week dosing period.

Table 12 TK data from the 4-week mouse study

Parameter / Dose Group (mg/kg/dose)		Mean C_{max} (µg/mL)		Mean AUC_{0-72} (µg*h/mL)		
		Day 1	Day 25 or 22	Day 1	Day 25 or 22	AR (Day 25 or 22/ Day 1)
Male	5 (SC)	18.8	62.2	915	3472	3.8
	25 (SC)	142	777	7654	48881	6.4
	100 (SC)	601	2194	32622	153627	4.7
	25 (IV)	188	404	9834	26308	2.7
Female	5 (SC)	22.4	32.6	1013	1689	1.7
	25 (SC)	157	588	8681	38351	4.4
	100 (SC)	658	2031	36150	142036	3.9
	25 (IV)	182	353	10546	23078	2.2
Combined	5 (SC)	20.6	47.4	964	2581	2.7
	25 (SC)	150	683	8168	43616	5.3
	100 (SC)	630	2112	34385	147831	4.3
	25 (IV)	185	379	10190	24693	2.4

AR= accumulation ratio

Dosing Solution Analysis

Dosing formulations were prepared on the day of dosing and used within 6 hours of formulation completion. The study protocol states that REGN844 was stable at concentrations of 1 to 50 mg/mL for 6 hours when stored refrigerated. It appears that dosing formulations were not analyzed to confirm test article concentrations.

Study title: A 4-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 9-Week Recovery Period

Study no.: REGN88-TX-06040 ((b) (4)-460003)
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: February 1, 2007
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: REGN88; Batch Lot # K06002D600X11A; 97.3% purity

Key Study Findings

- Groups of 5 monkeys/sex/group were intravenously administered REGN88 once weekly at doses of 0 (placebo control), 5, 10, or 40 mg/kg/dose for 5 weeks. Three monkeys/sex/group were sacrificed one week after a 5-week dosing period and the remaining 2 monkeys/sex/group were sacrificed after a 9-week recovery period.
- There was no mortality in the study.
- Decreased mean neutrophil counts were observed in males and females in all test article-dosed groups in Week 1 (except MD males) and in LD males and HD males and HD females in Week 4 compared to the respective control groups. Note that mean baseline value in males in the MD group was higher than the control group. Decreased mean levels of fibrinogen were observed in males and females in all test article-dosed groups in Weeks 1 and 13, which were reversible at the end of the recovery period. Decreased mean CRP levels were observed in females in all test article-dosed groups in Weeks 1 and 4. Percent lymphocytes was increased in males and females in all test article-dosed groups in Week 1 (except MD males).
- Peak serum levels of REGN88 increased proportionally with dose. Apparently, there was some accumulation of REGN88 in all three test article-dosed groups after each infusion as trough levels did not return to baseline before the next scheduled infusion. There was no gender difference. Detectable levels of ADA were observed in 2 monkeys at 5 mg/kg (LD) and 3 monkeys at 10 mg/kg (MD).

APPEARS THIS WAY ON ORIGINAL

Methods

Doses:	0 (Placebo; Group 1), 5 (LD), 10 (MD), and 40 (HD) mg/kg/week; Placebo is an aqueous buffered solution, pH 6.0, containing 6% sucrose, 0.13% polysorbate 20 and 10 mM histidine
Frequency of dosing:	Once weekly for 5 weeks (in Weeks 0, 1, 2, 3, and 4)
Route of administration:	Intravenous infusion (~30 min)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.9% sodium chloride was used to make an appropriate concentration for each dose group
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*) of Vietnamese origin from (b) (4)
Number/Sex/Group:	3 for the main study group; 2 for the recovery group
Age:	Approximately 2-3 years old at the initiation of dosing
Weight:	1.686 kg to 2.420 kg for males and 1.950 kg to 2.363 kg for females at the initiation of dosing
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	There were no deviations that affected the integrity of the study or impacted the study design.

Observations and Results

Mortality

Viability check was performed twice daily.

All animals survived to the scheduled primary or recovery necropsies.

Clinical Signs

Clinical examinations were performed daily (three times on the dosing days; once on the nondosing days and during the recovery period) and detailed physical examinations were performed weekly.

There were no test article-related clinical observations.

Watery diarrhea was observed in one LD male and two HD males with 1 and 6 incidences, respectively. Because the incidences were relatively low and other related fecal findings were found across the groups, including the control group, the finding was not considered test article-related. All other observations in the test article-dosed groups were also noted

in the control group with similar incidences, were not noted in a dose-related manner, and/or were common findings for laboratory monkeys.

Body Weights

Body weight was measured weekly. Final body weights (fasted) were recorded prior to each scheduled necropsy.

There were no test article-related effects on mean body weight or body weight gains.

Feed Consumption

Seven to nine biscuits (PMI Nutrition International, LLC, Certified Monkey LabDiet® 5048) were offered twice daily. The diet was supplemented with other nutrients (such as fresh fruits) that were presented to the animals as part of the environmental enrichment program. The study report did not state about that food consumption was measured.

Ophthalmoscopy

Ophthalmic examinations were performed during study weeks -2, 3, 12 (females), or 13 (males). Animals were anesthetized with ketamine prior to examination.

There were no abnormal ophthalmic findings.

ECG

Electrocardiograms, blood pressures, and body temperatures were recorded during study weeks -2, 4, and 12. During the dosing period, the ECGs were measured approximately 1-2 hours following completion of the dosing administration.

There were no test article-related effects on ECG, blood pressures, or body temperatures.

Hematology

Blood samples for hematology evaluation were collected prior to the initiation of dosing (study week -2) and during Weeks 1, 4, and 13 (recovery animals only). Animals were fasted overnight prior to blood collections. The following hematology parameters were evaluated.

Hematology parameters [taken directly from the study report pp. 32]:

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (Pro Time)
Activated partial thromboplastin time (APTT)
Reticulocyte count
Percent (Reticulocyte)
Absolute (Retic Absolute)

Differential leukocyte count -
Percent and absolute
-Neutrophil
-Lymphocyte
-Monocyte
-Eosinophil
-Basophil
-Large unstained cell
Platelet estimate[a]
Red cell morphology (RBC Morphology)[a]
Fibrinogen

( ) - Designates tabular abbreviation
[a] - Presented on individual tables if a manual differential was performed, and the manual data were accepted and reported instead of the automated differential data

Decreased mean neutrophil counts (percent and absolute counts) were observed in males and females in all test article-dosed groups in Week 1 (except MD males) and in LD males and HD males and HD females in Week 4 compared to the control group. Note that mean baseline value in males in the MD group was higher than the control group. Test article-dosed females appeared to have much higher levels of neutrophils at the end of the recovery period, however, these changes were due to an abnormally low mean value in control females.

Percent lymphocytes were increased in males and females in all test article-dosed groups in Week 1 (except MD males).

Decreased mean levels of fibrinogen were observed in males and females in all test article-dosed groups in Weeks 1 and 4 compared to the control group. The levels were still slightly lower in MD and HD males than the control males at the end of the recovery period.

Table 13 Changes in the hematology parameters from the 4-week IV monkey study

Dose Group (mg/kg/dose) / Parameter		Males				Females			
		0	5	10	40	0	5	10	40
Neutrophil (%)	Wk -2	48.1	39.6 (-18%)	56.1 (+17%)	60.4 (+26%)	62.4	61.6 (-1%)	62.9 (+0.8%)	68.6 (+10%)
	Wk 1	37.5	19.6 (-48%)	36.9 (-2%)	25.7 (-32%)	47.6	29.6 (-38%)	23.1 (-52%)	28.7 (-40%)
	Wk 4	26.6	14.0 (-47%)	26.8 (+0.8%)	21.2 (-20%)	28.4	28.2 (-0.7%)	25.9 (-9%)	22.1 (-22%)

	Wk 13	19.9	11.1 (-44%)	22.7 (+14%)	21.0 (+6%)	16.3	50.8 (+212%)	50.8 (+212%)	32.1 (+97%)
Abs neutrophil (103/µl)	Wk -2	6.16	6.38 (+4%)	7.94 (+29%)	12.71 (+106%)	9.71	8.21 (-15%)	9.26 (-5%)	8.40 (-14%)
	Wk 1	4.27	2.35 (-45%)	4.15 (-3%)	2.29 (-46%)	4.45	2.36 (-47%)	1.61* (-64%)	1.93 (-57%)
	Wk 4	2.81	1.19 (-58%)	3.50 (+25%)	1.93 (-31%)	2.30	2.32 (+0.9%)	2.65 (+15%)	1.36 (-41%)
	Wk 13	2.16	1.18 (-45%)	3.15 (+46%)	2.37 (+10%)	1.36	4.19 (+208%)	6.95 (+411%)	2.68 (+97%)
Lymphocyte (%)	Wk -2	48.0	56.2 (+17%)	39.0 (-19%)	36.5 (-24%)	35.0	35.9 (+2.6%)	35.0 (0%)	28.5 (-19%)
	Wk 1	57.1	74.7 (+31%)	55.5 (-2.8%)	67.6 (+18%)	48.3	66.6 (+38%)	72.3 (+50%)	65.2 (+35%)
	Wk 4	67.9	80.7 (+19%)	66.7 (-1.8%)	73.3 (+8%)	66.0	67.3 (+2%)	69.4 (+5%)	71.1 (+8%)
	Wk 13	73.8	83.9 (+14%)	73.8 (0%)	74.6 (+1%)	78.9	45.7 (-42%)	46.1 (-42%)	61.5 (-22%)
Fibrinogen (mg/dL)	Wk -2	215.6	213.0 (-1%)	251.8 (+17%)	281.6 (+31%)	213.0	238.8 (+12%)	245.6 (+15%)	229.0 (+8%)
	Wk 1	231.8	180.0* (-22%)	155.4** (-33%)	160.0** (-31%)	215.2	139.6** (-35%)	167.4** (-22%)	160.6** (-25%)
	Wk 4	240.0	167.8 (-30%)	189.0 (-21%)	170.0 (-29%)	236.2	168.6** (-29%)	198.6 (-16%)	178.8** (-24%)
	Wk 13	223.5	238.5 (+7%)	188.5 (-16%)	192.5 (-14%)	195.0	208.5 (+7%)	191.5 (-2%)	186.0 (-5%)

* P < 0.05; ** P < 0.01

Clinical Chemistry

Blood samples for serum chemistry parameters were collected prior to the initiation of dosing (study week -2) and during study weeks 1, 4, and 13 (recovery animals only). Animals were fasted overnight prior to blood collections. The following serum chemistry parameters were evaluated. In addition, C-reactive protein (CRP) was also evaluated.

Clinical chemistry parameters [taken directly from the study report pp. 33]:

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin Ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Gamma glutamyltransferase (GlutamylTransfer)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides (Triglyceride)

( ) - Designates tabular abbreviation

Decreased mean CRP levels were observed in females in all test article-dosed groups in Weeks 1 and 4 (Table 14). For males, baseline values in the MD and HD groups were much higher than other values, and thus, the effect of RENG88 could not be clearly determined.

Table 14 Changes in the clinical chemistry parameters from the 4-week IV monkey study

Dose Group (mg/kg/dose) / Parameter		Male				Female			
		0	5	10	40	0	5	10	40
CRP (mg/mL)	Wk -2	0.9	1.6 (+78%)	9.5 (+956%)	7.0 (+678%)	1.7	2.4 (+41%)	3.0 (+77%)	3.4 (+100%)
	Wk 1	1.4	0.4 (-71%)	1.1 (-21%)	1.8 (+29%)	1.9	0.4 (-79%)	0.6 (-68%)	0.8 (-58%)
	Wk 4	0.7	0.6 (-14%)	4.8 (+586%)	1.3 (+86%)	1.6	0.8 (-50%)	1.2 (-25%)	0.9 (-44%)
	Wk 13	0.4	1.0 (+150%)	2.3 (+475%)	0.8 (+100%)	0.6	0.8 (+33%)	0.4 (-33%)	1.1 (+83%)

Urinalysis

Urine samples for urinalysis were collected prior to the initiation of dose administration (study week -2) and during study weeks 1, 4, and 13 (recovery animals only). Animals were fasted overnight while using cage pans for urine collection. The following urinalysis parameters were evaluated.

Urinalysis parameters [taken directly from the study report pp. 33]:

Specific gravity (SG)	Ketones (KET)
pH	Bilirubin (BIL)
Urobilinogen (URO)	Occult blood (BLD)
Total volume (TVOL)	Leukocytes (LEU)
Color (COL)	Nitrites (NIT)
Clarity (CLA)	Microscopy of sediment
Protein (PRO)	[Tabular abbreviations appear on individual tables]
Glucose (GLU)	

( ) - Designates tabular abbreviation

There were no test article-related changes.

Gross Pathology

The main study animals were sacrificed 1 week following the 5-week dosing period.

A complete necropsy was performed on all animals. Animals were euthanized by an intravenous injection of sodium pentobarbital followed by exsanguination. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted) [taken directly from the study report, pp.36]:

Adrenal glands (2)
Aorta
Bone with marrow
  Sternum
Bone marrow smear[a]
Brain
  Cerebrum level 1
  Cerebrum level 2
  Cerebellum with medulla/pons
Cervix
Epididymides (2)[b]
Eyes with optic nerve (2)[c]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Infusion sites
Kidneys (2)[d,e]
Larynx
Liver (sections of 2 lobes)
Lungs [including bronchi, fixed by inflation with fixative (2)]
Lymph nodes
  Mandibular
  Mesenteric
Ovaries (2)
Oviducts (2)
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary glands
  [mandibular (2)]
Seminal vesicles
Skeletal muscle (rectus femoris)
Skin with mammary gland
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2)[b]
Thymus
Thyroid [with parathyroids (2)]
Trachea
Urinary bladder
Uterus
Vagina
Gross lesions (when possible)

[a] - Bone marrow smears were obtained at necropsy but not placed in formalin; slides from all control and high-dose group animals at the primary necropsy were evaluated for complete differentials by (b) (4) (b) (4) (Appendix I).
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - A small portion obtained from the anterior portion of the left kidney cortex was cut into 1-mm^3 sections and fixed in McDowell-Trump's Fixative for possible future electron microscopic analysis.
[e] - A section of the left posterior portion of the left kidney cortex was placed in OCT medium and stored in liquid nitrogen for possible future immunohistochemistry. The following GLP deviation occurred. The OCT medium manufacturer, lot number and expiration date were not recorded for any tissues collected during the scheduled necropsies. This deviation did not impact the quality or integrity of the study.

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies as listed below [taken directly from the study report, pp.37]. Paired organs were weighed together.

Adrenals	Pituitary
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus

Decreased mean thymus weights (absolute and relative to body weight) were observed in males and females in all test article-dosed groups in a non-dose dependent manner (Table 15). The decrease was reversible at the end of the recovery period.

Increased mean adrenal gland weights (absolute and relative to body weight) were observed in males at the high dose, whereas decreased mean adrenal gland weights were observed in females in all test article-dosed groups. There were no macroscopic or microscopic correlates to these weight changes. These changes appeared to be incidental.

Lower mean testes weights were observed in males in the high dose group, compared to the control group, at the end of the dosing period. However, histological data showed that these monkeys were sexually immature. Thus, the observed difference was most likely due to individual variability in monkeys at the peripubertal stage. Lower mean weights of ovaries and uterus were observed in females in all test article-dosed groups at the end of the dosing period. As there were no correlating histopathology findings and these females were peripubertal, these changes were also likely due to individual variability in monkeys at the peripubertal stage.

Table 15 Changes in organ weights from the 4-week IV monkey study

Dose Group (mg/kg/dose) / Organ		Males				Females			
		0	5	10	40	0	5	10	40
Adrenal Glands (g)	M	0.3990	0.3934 (-1%)	0.3572 (-11%)	0.4451 (+12%)	0.5033	0.4000 (-21%)	0.3411* (-32%)	0.4025 (-20%)
Adrenal Glands/ BW	M	0.018	0.019 (+6%)	0.017 (-6%)	0.022 (+22%)	0.024	0.017* (-29%)	0.015** (-38%)	0.019 (-21%)
Adrenal Glands (g)	R	0.3416	0.4244 (+24%)	0.4158 (+22%)	0.3621 (+6%)	0.3496	0.4856 (+39%)	0.4752 (+36%)	0.3508 (0.3%)
Adrenal Glands/ BW	R	0.015	0.017 (+13%)	0.017 (+13%)	0.015 (0%)	0.015	0.022 (+47%)	0.020 (+33%)	0.015 (0%)
Thymus (g)	M	6.07	2.63* (-57%)	3.06* (-50%)	2.89* (-52%)	4.34	3.41 (-21%)	2.42 (-44%)	2.51 (-42%)
Thymus/ BW	M	0.276	0.125* (-55%)	0.146 (-47%)	0.143* (-48%)	0.204	0.149 (-27%)	0.110 (-46%)	0.117 (-43%)
Thymus (g)	R	5.91	8.10 (+37%)	5.81 (-2%)	5.49 (-7%)	2.58	2.55 (-1%)	2.70 (+5%)	4.23 (+64%)

Thymus/ BW	R	0.260	0.322 (+24%)	0.232 (-11%)	0.228 (-12%)	0.112	0.111 (-1%)	0.112 (0%)	0.182 (+63%)
Testes (g)	M	0.86	0.74 (-14%)	0.84 (-2%)	0.48 (-44%)	-	-	-	-
Testes/ BW	M	0.039	0.035 (-10%)	0.040 (+3%)	0.024 (-39%)	-	-	-	-
Testes (g)	R	0.54	2.03 (+276%)	1.00 (+85%)	0.66 (+22%)	-	-	-	-
Testes/ BW	R	0.024	0.072 (+200%)	0.040 (+67%)	0.027 (+13%)	-	-	-	-
Ovaries (g)	M	-	-	-	-	0.2464	0.1935 (-22%)	0.2083 (-16%)	0.1802 (-27%)
Ovaries/ BW	M	-	-	-	-	0.012	0.008 (-33%)	0.010 (-17%)	0.008 (-33%)
Ovaries (g)	R	-	-	-	-	0.4592	0.2576 (-44%)	0.1589 (-65%)	0.1244 (-73%)
Ovaries/ BW	R	-	-	-	-	0.020	0.012 (-40%)	0.007 (-65%)	0.006 (-70%)
Uterus (g)	M	-	-	-	-	4.31	3.54 (-18%)	3.64 (-16%)	1.61 (-63%)
Uterus/ BW	M	-	-	-	-	0.196	0.159 (-19%)	0.168 (-14%)	0.076 (-61%)
Uterus (g)	R	-	-	-	-	2.85	4.30 (+51%)	2.36 (-17%)	1.97 (-31%)
Uterus/ BW	R	-	-	-	-	0.121	0.193 (+60%)	0.098 (-19%)	0.085 (-30%)

M = after the dosing period (N= 3/gp); R = after the recovery period (N=2/gp)
* P < 0.05; ** P < 0.01

Histopathology

Histopathologic examination was performed on all tissues listed above from all animals at the scheduled primary necropsy after the tissues were stained with hematoxylin and eosin. Histopathologic examination was not performed on recovery animals.

Adequate Battery: Yes

Peer Review: Not performed

Histological Findings

There were no test article-related histological findings, including findings in the injection sites (Table 16).

Histological data from male reproductive tissues from all male monkeys in the main study portion showed that these monkeys were sexually immature.

All preneoplastic (hyperplasia) and neoplastic findings are also listed in Table 16. One female in the MD group had cervical benign papilloma, but this finding was considered incidental as the finding was observed in a single female in the MD group and the finding is relatively common in monkeys. For more detailed explanations, see the carcinogenicity risk assessment under the Carcinogenicity section.

Table 16 Histological findings in the 4-week IV toxicity study in monkeys

Dose Group (mg/kg/dose) / Tissue	Male				Female			
	0	5	10	40	0	5	10	40
Cecum								
Intimal hyperplasia-mild	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
min	1	0	0	0	0	0	0	0
Lung								
Bronchiolo-alveolar hyperplasia	0/3	0/3	0/3	0/3	1/3	0/3	0/3	0/3
min	0	0	0	0	1	0	0	0
Mand lymph nodes								
Lymphoid hyperplasia	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
min	1	0	0	0	0	0	0	0
Mes lymph nodes								
Lymphoid hyperplasia	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3
min	0	0	0	0	0	0	0	1
Mammary gland								
Epithelial hyperplasia	0/3	1/3	0/3	0/3	0/3	0/3	0/3	0/3
min	0	1	0	0	0	0	0	0
Spleen								
Lymphoid hyperplasia	1/3	1/3	0/3	1/3	0/3	3/3	1/3	0/3
min	1	0	0	0	0	3	1	0
mild	0	1	0	1	0	0	0	0
Thymus								
Lymphoid depletion	0/3	1/3	0/3	1/3	0/3	0/3	0/3	0/3
min	0	1	0	1	0	0	0	0
Cervix								
Papilloma, benign neoplasm	-	-	-	-	0/3	0/3	1/3	0/3
Epididymides								
immature	3/3	3/3	3/3	3/3	-	-	-	-
Prostate								
immature	3/3	3/3	3/3	3/3	-	-	-	-

Seminal vesicles								
immature	3/3	3/3	3/3	3/3	-	-	-	-
Intimal hyperplasia	0/3	0/3	0/3	1/3	-	-	-	-
mild	0	0	0	1	-	-	-	-
Testes								
immature	3/3	3/3	3/3	3/3	-	-	-	-

Toxicokinetics

Blood samples for total levels of REGN88 were collected from all animals on each day of dosing (at pre-dose, 5 min, 24 hr and 72 hr postdose), once on the day of primary necropsy, once weekly during recovery, and once on the day of recovery necropsy (for recovery animals). Blood samples for serum antibody analysis were collected from all animals during pretest, weekly prior to dose administration, on the day of primary necropsy, during the fourth week of recovery, and on the day of recovery necropsy.

All toxicokinetic samples were analyzed. For anti-drug antibody (ADA) analysis, samples from all animals were analyzed at Day 1, 0 hr, primary necropsy, and recovery necropsy (for recovery animals). If ADAs were detected, then all remaining time points were also analyzed for that animal.

Total REGN88 concentrations in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) was 3.13 ng/mL of REGN88 in the assay (2% monkey serum) and 0.157 µg/mL (156.5 ng/mL) in neat serum.

ADAs in monkey serum were detected using a validated ELISA. Microplates coated with REGN88 were used to capture antibodies that are directed against REGN88 in serum samples. The LLOQ was 52.7 ng/mL of ADAs in the assay and 5270 ng/mL in neat serum.

REGN88 was not detected in any serum sample from control animals. Peak serum levels from the three REGN88-dosed groups increased proportionally with dose. Mean total REGN88 concentrations at 5 minutes postdose on Day 1 were 127, 273, and 1177 µg/mL in the 5, 10, and 40 mg/kg dose groups, respectively. Trough levels also increased with dose. Apparently, there was some accumulation of REGN88 in all three dose groups after each infusion as trough levels did not return to baseline before the next scheduled infusion (see Figure 6). There was no gender difference. All REGN88-dosed recovery animals, except one male at 10 mg/kg, had circulating levels of REGN88 during the recovery period, and 8 animals (2 females at 5 mg/kg, 2 males at 10 mg/kg and 2 males and 2 females at 40 mg/kg) still had detectable REGN88 levels at the end of the recovery period.

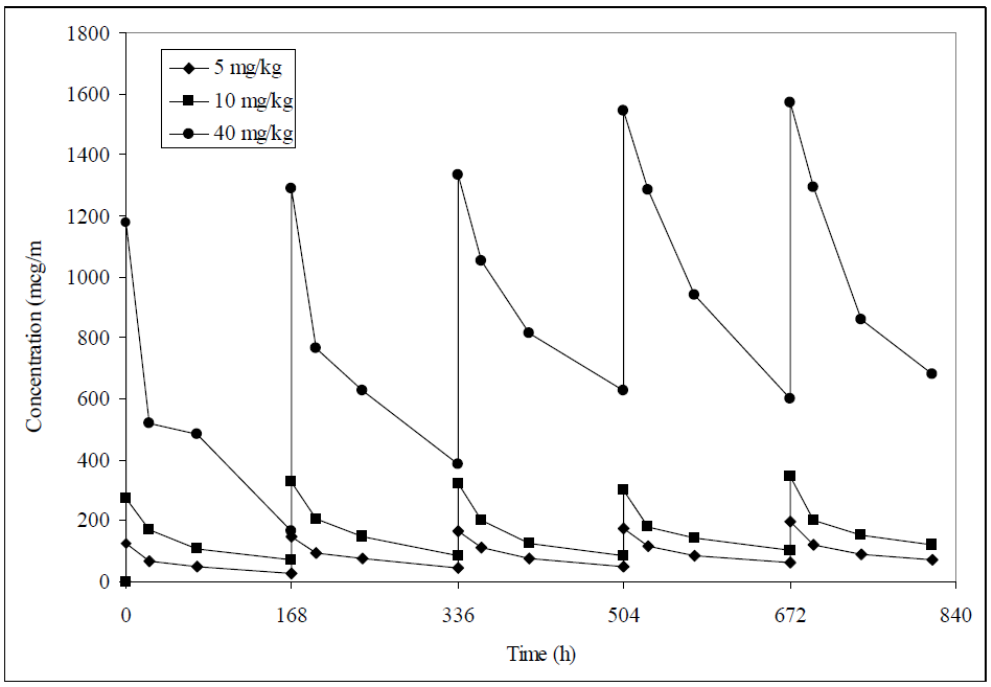


Figure 6 Concentration vs. Time Curves for the Three REGN88 Treated Cohorts during the Dosing Phase of the Study [taken directly from the study report, pp. 1098]

ADAs were not detected in any serum samples from control animals or in any predose (Day 1, 0h) sample collected from monkeys injected IV with REGN88. Detectable levels of ADA were observed in 2 monkeys at 5 mg/kg and 3 monkeys at 10 mg/kg. Although these animals had similar peak increases of REGN88 levels, substantially lower trough levels of REGN88 were observed at 24 hr postdose, suggesting increased clearance of REGN88.

Dosing Solution Analysis

Dosing formulations were prepared on the day of dosing and stored refrigerated. Samples for concentration analyses were collected from each prepared dosing formulation. In addition, 1 sample (2 mL) was collected on the day of the first female dose infusion from the formulation used for 1 animal in the 5 mg/kg group at the end of the infusion line for concentration analysis. The samples were analyzed using a validated spectrophotometric method measuring UV absorbance at wavelengths of 280 and 320 nm to confirm test article concentration in aqueous formulations ranging in test article concentration from 2.00 to 50.0 mg/mL.

The analyzed concentrations were within 90% to 110% of the nominal concentration.

Study title: A 13-Week Intravenous Infusion Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by an 8-Week Recovery Period

Study no.:	REGN88-TX-06037 ((b) (4)-460004)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 22, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	REGN88; Batch Lot # K07002J600X11A; 97.0% purity

Key Study Findings

- Groups of 6 monkeys/sex/group were intravenously administered REGN88 once weekly at doses of 0 (placebo control), 1, 10 or 50 mg/kg/dose for 13 weeks. Four monkeys/sex/group were sacrificed after a 13-week dosing period and the remaining 2 monkeys/sex/group were sacrificed after an 8-week recovery period.
- There was no test article-related mortality in the study.
- Slightly higher incidences of fecal findings were observed in REGN88-dosed male monkeys (soft feces in 6/3, 14/3, 21/3 and 27/3 males in the control, LD, MD, and HD groups, respectively, and diarrhea in 7/2 males in the MD group during the dosing period). However, control female monkeys had a similar incidence of soft feces and diarrhea. An increased incidence of swollen urogenital area was observed in female monkeys (12/2, 19/3, 95/5 and 77/5 monkeys in the control, LD, MD, and HD groups, respectively, during the dosing period). The higher incidence of swollen urogenital area was still observed in the MD and HD females during the recovery period.
- Decreased mean neutrophil counts were observed in males and females in all test article-dosed groups in Weeks 3 and 13, compared to the control group. Decreased mean levels of fibrinogen were observed in males and females in the 10 and 50 mg/kg groups in Weeks 3 and 13. Mean levels of CRP were decreased in males and females in all test article-dosed groups in Week 0 and in males and females in the 10 and 50 mg/kg groups in Week 3, and levels were lower in females in all test article-dosed groups in Week 13.
- Systemic exposure (peak serum levels and AUC_{all}) increased with dose. There was no gender difference at any dose levels. Accumulation was observed at 10 and 50 mg/kg over the dosing period, however, decreased exposure was observed at 1 mg/kg over the dosing period. A positive ADA response was observed in 11 of 12 monkeys in the 1 mg/kg group. Thus, the presence of ADAs correlated with decreases in exposure, suggesting an increased clearance rate at 1 mg/kg.

Methods

Doses:	0 (Placebo; Group 1), 1 (LD), 10 (MD), and 50 (HD) mg/kg/week; Placebo is an aqueous buffered solution, pH 6.0, containing 6% sucrose, 0.13% polysorbate 20, and 10 mM histidine
Frequency of dosing:	Once weekly
Route of administration:	Intravenous infusion (~30 min)
Dose volume:	5 mL/kg
Formulation/Vehicle:	0.9% sodium chloride (vehicle used in preparation of placebo and test article formulations)
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*) of Vietnamese origin from (b) (4)
Number/Sex/Group:	4 for the main study group; 2 for the recovery group
Age:	Approximately 2-3 years old at the initiation of dosing
Weight:	1.898 kg to 2.356 kg for males and 1.664 kg to 2.078 kg for females at the initiation of dosing
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Measurements of ECG recording were not readable due to excessive artifact or undetectable waveforms in some animals across the groups at different time points. However, ECG recording was readable in the remaining animals so this deviation did not greatly impact the outcome of the study. Other deviations also did not negatively impact the integrity of the study or the outcome of the study.

Observations and Results

Mortality

Viability check was performed twice daily.

There was no apparent test article-related mortality.

A single male in the HD group (Animal No. 2575) was found dead on Day 31, however, the death of this monkey was most likely due to a gavage error and was not attributed to test article. This animal was gavaged to provide nutritional supplementation due to occurrences of diarrhea and/or thin body from Day 21. The gavage error was evident by macro- and microscopic findings, including inflammation and subacute adventitia in the esophagus and

presence of white fluid, pleuritis, edema, fibroplasia, necrosis, and alveolar hemorrhage in the lung.

A single female in the MD recovery group (Animal No. 2621) died prior to euthanasia on Day 123. Clinical signs on Day 123 included hypoactivity, thin body, and dermal atonia. Histologic lesions included mucosal ulceration in the large intestine, associated with inflammation, bacterial overgrowth, and numerous amoebae. Small and large intestinal mucosal proliferation (epithelial hyperplasia) was noted in this animal and correlated with intestinal thickening that was observed grossly. Amoebiasis was likely the cause of death in this animal, but the relationship of this early death to the test article is unclear.

Clinical Signs

Clinical examinations were performed daily (three times on the dosing days [at the start of infusion, the end of infusion, and ~ 1 to 2 hrs postdose; once on the nondosing days] and during the recovery period) and detailed physical examinations were performed weekly.

Slightly higher incidences of fecal findings in REGN88-dosed male monkeys (soft feces in 6/3, 14/3, 21/3 and 27/3 males in the control, LD, MD and HD groups, respectively, and diarrhea in 7/2 males in the MD group during the dosing period). However, control female monkeys had a similar incidence of soft feces and diarrhea. An increased incidence of swollen urogenital area was observed in female monkeys (12/2, 19/3, 95/5 and 77/5 monkeys in the control, LD, MD and HD groups, respectively, during the dosing period). The higher incidence of swollen urogenital area was still observed in the MD and HD females during the recovery period.

Body Weights

Body weight was measured at least weekly. Final body weights (fasted) were recorded prior to each scheduled necropsy.

There were no test article-related changes in mean body weight or body weight gain.

At the end of dosing period, there was an increase in mean body weight gain in the HD group (+35% in males and +23% in females, relative to the respective control groups), however, the changes were not substantial and thus not considered test article-related.

Feed Consumption

Eight biscuits (PMI Nutrition International, LLC, Certified Monkey LabDiet® 5048) were offered twice daily. The diet was supplemented with other nutrients (such as fresh fruits) that were presented to the animals as part of the environmental enrichment program. Food consumption was qualitatively assessed daily and recorded as part of the daily observations.

Ophthalmoscopy

Ophthalmic examinations were performed during study weeks -2, 11, and 20. Animals were fasted overnight and anesthetized with ketamine prior to examination.

There were no abnormal ophthalmic findings.

ECG

Electrocardiograms, blood pressures, and body temperatures were recorded during study weeks -4 (females), -3 (males), 4, 12, and 20. During the dosing period, ECGs were measured approximately 1-2 hours at the end of infusion.

There were no test article-related effects on ECG, blood pressures and body temperatures.

A decrease in mean heart rate was observed in males in the MD and HD groups (7% to 16% decreased in Weeks 4 and 12, relative to the control group). Decreased heart rate was still observed in these groups in Week 20. Increases in mean PR (8-18%), QT (9-16%), and QTc (3-10%) intervals were observed in males in the MD and HD groups in week 4, 12, and/or 20. The changes only occurred in males, not in females, and were not observed in other studies. Thus, the findings were not considered test article-related.

Hematology

Blood samples for hematology evaluation were collected prior to the initiation of dosing (study week -2) and during study weeks 3, 13 (at end of the dosing period), and 21 (at the end of the recovery period). Animals were fasted overnight prior to blood collections. The following hematology parameters were evaluated.

Hematology parameters [taken directly from the study report pp.33]:

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (Pro Time)
Activated partial thromboplastin time (APTT)
Reticulocyte count
Percent (Reticulocyte)
Absolute (Retic Absolute)
Fibrinogen
Differential leukocyte count -
Percent and absolute
-Neutrophil
-Lymphocyte
-Monocyte
-Eosinophil
-Basophil
-Large unstained cell
Platelet estimate[a]
Red cell morphology (RBC Morphology)[a]

( ) - Designates tabular abbreviation
[a] - Presented on individual tables if a manual differential was performed, and the manual data were accepted and reported instead of the automated differential data

Decreased mean neutrophil counts were observed in males and females in all test article-dosed groups in Weeks 3 and 13 compared to the control group (Table 17). The values of neutrophil counts (percent and absolute counts) at the end of the recovery period was very variable, and thus reversibility of the changes could not be concluded.

Decreased mean levels of fibrinogen were observed in males and females in MD and HD groups in Weeks 3 and 13. Decreased mean levels of fibrinogen were still observed in HD males and MD and HD females at the end of the recovery period.

Table 17 Changes in the hematology parameters from the 13-week IV monkey study

Parameter		Male				Female			
Dose Group (mg/kg/dose)		0	1	10	50	0	1	10	50
Neutrophil (%)	Wk -2	45.8	46.5 (+2%)	48.8 (+7%)	59.5 (+30%)	63.5	45.0 (-29%)	57.4 (-10%)	61.3 (-4%)
	Wk 3	42.4	25.6 (-40%)	19.7* (-54%)	36.8 (-13%)	48.1	32.6 (-32%)	30.3 (-37%)	41.3 (-14%)
	Wk 13	36.5	28.5 (-22%)	21.7 (-41%)	30.6 (-16%)	39.0	35.0 (-10%)	33.1 (-15%)	30.0 (-23%)
	Wk 21	22.2	19.7 (-11%)	30.1 (+36%)	8.7 (-61%)	36.3	50.4 (+39%)	48.3 (-33%)	61.4 (+69%)
Abs neutrophil (10^3/µl)	Wk -2	4.43	4.67 (+5%)	6.20 (+40%)	9.89* (+123 %)	7.66	3.08 (-60%)	5.54 (-28%)	5.69 (-26%)
	Wk 3	4.24	2.09 (-51%)	1.72 (-59%)	3.19 (-25%)	3.17	1.81 (-43%)	1.75 (-45%)	2.55 (-20%)
	Wk 13	3.01	2.32 (-23%)	1.39 (-54%)	2.54 (-16%)	3.21	2.72 (-15%)	2.75 (-14%)	2.03 (-37%)
	Wk 21	2.82	1.82 (-36%)	3.16 (+12%)	1.10 (-61%)	2.28	6.01 (+164%)	6.65 (+192%)	4.64 (+104%)
Fibrinogen (mg/dL)	Wk -2	191.3	176.7 (-8%)	248.3 (+30%)	201.8 (+6%)	241.3	237.2 (-2%)	188.7 (-22%)	221.7 (-8%)
	Wk 3	221.5	211.3 (-5%)	173.5* (-22%)	160.2** (-28%)	237.0	228.8 (-4%)	145.2** (-39%)	181.8* (-23%)
	Wk 13	222.7	215.0 (-4%)	188.8 (-15%)	183.6 (-18%)	261.5	222.5 (-15%)	163.5** (-38%)	195.0** (-25%)
	Wk 21	245.0	237.5 (-3%)	252.0 (+3%)	149.0 (-39%)	267.0	283.5 (+6%)	221.0 (-17%)	227.5 (-15%)

* $P < 0.05$

Clinical Chemistry

Blood samples for serum chemistry and C-reactive protein evaluations were collected prior to the initiation of dose administration (study week -2) and during study weeks 3, 13 (at the end of dosing period), and 21 (at the end of recovery period). Animals were fasted overnight prior to blood collections. The following serum chemistry parameters were evaluated [taken directly from the study report pp.34]:

Albumin	Aspartate aminotransferase (AspartatTransfer)
Total protein	Gamma glutamyltransferase (GlutamylTransfer)
Globulin [by calculation]	Glucose
Albumin/globulin Ratio (A/G Ratio) [by calculation]	Total cholesterol (Cholesterol)
Total bilirubin (Total Bili)	Calcium
Urea nitrogen	Chloride
Creatinine	Phosphorus
Alkaline phosphatase (AlkalinePhos'tse)	Potassium
Alanine aminotransferase (Alanine Transfer)	Sodium
	Triglycerides (Triglyceride)

( ) - Designates tabular abbreviation

Mean levels of CRP were decreased in males and females in all test article-dosed groups in Week 0 and in males and females in MD and HD groups in Week 3, and mean levels were still lower in females in all test article-dosed groups in Week 13 (Table 18). As the values were variable at the end of recovery period, reversibility could not be concluded.

Mean levels of globulin were slightly lower in males and females in the HD group in Week 13 (-11 and -10%, respectively) and remained low at the end of the recovery period. This decrease resulted in slightly higher A/G ratios in this group of males and females. Because these changes are minor in magnitude, the toxicological significance is unknown.

Table 18 Changes in the clinical chemistry parameters from the 13-week IV monkey study

Parameter	Dose Group (mg/kg/dose)	Male				Female			
		0	1	10	50	0	1	10	50
Globulin (g/dL)	Wk -2	3.0	3.0 (0%)	3.2 (+7%)	2.9 (-3%)	3.2	3.1 (-3%)	3.2 (0%)	3.1 (-3%)
	Wk 3	2.6	2.9 (+12%)	2.7 (+4%)	2.6 (0%)	2.8	2.8 (0%)	2.7 (-4%)	2.6 (-7%)
	Wk 13	2.8	2.8 (0%)	2.6 (-7%)	2.5 (-11%)	2.9	2.8 (-3%)	2.7 (-7%)	2.6 (-10%)
	Wk 21	2.8	2.8 (0%)	2.9 (+4%)	2.2 (-21%)	2.7	3.3 (+22%)	3.4 (+26%)	2.5 (-7%)
A/G ratio	Wk -2	1.51	1.51	1.45 (-4%)	1.56 (+3%)	1.49	1.55 (+4%)	1.53 (+3%)	1.57 (+5%)
	Wk 3	1.60	1.48 (-8%)	1.60 (0%)	1.61 (+0.6%)	1.54	1.60 (+4%)	1.68 (+9%)	1.72 (+12%)
	Wk 13	1.62	1.54 (-5%)	1.77 (+9%)	1.77 (+9%)	1.48	1.59 (+7%)	1.70 (+15%)	1.71 (+16%)
	Wk 21	1.63	1.66 (+2%)	1.48 (-9%)	2.00 (+23%)	1.69	1.46 (-14%)	1.23 (-27%)	1.85 (+10%)
CRP (ng/mL)	Wk -2	10389.3	10152.6 (-2%)	32231.3 (+210%)	10900.4 (+5%)	14411.1	7878.1 (-45%)	10244.8 (-29%)	13666.6 (-5%)
	Wk 0	15254.8	6279.7 (-59%)	9460.8 (-38%)	4800.2* (-69%)	43618.5	5392.2 (-88%)	4684.2 (-89%)	5770.7 (-87%)
	Wk 3	9698.7	17543.4 (+81%)	3847.0 (-60%)	3213.6 (-67%)	20688.4	35948.8 (+74%)	3974.3 (-81%)	3233.7 (-84%)
	Wk 13	4629.2	3519.8 (-24%)	6566.9 (+42%)	6378.3 (+38%)	8025.6	3417.1 (-57%)	3439.2 (-57%)	3323.7 (-59%)
	Wk 21	6276.4	3114.8 (-50%)	7264.0 (+16%)	2577.2 (-59%)	4739.0	6384.7 (+35%)	2018.4 (-57%)	4192.4 (-12%)

* P < 0.05; ** P < 0.01

Urinalysis

Urine samples for urinalysis were collected prior to the initiation of dose administration (study week -2) and during study weeks 3, 13 (end of dosing period), and 21 (end of recovery period). Animals were fasted overnight while using cage pans for urine collection. The following urinalysis parameters were evaluated [taken directly from the study report pp.34]:

Specific gravity (SG)	Ketones (KET)
pH	Bilirubin (BIL)
Urobilinogen (URO)	Occult blood (BLD)
Total volume (TVOL)	Leukocytes (LEU)
Color (CLOR)	Nitrites (NIT)
Clarity (CLA)	Microscopy of sediment
Protein (PRO)	[Tabular abbreviations appear
Glucose (GLU)	on individual tables]

( ) - Designates tabular abbreviation

There were no test article-related changes.

Gross Pathology

Complete necropsies were performed on all animals. Animals were euthanized by an intravenous injection of sodium pentobarbital followed by exsanguination. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted) [taken directly from the study report pp. 37]:

Adrenal glands (2)
Aorta
Bone with marrow
  Sternum
Bone marrow smear[a]
Brain
  Cerebrum level 1
  Cerebrum level 2
  Cerebellum with medulla/pons
Cervix
Epididymides (2)[b]
Eyes with optic nerves (2)[c]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Infusion sites
Kidneys (2)[d,e]
Larynx
Liver (sections of 2 lobes)
Lungs [including bronchi, fixed by inflation with fixative (2)]
Lymph nodes
  Mandibular
  Mesenteric
Ovaries (2)
Oviducts
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary glands [mandibular (2)]
Seminal vesicles
Skeletal muscle (rectus femoris)
Skin with mammary gland
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2)[b]
Thymus
Thyroid [with parathyroids (2)]
Trachea
Urinary bladder
Uterus
Vagina
Gross lesions (when possible)

[a] - Bone marrow smears were obtained at necropsy but not placed in formalin; slides were not examined.
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - A small sample obtained from the anterior portion of the left kidney cortex was cut into 1 mm^3 sections and was fixed in McDowell-Trump's Fixative for future EM Analysis.
[e] - A small sample taken from the anterior of the left kidney cortex was placed in OCT medium (exp. date: January 2011) and placed in liquid nitrogen for possible potential future immunohistochemistry.

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies. Paired organs were weighed together.

Organ list [taken directly from the study report pp.38]

Adrenals	Pituitary
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus

There were increased spleen weights in the males in the HD group and females in all test article-dosed groups compared to the respective control groups. Increased thymus weights were also observed in males and females in all test article-dosed groups.

The increased mean adrenal gland weights were observed in females in the MD and HD groups. There were no macroscopic or microscopic correlates to these weight changes.

Higher mean testes weights were observed in males in the high dose group than in males in other groups at the end of the dosing period, however, histological data showed that all these monkeys were sexually immature. Thus, the observed difference was most likely due to individual variability in monkeys at the peripubertal stage. Lower mean weights of ovaries in females in the LD and HD groups and higher mean weights of uterus in females in all test article-dosed groups were observed at the end of the dosing period. As there were no correlated histopathology findings and these females were peripubertal, these changes were also likely due to individual variability in monkeys at the peripubertal stage. In addition, normal reproductive cycling also causes notable inter-animal variation in uterine and ovarian weights (Sellers et al., 2007. Toxicologic Pathology, 35:751-5).

A higher mean pituitary gland weight was observed in males in the HD group. The higher weight was due to one male with hyperplasia in the pituitary gland, but the hyperplasia finding was considered incidental.

Table 19 Changes in organ weights from the 13-week IV monkey study at the primary necropsy

Dose Group (mg/kg/dose) / Organ	Male				Female			
	0	1	10	50	0	1	10	50
Adrenal Glands (g)	0.4155	0.4161 (0.1%)	0.4260 (+2.5%)	0.4501 (+8.3%)	0.3146	0.3169 (+0.7%)	0.3808 (+21%)	0.4111 (+31%)
Adrenal Glands/BW	0.018	0.018 (0%)	0.018 (0%)	0.018 (0%)	0.016	0.016	0.019 (+19%)	0.020 (+25%)
Pituitary gland (g)	0.0280	0.0338 (+21%)	0.0353 (26%)	0.0456 (+63%)	0.0392	0.0436 (+11%	0.0454 (+16%)	0.0361 (-8%)
Pituitary gland/BW	0.001	0.001	0.001	0.002 (100%)	0.002	0.002	0.003 (+50%)	0.002
Spleen (g)	3.32	3.21 (-3.3%)	3.56 (+7%)	4.12 (+24%)	1.80	2.36 (+31%)	3.14 (+74%)	2.60 (+44%)
Spleen/BW	0.142	0.136 (-4%)	0.147 (+4%)	0.165 (+16%)	0.091	0.119 (+31%)	0.152 (+67%)	0.124 (+36%)
Thymus (g)	2.71	3.41 (+26%)	5.70 * (+110%)	4.45 (+64%)	2.08	3.25g (+56%)	2.81 (+35%)	2.88 (+39%)
Thymus/BW	0.116	0.144 (+24%)	0.234** (+102%)	0.179 (+54%)	0.105	0.164 (+56%)	0.135 (+29%)	0.136 (+30%)
Testes (g)	0.80	0.80	0.85 (+6%)	1.48 ** (+85%)	-	-	-	-
Testes/BW	0.034	0.034	0.035 (+3%)	0.059** (+74%)	-	-	-	-
Ovaries (g)	-	-	-	-	0.1884	0.1446 (-23%)	0.1785 (-5%)	0.1351 (-28%)
Ovaries/BW	-	-	-	-	0.010	0.008 (-20%)	0.009 (-10%)	0.007 (-30%)
Uterus (g)	-	-	-	-	1.24	1.99 (+61%)	2.31 (+86%)	2.26 (+82%)
Uterus/BW	-	-	-	-	0.062	0.101 (+63%)	0.114 (+84%)	0.106 (+71%)

* P < 0.05; ** P < 0.01

Histopathology

After fixation, sectioned tissues were stained with hematoxylin and eosin.

Collected tissues were examined microscopically from all animals at the scheduled primary necropsy and animals that were found dead.

Adequate Battery: Yes

Peer Review
The histopathology peer review was performed a Sanofi-Aventis pathologist.

Histological Findings

There were no test article-related histological findings.

Histological data from male reproductive tissues from all male monkeys in the main study portion showed that these monkeys were sexually immature.

Several findings from injection sites are listed in Table 20. Although muscle degeneration and vascular degeneration were only observed in HD males (one male with muscle degeneration and another male with vascular degeneration), overall, there were no test article-related findings in the injection sites.

All preneoplastic (hyperplasia) and neoplastic findings are also listed in Table 20. One female in the LD group had cervical epithelial dysplasia, which was considered incidental because this finding was observed in the only low dose female.

Other findings listed in Table do not appear test article-related as the incidence and/or severity of findings are low.

Table 20 Histological findings from the 13-week IV monkey study at the primary necropsy

Dose group (mg/kg/dose) / Parameter	Male				Female			
	0	1	10	50	0	1	10	50
Kidney								
Cortical cyst	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4
Crystals	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Fibrosis	0/4	0/4	1/4	1/4	0/4	1/4	1/4	1/4
Medullary mineralization	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4
Renal tubular regeneration	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Pancreas								
Islet cell hyperplasia	0/3	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Pituitary gland								
Hyperplasia	0/3	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Skin								
Edema	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
Erosion	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Subacute inflammation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Spleen								
Lymphoid hyperplasia	0/4	1/4	0/4	0/4	1/4	1/4	1/4	0/4
Cervix								
Epithelial Dysplasia	-	-	-	-	0/4	1/4	0/4	0/4
Epididymides								
Immature	4/4	4/4	4/4	4/4	-	-	-	-
Prostate								
Immature	4/4	4/4	4/4	4/4	-	-	-	-
Seminal vesicles								
Immature	4/4	4/4	4/4	4/4	-	-	-	-
Testes								

Immature	4/4	4/4	4/4	4/4	-	-	-	-
Injection site, left								
Muscle degeneration	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Fibroplasia	0/4	0/4	0/4	0/4	2/4	1/4	0/4	1/4
Perivascular lymphocyte infiltrate	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4
Chronic inflammation	0/4	1/4	0/4	2/4	0/4	0/4	0/4	1/4
Subacute inflammation	1/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Injection site, right								
Epithelial hyperplasia	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4

Toxicokinetics

Blood samples for toxicokinetic evaluation were collected from all animals on each day of dosing (at predose, approximately 5 minutes after the end of infusion, and 24 hours postdose), once on the day of primary necropsy (Week 13), once during the 4th week of recovery period (Week 17), and once on the day of recovery necropsy (Week 21). Total REGN88 levels in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) was 3.13 ng/mL of REGN88 in the assay and 156.5 ng/mL in neat serum.

Blood samples for serum anti-drug antibody (ADA) analysis were collected from all animals on Days 0, 7, 14, 21, 35, 49, 63, and 77 prior to dose administration, on the day of primary necropsy (Week 13), during the 4th week of recovery period (Week 17), and on the day of recovery necropsy (Week 21). ADA levels in monkey serum were measured using a validated ELISA. Samples from all animals were analyzed at the following time points: Day 0 (Week 1), Day 35 (Week 6), Day 77 (Week 12), Week 13 (Primary Necropsy), and Week 21 (Recovery Necropsy). If ADAs were detected (e.g., serum levels of ADA above the LLOQ), then all remaining time points were analyzed for that animal. The LLOQ of ADAs was 52.7 ng/mL in the assay and 5270 ng/mL in neat serum.

REGN88 was not detected in serum samples from control animals or in pre-dose (0h on Day 0) samples in all REGN88-dosed groups, except one occasion. One control female had a measurable REGN88 level (165 ng/mL, which was slightly above LLOQ of 156.5 ng/mL) at 24 hr (Day 50) after the IV infusion of placebo on Day 49.

Peak serum levels from three REGN88-dosed groups increased approximately proportionally with dose. Mean concentrations of REGN88 (combined males and females) from serum samples obtained 5 minutes after the first IV infusion were 28.3, 259, and 1141 µg/mL in the 1, 10, and 50 mg/kg groups, respectively.

Mean AUC_{all} values also increased with dose (Table 21). There was no gender difference at all three dose levels. Accumulation was observed in the 10 and 50 mg/kg groups over the dosing period, however, decreased exposure was observed at 1 mg/kg over the dosing period. In the 1 mg/kg group, only two monkeys (#2584 and 2607) maintained consistent AUC exposure values throughout the dosing period and values in Week 13 were greater than those in Week 1, indicating some accumulation of the test article in these monkeys. The remaining monkeys in the 1 mg/kg group showed decreased AUC exposures over the dosing period and these monkeys had detectable ADAs as described in the paragraph below.

No detectable levels of REGN88 were observed in serum samples from the 4 recovery animals in the LD group in Week 17 or Week 21, whereas REGN88 was detected in all serum samples from recovery animals in both MD and HD groups during the 8-week recovery period.

Table 21 TK data from the 13-week IV monkey study

Parameter \ Dose Group (mg/kg/dose)		Males			Females			Combined		
		G2 1	G3 10	G4 50	G2 1	G3 10	G4 50	G2 1	G3 10	G4 50
AUC_{all} (h*µg/mL)	Wk 1	1347	20833	112736	1622	23521	114978	1484	22177	113857
	Wk 13	393	56448	263856	384	66652	252770	389	61550	257809
	AR (wk13/ wk 1)	0.3	2.7	2.3	0.2	2.8	2.2	0.3	2.8	2.3

AR= accumulation ratio

ADAs were not detected in any serum samples from control animals or in any pre-dose (0h on Day 0) samples collected from monkeys in REGN88-dosed groups.

ADAs were not detected in monkeys in the 10 and 50 mg/kg groups, however, detectable levels of ADAs were observed in 11 of 12 monkeys in the 1 mg/kg group. At 1 mg/kg, ADAs were detected in five monkeys from Day 14 and in five other monkeys from Day 21. One monkey (#2584) did not have detectable ADAs until Day 49, and Animal No. 2607 did not have any detectable ADAs. As described above, Animal Nos. 2584 and 2607 maintained systemic exposure of REGN88 throughout the dosing period, whereas other 10 monkeys showed decreased exposure. Thus, the presence of ADAs correlated with decreases in exposure, suggesting an increased clearance rate at 1 mg/kg. ADAs were also present in recovery animals in the 1 mg/kg group.

Dosing Solution Analysis

Dosing formulations were prepared on the day of dosing. Samples were collected from each male dosing formulation during study weeks 0, 1, 3, 7, and 12 and analyzed prior to use by UV for concentration confirmation.

The analyzed concentrations were within 90% to 110% of the nominal concentration, and no test article was detected in the vehicle formulations.

6.2 Repeat-Dose Toxicity

Study title: A 13-Week Subcutaneous Injection Study of REGN88 (Anti-Interleukin-6 Receptor Monoclonal Antibody) in Cynomolgus Monkeys Followed by a 12-Week Recovery Period

Study no.:	REGN88-TX-06038 ((b) (4)-460005)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	February 22, 2007
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	REGN88; Batch Lot # K07002J600X11A; 97.0% purity

Key Study Findings

- Groups of 6 monkeys/sex/group were administered REGN88 twice weekly by subcutaneously injection at doses of 0 (placebo control), 1, 5, 15, or 50 mg/kg/dose for 13 weeks. Four monkeys/sex/group were sacrificed 1 week after a 13-week dosing period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period.
- There was no mortality in the study.
- Decreased mean neutrophil counts were observed in males and females in all test article-dosed groups in Weeks 3 and 13 (except females in 15 mg/kg/dose group in Week 3), compared to the control group. Decreased mean levels of fibrinogen were observed in males and females in ≥5 mg/kg/dose groups in Weeks 3 and 13. These effects appeared to be reversible as the values were similar or closer to those in the control group at the end of the recovery period. Mean levels of CRP were decreased in males and females in ≥5 mg/kg/dose groups in Weeks 0 and 3, and levels were still lower than in the control group at the end of the recovery period, except the mean values in males in the 50 mg/kg/dose group and females in the 15 mg/kg/dose group.
- Mean concentrations of REGN88, but not AUC values, were reported in the study report. Mean peak serum concentrations of REGN88 increased approximately proportionally with dose following the first week of administration, although levels in the 15 mg/kg/dose group were greater than expected. While peak levels increased at ≥5 mg/kg/dose over the dosing period, peak levels were decreased at 1 mg/kg/dose.

Since all animals in this dose group had ADAs, the presence of ADAs might increase clearance of the drug.

Methods

Doses:	0 (Placebo; Group 1), 1 (Group 2), 5 (Group 3), 15 (Group 4), and 50 (Group 5) mg/kg/dose; Placebo is an aqueous buffered solution, pH 6.0, containing 6% sucrose, 0.13% polysorbate 20 and 10 mM histidine
Frequency of dosing:	Twice weekly
Route of administration:	Subcutaneous injection
Dose volume:	0.74, 0.77, 0.07, 0.22 and 0.74 mL/kg in Groups 1, 2, 3, 4 and 5, respectively
Formulation/Vehicle:	0.9% sodium chloride (vehicle used in preparation of test article formulation for injection for Group 2); For the control group and Groups 3-5, formulations as supplied by Regeneron Pharmaceuticals, Inc. were administered without dilution.
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis*) from (b) (4)
Number/Sex/Group:	4 for the main study; 2 for the recovery group
Age:	Approximately 2-3 years old at the initiation of dosing
Weight:	1.592 kg to 2.084 kg for males and 1.571 kg to 1.931 kg for females at the initiation of dosing
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Measurements of ECG recording were not readable due to excessive artifact or undetectable waveforms in some animals across the groups at different time points. However, ECG recording was readable in the remaining animals so this deviation did not greatly impact the outcome of the study. Other deviations also did not negatively impact the integrity of the study or the outcome of the study.

Observations and Results

Mortality

Viability check was performed twice daily.

All animals survived to the scheduled primary or recovery necropsies.

Clinical Signs

Clinical examinations were performed daily (twice on the dosing days; once on the nondosing days, and during the recovery period) and detailed physical examinations were performed weekly.

There were no test article-related clinical observations.

All observations in the test article-dosed groups were also noted in the control group with similar incidences, were not noted in a dose-related manner, and/or were common findings for laboratory monkeys.

Body Weights

Body weight was measured at least weekly.

There were no test article-related changes in mean body weight or body weight gain.

At the end of dosing period, there was a slight decrease in mean body weight gain in the 50 mg/kg/dose group. As the magnitude of the change was not substantial, the change was considered incidental.

Feed Consumption

Eight biscuits (PMI Nutrition International, LLC, Certified Monkey LabDiet® 5048) were offered twice daily. The diet was supplemented with other nutrients (such as fresh fruits or vitamin tablets) that were presented to the animals as part of the environmental enrichment program. Food consumption was not recorded.

Ophthalmoscopy

Ophthalmic examinations were performed during study weeks -2, 11 (females), 12 (males), and 20 (recovery animals). Animals were fasted overnight and anesthetized with ketamine prior to examination.

There were no abnormal ophthalmic findings.

ECG

Electrocardiograms, blood pressures, and body temperatures were recorded during study weeks -4 (females), -3 (males), 0, 3, 12, 20 (recovery females), and 21 (recovery males). During the dosing period, ECGs were measured approximately 1-2 hours following completion of the second weekly dosing administration.

There were no test article-related effects on ECG, blood pressures, or body temperatures.

Hematology

Blood samples for hematology evaluation were collected prior to the initiation of dose administration (study week -2) and during study weeks 3, 13, 21, and 25. Animals were fasted overnight prior to blood collections.

Hematology parameters [taken directly from the study report, pp.36]

Total leukocyte count (White Cells)	Reticulocyte count
Erythrocyte count (Red Cells)	Percent (Reticulocyte)
Hemoglobin	Absolute (Retic Absolute)
Hematocrit	Differential leukocyte count -
Mean corpuscular volume (MCV)	Percent and absolute
Mean corpuscular hemoglobin	-Neutrophil
(MCH)	-Lymphocyte
Mean corpuscular hemoglobin	-Monocyte
concentration (MCHC)	-Eosinophil
Platelet count (Platelet)	-Basophil
Prothrombin time (Pro Time)	-Large unstained cell
Activated partial thromboplastin time	Platelet estimate[a]
(APTT)	Red cell morphology
Reticulocyte count	(RBC Morphology)[a]
Percent (Reticulocyte)	Fibrinogen
Absolute (Retic Absolute)	

( ) - Designates tabular abbreviation
[a] - Presented on individual tables if a manual differential was performed, and the manual data were accepted and reported instead of the automated differential data

Decreased mean neutrophil counts were observed in males and females in all test article-dosed groups in Weeks 3 and 13 (except females in 15 mg/kg/dose in Week 3), compared to the control group (Table 22). Decreased mean levels of fibrinogen were observed in males and females in the ≥5 mg/kg/dose groups in Weeks 3 and 13. These effects appeared to be reversible as the values were similar or closer to the control group at the end of the recovery period.

Table 22 Changes in hematology parameters from the 13-week SC monkey study

Parameter \ Dose Group (mg/kg/dose)		Males					Females				
		0	1	5	15	50	0	1	5	15	50
Neutrophil (%)	Wk -2	46.6	45.5 (-3%)	38.2 (-18%)	46.2 (-0.9%)	48.1 (+3%)	52.7	46.1 (-13%)	46.0 (-13%)	60.9 (+16%)	52.3 (-0.8%)
	Wk 3	53.8	41.4 (-23%)	23.3 (-57%)	32.2 (-40%)	32.8 (-39%)	52.9	28.7 (-46%)	37.9 (-28%)	43.6 (-18%)	35.3 (-33%)
	Wk 13	46.6	45.6 (-2%)	20.2* (-57%)	26.2 (-44%)	26.3 (-44%)	30.5	26.3 (-14%)	30.1 (-1%)	29.2 (-4%)	32.6 (+7%)
	Wk 25	41.3	53.8 (+30%)	18.4 (-55%)	52.0 (+26%)	51.0 (+24%)	12.3	28.7 (+133%)	13.5 (+10%)	25.7 (+109%)	34.0 (+176%)
Abs neutrophil (10^3/µl)	Wk -2	3.87	4.06 (+5%)	2.51 (-35%)	3.84 (-0.8%)	3.79 (-2%)	4.42	3.82 (-14%)	3.70 (-16%)	5.90 (+34%)	5.83 (+32%)
	Wk 3	4.36	3.13 (-28%)	1.16 (-73%)	1.71 (-61%)	2.13 (-51%)	2.88	1.83 (-37%)	1.67 (-42%)	3.49 (+21%)	2.24 (-22%)
	Wk 13	5.46	4.02 (-26%)	1.14* (-79%)	1.63* (-70%)	1.34* (-76%)	3.15	2.36 (-25%)	2.60 (-18%)	2.47 (-22%)	3.10 (-2%)
	Wk 25	2.57	5.66 (+120%)	1.89 (-27%)	4.14 (+61%)	5.41 (+111%)	0.80	3.75 (+369%)	0.96 (+20.0%)	1.73 (+116.3%)	3.12 (+290%)
Fibrinogen (mg/dL)	Wk -2	215	215	208 (-3%)	215	235 (+9%)	209.2	207.3 (-0.9%)	214.5 (+3%)	195.7 (-7%)	173.0 (-17%)
	Wk 3	225	196 (-13%)	170** (-24%)	171** (-24%)	195 (-13%)	225.4	214.2 (-5%)	181.2* (-15%)	164.8** (-22%)	155.8** (-27%)
	Wk 13	225	223 (-0.9%)	180 (-20%)	197 (-12%)	199 (-12%)	212.3	201.2 (-5%)	181.2 (-15%)	164.8** (-22%)	155.8** (-27%)
	Wk 25	245	221 (-10%)	244 (-0.4%)	217 (-11%)	230 (-6%)	208.5	225.5 (+8%)	193.5 (-7%)	209.5 (+0.5%)	183.0 (-12%)

* $P < 0.05$; ** $P < 0.01$

Clinical Chemistry

Blood samples for serum chemistry and C-reactive protein (CRP) analysis were collected prior to the initiation of dose administration (study week -2) and during study weeks 0 (CRP analysis only), 3, 13, 21, and 25. Animals were fasted overnight prior to blood collection.

Serum chemistry parameters [taken directly from the study report, pp.37]

Albumin	Aspartate aminotransferase (AspartatTransfer)
Total protein	
Globulin [by calculation]	Gamma glutamyltransferase (GlutamylTransfer)
Albumin/globulin Ratio (A/G Ratio) [by calculation]	Glucose
Total bilirubin (Total Bili)	Cholesterol
Urea nitrogen	Calcium
Creatinine	Chloride
Alkaline phosphatase (AlkalinePhos'tse)	Phosphorus
	Potassium
Alanine aminotransferase (Alanine Transfer)	Sodium
	Triglycerides (Triglyceride)

( ) - Designates tabular abbreviation

Mean levels of CRP were decreased in males and females in the ≥5 mg/kg/dose groups in Weeks 0 and 3, and levels were still lower than in the control group at the end of the recovery period, except the mean values in males in the 50 mg/kg/dose group and females in the 15 mg/kg/dose group (Table 23).

Table 23 Changes in the clinical chemistry parameters from the 13-week SC monkey study

Dose Group (mg/kg/dose) / Parameter		Males					Females				
		0	1	5	15	50	0	1	5	15	50
CRP (ng/mL)	Wk -2	6395	16498 (+158%)	10197 (+59%)	7306 (+14%)	10042 (+57%)	10350	4978 (-52%)	10750 (+4%)	12475 (+21%)	10755 (+4%)
	Wk 0	9269	8307 (-10%)	4403 (-53%)	3220 (-65%)	5325 (-43%)	10362	2378 (-77%)	2071 (-80%)	3950 (-62%)	2816 (-73%)
	Wk 3	10938	14708 (+35%)	7674 (-30%)	6404 (-42%)	5262 (-52%)	8804	5950 (-32%)	5051 (-43%)	7494 (-15%)	5794 (-34%)
	Wk 13	6146	12994 (+111%)	5642 (-8%)	3932 (-36%)	6465 (+5%)	4992	4925 (-1%)	3977 (-20%)	4777 (-4%)	4780 (-4%)
	WK 25	9081	10037 (+11%)	3046 (-67%)	3131 (-66%)	13221 (+46%)	5112	2575 (-50%)	2479 (-52%)	8095 (+58%)	2366 (-54%)

Urinalysis

Urine samples for urinalysis were collected prior to the initiation of dose administration (study week -2), 3, 13, 21, and 25. Animals were fasted overnight prior to urine collection.

Urinalysis parameters [taken directly from the study report, pp. 37]

Specific gravity (SG)	Ketones (KET)
pH	Bilirubin (BIL)
Urobilinogen (URO)	Occult blood (BLD)
Total volume (TVOL)	Leukocytes (LEU)
Color (COL)	Nitrites (NIT)
Clarity (CLA)	Microscopy of sediment
Protein (PRO)	[Tabular abbreviations appear
Glucose (GLU)	on individual tables]

( ) - Designates tabular abbreviation

There were no test article-related changes.

Gross Pathology

Complete necropsies were performed on all animals. Animals were euthanized by an intravenous injection of sodium pentobarbital followed by exsanguination. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

Adrenal glands (2)
Aorta
Bone with marrow
  Sternum
Bone marrow smear[a]
Brain
  Cerebrum level 1
  Cerebrum level 2
  Cerebellum with medulla/pons
Cervix
Epididymides (2)[b]
Eyes with optic nerve (2)[c]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
Heart
Injection site
Kidneys (2)[d,e]
Larynx
Liver (sections of 2 lobes)
Lungs [including bronchi, fixed by
  inflation with fixative (2)]
Lymph nodes
  Mandibular
  Mesenteric
Ovaries (2)
Oviducts (2)
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary glands
  [mandibular (2)]
Seminal vesicles
Skeletal muscle (rectus femoris)
Skin with mammary gland
Spinal cord (cervical, thoracic, lumbar)
Spleen
Testes (2)[b]
Thymus
Thyroid [with parathyroids (2)]
Trachea
Urinary bladder
Uterus
Vagina
Gross lesions (when possible)

[a] - Bone marrow smears were obtained at necropsy but not placed in formalin; slides were examined only if scientifically warranted.
[b] - Fixed in Bouin's solution
[c] - Fixed in Davidson's solution
[d] - A small sample obtained from the anterior portion of the left kidney cortex was cut into 1-mm^3 sections and fixed in McDowell-Trump's Fixative for possible future electron microscopic analysis.
[e] - A small sample taken from the anterior portion of the left kidney cortex was placed in OCT medium and stored in liquid nitrogen at approximately -70°C for possible future immunohistochemistry.

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies. Paired organs were weighed together.

Adrenal glands	Pituitary
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus

Slightly decreased mean weights of the adrenal gland and thymus were observed in males in the 50 mg/kg/dose group, compared to the control group (Table 24). Increased thymus weights were observed in females in all test article-dosed groups relative to the control group. As there were not consistent changes between males and females, the toxicological significance of these changes is not clear.

Table 24 Changes in organ weights from the 13-week SC monkey study at the primary necropsy

Dose Group (mg/kg/dose) / Organ	Males					Females				
	0	1	5	15	50	0	1	5	15	50
Adrenal Glands (g)	0.4033	0.3661 (-9%)	0.3817 (-5%)	0.3817 (-5%)	0.3835 (-21%)	0.3400	0.3574 (+5%)	0.4137 (+22%)	0.3330 (-2%)	0.3449 (+1%)
Adrenal Glands/BW	0.020	0.018 (-10%)	0.019 (-5%)	0.018 (-10%)	0.016 (-20%)	0.018	0.017 (-6%)	0.021 (+17%)	0.017 (-6%)	0.018 (0%)
Thymus (g)	4.84	4.77 (-1%)	6.09 (+26%)	5.58 (+15%)	3.73 (-23%)	2.66	5.10 (+92%)	5.94 (+123%)	4.80 (+81%)	4.33 (+63%)
Thymus/BW	0.237	0.223 (-6%)	0.305 (+29%)	0.255 (+8%)	0.192 (-19%)	0.136	0.241 (+77%)	0.292 (+115%)	0.245 (+80%)	0.219 (+61%)

Histopathology

Collected tissues were examined microscopically from all animals at the scheduled primary necropsy. Target tissues for microscopic evaluation from all animals euthanized at the recovery necropsy included the brain, heart, and injection sites.

Giemsa staining was performed on sections of heart from a female in the 5 mg/kg/dose group (# 2705) to further evaluate myocardial changes in this monkey. Gram stain was performed on a section of brain from a male in the 1 mg/kg/dose group (# 2700) to further evaluate brain changes in this monkey.

Adequate Battery: **Yes**

Peer Review: The histopathology peer review was performed by (b) (4)

Histological Findings
Non dose-dependent minimal to moderate perivascular mixed inflammatory cell infiltrates were observed in dermis and/or subcutis in test article-dosed monkeys at the end of the dosing period (Table 25). Perivascular infiltrates were composed predominately of lymphocytes and plasma cells admixed with occasional granulocyte. Increased severity of fibrosis was also observed in test article-dosed monkeys. Muscle regeneration was only observed in test article-dosed monkeys. These findings tended to be reversible in recovery monkeys (Table 26).

Histological data from testes from all male monkeys in the main study portion showed that these monkeys were sexually immature.

All preneoplastic (hyperplasia) findings are also listed in Table 25. Occasional findings of hyperplasia in different tissues appeared incidental.

Table 25 Histopathologic findings in the 13-week SC toxicity study in monkeys at the end of the dosing period

Dose Group (mg/kg/dose) / Tissue	Males					Females				
	0	1	5	15	50	0	1	5	15	50
Larynx										
Lymphoid Hyperplasia, peribronchial	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
min	0	1	0	0	0	0	0	0	0	0
Lung										
Lymphoid Hyperplasia, peribronchial	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
min	0	1	0	0	0	0	0	0	0	0
Pancreas										
Hyperplasia, islet cell	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
min	0	0	1	0	0	0	0	0	0	0
Testes										
immature	4/4	4/4	4/4	4/4	4/4	-	-	-	-	-
Injection site										

Fibrosis	2/4	3/4	2/4	2/4	4/4	2/4	0/4	0/4	2/4	1/4
min	1	2	2	2	0	2	0	0	1	0
mild	1	1	0	0	4	0	0	0	1	0
moderate	0	0	0	0	0	0	0	0	0	1
Infiltrate, Mixed inflammatory cell, perivascular	0/4	**2/4**	**2/4**	**2/4**	**3/4**	1/4	**3/4**	**3/4**	**3/4**	**3/4**
min	0	2	1	2	3	1	2	0	3	3
mild	0	0	1	0	0	0	1	2	0	0
moderate	0	0	0	0	0	0	0	1	0	0
Muscle regeneration	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4	2/4	0/4
min	0	0	0	0	1	0	1	0	2	0

Table 26 Histopathologic findings in the 13-week SC toxicity study in monkeys at the end of the recovery period

Dose Group (mg/kg/dose) / Tissue	Male					Female				
	0	1	5	15	50	0	1	5	15	50
Injection site										
Infiltrate, Mixed inflammatory cell, perivascular	0/2	0/2	0/2	0/2	0/2	0/2	2/2	0/2	0/2	0/2
min	0	0	0	0	0	0	2	0	0	0
Muscle degeneration	0/2	1/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
min	0	1	0	0	0	0	0	0	0	0

Toxicokinetics

Blood samples for toxicokinetic evaluation were collected from all animals during study weeks 0, 1, 2, 3, 5, 7, 9, and 11 (at pre-dose and 24 hours after the second weekly dosing administration), on the day of primary necropsy (Week 13), and once each during the fourth (Week 17), eighth (Week 21), and eleventh (Week 24) weeks of recovery. Although samples were scheduled to be collected on the day of the recovery necropsy (Week 25), samples were not collected on that day (noted by a deviation). No toxicokinetic samples

were collected prior to the initial dosing of the animals. Total REGN88 levels in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) of REGN88 was 3.13 ng/mL in the assay and 156.5 ng/mL in neat serum.

Blood samples (approximately 1 mL each) for serum antibody analyses were collected from all animals during pretest, prior to the second weekly dose administration during study weeks 0, 1, 2, 3, 5, 7, 9, and 11, on the day of primary necropsy, once each during the fourth and eighth weeks of recovery, and once during the week prior to the recovery necropsy.

Blood samples for serum anti-drug antibody (ADA) analysis were collected predose from all animals, prior to the second weekly dose administration during weeks 0, 1, 2, 3, 5, 7, 9, and 11, on the day of primary necropsy (Week 13), and once each during the fourth (Week 17), eighth (Week 21), and eleventh (Week 24) weeks of recovery. Although samples were scheduled to be collected on the day of the recovery necropsy (Week 25), samples were not collected on that day (noted by a deviation). Samples from all animals were analyzed at the following time points: Weeks 0, 2, 5, 9, 11, 13, 17, 21, and 24. If ADAs were detected (e.g., serum levels of ADAs above the LLOQ), then all remaining time points were analyzed for that animal. The levels of ADAs in monkey serum were measured using a validated ELISA. The LLOQ of ADAs was 52.7 ng/mL in the assay and 5270 ng/mL in neat serum.

REGN88 was not detected in serum samples from control animals, except two occasions. One male and one female in the control group had measurable REGN88 levels [5.66 µg/mL at 24 hr after the first week of injection (Week 0) and 1.49 µg/mL prior to dosing (0h) in Week 11, respectively].

Mean concentrations of REGN88, but not AUC values, were reported in the study report. Mean peak serum concentrations of total REGN88 increased approximately proportionally with dose following the first week of administration, although levels in the 15 mg/kg dose group were greater than expected. Mean serum concentrations (combined males and females) collected at 24 hours after the second dose of the first week were 9.15, 56.8, 262, and 716 ng/mL in the 1, 5, 15, and 50 mg/kg/dose groups, respectively (Table 27). While peak levels increased at 5, 15, and 50 mg/kg/dose over the dosing period, peak levels were decreased at 1 mg/kg/dose. Since all animals in this dose group had ADAs, the presence of ADAs might increase clearance of drug thereby minimizing drug accumulation during the dosing period.

REGN88 was detected in the majority of serum samples from the 5, 15, and 50 mg/kg/dose groups during the recovery period, but REGN88 was not detected in samples collected from the 1 mg/kg/dose group during the 12-week recovery period.

ADAs were not detected in control, 15, and 50 mg/kg groups, but ADAs were detected in all animals in the 1 mg/kg/dose group and 4 (1 male and 3 females) out of 12 in the 5 mg/kg/dose group. As mentioned above, mean peak serum concentrations decreased over the dosing period in the 1 mg/kg/dose group. Four monkeys that had ADAs in the

5 mg/kg/dose group also had lower REGN88 levels of relative to animals that did not have ADAs in this group. These results suggest that the presence of ADAs may have increased the clearance rate of REGN88.

Table 27 TK data from the 13-week SC toxicity study in monkeys

Dose Group (mg/kg/dose)		Mean Serum Concentration (µg/mL)		
		Week 0, 24h	Week 11, 24h	AR (Wk11/ Wk0)
Male	1	9.02	3.91	0.43
	5	61.8	223	3.6
	15	314	1052	3.4
	50	672	2623	3.9
Female	1	9.27	1.43	0.15
	5	51.9	104	2.0
	15	209	796	3.8
	50	761	2843	3.7
Combined	1	9.15	2.67	0.29
	5	56.8	164	2.9
	15	262	924	3.5
	50	716	2733	3.8

Dosing Solution Analysis

For the Group 2 (1 mg/kg/dose) dosing formulation, the REGN88 formulation supplied by the sponsor was diluted with 0.9% sodium chloride. The control (Group 1) and Groups 3-5 formulations were administered as supplied by the sponsor without dilution. Dosing formulations were prepared on the day of dosing. The samples collected from the first male weekly dosing formulations of Group 2 for study weeks 0, 1, 2, 3, 7, and 12 were analyzed using a validated spectrophotometric method measuring UV absorbance at wavelengths of 280 and 320 nm to confirm test article concentration.

The analyzed concentrations were within 90% to 110% of the nominal concentration.

Study title: A 13-Week Bridging Subcutaneous Study of REGN88 in Cynomolgus Monkeys

Study no.:	REGN88-TX-09053 ((b) (4)-460024)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 4, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	Test Article 1: REGN88 (P3); Batch Lot # K09012D660I11A; 97% purity (at timepoint 0; and 96% purity at 5 months). Test Article 2: REGN88 (P1); Batch Lot # K08003D600A11A; 99% purity (at timepoint 0; and 97% purity at 5 months).

Key Study Findings

- Groups of 4 monkeys/sex/group were administered REGN88 twice weekly by subcutaneously injection at doses of 0 (P3 placebo control), 5 (P3), 50 (P3) or 50 (P1) mg/kg/dose for 13 weeks to evaluate and compare potential adverse effects of REGN88 manufactured by and formulated with the original process (P1) compared to the newer process (P3).
- There was no mortality in the study.
- Mean neutrophil counts were decreased in males and females of all test article-dosed groups in Week 4 when compared to baseline values and/or the control group. Decreased mean levels of fibrinogen were observed in males in 50 mg/kg/dose P3 and P1 groups and in females 5 and 50 mg/kg/dose P3 and 50 mg/kg/dose P1 group in Weeks 4 and 12.
- Mean systemic exposure of REGN88 (serum concentrations and $AUC_{0\text{-}24h}$ estimates) in the 5 and 50 mg/kg/dose P3 groups increased in an approximate dose-proportional manner. $AUC_{0\text{-}24h}$ estimates in the 50 mg/kg/dose P3 group was approximately 16% lower than the 50 mg/kg/dose P1 group over the course of the study. There was no sex difference. There was accumulation of the test article over the dosing period. ADAs were not detected in either the 50 mg/kg/dose P1 or P3 groups, and 2/8 animals in the 5 mg/kg/dose P3 group exhibited a positive ADA response during the course of the study.
- Overall, toxicity profiles between P3 and P1 dose groups were similar, but systemic exposure of REGN88 from the P3 process was slightly lower than REGN88 from the P1 process.

Methods

Doses:	0 (P3 placebo; Group 1), 5 (Group 2; P3 process), 50 (Group 3; P3 process) and 50 (Group 4; P1 process) mg/kg/dose; P3 placebo is an aqueous buffered solution, pH 6.0, containing 25 mM histidine, 0.2% polysorbate 20, 50 mM arginine, and 5% sucrose.
Frequency of dosing:	Twice weekly (approximately 3 to 4 days apart)
Route of administration:	Subcutaneous injection
Dose volume:	0.76, 0.08, 0.76, and 0.76 mL/kg in Groups 1, 2, 3, and 4, respectively
Formulation/Vehicle:	0.9% sodium chloride (vehicle used in preparation of placebo and test article formulation for injection for Group 2)
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis;* Vietnamese origin) from (b) (4)
Number/Sex/Group:	4 for the main study
Age:	Approximately 2.8 years old at the initiation of dosing
Weight:	2.088 kg to 2.689 kg for males and 2.008 kg to 2.919 kg for females at the initiation of dosing
Satellite groups:	None
Unique study design:	The objective of this study was to evaluate and compare potential adverse effects of REGN88 manufactured by and formulated with the original process (P1) compared to the newer process (P3). (b) (4) Monkeys were pair-housed. The first day of dosing was Day 0; the first week of dosing was Week 0.
Deviation from study protocol:	There were no deviations that affected the integrity of the study.

Observations and Results

Mortality

Viability check was performed twice daily.

All animals survived to the scheduled necropsy.

Clinical Signs

Clinical examinations were performed daily (twice on the dosing days; once on the nondosing days and during the recovery period) and detailed physical examinations were performed weekly.

There were no test article-related clinical observations.

All observations in the test article-dosed groups were also noted in the control group with similar incidences, and/or were common findings for laboratory monkeys.

Body Weights

Body weight was measured at least weekly.

There were no test article-related effects on mean body weight or body weight gain.

Apparent increases in mean body weight gain in all test article-dosed female groups (Groups 2-4) during the dosing period were due to low body weight gain in the control female group and thus, it was not considered test article-related.

Feed Consumption

Approximately 3 PMI Nutrition International, LLC, Certified Hi-Fiber Diet 5K91 LabDiet® biscuits were offered twice daily. The diet was supplemented with other nutrients (such as fresh fruits) which were presented to the animals as part of the environmental enrichment program. Food consumption was assessed qualitatively daily and recorded as part of the daily observations.

Ophthalmoscopy

Ophthalmic examinations were performed on all animals during study weeks -3 and 12. Animals were anesthetized with ketamine prior to examination.

The study report states that during the study, the ketamine manufacturer (Teva Animal Health) issued a recall for several lot numbers of ketamine due to adverse reactions (including respiratory depression, emesis, salivation, vocalization, erratic and/or prolonged recoveries, spastic jerking movements, convulsions, muscular tremors, hypertonicity, opisthotonos, dyspnea, and cardiac arrest). One of the recalled lots (no. 540197P) was utilized for ophthalmic examinations and/or electrocardiographic evaluations on this study. However, there were no adverse reactions to ketamine anesthesia noted during this study.

There were no abnormal ophthalmic findings.

ECG

Electrocardiograms, blood pressures, and body temperatures were recorded during study weeks -4 (females), -3 (males), and 12. During the dosing period, The ECGs were

measured approximately 1-2 hours postdose after the final dose administration in Week 12. Animals were fasted and anesthetized with ketamine prior to examination.

There were no test article-related effects on heart rate, ECG, blood pressures, or body temperatures.

Hematology

Blood samples for hematology and coagulation evaluation were collected from all monkeys prior to the initiation of dose administration (study week -3), once during Week 4, and within 1 week of the scheduled necropsy (Week 12). The animals were fasted overnight prior to blood collection. Below hematology parameters were evaluated.

Hematology parameters [taken directly from the study report pp. 35]

- Total leukocyte count (WBC)
- Erythrocyte count (RBC)
- Hemoglobin (HGB)
- Hematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular hemoglobin (MCH)
- Mean corpuscular hemoglobin concentration (MCHC)
- Platelet count (PLATELET)
- Prothrombin time (PT)
- Activated partial thromboplastin time (APTT)
- Reticulocyte count
  - Percent (RETIC)
  - Absolute (RETIC ABSOLUTE)
- Differential leukocyte count -
  - Percent and absolute
    - -Neutrophil (NEU)
    - -Lymphocyte (LYMPH)
    - -Monocyte (MONO)
    - -Eosinophil (EOS)
    - -Basophil (BASO)
    - -Large unstained cell (LUC)
- Fibrinogen
- Hemoglobin Distribution Width (HDW)
- Platelet estimate
- Red cell morphology (RBC Morphology)

( ) = Designates tabular abbreviation

Mean neutrophil counts were observed in males and females in all test article-dosed groups in Week 4 when compared to baseline values and/or the control group (Table 28). Decreased mean levels of fibrinogen were observed in males in 50 mg/kg/dose P3 and P1 groups and in females 5 and 50 mg/kg/dose P3 and 50 mg/kg/dose P1 groups in Weeks 4 and 12.

Decreased mean white cell counts were observed in males in all test article-dosed groups in Week 4 when compared to baseline values and the control group.

Table 28 Changes in the hematology parameters from the 13-week bridging SC monkey study

Parameter \ Dose Group mg/kg/dose		Males				Females			
		G1 0	G2 5	G3 50	G4 50	G1 0	G2 5	G3 50	G4 50
White cells (10^3/µl)	Wk -3	14.12	12.01 (-15%)	13.29 (-6%)	9.30 (-34%)	12.02	14.70 (+22%)	15.04 (+25%)	19.99** (+66%)
	Wk 4	11.35	6.71* (-41%)	8.32 (-27%)	6.65* (-41%)	7.98	5.75 (-28%)	8.98 (+13%)	8.79 (+10%)
	Wk 12	8.48	9.32	8.05 (-5%)	7.32 (-14%)	6.31	5.26 (-17%)	8.11 (+29%)	8.32 (+32%)
Neutrophil (%)	Wk -3	39.0	34.4 (-12%)	38.1 (-2%)	31.2 (-20%)	45.0	66.0* (+47%)	52.1 (+16%)	65.0 (+44%)
	Wk 4	59.9	26.4** (-56%)	28.8** (-52%)	28.8** (-52%)	31.9	27.8 (-13%)	24.1 (-25%)	24.0 (-25%)
	Wk 12	45.3	50.3 (+11%)	42.8 (-6%)	29.7 (-34%)	40.5	36.9 (-9%)	47.0 (+16%)	43.9 (+8%)
Abs neutrophil (10^3/µl)	Wk -3	5.38	4.20 (-22%)	5.17 (-4%)	2.87 (-47%)	5.43	9.88 (+82%)	7.91 (+46%)	13.01** (+140%)
	Wk 4	6.80	1.74** (-74%)	2.44** (-64%)	1.96** (-71%)	2.42	1.54 (-36%)	2.08 (-14%)	2.26 (-7%)
	Wk 12	3.70	5.17 (+40%)	3.57 (-4%)	2.01 (-46%)	2.52	1.96 (-22%)	3.92 (+56%)	3.39 (+35%)
Fibrinogen (mg/dL)	Wk -3	223.0	225.0 (+0.9%)	215.5 (-3%)	200.0 (-10%)	201.8	193.8 (-4%)	204.8 (+2%)	191.0 (-5%)
	Wk 4	198.8	230.0 (+16%)	176.5 (-11%)	170.8 (-14%)	218.3	165.8* (-24%)	169.0* (-23%)	171.5* (-21%)
	Wk 12	219.0	209.3 (-4%)	174.3 (-20%)	164.8* (-25%)	213.3	162.5** (-24%)	163.3** (-24%)	159.3** (-25%)

* P < 0.05; ** P < 0.01

Clinical Chemistry

Blood samples for clinical chemistry evaluation were collected from all monkeys prior to the initiation of dose administration (Week -3), once during Week 4, and within 1 week of the scheduled necropsy (Week 12). The animals were fasted overnight prior to blood collection. Below serum chemistry parameters were evaluated.

Serum chemistry parameters [taken directly from the study report pp. 35]

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (ALP)
Alanine aminotransferase (ALT)
Aspartate aminotransferase (AST)
Gamma glutamyltransferase (GGT)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides (Triglyceride)
C-reactive protein (CRP) [a]

( ) = Designates tabular abbreviation
[a] = Presented in special chemistry tables

There were no apparent test article-related effects.

As the baseline levels (Week -3) of CRP in males and females in REGN88-dosed groups were lower than the control group, the effect of REGN88 on CRP levels could not be determined in this study. Other changes were considered incidental because there were inconsistent changes between males and females and changes appeared to be isolated occurrences.

Table 29 Changes in the clinical chemistry parameters from the 13-week bridging SC monkey study

Parameter \ Dose Group (mg/kg/dose)		Males				Females			
		G1 0	G2 5	G3 50	G4 50	G1 0	G2 5	G3 50	G4 50
CRP (ng/mL)	Wk -3	64.4	36.0 (-44%)	47.8 (-26%)	24.5 (-62%)	64.0	34.2 (-47%)	47.9 (-25%)	37.3 (-42%)
	Wk 4	13.3	9.9 (-26%)	11.6 (-13%)	9.1 (-32%)	7.1	5.3 (-25%)	5.3 (-25%)	7.4 (+4%)
	Wk 12	17.2	10.9 (-37%)	11.6 (-33%)	8.4 (-51%)	14.3	6.3 (-56%)	9.3 (-35%)	11.2 (-22%)

Urinalysis

Urine samples for urinalysis were collected from all monkeys prior to the initiation of dose administration (Week -3), once during Week 4, and within 1 week of the scheduled necropsy (Week 12). The animals were fasted overnight while using cage pans for urine collection. The following urinalysis parameters were evaluated:

Urinalysis parameters [taken directly from the study report pp.36]

Specific gravity (SG)	Ketones (KET)
pH	Bilirubin (BIL)
Urobilinogen (URO)	Occult blood (BLD)
Total volume	Leukocytes (LEU)
Color (COL)	Nitrites (NIT)
Clarity (CLA)	Microscopy of sediment
Protein (PRO)	[Tabular abbreviations appear
Glucose (GLU)	on individual tables]

( ) = Designates tabular abbreviation

There were no test article-related changes.

Gross Pathology

A complete necropsy was performed on all animals. Animals were anesthetized with ketamine prior to euthanasia by an intravenous injection of sodium pentobarbital followed by exsanguination. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

Tissues collected [taken directly from the study report, pp.39]

Adrenal glands (2)
Aorta
Bone with marrow
  Sternum
  Joint: Femoral (Distal)
Bone marrow smear [a]
Brain
  Cerebrum (2 levels)
  Cerebellum with medulla/pons
Cervix
Epididymides (2) [b]
Eyes with optic nerves (2) [c]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
  Gut Associated Lymphoid tissue [d]
Heart
Injection site
Kidneys (2)
Larynx
Liver (sections of 3 lobes) [e]
Lungs [including bronchi, fixed by inflation with fixative (2)]
Lymph node
  Axillary (2)
  Cervical
  Mesenteric
Nasal tissue (skull/nasal cavity)
Ovaries (2)
Oviducts (2)
Pancreas
Peripheral nerve (sciatic)
Pituitary
Prostate
Salivary glands
  Mandibular (2)
  Parotid (2)
Seminal vesicles
Skeletal muscle (rectus femoris)
Skeletal muscle (diaphragm)
Skin with mammary gland [f]
Spinal cord (cervical, thoracic, lumbar) [g]
Spleen
Testes (2) [b]
Thymus
Thyroid [with parathyroids (2)]
Tongue
Trachea
Ureters (2)
Urinary bladder
Uterus (body and horn)
Vagina
Gross lesions (when possible)

[a] = Collected at the time of the scheduled necropsy but not placed in formalin. Bone marrow smears were not examined.
[b] = Fixed in Bouin's solution.
[c] = Fixed in Davidson's solution.
[d] = Included in section of jejunum or ileum.
[e] = The 3 lobes included right and left lobes and the right portion of the median lobe.
[f] = For females, a corresponding section of skin was collected from the same anatomical area for males.
[g] = Cervical, thoracic, and lumbar spinal cord were designated as "spinal cord" in the raw data.

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies. Paired organs were weighed together.

Organs listed [taken directly from the study report, pp.40]

Adrenal glands	Pituitary
Brain	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid gland with parathyroids
Ovaries without oviducts	Uterus (without oviduct)

There were no test article-related changes in organ weights.

An increase in the mean thymus weight was observed in males in the 50 mg/kg/dose P1 group (Group 4), whereas a decrease was observed in females in Group 4 (Table 30). These disparate changes between males and females in Group 4 were considered incidental.

Table 30 Changes in organ weights from the 13-week SC bridging monkey study

Dose Group (mg/kg) / Organ	Males				Females			
	G1	G2	G3	G4	G1	G2	G3	G4
	0	5	50	50	0	5	50	50
Thymus (g)	3.42	3.69 (+8%)	3.30 (-4%)	4.87 (+42%)	2.57	2.87 (+12%)	2.91 (+13%)	1.91 (-26%)
Thymus/BW	0.134	0.145 (+8%)	0.135 (+0.7%)	0.200 (+49%)	0.112	0.117 (+5%)	0.122 (+9%)	0.081 (-28%)

Histopathology

Microscopic examination was performed on all tissues above from all animals.

Adequate Battery: Yes

Peer Review: The histopathology peer review was performed by (b) (4) and consisted of examination of the following tissues: (1) all injection site, heart, and brain slides from all primary necropsy animals and (2) all tissue slides from the animal with myocardial inflammation (Female No. 2709 in the 5 mg/kg group).

Histological Findings

All findings from injection sites and some other findings are listed in Table 31. Minimal perivascular mononuclear infiltrates were observed in subcutaneous injection sites of test article-dosed monkeys at the end of the dosing period. However, incidences of inflammation (chronic, subacute and granulomatous) or ulceration in the test article-dosed groups were similar to those in the control group.

Histological data from the reproductive tissues from all male monkeys showed that these monkeys were sexually immature.

All preneoplastic (hyperplasia) and neoplastic findings are also listed in Table 31. Adenoma in the adrenal cortex was observed in one male in 50 mg/kg/dose P1 group. In addition, moderate squamous metaplasia in the cervix of one female 5 mg/kg/dose P3 group was noted. Occasional findings of hyperplasia in different tissues appeared incidental. For more detailed explanations on these findings, see the carcinogenicity risk assessment under the Carcinogenicity section.

Other findings listed in Table 31 do not appear test article-related as the incidence and/or severity of findings are low.

Table 31 Histopathological findings in the 13-week SC bridging toxicity study in monkeys

Dose Group (mg/kg) / Tissue	Male				Female			
	G1 0	G2 5	G3 50	G4 50	G1 0	G2 5	G3 50	G4 50
Adrenal cortex								
Adenoma (benign neoplasm)	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
Diaphragm								
Mononuclear infiltrate	0/4	0/4	1/4	2/4	0/4	1/4	0/4	0/4
min	0	0	1	2	0	1	0	0
Eyes								
Cyst, ciliary body present	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Liver								
Bile duct hyperplasia	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
min	1	0	0	0	0	0	0	0
Chronic inflammation	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
min	0	0	0	1	0	0	0	0
Lungs								
Bronchiolo-alveolar Hyperplasia	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
min	0	0	0	1	0	0	0	0
Nasal level 1								
Lymphoid hyperplasia	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4

min	1	0	0	0	0	0	0	0
Nasal level 2								
Lymphoid hyperplasia	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
mild	0	1	0	0	0	0	0	0
Nasal level 3								
Lymphoid hyperplasia	0/4	0/4	0/4	1/4	0/4	0/4	1/4	0/4
min	0	0	0	0	0	0	1	0
mild	0	0	0	1	0	0	0	0
Spleen								
Capsular fibrosis	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
mild	0	0	0	1	0	0	0	0
Lymphoid hyperplasia	1/4	2/4	0/4	1/4	1/4	0/4	1/4	0/4
min	0	2	0	1	0	0	1	0
mild	1	0	0	0	1	0	0	0
Thymus								
Epithelial hyperplasia	0/4	1/4	1/4	0/4	0/4	1/4	1/4	0/4
min	0	1	1	0	0	1	0	0
mild	0	0	0	0	0	0	1	0
Thyroid glands								
Alteration, colloid	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
Tongue								
Epithelial degeneration	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
mild	0	0	0	1	0	0	0	0
Cervix								
Squamous metaplasia	-	-	-	-	0/4	1/4	0/4	0/4
moderate					0	1	0	0
Uterus								
Hemorrhage	-	-	-	-	0/4	0/4	1/4	2/4
min					0	0	1	2
Epididymides								
immature	4/4	4/4	4/4	4/4	-	-	-	-
Prostate								
immature	4/4	4/4	4/4	4/4	-	-	-	-
Seminal vesicles								
immature	4/4	4/4	4/4	4/4	-	-	-	-
Testes								
immature	4/4	4/4	4/4	4/4	-	-	-	-

Injection site								
Fibrosis	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
min	0	0	0	1	0	0	0	0
Perivascular mononuclear infiltrate	0/4	**1/4**	**3/4**	**3/4**	0/4	0/4	**2/4**	**4/4**
min	0	1	3	3	0	0	2	4
Acute inflammation	1/4	0/4	0/4	0/4	1/4	0/4	0/4	1/4
min	1	0	0	0	1	0	0	1
Chronic inflammation	2/4	3/4	1/4	2/4	3/4	0/4	2/4	2/4
min	2	3	1	2	3	0	2	2
Granulomatous inflammation	0/4	1/4	0/4	1/4	0/4	0/4	0/4	0/4
min	0	1	0	1	0	0	0	0
Subacute inflammation	0/4	0/4	0/4	1/4	2/4	1/4	0/4	1/4
min	0	0	0	1	2	1	0	1

Toxicokinetics

Blood samples for toxicokinetic evaluation were collected from all monkeys prior to dosing and at approximately 24 hours after the second weekly dose administration during Weeks 0, 1, 2, 3, 5, 7, 9, and 11, and once on the day of the scheduled necropsy. Blood samples for anti-drug antibody (ADA) evaluation were collected prior to dose administration on Day 0 and prior to each second weekly dose during Weeks 0, 1, 2, 3, 5, 7, 9, and 11, and on the day of scheduled necropsy.

Total REGN88 concentrations in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) of REGN88 was 3.13 ng/mL in the assay (2% monkey serum) and 156.5 ng/mL in neat serum.

ADAs in monkey serum were detected using a validated bridging immunoassay, which was a non-quantitative, titer-based assay that used a floating cutpoint to determine positive response levels in samples, based on a negative control. Three positive quality controls (PQCs) were included in each assay run to monitor the performance and acceptability of the assay, and the PQC was REGN593, a mouse anti-human IgG, CH1 domain specific, monoclonal antibody. The lower limit of detection (LLOD) using the mouse anti-human IgG positive control was about 26.9 ng/mL in the absence of REGN88 and 123.8 ng/mL in the presence of 20 µg/mL of REGN88.

REGN88 was not detected in any serum samples from animals in the control group. Serum concentrations and $AUC_{0\text{-}24h}$ estimates in the 5 and 50 mg/kg P3 groups (G2 and G3) increased in an approximate dose-proportional manner (Table 32). $AUC_{0\text{-}24h}$ estimates in the

50 mg/kg/dose P3 group was approximately 16% lower than the 50 mg/kg/dose P1 group over the course of the study (20% lower in Week 0 and 11% lower in Week 11; ranged between 10% to 20% lower for individual weeks). There were no sex differences in REGN88 exposure. There was accumulation of the test article over the dosing period.

Table 32 TK data from the 13-week bridging SC toxicity study

Parameter \ Dose Group (mg/kg/dose)		Males			Females			Combined		
		G2	G3	G4	G2	G3	G4	G2	G3	G4
		5	50	50	5	50	50	5	50	50
Mean serum conc. (µg/mL)	Wk 0 24h	91.3	846	1107	96.8	825	908	94.0	836	1007
	Wk 11 24h	257	2628	3165	296	3185	3325	276	2906	3245
$AUC_{0\text{-}24h}$ (h*µg/mL)	Wk 0	1498	15078	20511	1711	15168	17154	1605	15123	18833
	Wk 11	5904	59100	74640	7533	75000	75990	6719	67050	75315
	AR (wk11 /wk 0)	3.9	3.9	3.6	4.4	4.9	4.4	4.1	4.4	4.0

ADAs were not detected in any serum samples from the control group. In the 5 mg/kg/dose P3 group, 2/8 animals (one male and one female) exhibited a positive ADA response during the course of the study. The male was ADA-positive from Week 2 and the female was ADA-positive from Week 9; the positive ADA response was maintained in both animals for the remainder of the study. The male with the positive ADA response had decreased REGN88 concentrations from Week 2. ADAs were not detected in either 50 mg/kg/dose P1 or P3 group.

Dosing Solution Analysis

The control (Group 1) and REGN88 P1 (Group 4) formulations were administered as supplied by the sponsor without dilution. For the REGN88 P3 groups (Groups 2 and 3) dosing formulation, the REGN88 P3 formulation supplied by the sponsor was diluted with REGN88 P3 Placebo to achieve 66 mg/mL. Dosing formulations were prepared on the day of dosing and used within 6 hours of preparation. The samples were collected once monthly on the days of solution preparation (prior to use) and analyzed using a validated spectrophotometric method measuring UV absorbance at wavelengths of 280 and 320 nm to confirm test article concentration.

The analyzed dosing formulations prepared by the P3 process as well as the P1 process were within 90% to 110% of the nominal concentration.

Study title: A 6-Month Once Weekly 30-Minute Intravenous Injection Study of REGN88 in Cynomolgus Monkeys Followed by a 12-Week Recovery Period

Study no.:	REGN88-TX-08031 ((b) (4)-460012)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	January 14, 2009
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	REGN88; Batch Lot # K08003D600A11A; 97.8% purity.

Key Study Findings

- Groups of 6 monkeys/sex/group were intravenously administered REGN88 once weekly at doses of 0 (placebo control), 0.5, 5, 15, or 50 mg/kg/week. Four monkeys/sex/group were sacrificed after a 26-week dosing period and the remaining 2 monkeys/sex/group were sacrificed after a 12-week recovery period.
- The relationship to the test article was unclear for preterminally sacrificed monkey in the 15 mg/kg group due to typhlocolitis.
- There was a trend toward minimally to slightly lower mean neutrophil counts and fibrinogen values. Mean Interleukin-6 (IL-6) values were markedly higher in the ≥ 5 mg/kg groups of males and females in Weeks 4, 12, and 25, compared to the control group. These elevations were considered to be due to test article administration and were an expected pharmacological effect, although there was much individual variation without dose-related response. These effects resolved at the end of the recovery period.
- In a TDAR assay, slightly but statistically significant decreases primary and secondary IgG responses to KLH administration were noted in males and females in the ≥ 5 mg/kg dose groups. The decreases were not generally dose-dependent.
- Mean systemic exposure of REGN88 (AUCs and peak serum levels) increased with dose. There was no sex difference, but accumulation was observed at ≥ 5 mg/kg. Mean peak levels of REGN88 in the 0.5 mg/kg group were decreased from Week 2 of the dosing period, which was associated with the presence of ADAs in 10 out of 12 animals in this group by Day 14. The presence of ADAs in this group appeared to increase clearance of drug, thereby minimizing drug accumulation during the dosing period and decreasing overall exposure to REGN88.

Methods

Doses:	0 (Placebo; Group 1), 0.5 (Group 2), 5 (Group 3), 15 (Group 4), and 50 mg/kg/week (Group 5); Placebo is an aqueous buffered solution, pH 6.0, containing 6% sucrose, 0.13% polysorbate 20 and 10 mM histidine
Frequency of dosing:	Once weekly for 26 doses
Route of administration:	Intravenous infusion (approximately 30 minutes)
Dose volume:	5 mL/kg for Group 1 & Groups 3-5 and 2.5 mL/kg for Group 2
Formulation/Vehicle:	0.9% sodium chloride (vehicle used in preparation of placebo and test article formulations)
Species/Strain:	Cynomolgus monkeys (*Macaca fascicularis;* of Vietnamese origin) from (b) (4)
Number/Sex/Group:	4 for the main study; 2 for the recovery group
Age:	Approximately 3-5 years old at the initiation of dosing
Weight:	2.015 to 4.303 kg for the males and 2.078 to 3.262 kg for females at the initiation of dosing
Satellite groups:	None
Unique study design:	Monkeys were pair-housed.
Deviation from study protocol:	There were no deviations that affected the integrity of the study.

Observations and Results

Mortality

Viability check was performed twice daily.

There was no test article-related mortality since there was no death at the highest dose tested in the study.

One male in the 0.5 mg/kg (Animal No. 3928) was found dead on Day 159, and one male in the 15 mg/kg group (Animal No. 3927) was preterminally sacrificed on Day 133.

The death of Animal No. 3928 appeared to be an accidental choking incidence since foreign material (apparently “clump of biscuits”) was found in the esophagus (macroscopic) and severe pharyngeal dilatation and foreign material/plant (luminal) debris was found within the lumen of the pharynx, larynx, and bronchi and bronchioles.

Microscopic findings in Animal No. 3927 included ulceration, hemorrhage, mucosal abscessation, and generalized inflammation of the large intestine, consistent with the antemortem clinical signs of diarrhea, inappetence, and body weight loss. This animal had masses in the esophagus and choristoma, comprised of tracheal mucosa. This unscheduled

death was considered to be due to moderate typhlocolitis, and the relationship to the test article was unclear.

Clinical Signs

Clinical examinations were performed daily (twice [prior to dosing and 1 to 2 hours postdose] on the dosing days; once on the nondosing days and during the recovery period) and detailed physical examinations were performed at least weekly.

There were no test article-related clinical observations.

All observations in the test article-dosed groups were also noted in the control group with similar incidences, were not noted in a dose-related manner and/or were common findings for laboratory monkeys.

Body Weights

Body weight was measured at least weekly, beginning during pretest, at randomization, and on the day prior to the scheduled necropsy.

There were no test article-related effects on mean body weights or body weight gains in both males and females.

Feed Consumption

Approximately 4 biscuits were offered twice daily. The diet was supplemented with other nutrients (such as fresh fruits) as part of the environmental enrichment program. Food consumption was not recorded.

Ophthalmoscopy

Ophthalmic examinations were performed on all animals during study weeks -2, 25 and/or 37. Animals were anesthetized with ketamine prior to examination.

There were no abnormal ophthalmic findings.

ECG

Electrocardiograms, blood pressures, and body temperatures were recorded during study weeks -2, 13, 25, and/or 37. During the dosing period, ECGs were measured approximately 1-2 hours postdose. Animals were anesthetized with ketamine prior to examination.

There were no test article-related effects on ECG, blood pressures, and body temperatures.

Hematology

Blood samples for hematology evaluation were collected from all animals prior to the initiation of dose administration (study weeks -1 and -2 for males and females, respectively) and during study weeks 4, 12 (prior to KLH administration), and within 1 week of each scheduled necropsy (Weeks 25 and 37). Additional blood samples for hematology evaluations were collected for Male No. 3927 (15 mg/kg group) during study weeks 17 and 19 prior to euthanasia *in extremis*. Animals were fasted overnight prior to blood collections.

List taken directly from the study report pp. 40

Total leukocyte count (White Cells)
Erythrocyte count (Red Cells)
Hemoglobin
Hematocrit
Mean corpuscular volume (MCV)
Mean corpuscular hemoglobin (MCH)
Mean corpuscular hemoglobin concentration (MCHC)
Platelet count (Platelet)
Prothrombin time (Pro Time)
Activated partial thromboplastin time (APTT)
Reticulocyte count
Percent (Reticulocyte)
Absolute (Retic Absolute)
Red cell distribution width
Red CellWidth
Differential leukocyte count -
Percent and absolute
-Neutrophil
-Lymphocyte
-Monocyte
-Eosinophil
-Basophil
-Large unstained cell
Fibrinogen
Platelet estimate[a]
Red cell morphology[a]
(RBC Morphology)

( ) - Designates tabular abbreviation
[a] - The manual data were accepted and reported instead of the automated differential data

There was a trend toward minimally to slightly lower mean neutrophil counts and fibrinogen values in the ≥ 5mg/kg groups without a dose-related response during the dosing period.

Decreased mean white cell counts were observed females in the ≥5 mg/kg dose groups without a dose-related response in Weeks 25 and 37.

Table 33 Changes in the hematology parameters from the 6-month monkey study

Parameter / Dose Group (mg/kg/wk)		Males					Females				
		0	0.5	5	15	50	0	0.5	5	15	50
White cells (10^3/µL)	Wk -1/ -2	14.49	12.60 (-13%)	12.86 (-11%)	12.41 (-14%)	9.62 (-34%)	10.17	11.88 (+17%)	11.47 (+13%)	12.05 (+19%)	13.07 (+29%)
	Wk 4	12.75	10.22 (-20%)	8.22* (-36%)	11.98 (-6%)	7.56* (-41%)	8.98	7.98 (-11%)	8.39 (-7%)	9.53 (+6%)	9.52 (+6%)
	WK 12	11.06	11.86 (+7%)	9.41 (-15%)	10.72 (-3%)	7.81 (-29%)	10.16	10.45 (+3%)	9.33 (-8%)	10.45 (+3%)	9.94 (-2%)
	Wk 25	12.40	12.68 (+2%)	10.88 (-12%)	9.61 (-23%)	8.29 (-33%)	10.98	9.26 (-16%)	8.25 (-25%)	8.84 (-20%)	8.20 (-25%)
	Wk	10.28	7.81	11.97	10.43	8.49	13.61	8.67	9.77	9.57	8.95

	37		(-24%)	(+16%)	(+1%)	(-17%)		(-36%)	(-28%)	(-30%)	(-34%)
Neutrophil (%)	Wk -1/ -2	46.0	30.4 (-34%)	38.3 (-17%)	42.7 (-7%)	53.5 (+16%)	38.9	45.9 (+18%)	45.2 (+16%)	42.0 (+8%)	50.6 (+30%)
	Wk 4	39.3	30.0 (-24%)	32.4 (-18%)	44.8 (+14%)	38.9 (-1%)	39.9	40.3 (+1%)	32.3 (-19%)	29.1 (-27%)	47.8 (+20%)
	Wk 12	28.9	27.5 (-5%)	21.9 (-24%)	33.2 (+15%)	28.0 (-3%)	41.8	43.7 (+5%)	32.6 (-22%)	28.1 (-33%)	40.5 (-3%)
	Wk 25	33.6	30.4 (-10%)	28.0 (-17%)	26.1 (-22%)	29.3 (-13%)	48.4	44.7 (-8%)	30.3 (-37%)	23.7** (-51%)	40.9 (-16%)
	Wk 37	38.0	21.8 (-43%)	37.1 (-2%)	49.2 (+30%)	42.3 (+11%)	42.8	50.0 (+17%)	29.6 (-31%)	39.9 (-7%)	59.4 (+39%)
Neutrophil (10^3/µL)	Wk -1/ -2	6.67	3.80 (-43%)	5.02 (-25%)	5.32 (-20%)	5.18 (-22%)	3.92	5.98 (+53%)	5.17 (+32%)	4.91 (+25%)	6.42 (+64%)
	Wk 4	5.06	3.12 (-38%)	2.75 (-46%)	5.26 (+4%)	3.08 (-39%)	3.77	3.43 (-9%)	2.66 (-29%)	2.64 (-30%)	4.45 (+18%)
	Wk 12	3.04	3.34 (+10%)	2.10 (-31%)	3.68 (+21%)	2.31 (-24%)	4.41	5.03 (+14%)	3.12 (-29%)	2.84 (-36%)	4.05 (-8%)
	Wk 25	4.16	3.81 (-8%)	2.97 (-29%)	2.44 (-41%)	2.60 (-38%)	5.56	4.23 (-24%)	2.43** (-56%)	2.13** (-62%)	3.31 (-41%)
	Wk 37	3.84	1.70 (-56%)	4.46 (+16%)	5.14 (+34%)	3.63 (-6%)	5.87	4.64 (-21%)	2.91 (-50%)	3.87 (-34%)	5.30 (-10%)
Fibrinogen (mg/dL)	Wk -1/ -2	214.0	213.7 (+8%)	219.7 (+3%)	242.5 (+13%)	249.7 (+17%)	194.3	205.2 (+6%)	205.2 (+6%)	227.7 (+17%)	247.3 (+27%)
	Wk 4	240.3	262.8 (+9%)	213.2 (-11%)	197.0 (-18%)	205.8 (-14%)	220.3	236.8 (+8%)	182.0* (-17%)	183.5* (-17%)	201.5 (-9%)
	Wk 12	225.3	240.3 (+7%)	202.2 (-10%)	199.5 (-12%)	198.5 (-12%)	205.0	216.5 (+6%)	170.5* (-17%)	173.7 (-15%)	190.5 (-7%)
	Wk 25	229.7	227.8 (-1%)	204.2 (-11%)	194.8 (-15%)	208.5 (-9%)	208.2	220.7 (+6%)	201.0 (-4%)	172.3 (-17%)	197.5 (-5%)
	Wk 37	255.0	250.0 (-2%)	256.0 (+0.4%)	201.0 (-21%)	287.0 (+13%)	214.5	237.5 (+11%)	235.0 (+10%)	255.5 (+19%)	200.0 (-7%)

* P≤0.05, ** P≤0.01

Clinical Chemistry

Blood samples for serum chemistry evaluation were collected from all animals prior to the initiation of dose administration (study weeks -1 and -2 for males and females, respectively), during study weeks 4, 12 (prior to KLH administration), and within 1 week of each scheduled necropsy (Weeks 25 and 37). Additional blood samples for serum chemistry evaluations were collected for Male No. 3927 (15 mg/kg group) during study weeks 17 and 19 prior to euthanasia in extremis. Animals were fasted overnight prior to blood collections.

List taken directly from the study report pp. 40

Albumin
Total protein
Globulin [by calculation]
Albumin/globulin Ratio (A/G Ratio) [by calculation]
Total bilirubin (Total Bili)
Urea nitrogen
Creatinine
Alkaline phosphatase (AlkalinePhos'tse)
Alanine aminotransferase (Alanine Transfer)
Aspartate aminotransferase (AspartatTransfer)
Gamma glutamyltransferase (GlutamylTransfer)
Glucose
Total cholesterol (Cholesterol)
Calcium
Chloride
Phosphorus
Potassium
Sodium
Triglycerides (Triglyceride)
C-Reactive protein (CRP)[a]
IgA[a, b]
IgE[a, b]
IgG[a, b]
IgM[a, b]
IL-6 (IL6)[a, b]

( ) - Designates tabular abbreviation
[a] - Presented on special chemistry tables
[b] - Shipped frozen to (b) (4) for analysis.

Mean Interleukin-6 (IL-6) values were markedly higher in males and females in the ≥5 mg/kg groups than the control group in Weeks 4, 12, and 25. These elevations were considered to be due to test article administration and were an expected pharmacological effect, although there was high individual variation, without a dose-related response. These effects resolved during the 12-week recovery period.

Decreased mean CRP levels were observed in males in the ≥15 mg/kg dose groups in Weeks 4, 12, and 25 and females in the ≥5 mg/kg dose groups in Weeks 4 and 12.

Table 34 Changes in the clinical chemistry parameters from the 6-month monkey study

Dose Group (mg/kg/wk) / Parameter		Males					Females				
		0	0.5	5	15	50	0	0.5	5	15	50
CRP (mg/L)	Wk -1/ -2	6.0	7.5 (+25%)	10.5 (+75%)	9.8 (+63%)	6.9 (+15%)	5.3	11.1 (+109%)	13.1 (+147%)	13.7 (+159%)	6.5 (+23%)
	Wk 4	12.5	26.9 (+115%)	15.2 (+22%)	4.0 (-68%)	4.3 (-66%)	10.7	26.4 (+147%)	4.2 (-61%)	4.5 (-58%)	3.1 (-71%)

	Wk 12	17.6	16.0 (-9%)	28.5 (+62%)	5.0 (-72%)	5.0 (-72%)	19.2	20.6 (+7%)	10.2 (-47%)	9.4 (-51%)	8.6 (-55%)
	Wk 25	15.7	13.6 (-13%)	21.6 (+38%)	10.0 (-36%)	7.1 (-55%)	9.4	11.4 (+21%)	14.1 (+50%)	10.3 (+10%)	7.6 (-19%)
	Wk 37	10.4	22.4 (+115%)	13.3 (+28%)	24.7 (+138%)	12.8 (+23%)	4.5	8.4 (+87%)	19.8 (+340%)	37.2 (+727%)	10.7 (+138%)
IL6 (pg/dL)	Wk -1/ -2	0.75	1.75 (+133%)	1.00 (+33%)	1.78 (+138%)	1.01 (+35%)	0.49	5.96 (+1116%)	1.58 (+222%)	0.69 (+41%)	1.28 (+161%)
	Wk 4	7.78	16.67 (+114%)	46.01 (+491%)	78.69** (+911%)	63.72* (+719%)	5.44	9.18 (+69%)	81.91** (+1406%)	73.45** (+1250%)	37.91 (+597%)
	Wk 12	3.18	4.22 (33%)	19.75 (+521%)	62.39* (+1862%)	42.16 (+1226%)	2.66	2.99 (+12%)	30.93 (+1063%)	55.17** (+1974%)	30.01 (+1028%)
	Wk 25	2.69	1.94 (-28%)	9.41 (+250%)	22.63** (+741%)	26.92** (+901%)	2.06	1.65 (-20%)	12.57 (+510%)	24.56** (+1092%)	15.46* (+651%)
	Wk 37	1.59	0.00 (-100%)	1.83 (+15%)	0.58 (-64%)	1.63 (+3%)	0.00	0.00	0.00	0.00	6.19

* P < 0.05; ** P < 0.01

Urinalysis

Urine samples for urinalysis were collected from all animals prior to the initiation of dose administration (study weeks -1 and -2 for males and females, respectively), during study weeks 4, 12 (prior to KLH administration), and within 1 week of each scheduled necropsy (Weeks 25 and 37). Animals were fasted overnight while using cage pans for urine collection. On Day 85, the automatic watering system for Animal No. 3960 (5 mg/kg group female) was found to be leaking and the urine that had been collected overnight was contaminated by water. Urine collection was restarted for this animal, and a sample was collected over the course of approximately 4 hours on the same day.

List taken directly from the study report, pp. 41

Specific gravity (SG)
pH
Urobilinogen (URO)
Total volume (TVOL)
Color (CLOR)
Clarity (CLA)
Protein (PRO)
Glucose (GLU)
Ketones (KET)
Bilirubin (BIL)
Occult blood (BLD)
Leukocytes (LEU)
Nitrites (NIT)
Microscopy of sediment
[Tabular abbreviations appear on individual tables]

( ) - Designates tabular abbreviation

There were no test article-related changes.

There was a lower pH in males in the 50 mg/kg group in Week 12 (pH 7.3), compared to control or baseline. It occurred only one time in only males and thus, this finding was not considered test article-related.

Gross Pathology

Complete necropsies were performed on all animals. Animals were euthanized by an intravenous injection of sodium pentobarbital followed by exsanguination. The following tissues and organs were collected and placed in 10% neutral-buffered formalin (except as noted):

List taken directly from the study report pp.45

Adrenal Glands (2)
Aorta
Bone with marrow
  Sternum
Bone marrow smear [a]
Brain
  Cerebrum (2 levels)
  Cerebellum with pons/medulla
Cervix
Epididymides (2) [b]
Eyes with optic nerves (2) [b]
Gallbladder
Gastrointestinal tract
  Esophagus
  Stomach
  Duodenum
  Jejunum
  Ileum
  Cecum
  Colon
  Rectum
  Gut-Associated Lymphoid tissue (Peyer's patches) [c]
Heart
Infusion site
Joint: Femoral (Distal)
Kidneys (2)
Larynx
Liver (sections of three lobes; right lobe, left lobe and left portion of the median lobe) [d]
Lungs [including bronchi, fixed by inflation with fixative (2)]
Lymph node
  Cervical
  Mandibular [d]
  Mesenteric
Nasal tissue (skull/nasal cavity) [e]
Ovaries (2)
Oviducts (2)
Pancreas
Peripheral nerve (sciatic)
Pharynx
Pituitary
Prostate
Salivary gland [mandibular (2)]
Salivary gland [parotid (2)]
Seminal vesicles
Skeletal muscle (rectus femoris)
Skeletal muscle (diaphragm)
Skin with mammary gland [f]
Spinal cord (cervical, thoracic, lumbar) [g]
Spleen [d]
Testes (2) [b]
Thymus [d]
Thyroids [with parathyroids (2)]
Tongue
Trachea
Ureters (2)
Urinary bladder
Uterus (body and horn)
Vagina
Gross lesions (when possible)

[a] - Bone marrow smears were obtained at the scheduled necropsies and from the animal euthanized *in extremis* but not placed in formalin; slides were not examined.
[b] - Fixed in Davidson's solution.
[c] - Included in section of jejunum or ileum.
[d] - A portion of the thymus (anterior), anterior portion of the spleen, section of the right lobe of liver and right mandibular lymph nodes from similar anatomical locations from all animals were fixed for a minimum of 48 hours in formalin and then embedded in paraffin for potential immunostaining for PCNA and evaluation of proliferation indices in these tissues. Immunostaining of these tissues was not performed. .
[e] - Retained in fixative, no microscopic examination.
[f] - For females only; a corresponding section of skin was taken from the same anatomic area for males.
[g] - Cervical, thoracic, and lumbar spinal cord were designated as "spinal cord" in the raw data.

There were no test article-related macroscopic findings.

Organ Weights

Selected organs were weighed at the scheduled necropsies. Paired organs were weighed together.

Adrenal glands	Prostate
Brain	Seminal vesicles
Epididymides	Spleen
Heart	Testes
Kidneys	Thymus
Liver	Thyroid with parathyroids
Ovaries	Uterus
Pituitary	

Increased mean thymus weights (absolute and relative to body weight) were observed in males in all test article-dosed groups and females in the ≥5 mg/kg dose groups in a non-dose-dependent manner. The change was reversible at the end of the recovery period.

Increased mean adrenal gland weights (absolute and relative to body weight) were observed in males in all test article-dosed groups, whereas decreased mean adrenal gland weights were observed in females in all test article-dosed groups. These weight changes were not dose-related and there were no macroscopic or microscopic correlates.

Lower mean weights of male reproductive organs were observed in all test article-dosed groups in a non-dose-dependent manner relative to the control group at the end of the dosing period. However, histological data showed that these monkeys were sexually immature. Thus, the observed difference was most likely due to individual variability at the peripubertal stage. Lower mean uterus weights were observed in the ≥5 mg/kg dose groups in a non-dose dependent manner at the end of the dosing period. As there were no correlated histopathology findings and these females were peripubertal, these changes were also likely due to individual variability at the peripubertal stage. In addition, normal reproductive cycling also causes notable interanimal variation in uterine and ovarian weights (Sellers et al., 2007. Toxicologic Pathology, 35:751-5).

Table 35 Changes in organ weights from 6-month monkey study at the end of the dosing period

Dose Group (mg/kg/wk) / Organ		Males					Females				
		0	1	5	15	50	0	1	5	15	50
Adrenal Glands (g)	M	0.4118	0.4589 (+11%)	0.4570 (+11%)	0.4841 (+18%)	0.5329 (+29%)	0.5099	0.4253 (-15%)	0.4237 (-17%)	0.4598 (-10%)	0.4077 (-20%)
Adrenal Glands/BW	M	0.011	0.014 (+27%)	0.013 (+18%)	0.016 (+46%)	0.015 (+36%)	0.019	0.016 (-16%)	0.014 (-26%)	0.016 (-16%)	0.015 (-21%)
Adrenal Glands (g)	R	0.5570	0.4097	0.5819 (+5%)	0.4906 (-12%)	0.4443 (-20%)	0.3582	0.5277 (+47%)	0.4040 (+13%)	0.4170 (+16%)	0.4421 (+23%)
Adrenal Glands/BW	R	0.016	0.011	0.017 (+6%)	0.013 (-19%)	0.013 (-19%)	0.012	0.017 (+42%)	0.015 (+25%)	0.016 (+33%)	0.015 (+25%)
Thymus (g)	M	1.96	2.57 (+31%)	2.68 (+37%)	2.44 (+25%)	2.79 (+42%)	1.21	1.18 (-3%)	2.21 (+83%)	1.67 (+38%)	2.03 (+68%)
Thymus/BW	M	0.052	0.079 (+52%)	0.084 (+62%)	0.077 (+48%)	0.081 (+56%)	0.043	0.043 (0%)	0.072 (+67%)	0.059 (+37%)	0.074 (+72%)
Thymus (g)	R	1.85	0.68 (-63%)	2.88 (+56%)	0.23 (-88%)	2.03 (+10%)	2.76	1.41 (-49%)	1.31 (-53%)	1.15 (-58%)	0.93 (-66%)
Thymus/BW	R	0.055	0.018 (-67%)	0.088 (+60%)	0.006 (-89%)	0.051 (-7%)	0.091	0.046 (-50%)	0.048 (-47%)	0.044 (-52%)	0.031 (-66%)
Epididymides (g)	M	1.67	1.35 (-19%)	1.27 (-24%)	0.81 (-52%)	1.49 (-11%)	-	-	-	-	-
Epididymides /BW	M	0.040	0.040 (0%)	0.034 (-15%)	0.027 (-33%)	0.043 (+8%)	-	-	-	-	-
Prostate (g)	M	0.85	0.59 (-31 %)	0.42 (-51%)	0.22 (-74%)	0.54 (-37%)	-	-	-	-	-
Prostate/BW	M	0.020	0.018 (-10%)	0.012 (-40%)	0.008 (-60%)	0.015 (-25%)	-	-	-	-	-
Seminal vesicles (g)	M	4.83	3.02 (-38%)	2.58 (-47%)	0.86 (-82%)	3.18 (-34%)	-	-	-	-	-
Seminal vesicles/BW	M	0.109	0.091 (-17%)	0.066 (-39%)	0.028 (-74%)	0.092 (-16%)	-	-	-	-	-
Testes (g)	M	8.52	5.52 (-35%)	3.97 (-53%)	1.91 (-78%)	3.36 (-61%)	-	-	-	-	-
Testes/BW	M	0.196	0.164 (-16%)	0.099 (-50%)	0.061 (-69%)	0.093 (-53%)	-	-	-	-	-
Uterus (g)	M	-	-	-	-	-	4.46	4.82 (+8%)	6.28 (+41%)	7.06 (+58%)	5.73 (+29%)
Uterus/BW	M	-	-	-	-	-	0.161	0.176 (+9%)	0.213 (+32%)	0.242 (+50%)	0.208 (+29%)

M = after the dosing period (N= 4/gp); R = after the recovery period (N=2 or 1/gp)
"-" represents not applicable.

Histopathology

Collected tissues were stained with hematoxylin and eosin and examined microscopically. Special stains were employed for one early death animal (# 3927) in an effort to determine an etiologic diagnosis; these included Gram, Heidenhain, Periodic Acid-Schiff, Wheatley's, Giemsa, and Gridley's stains. Microscopic examination was performed on all tissues listed above from all animals found dead or euthanized in extremis, and at the Week 26 primary necropsy, but not at the Week 38 recovery necropsy.

Adequate Battery: Yes

Peer Review: The histopathology peer review was performed by a pathologist from Sanofi.

Histological Findings

Histopathologic findings, including all preneoplastic (hyperplasia) lesions, are listed in Table 36. Minimal hyperplsia in the adrenal cortex was observed in one male in the 50 mg/kg group, but not considered test article-related. Occasional findings of hyperplasia in different tissues appeared incidental. For more explanations on the hyperplasia finding in the adrenal cortex, see the carcinogenicity risk assessment under the Carcinogenicity section.

Although necrosis (minimal) was observed in one male in the 50 mg/kg/dose group, overall, there were no test article-related findings in the injection sites. Other injection site findings were not listed in the table.

Histological data from the reproductive tissues from a majority of male monkeys in the main study portion showed that these monkeys were sexually immature.

Other findings listed in the table did not appear test article-related as the incidence and/or severity of findings are low.

Table 36 Histopathological findings in the 6-month IV toxicity study in main study monkeys

Dose Group (mg/kg/wk) / Tissue	Males					Females				
	0	0.5	5	15	50	0	0.5	5	15	50
Adrenal Gland										
Hyperplasia, cortex	0/4	0/4	0/4	0/4	**1/4**	0/4	0/4	0/4	0/4	0/4
min	0	0	0	0	1	0	0	0	0	0
Cecum										

Abscess, crypts	0/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4	2/4	2/4
min	0	0	0	1	1	0	0	0	2	2
Brown pigment	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	1/4
min	0	0	0	0	0	0	0	0	1	1
Larynx										
Chronic inflammation	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
moderate	0	0	0	0	1	0	0	0	0	0
Subacute inflammation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
min	0	0	0	0	0	0	0	0	0	1
Lymph node, mesenteric										
Sinus histiocytosis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4
mild	0	0	0	0	0	0	0	0	0	1
Sciatic nerve										
Hemorrhage	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4[a]
severe	0	0	0	0	0	0	0	0	0	1
Acute inflammation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4[a]
min	0	0	0	0	0	0	0	0	0	1
Pharynx										
Mononuclear infiltrate	1/4	0/4	2/4	1/4	2/4	0/4	0/4	3/4	2/4	3/4
min	1	0	2	1	1	0	0	3	0	3
mild	0	0	0	0	1	0	0	0	2	0
Chronic inflammation	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
mild	0	0	0	0	1	0	0	0	0	0
Skeletal muscle										
Parasite, protozoa present	0/4	0/4	0/4	0/4	1/4[b]	0/4	0/4	0/4	0/4	0/4
Skin										

Epithelial hyperplasia	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4
min	0	0	0	0	0	1	0	0	0	0
Spleen										
Lymphoid hyperplasia	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
min	0	0	1	0	0	0	0	0	0	0
Stomach										
Abscess, crypts	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4	1/4
min	0	0	0	0	0	0	0	0	2	1
Thymus										
Epithelial hyperplasia	2/4	1/4	1/4	3/4	0/4	0/4	1/4	1/4	2/4	1/4
min	2	1	1	3	0	0	1	1	2	1
Tongue										
Parasite, protozoa present	0/4	0/4	0/4	0/4	1/4[b]	0/4	1/4	0/4	0/4	0/4
Epididymides										
immature	2/4	3/4	3/4	4/4	4/4	-	-	-	-	-
Prostate										
immature	2/4	3/4	3/4	4/4	4/4	-	-	-	-	-
Seminal vesicles										
immature	2/4	3/4	3/4	4/4	4/4	-	-	-	-	-
Testes										
immature	2/4	3/4	3/4	4/4	4/4	-	-	-	-	-
Injection site saph, left										

Intimal hyperplasia	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
moderate	0	0	0	0	1	0	0	0	0	0
Epithelial hyperplasia	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4	0/4
mild	0	0	0	0	0	1	0	1	0	0
Necrosis	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4
min	0	0	0	0	1	0	0	0	0	0
Injection site saph, right										
Epithelial hyperplasia	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4	0/4
mild	0	0	0	0	0	1	0	1	0	0

[a]One female monkey in the 50 mg/kg/dose group had both severe hemorrhage and minimal acute inflammation in the sciatic nerve.
[b]One male monkey in the 50 mg/kg/dose group had protozoa present in both skeletal muscle and tongue.

Special Evaluation

Immunophenotying

Blood samples for peripheral blood phenotyping were collected from all animals during study weeks -2, -1, 13, 25, and/or 37. Samples were examined for the antigen markers listed in Table 37.

Table 37 [taken directly from the study report pp.42]

Antigen Marker (s)Cell	Population Identified
$CD45^+$	All lymphocytes
$CD20^+$	B-lymphocytes
$CD3^+$	T-lymphocytes
$CD3^+/CD4^+$	T-helper lymphocytes
$CD3^+/CD8^+$	T-cytotoxic/suppressor lymphocytes
$CD3^-/CD16^+$	Natural-killer cells
$CD3^-/CD14^+$	Monocytes

There were no test article-related effects.

Slightly lower percent values of natural killer cells was observed in males in the 50 mg/kg group on Day 176, however, there was high variability in the natural killer cell values across the groups of males and females at different time points. Thus, the change observed for natural killer cell values was not considered test article-related.

Mean percent values of monocytes in males in the 50 mg/kg dose group were statistically higher than those in the control in Weeks 13 and 25, however, the change was not seen in females. Thus, the change in males was not considered test article-related.

T-cell Dependent Antibody Response (TDAR) Assay

Keyhole Limpet Hemocyanin (KLH) was administered via intramuscular injection (bolus) to all animals at a dose level of 2 mg and a dose volume of 0.2 mL on Days 57 and 85 (within 24 hours of the previous day's test article or placebo dose). Blood samples for TDAR assays were collected during study weeks -1, 9 (once daily on Days 64 through 67), 13, 17, 22, and 25. The samples were analyzed primary IgM and IgG, and secondary IgG responses to KLH using ELISA.

Pretest	Baseline
Study day 64 through 67 (once daily, study week 9)	Primary response
Study day 93 (study week 13)	Secondary response
Study day 121 (study week 17)	Secondary response
Study day 156 (study week 22)	Secondary response
Study day 177 (study week 25)	Secondary response

Slightly, but statistically significant decreases primary and secondary IgG responses to KLH administration were noted in males and females in the 5, 15, and 50 mg/kg groups (for the primary IgG response, ranges of 4.5 to 13.3% for males and 11.9 to 20.8% for females lower relative to the respective control values; for the secondary IgG response, ranges of 6.7 to 19.9% for males and 12.1 to 25.4% for females lower relative to the respective control values). The decreases were not generally dose-dependent.

Toxicokinetics

Blood samples for toxicokinetic evaluation were collected from all animals at the following time points: predose, 5 minutes, and 24 hours following the 1st, 2nd, and 3rd infusion; predose, 5 minutes, and 24 and 168 (prior to the next dose) hours following the 4th, 8th, 12th, 17th, 21st, and 25th infusions; and once on the day of the primary necropsy (Week 26), during Week 32, and on the day of the recovery necropsy (Week 38). Total REGN88 levels in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) of REGN88 was 3.13 ng/mL in the assay (2% monkey serum) and 156.5 ng/mL in neat serum.

Blood samples for anti-drug antibody (ADA) evaluation were collected from all animals at the following time points: prior to the 1st, 2nd, 3rd, 4th, 8th, 12th, 17th, 21st, and 25th doses, and once on the days of the scheduled necropsies (Weeks 26 and 38). ADAs in monkey serum were detected using a validated bridging immunoassay. This method was a non-quantitative, titer-based assay that uses a floating cutpoint to determine positive response levels in samples, based on a negative control. Three positive quality controls were included in each assay run to monitor the performance and acceptability of the assay. The lower limit of detection (LLOD) using mouse anti-human IgG positive control was about 26.9 ng/mL in the absence of REGN88 and 123.8 ng/mL in the presence of 20 µg/mL of REGN88.

REGN88 was not detected in any serum samples from animals in the control group or in predose (0h on Day 0) samples from monkeys in REGN88-dosed groups. Mean systemic exposure of REGN88 (AUCs and peak serum levels) increased with dose. Mean peak serum concentrations of total REGN88 increased approximately proportionally with dose following the first dose. There was no sex difference, but accumulation was observed at ≥5 mg/kg. While mean REGN88 systemic exposure increased throughout the study in the ≥5 mg/kg groups, mean peak REGN88 serum levels were not maintained in the 0.5 mg/kg group. Mean peak levels of REGN88 in the 0.5 mg/kg group were decreased from Week 2 of the dosing period, which was associated with the presence of ADAs in 10 out of 12 animals in this group by Day 14. The presence of ADAs in this group appeared to increase clearance of drug, thereby minimizing drug accumulation during the dosing period and decreasing overall exposure to REGN88.

Ten of the 14 recovery animals (3/4 at 5 mg/kg, 3/3 at 15 mg/kg, and 4/4 at 50 mg/kg) had circulating levels of REGN88 throughout the entire 12-week recovery period. Only 4 animals (3/3 at 0.5 mg/kg and 1/4 at 5 mg/kg) had no detectable REGN88 during the recovery period.

Table 38 TK data from the 6-month IV toxicity study in monkeys

Parameter / Dose Group (mg/kg/wk)		Mean REGN88 levels (µg/mL)		AUC_{all} (h*mg/mL)		
		1st dose at 5 min	25th dose at 5 min	1st dose	25th dose	AR (25th dose/ 1st dose)
Male	0.5 (G2)	12.8	7.72	0.662	0.338	0.5
	5 (G3)	155	159	13.0	14.7	1.1
	15 (G4)	345	857	33.2	109	3.3
	50 (G5)	1378	3020	123	371	3.0
Female	0.5 (G2)	11.5	2.55	0.626	0.0331	0.05
	5 (G3)	136	247	11.1	31.1	2.8
	15 (G4)	435	596	32.3	71.3	2.2
	50 (G5)	1343	3203	146	391	2.7
Combined	0.5 (G2)	12.2	4.90	0.644	0.172	0.3
	5 (G3)	145	203	12.1	22.9	1.9
	15 (G4)	390	715	32.8	88.6	2.7
	50 (G5)	1361	3112	134	381	2.8

ADAs were not detected in any serum samples from control animals or in any predose samples collected on Day 0 from monkeys in REGN88-dosed groups, with one exception. ADAs were detected in Monkey No. 3954 in the 0.5 mg/kg group prior to receiving any REGN88 and at the other time points examined. All animals in the 0.5 mg/kg dose group and 5/12 (4 males and 1 female) animals in the 5 mg/kg group had detectable ADAs. These findings were associated with decreased mean peak serum concentrations at

0.5 mg/kg over the dosing period and lower levels of REGN88 in all monkeys that had ADAs in the 5 mg/kg group relative to ADA-negative animals within the same group, suggesting that the presence of the ADAs may have decreased exposure to REGN88.

ADAs were not detected in the 15 and 50 mg/kg dose groups, except one male in the 15 mg/kg dose group at one time point (following the third dose on Day 14).

Dosing Solution Analysis

Samples for concentration analysis were collected approximately once monthly from all dosing formulations, including the control formulation. Concentrations were analyzed using a validated spectrophotometric method using UV absorbance. Following the administration of the last dose, 2 test article vials and 2 control vials were shipped frozen for end-of-study stability analysis.

The analyzed concentrations that were within 90% to 110% of the nominal concentration. Adequate stability of the test article and the placebo, when frozen, was demonstrated for the entire dosing period of the study.

8 Carcinogenicity

Carcinogenic risk proposal for Sarilumab

The sponsor submitted a nonclinical question about the adequacy of their plan for assessment of the carcinogenic risk associated with sarilumab in the EOP2 briefing document. The sponsor does not plan to conduct nonclinical studies regarding carcinogenic potential of sarilumab, based on the weight of evidence for the available in vitro and in vivo data related to inhibition of IL-6 in tumor promotion, and the anti-tumor effects specifically observed with sarilumab. The sponsor believes no additional nonclinical studies are needed to evaluate the carcinogenic potential of sarilumab in patients. However, note that three monkeys in toxicology studies using sarilumab exhibited tumors (adenoma in the adrenal cortex of a male at the high dose in a 13-week SC study, papilloma (benign neoplasm) in the cervix of a female at the mid dose in a 5 week IV study, and squamous metaplasia in the cervix and uterus of a female at the low dose in a 13-week SC study). In addition, one female mouse at the high dose in the fertility study using REGN844, a mouse surrogate antibody (IgG2a) against IL-6, had squamous cell metaplasia in the cervix along with squamous cell hyperplasia in the vagina. The sponsor provided the following rationale to support their position that additional carcinogenic evaluation is not needed:

1) Based on the weight of literature evidence, inhibition of IL-6 leads to inhibition of p-STAT3 activation and this inhibition decreases the potential for tumor growth, differentiation, and proliferation of different tumor cell types. Inhibition of p-STAT3 is also known to inhibit the expression and activity of

immunosuppressive factors, thereby resulting in increased immunity against tumors.

2) In vitro and/or in vivo studies with sarilumab using tumor cell lines (prostate [Du145] and lung [NCI-H1650]) showed inhibition of p-STAT3 activation and inhibition of tumor growth in tumor xenograft animal models. Inhibition of p-STAT3 activation and tumor growth by sarilumab were associated with induction of cleaved caspase-3 in the tumors [Du145] suggesting a possible mechanism related to apoptosis for anti-tumor effects of sarilumab.
3) Tumors that occurred in monkey studies using sarilumab and the mouse study using REGN844 were considered not test article-related. See below for details.
4) Although there is some evidence in the literature indicating a role for IL-6 in tumor immunity and that IL-6Rα inhibition may lead to tumor promotion, the evidence in the literature for the lack of IL-6 inhibition in promoting tumor formation outweighs the evidence for a role of IL-6 inhibition to induce tumors. However, because sarilumab is expected to modulate the immune system, any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion are better managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches than any additional nonclinical studies [ICH S6(R1)].

The sponsor's assessment included a literature evaluation of the role of the IL-6R pathway in tumor promotion and immune suppression, as well as the available data for similar biotherapeutic compounds that have been approved for commercial use. This, together with pharmacology data on sarilumab in xenograft models, tissue-cross reactivity data, and evaluation of data from the repeat-dose toxicity studies in cynomolgus monkeys, forms the basis for this assessment for sarilumab. Based on the results of this evaluation, other additional in vitro or in vivo studies are not considered necessary to evaluate the carcinogenic potential of sarilumab. The sponsor also notes that animal carcinogenicity assessment was not needed for the approved anti-IL-6Rα mAb (tocilizumab) at the time of BLA review. Because the target of sarilumab is the same target as that of tocilizumab and has an identical mechanism of action, the sponsor expects no differences with respect to carcinogenic potential between sarilumab and tocilizumab.

Sarilumab is a fully human antibody specific for IL-6Rα that binds IL-6, and prevents IL 6 mediated signal transduction and downstream biological effects mediated by IL-6. IL-6 is a pleiotropic cytokine, which has a wide range of biological activities including modulation of the immune system, inflammation, hematopoiesis, neural development, reproduction, and bone metabolism. In addition, it has been reported that IL-6 is also involved in the induction of B-cell, T-cell, and astrocyte differentiation and the induction of acute phase proteins in hepatocytes, such as C-reactive protein (CRP). Serum levels of IL-6 are low or undetectable under normal physiological conditions. The production of IL-6 is regulated by several physiological factors like diet, exercise, and stress. IL-6 production by skeletal muscle increases 100-fold during physical activity and adipose tissues are another main source of IL-6.

IL-6 signaling involves a specific receptor (IL-6Rα) and a central, ubiquitous signal-transducing protein, glycoprotein 130 (gp130). A soluble form of the IL-6Rα receptor is also known to be expressed and can mediate IL-6 signaling. Signaling occurs through two receptor-activating pathways: 1) binding of IL-6 to membrane bound IL-6Rα on cells expressing both this component and gp130, and 2) binding of IL-6 to soluble IL-6Rα and subsequent binding to gp130 on cells lacking IL-6Rα and cell activation. On target cells, the complex of IL-6 and IL-6R associates with the signal-transducing membrane protein gp130, thereby inducing its dimerization and initiation of signaling. Glycoprotein 130 is expressed by all cells in the body, whereas IL-6Rα is mainly expressed by hepatocytes, neutrophils, monocytes/macrophages, and lymphocytes.

IL-6 activates several intracellular signaling pathways. Binding of IL-6 to its receptor activates the Janus family of kinases (JAK1, JAK2, and TYK2) bound to the cytoplasmic domain of gp130. These kinases phosphorylate signal transducer and activator of transcription (STAT)-3, promoting its nuclear transfer and transcriptional function. IL-6 also activates Ras and promotes its translocation to the plasma membrane where it activates Raf, mitogen activated protein kinase kinase (MEK), and MAP (Erk1/2). A third pathway activated by IL-6 is the phosphoinositol 3 kinase (PI3K)–protein kinase B (PkB/Akt) pathway, as JAK can phosphorylate PI3K.

Specific information from available literature and toxicology data to support the sponsor's rationale is included herein.

1) Activation of the Il-6 pathway in tumor development
   a) Activation of STAT3 in a tumor environment can contribute to tumor promotion either directly by activating tumor cells or indirectly by inactivating immune effector cells.
      i) STAT3 activation produces immunosuppressive factors that inhibit anti-tumor responses by the immune system.
      ii) Inhibition of STAT3 enhances the effector function of dendritic cells, T cells, NK cells, and neutrophils, and reduces the number of infiltrating Treg cells at the tumor site, thereby enhancing immune responses against the tumor.
      iii) STAT3 can be activated by growth factors, including epidermal growth factors, insulin-like growth factor, platelet-derived growth factor, and vascular endothelial growth factor receptor.
   b) IL-6 has been shown to be critical for the growth and differentiation of various hematopoietic cell lineages, and it is particularly important for B cell differentiation and generation of antibody-mediated immune responses.
      i) Mice overexpressing IL-6 develop massive polyclonal plasmacytosis and mesangial cell proliferation.
      ii) Many lymphomas and leukemias derived from hematopoietic lineages express IL-6Rα and are IL-6-responsive.
   c) IL-6 has been identified as a critical NF-κB-dependent pro-tumorigenic cytokine produced by lamina propria myeloid cells that, in turn, stimulates the survival and proliferation of premalignant intestinal epithelial cells in colitis-associated cancer.
   d) The production of IL-6 may lead to 'cancer cachexia', a wasting syndrome related to catabolism of proteins and lipids throughout the body.

2) Inhibition of the IL-6 pathway in tumor development
   a) Regression of tumor size was observed with treatment of nude mice with an anti-IL-6 mAb in mouse prostate cancer cell tumor xenograft models.
   b) IL-6 knockout mice are resistant to induction of tumors derived from a B-cell lineage. Also, IL-6 knockout mice did not form tumors in a model of mice predisposed to spontaneous astrocytoma formation.
   c) In a periportal model of liver injury using normal and IL-6 knockout mice, the proliferative response in hepatocytes and bile duct cells was lower in IL-6 knockout mice than normal mice, although overall repair of the injury was accomplished in the same time period in both groups.
   d) In another model, IL-6 knockout mice and treatment with anti-IL-6 receptor antibody have also been shown to have a lesser degree of metastatic liver tumor after intrasplenic administration of a lung carcinoma cell line, LLC.

3) Studies showed IL-6 had antitumor activity in some experimental animal models.
   a) Reduced tumorigenicity was observed in normal mice when injected with mouse fibroblasts transduced to constitutively secrete IL-6, but this reduced tumorigenicity was not seen with nude or irradiated mice, suggesting that IL-6 may mediate its anti-tumor effects via activation of immune cells not present in these animals.
   b) Melanoma cells transfected with human IL-6 DNA demonstrated slower tumor growth in vivo when compared to control animals.

4) Tumor xenograft growth assay using sarilumab

To assess effects of sarilumab on the growth of human tumor xenografts in immune-compromised mice and the activation of STAT3 in tumor cells, the sponsor conducted a human tumor xenograft assay. The effects of sarilumab on tumor growth were evaluated in Du145 (human prostate cancer) or NCI-H1650 (human lung carcinoma)-tumor bearing mice by measuring tumor sizes. Immunohistochemistry was conducted with tumor sections with antibodies against cleaved caspase3 (apoptosis), Ki67 (proliferation), or PECAM-1 (angiogenesis). The effect of sarilumab (REGN88) on the activation of STAT3 was also tested in tumor cells treated with REGN88 by western blot analysis using antibodies against phospho-STAT3. The results showed that treatment with REGN88 inhibited the growth of Du145 and NCI-H1650 tumor xenografts, compared to control mice. REGN88 caused increased apoptosis in the Du145 xenograft. Furthermore, REGN88 inhibited IL-6-induced STAT3 activation in cultured Du145 and NCI-H1650 tumor cells. Phospho-STAT3 levels were also reduced in Du145 tumor xenografts in mice treated with a single dose of REGN88, compared to control mice treated with hFc protein.

5) Findings from toxicity studies using sarilumab or REGN844

In the general toxicity studies (one 5-week IV, one 13-week IV, two 13-week SC, and one 6-month IV), a total of 54 male and female monkeys were treated with placebo and a total of 186 male and female monkeys were treated with sarilumab at doses ranging from 0.5 to 100 mg/kg/week. See Table 39. In an enhanced pre/postnatal developmental study (ePPND), an

additional 12 placebo-treated pregnant monkeys and 24 sarilumab-treated pregnant monkeys received intravenous administrations weekly from Gestation Day (GD) 20-22 through natural birth (~GD160-165). However, the F0 maternal monkeys in the ePPND study were not euthanized unless moribund, and thus, only three monkeys that were preterminally sacrificed or found dead were macroscopically examined in this study. Lastly, more than 220 CD-1 mice (excluding TK animals) treated with REGN844 were used in both a 4-week exploratory SC and IV toxicity study and a GLP SC fertility study.

Table 39 Total Animals/Dose in Toxicity Studies with sarilumab and REGN844a [taken directly from the Sponsor's carcinogenicity risk assessment position paper]

Sarilumab Toxicity Study Types	Dose (mg/kg/week)													
	0	0.5	1	2	5	10	15	20	25	30	40	50	100	200
Total Monkeys in General Toxicity Studies	54	12	12	12	22	42	12			12	10	24	28	
Total Monkeys in ePPND Study	12				12		12					12		
Total Mice in General Toxicity Study [b]	20					20			20			20		20
Total Mice in Fertility Study	48							48				48		48

a = monkeys were administered sarilumab and mice were administered REGN844
b = excluding mice for toxicokinetic determinations

In these nonclinical studies, there were 4 animals (3 monkeys and 1 mouse) with tumors. See Table 40. In addition, there were a number of incidences of hyperplasia in various organs (Tables 41-42), however, some occurred in the control animals. Overall, hyperplasia findings in these studies appear not test article-related, but incidental.

Table 40 Tumor Findings in Mouse and Monkey Studies

	Species	Duration/ Dosing Route	Group	Animal No.	Exposure
Adrenal gland					
Adenoma, cortex, unilateral	**Monkey**	**13-week SC (bridging)**	**HD-P1* (100 mg/kg/week)**	**Male no. 4703**	**(24 h after Dose # 24, week11) C_{max} of 4650 µg/mL; AUC_{0-24} of 101640 h*µg/mL)**
Hyperplasia, cortex	Monkey	6-month IV	HD (50 mg/week)	Male no. 3925	(5 min after Dose #25) C_{max} of 2929 µg/mL; AUC_{all} of 355 h*mg/mL
Cervix					

Papilloma, benign neoplasm	**Monkey**	**4-week IV**	**MD (10 mg/kg/week)**	**Female no. 2528**	**(Day 29, 5-min) C_{max} of 451 µg/mL**
Squamous metaplasia-moderate	**Monkey**	**13-week SC (bringing)**	**LD-P3* (10 mg/kg/week)**	**Female no. 4716**	**(0 h after Dose # 24, week11) C_{max} of 451 µg/mL; AUC_{0-24} of 7380 h*µg/mL)**
Squamous Metaplasia	**Mouse**	**Fertility SC (~ 3-5 weeks)**	**HD (200 mg/kg/week)**	**Female no. 183**	**NA**
Uterus					
Metaplasia, squamous	**Monkey**	**13-week SC (bridging)**	**LD-P3* (10 mg/kg/week)**	**Female no. 4716**	**(0 h after Dose # 24, week11) C_{max} of 451 µg/mL; AUC_{0-24} of 7380 h*µg/mL)**

Table 41 Summary of Hyperplasia in General Toxicity Studies with Sarilumab in Monkeys [taken directly from the Sponsor's carcinogenicity risk assessment position paper]

	Study				
	5-Week IV	13-Week IV	13-Week SC	13-Week SC Bridging**	26-Week IV
Dose (mg/kg/week)	0, 5, 10, 40	0, 1, 10, 50	0, 2, 10, 30, 100	0, 10, 100	0, 0.5, 5, 15, 50
Microscopic Diagnosis					
Adrenal gland, minimal					50 mkw: 1M
Cecum, mild	0 mkw: 1M				
Infusion site, minimal – moderate		1 mkw: 1F 50 mkw: 1M			0 mkw: 1F 5 mkw: 1F 50 mkw: 1M
Larynx, lymphoid, minimal			2 mkw: 1M		
Liver, bile duct, minimal				0 mkw: 1M	
Lung, bronchiole-alveolar, minimal	0 mkw: 1F			100 mkw: 1M	
Lung, lymphoid, minimal			2 mkw: 1M 10 mkw: 1F		
Lymph node, mandibular, minimal	0 mkw: 1M				
Lymph node, mesenteric, minimal	40 mkw: 1F				
Mammary gland, minimal-mild	5mkw: 1M	10mkw: 1F			
Nasal Cavity, lymphoid, minimal – mild				0 mkw: 1M 10 mkw: 1M 100 mkw: 1M	
Pancreas, islet cell, minimal		50 mkw: 1F	10 mkw:1M		
Pituitary, mild		50 mkw: 1M			
Seminal vesicle, mild	40mkw: 1M				
Skin, minimal					0 mkw: 1F
Spleen, lymphoid, minimal - mild	0 mkw: 1M 5 mkw: 1M, 3F 10 mkw: 1F 40 mkw: 1M	0 mkw: 1F 1 mkw: 1M, 1F 10 mkw: 1F		0 mkw: 1M, 1F 10 mkw: 2 M 100 mkw: 1 M, 1F	5 mkw: 1M
Thymus, cystic epithelial, minimal – mild				10 mkw: 1M, 1F 100 mkw: 1M, 1F	0 mkw: 2M 0.5 mkw: 1M, 1F 5 mkw: 1M, 1F 15 mkw: 3M, 2F 50 mkw: 1F

Values are number of monkeys with finding / sex
IV = Intravenous; SC = Subcutaneous; M=male; F = female; mkw = mg/kg/week
* = Because no incidence of hyperplasia were noted in the ePPND study; this study is not listed in table
** = dose of 100 mkw is combined listing of P1 and P3 groups

Table 42 Summary of Hyperplasia in Toxicity Studies with REGN844 (a mouse surrogate) in Mice [taken directly from the Sponsor's carcinogenicity risk assessment position paper]

	Study	
Microscopic Diagnosis	4-Week Exploratory SC & IV	SC Fertility
Dose (mg/kg/week)	0, 10, 50, 200 SC 25 IV	0, 20, 50, 200
Adrenal gland, subcapsular, cell type A, minimal	0 mkw: 2 F 200 mkw: 2 M, 2 F	
Kidney, tubular, simple, minimal	0 mkw: 5M, 2 F 200 mkw: 2 F	
Vagina, squamous epithelial cell, mild - moderate		20 mkw: 1 F 50 mkw: 2 F 200 mkw: 3 F

Values are number of mice with finding / sex
IV = Intravenous; SC = Subcutaneous; M = male, F = female
mkw = mg/kg/week

Specific neoplastic findings are addressed below.

Adrenal cortical adenoma:
An adrenal cortical adenoma was noted in the 3-month SC bridging toxicity monkey study comparing different cell lines, and the finding was noted in a single male in the 100 mg/kg/week group using the P1 process ((b) (4) formulation 1). Although there were no neoplastic findings in the adrenal glands of any other studies with sarilumab, hyperplasia was also observed in one male monkey in the 50 mg/kg/week group in the 6 month IV study. The presence of IL-6 and IL-6R has been noted in human adrenal gland by immunohistochemistry and polymerase chain reaction in the literature. Staining in the adrenal gland was noted in monkeys with sarilumab in the tissue cross-reactivity study; specifically, the staining was noted in the adrenal epithelium and adipocytes. No staining was noted with another antibody directed against IL-6R (Kato et al., 2009 Regul Tox and Pharm 53; 46-51).

The sponsor stated that adrenal cortical adenomas have been reported as one of the more common spontaneous neoplasms in cynomolgus monkeys with references of several published papers. In addition, the sponsor stated that based on personal communication with the conducting laboratory, as of June 2011, (b) (4) reported having at least 6 occurrences of this finding. Therefore, the sponsor considers the finding of adrenal cortical adenomas to be spontaneous and unrelated to sarilumab administration. Hyperplasia in the adrenal gland is also a background finding noted in cynomolgus monkeys (Ito Ta et al 1992. Exp. Anim. 41(4). 455-469), and the incidence in the 6-month study was, thus, considered not related to sarilumab.

As a follow-up, an Information Request was sent to the sponsor to provide historical control data for any tumor and preneoplastic findings in the adrenal gland of cynomolgus monkeys from the conducting laboratories (IR dated July 24, 2013). In the sponsor's response (dated October 1, 2013) to the IR, the sponsor elaborated on their earlier statement regarding the 6 occurrences of adrenal adenomas from (b) (4) and they also provided historical control data of tumors and hyperplastic findings in the adrenal gland of cynomolgus monkeys from four different CROs. In addition, the sponsor provided a summary table of adrenal pre- and neoplastic findings in monkeys from published papers. These papers were initially cited in the sponsor's carcinogenic risk assessment of sarilumab that was submitted on August 16, 2011.

Data regarding the 6 adrenal cortical adenomas were included in a recent poster that describes a total of 7 spontaneous adrenal cortical adenomas in cynomolgus monkeys from studies performed at the (b) (4). These 7 occurrences were from both control groups and test article-dosed groups (2 from control groups and 5 from test article-dosed groups). However, the lead author of the poster concluded that the 5 adenomas from the test article-dosed monkeys were highly unlikely to be related to test article as all occurrences of the adenomas from test article-dosed groups were from different studies using different test articles with ≤ 5 weeks of dosing duration.

A summary of adrenal gland tumors and hyperplastic findings in cynomolgus monkeys in the currently available CRO databases is shown in Table 43. Relatively high incidences of hyperplasia in the adrenal gland are noted in some sites (11% in (b) (4) and 2% in (b) (4)). Although the incidences of cortical adenomas are not high, several occurrences were observed at these CROs.

Table 43 Incidence of neoplastic and nonneoplastic proliferative findings in the adrenal gland cortex of cynomolgus monkeys from the historical control databases of four Contract Research Organizations (CROs) [taken directly from the document of Response to Agency Request, pp. 6, SD 331 submitted October 4, 2013]

Source	Date range for of data	Sex	Number of Cortical Adenoma Reported	Number of Cortical/Zona Fasciculata Hyperplasia Reported	Number of tissues/animals examined [a]
(b) (4)	2006-2011	M	0	1	182
		F	0	0	191
	2005-2013	M	0	4	1219
		F	1	4	1221
	1999-2010 [c]	M	1	0	401
		F	3	0	406
	2004-2010	Both	0	36	320
	2005-2012	M	0	1	128
		F	0	0	127
	2005-2012	M	0	28	1349
		F	1	19	1179

a = Some databases referred to number of tissues examined while other databases referred to the number of animals
b = US only and went back to 2005 for (b) (4) site
c = Available historical control database extract (b) (4) Database for Mauritius-origin monkeys is for 1999-2009 and for Asian-origin monkeys is for 2000-2010.
d = The incidence of adenoma, hyperplasia, and number of animals was derived using the values for the cynomolgus monkeys from Indonesia (adrenal left & right), Cambodia (adrenal, adrenal left & right), and China (adrenal, adrenal bilateral, adrenal left & right). For those monkey where hyperplasia was recorded in the right and left adrenal gland separately, it could not be ascertained how many monkeys may have had bilateral changes, and therefore, may have been counted twice.

The literature usually describes adrenal adenomas as a common tumor finding in cynomolgus monkeys. However, these reports raise some questions. For example, it is difficult to follow how some of these studies categorized data (e.g. spontaneous lesions from both control and dosed groups, or just control groups). Some studies also do not provide all essential information (e.g., the number of total monkeys evaluated). Thus, the findings in these published papers cannot serve as historical control data or background occurrence in the laboratory monkeys. However, several incidence reports are worth noting. From autopsy records of 157 breeders and untreated controls of cynomolgus monkeys in chemical carcinogenesis studies performed by the National Cancer Institute, spontaneous malignant tumors were observed in 3 monkeys (1.9%), and spontaneous benign tumors were observed in 2 (1.3%) monkeys (Takayama et al, 2008 Proc. Jpn Acad., Ser B 84(6):176-187). Endocrine tumors were frequently reported in macaques, and as in humans in which adrenal adenomas are found in 2% adult necropsies, most are nonsteroid producing (Lowenstine, LJ. Chapter 53: Neoplasms and proliferative disorders in nonhuman

primates. In Primates - the road to self-sustaining populations. Benirschke, K editor. Springer-Verlag, New York, NY. p.781-814, 1986). When spontaneous lesions were examined in 442 cynomolgus monkeys (caught in Indonesia, the Philippines, or Malaysia) used in 17 toxicity studies performed in (b) (4), nodular hyperplasia of zona fasicularis cells in the adrenal gland was observed in 10/221 (4.5%) male cynomolgus monkeys and 3/221 (1.4%) female cynomolgus monkeys (Ito Ta et al 1992. Exp. Anim. 41(4). 455-469). For cynomolgus monkeys, 1.25% of any neoplasms occurred between the ages of 1 and 4 years (see table below). The age of the monkey with adrenal cortical adenomas in the 13-week SC study was approximately 3 years old at the time of necropsy. These data may support that adrenal adenomas and hyperplasia observed in monkeys treated with sarilumab could be incidental findings.

TABLE 53.22. Effect of age on the incidence (%) of neoplasms in nonhuman primates in the literature.

COPYRIGHT MATERIAL WITHHELD:
Lowenstine, LJ. Chapter 53: Neoplasms and proliferative disorders in nonhuman primates. In Primates - the road to self-sustaining populations. Benirschke, K editor.Springer-Verlag, New York, NY. p.781-814, 1986

Historical control data from several different CROs show occurrences of adrenal cortical carcinomas in cynomolgus monkeys, but with relatively low incidences. The cortical adenoma seen with sarilumab was in the high-dose male, but the duration of the treatment was only 3 months. Even though adrenal cortical hyperplasia was also observed in another monkey treated with sarilumab, the incidence of the spontaneous adrenal hyperplasia could be high in cynomolgus monkeys. Collectively, based on evidence from the overall findings from sarilumab-treated animals, historical control databases from the CROs and findings described in the literature, the Reviewer considers that the findings in the adrenal gland are not related to sarilumab exposure.

Cervical Papilloma:
The second benign neoplasm was a papilloma of the cervix noted in one female at 10 mg/kg/week in the 5-week IV monkey toxicity study.

Cervical papillomas have been reported as common spontaneous neoplasms in cynomolgus monkeys (Cline JM et al., 2008 Tox Path 36;142S-163S). Similar to humans, the pathogenesis has been associated with the papillomavirus which has been demonstrated in monkeys (Wood et al., 2007 J. Virology 81:6339-45; Wood et al., 2004 Vet Pathol 41:108-115). Thus, the sponsor considers that papilloma was not related to sarilumab. In addition, one occurrence of mild, focal epithelial dysplasia was noted in the

cervix of one female at 1 mg/kg/week in the 13-week IV toxicity study. In a related fashion, the dysplasia in the cervical epithelium is a common finding (Cline JM et al., 2008 Tox Path 36;142S-163S; Wood et al., 2007 J. Virology 81:6339-45; Wood et al., 2004 Vet Pathol 41:108-115) and has been associated with papillomavirus. Therefore, the dysplasia finding was considered to be unrelated to sarilumab exposure.

As papillomarvirus-associated cervical dysplasia and papillomas have been occasionally encountered in cynomolgus monkeys, and the findings of cervical dysplasia and papillomas have not been observed in monkeys that received higher doses of sarilumab and/or longer durations of dosing, these cervical findings are considered incidental by the Reviewer.

Squamous metaplasia in the cervix and uterus:
Squamous metaplasia was observed in the cervix and uterus of one female monkey at 10 mg/kg/week in the 13-week SC bridging toxicity study, which the sponsor considers to be normal variation in tissue morphology associated with the estrous cycle.

Although diethylstilbestrol (DES)-induced squamous metaplasia of the cervix and uterus has been well-documented in the literature, squamous metaplasia of the endocervical glands is a common incidental finding in peripubertal monkeys. Because peripubertal animals are in a state of relative estrogen excess normally, some degree of cervical squamous metaplasia in 2- to 4-year-old monkeys is considered a normal finding (Cline JM et al., 2008 Tox Path 36;142S-163S). The age of the monkey with squamous metaplasia in the cervix and uterus in the 13-week SC study was approximately 3 years old at the time of necropsy, and thus, was peripubertal.

Squamous cell metaplasia was also observed in the cervix of one female mouse in the high dose group in the fertility study, and this mouse also had squamous cell hyperplasia in the vagina. This mouse was pregnant, but had only one implantation site and it was resorbed early. In addition to this mouse, five other mice dosed with REGN844 in the fertility study also had squamous cell hyperplasia in the vagina, and none of these mice were pregnant. The sponsor considers all these findings are also normal variation with the estrous cycle.

Pregnant animals have vaginal morphology that is thin and mucified. Nonpregnant animals have very different vaginal morphology (significantly thicker, especially if the animal is in proestrus or estrus). This is particularly true in the mouse, where thick squamous epithelium is a very common appearance throughout most of the normal cycle [Reviewer's personal communication with (b) (4)]. As the mouse with squamous cell metaplasia had early resorption, this mouse would have returned to or would be returning to normal cycling. Thus, the relatively thick epithelium in the vagina and cervix of this mouse probably reflected a synchronized return to normal cycling. Therefore, the Reviewer considers these findings to be incidental.

Other findings noted in monkey and mouse studies:
Neutrophil counts were decreased, often in a non-dose-dependent manner in most of the monkey studies. Additionally, decreased levels of fibrinogen were also observed in most of the monkey studies.

Decreased levels of CRP were observed during the first several weeks of the dosing period in most of the monkey studies. Serum levels of IL-6 were measured in the 6-month monkey study and markedly increased levels were seen in all test article-dosed groups; however, the levels declined to the baseline during the recovery period.

Immunophenotyping by flow cytometry was evaluated in monkeys in the 26-week IV toxicity study and in the ePPND toxicity study. Immnunophenotyping results indicated no apparent test article-related changes. TDAR was also evaluated in the 6-month monkey study, and slightly, but statistically significant decreases in primary and secondary IgG responses to KLH administration were noted in males and females at ≥5 mg/kg/week. The decreases were not generally dose-dependent.

Reviewer's summary and recommendation: The available literature data imply that IL 6 interacts with several pathways that contribute to its pro-tumorigenic activity due to its multiple effects on tumor cell proliferation and survival, angiogenesis, and inflammation. However, the literature data also show that IL-6 has anti-tumorigenic activity as well. The sponsor showed anti-tumorigenic effects of sarilumab in human tumor xenografts (Du145 or NCI-H1650) in mice. Although several incidences of tumors occurred in monkey and mouse studies using sarilumab and REGN844, respectively, the sponsor considers these findings to be not test article-related, and the Reviewer agrees with the sponsor's rationale and conclusion. Thus, no additional nonclinical studies are recommended to evaluate carcinogenic potential of sarilumab. However, the potential carcinogenic role of IL-6 in the endocrine glands and female reproductive tracts cannot be ruled out, as IL-6 is produced in the endocrine glands (such as the adrenal cortex) and elevated IL-6 levels have been reported in DES-induced vaginal metaplasia in animal studies. Therefore, the sponsor should manage any potential carcinogenic risks in patients by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches.

The sponsor's plan for carcinogenic risk assessment of sarilumab was discussed with Executive Carcinogenicity Assessment Committee (Exec CAC) members via emails and the Exec CAC members agreed with the Reviewer's recommendation. A response to the sponsor's proposed carcinogenic risk plan was as follows:

Carcinogenicity Assessment:
Question 13: The Sponsor conducted a weight-of-evidence based carcinogenicity risk assessment based on results from toxicology studies with sarilumab and REGN844 (the fully-murine surrogate against mouse IL-6R), effects of sarilumab in antitumor pharmacology models, and a review of published literature following IL-6R activation and inhibition. Does the Agency agree that, given the available data with sarilumab and data in the literature on IL-6 inhibition, no additional in vitro and in vivo studies (e.g., in vitro cell-based assays, xenograft models, tumor promotion models, 2-year bioassays) with sarilumab are necessary for registration?

Response: We agree that no additional nonclinical studies are needed to address the carcinogenic potential of sarilumab. We acknowledge your statement in the briefing

package that any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion are better managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. This appears to be a reasonable approach, and you should provide an appropriate proposal. The carcinogenic risk to humans for the chronic use of sarilumab in product label should include a balanced description of the literature information available to address the carcinogenic potential of sarilumab.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: SAR153191 (REGN88): Subcutaneous Fertility Study in Mice with REGN844

Study no.:	REGN844-TX-09048 or FER0480
Study report location:	EDR
Conducting laboratory and location:	sanofi-aventis U.S. Inc. Disposition, Safety, and Animal Research 1041 Route 202-206 Bridgewater, NJ 08807 USA
Date of study initiation:	March 10, 2010
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	REGN844 (mouse surrogate antibody for REGN88); lot # T09001D600X11A; 98.3% purity by SEC-HPLC

Key Study Findings

- In a subcutaneous study of fertility and early embryonic development to implantation in mice, male and female mice (24/sex/group) were administered REGN844 twice weekly at doses of 0 (placebo control), 10 (LD), 25 (MD), and 100 (HD) mg/kg/dose. The dosing period encompassed 4 weeks prior to mating and continuously until each animal's necropsy for males and 2 weeks prior to mating and through Gestation Day 7 for females.
- REGN844 concentrations increased with dose in males and females.
- There was no test article-related parental toxicity.
- There were no test article-related effects on reproductive and fertility parameters examined in the study, including number of corpora lutea, implantation sites, resorption and viable fetuses. However, estrous cycle observation was not included in the study.

Methods

Doses:	0 (REGN844 placebo), 10, 25, and 100 mg/kg/dose
Frequency of dosing:	twice weekly
Dose volume:	5 mL/kg
Route of administration:	Subcutaneous injection
Formulation/Vehicle:	REGN844 placebo was used as a diluent for REGN844 stock solution to make dose formulations for Groups 2-4.
Species/Strain:	CD-1(ICR) mice
Number/Sex/Group:	24
Satellite groups:	None
Study design:	Males were dosed for 4 weeks prior to cohabitation, during the cohabitation period, and until each animal's necropsy. Females were dosed 2 weeks prior to cohabitation, during cohabitation, and until Gestation Day (GD7). The cohabitation period occurred for up to a maximum of 14 days. The day on which evidence of mating was observed was designated as GD0.
Deviation from study protocol:	The protocol, amendments, and deviations (except one deviation) were not included in the study report. The study report states that there were no deviations that were judged to have had any significant impact on the validity or interpretation of the data. The only deviation included in the appendix of the formulation analysis phase report detailed samples for dosing formulation analysis that were analyzed 2 days after receipt of the samples at the conducting laboratory, instead of on the day of receipt. The samples were stored frozen and frozen stability and 2 cycles of freeze-thaw stability had been established previously. There were no apparent major issues in the study and thus, the protocol, amendments, and deviations were not requested from the sponsor.

Observations and Results

Mortality

Viability was checked at least twice daily.

Three mice were found dead during the study (one male [Animal No. 13] in the control group on Day 36 [post-cohabitation], one male at HD [Animal No. 89] on Day 17 [pre-cohabitation], and one female mouse at LD [Animal No. 130] on Day 14 [pre-cohabitation]).

The macroscopic examination showed that the male in the HD group had gelatinous, dark, red material in the thoracic body cavity, however the causes of all three deaths were not determined. As a single death in the high dose group occurred relatively early during the dosing period and a death also occurred in the control group, the deaths in the REGN844-treated groups were not considered test article-related.

Clinical Signs
Clinical observations were performed at least twice on dosing days (predose and 1-2 hours postdose) and once daily on nondosing days.

There were no test article-related clinical signs.

Body Weight

For males, body weights were measured twice weekly and prior to scheduled necropsy. For presumed pregnant females, body weights were measured twice weekly during the premating period (including the first day of cohabitation) and presumed GDs 0, 4, 8, and 14, and for females with no evidence of mating, body weights were measured twice weekly during the premating period and contining twice weekly after the completion of the cohabitation period.

There were no test article-related changes in mean body weight or body weight gain.

Feed Consumption

Food consumption was measured on Days 3, 7, and twice weekly thereafter, except during cohabitation. For females with no evidence of mating, food consumption was not measured after the cohabitation period.

There were no test article-related changes in mean food consumption.

A slight increase in mean food consumption in all dose groups during GDs 0-4 (11.9%, 10.2%, and 9.5% in the LD, MD, and HD groups relative to the control group) was not considered test article-related since the increase was observed transiently.

Toxicokinetics

Blood samples for toxicokinetic evaluation were collected from all female mice on GD 14 and the first 10 male mice per group prior to scheduled necropsy (2-3 hours postdose).

REGN844 concentrations in mouse serum were measured using a validated ELISA. The lower limit of quantitation (LLOQ) of REGN844 was 1.56 ng/mL in the assay and 78 ng/mL in neat mouse serum.

Mean serum concentrations of REGN844 at 2-3 hour after the last dose in males and approximately 7 days after the last dose in females are shown in Table 44. In male mice, REGN844 concentrations increased in an approximately dose-proportional manner. In female mice, exposure was greater than dose-proportional from 25 mg/kg/dose to 100 mg/kg/dose. In the 10 mg/kg/dose group, the majority of samples did not have measurable

levels of REGN844 on GD14. Measured exposure values were lower in females as samples were collected 7 days after the last dose in females (vs. 2-4 hours after the last dose in males).

Table 44 Mean REGN844 Serum Concentrations (µg/mL) from the fertility mouse study [taken directly from the study report, pp. 30]

	Group 1: 0 mg/kg/dose	Group 2: 10 mg/kg/dose	Group 3: 25 mg/kg/dose	Group 4: 100 mg/kg/dose
		Male Mice		
Mean±SD	BLQ	184±48.4	600±115	2187±291
		Female Mice		
Mean±SD	BLQ	BLQ	9.16±7.29	60.6*± 28.2*

BLQ = Below Limit of Quantification (<0.0780 µg/mL)
*values exclude results from apparent outlier

Dosing Solution Analysis

Placebo and REGN844 dose solutions were prepared on the day of test article administration and used within 6 hours of formulation completion. Samples from each concentration were collected three times (Weeks 1, 5 and 8) and analyzed for protein concentration by a validated UV spectrophotometric assay.

The analyzed concentrations were within 90% to 110% of the nominal concentration.

Necropsy

Males were sacrificed following completion of a majority of female cesarean sections. Females with confirmed mating were sacrificed on GD14 and females with no evidence of mating were sacrificed 15 days following the completion of the cohabitation period or sooner if the female appeared pregnant.

A necropsy (abdominal and thoracic cavities) was performed and macroscopic observations were recorded for all animals found dead or euthanized during the course of the study, and for all those surviving to scheduled necropsy.

The following organs were weighed: epididymides, testes, and prostate gland and seminal vesicle (prostate gland and seminal vesicle were weighed and reported together).

Microscopic examinations were performed on the following tissues: testes and epididymides for males, and ovaries, oviducts, uterus, cervix, and vagina for females. These tissues from all mice found dead or preterminally sacrificed were examined. In addition, the tissues from control and high dose males were also examined, whereas the tissues from all females in all dose groups were examined.

The pregnancy status of apparently non-gravid animals (no visible implantation sites present) was verified by staining of the uterus with 10% ammonium sulfide.

There were no test article-related effects on organ weights, and no test article-related macroscopic findings.

The study report stated that microscopic observations in the ovary and vagina were generally consistent with the gravid or nongravid state. Squamous cell metaplasia was observed in the cervix of one female mouse in the high dose group, and this mouse also had squamous cell hyperplasia in the vagina (Table 45). This mouse was pregnant, but had only one implantation site and it was resorbed early. In addition to this mouse, five other mice dosed with REGN844 in the fertility study also had squamous cell hyperplasia in the vagina, and none of these mice were pregnant. The sponsor considered all these findings normal variations of the estrous cycle.

Pregnant animals have vaginal morphology that is thin and mucified. Nonpregnant animals have very different vaginal morphology (significantly thicker, especially if the animal is in proestrus or estrus). This is particularly true in the mouse, where thick squamous epithelium is a very common appearance throughout most of the normal cycle [Reviewer's personal communication with (b) (4)]. As the mouse with squamous cell metaplasia had early resorption, this mouse would have returned to or would be returning to normal cycling. Thus, the relatively thick epithelium in the vagina and cervix of this mouse probably reflected a synchronized return to normal cycling. Therefore, the Reviewer considers these findings to be incidental.

Table 45 Histopathology Findings from the fertility study in the female mice

	Females			
	0 (control)	10 mg/kg/dose	25 mg/kg/dose	100 mg/kg/dose
Uterus				
Degeneration: implantation site	1/23	0/22	2/19	6/20
Cervix				
Metaplasia, squamous cell, mild	0/23	0/22	0/19	1/20*
Vagina				
Hyperplasia, squamous cell	0/24	1/24	2/24	3/21
Mild	0	1	2	1
moderate	0	0	0	2

*The female also had squamous cell hyperplasia in the vagina.

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

Mating/fertility indices, number of corpora lutea, implantations, resorptions, live and dead fetuses, and placental anomalies (only recorded if abnormal) were examined. Estrous cycle observation was not included in the study.

There were no test article-related effects on any fertility parameters examined in the study.

Table 46 Cesarean section data from the fertility mouse study

Parameter	0 (control)	10 mg/kg/dose	25 mg/kg/dose	100 mg/kg/dose
Mating Index (%)[a]	100 (24/24)	100 (23/23)	100 (24/24)	100 (24/24)
Fertility Index (%)[b]	100 (24/24)	95.7 (22/23)	91.7 (22/24)	91.7 (22/24)
No. of pregnant rats	24	22	22	22
Mean no. of days to mate	3.4	3.3	2.7	2.0
No. of litters with no live fetuses	0	0	0	1
Litter mean no. of corpora lutea [c]	13.8	13.5	14.7	13.7
Litter mean no. of implantation [c]	13.5	13.3	14.5	13.6
Litter mean no. of early resorption [c]	0.5	0.9	0.5	0.3
Litter mean no. of late resorption [c]	0.0	0.0	0.0	0.0
Litter mean no. of dead fetuses[c]	0.0	0.0	0.1	0.1
Litter mean no. of live fetuses[c]	13.1	12.4	13.9	13.2
Preimplantation loss [c] (litter mean % ± S.D.)	1.5 ± 3.3	1.4 ± 2.9	0.9 ± 2.2	0.3 ± 1.4
Postimplantation loss [c] (litter mean % ± S.D.)	3.0 ± 5.0	6.8 ± 11.0	4.2 ± 6.2	7.4 ± 21.1

[a]Mating Index (%) = (No. of pairs mated/No. of pairs cohabited)
[b]Fertility Index (%) = (No. of pregnant females/No. of mated females)
[c]including litter with no live fetuses

9.2 Enhanced Pre- and Postnatal Toxicity (ePPND) Study in NHPs

Study title: Study of the Effects of REGN88 on Embryo-Fetal Development and Pre- and Post-Natal Development When Administered Weekly by Intravenous Infusion to Pregnant Cynomolgus Monkeys

Study no.:	(b) (4).223.33 (Sponsor Reference no. REGN88-TX-08030)
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 16, 2008
GLP compliance:	Yes, except for the long-term stability data for hormone samples (estradiol, progesterone, and prolactin) and anti-drug antibody samples
QA statement:	Yes
Drug, lot #, and % purity:	REGN88, lot # K08003D600A12A, 97.7% purity by SE-HPLC

Key Study Findings

- Groups of 12 pregnant monkeys were intravenously administered REGN88 once weekly at doses of 0 (placebo control), 5 (LD), 15 (MD), or 50 (HD) mg/kg/week from gestation Day (GD) 20 through natural delivery (approximately GD 165), with subsequent maternal and offspring monitoring to approximately 28 days after delivery.
- There was no test article-related mortality.
- In maternal animals, there were decreased mean counts of lymphocyte, neutrophils, and white blood cells in all test article-dosed groups in a non-dose-related manner on GD 153 and/or LD 7. Slightly decreased mean levels of fibrinogen were also observed in all test article-dosed groups in a non-dose-related manner on GD 153, LD -7, and/or LD 30. These changes were similar to what have been observed in general toxicity studies in monkeys administered REGN88. Decreases in neutrophil counts and fibrinogen levels were consistently observed in general toxicity studies, but changes in lymphocyte and white blood cell counts were not consistent across the general toxicity studies, and thus, the toxicological significance of decreased counts of lymphocyte and white blood cells in maternal animals in this study is still unclear.
- In F1 neonates, there were no test article-related effects on birth examinations, clinical observation, mean body weights, functional and morphological development, and skeletal examination. No test article-related effects were noted on serum chemistry and immunophenotyping of peripheral lymphocytes. Decreased mean counts of lymphocytes (-34%) and white blood cells (-29%) were observed in the HD group on DB 30 (+/-2 days), compared to the control group. However, the values

exhibited large individual variations within the groups and thus, the toxicological significance of these changes was unknown. There were no test article-related changes in mean neutrophil counts and fibrinogen levels.

- Exposure to REGN88 increased with dose in maternal and F1 animals. REGN88 concentrations were still present in the F1 neonate animals on the day of their scheduled sacrifice.
- There were some concerns about the adequacy of this ePPND study. First, numbers of neonates in each group at scheduled necropsy were relatively small (3 males and 3 females at the control; 1 male and 6 females at LD; 2 males and 3 females at MD; 2 males and 2 females at HD). Second, immune function testing (e.g., TDAR) in the offspring during the postnatal phase was not conducted although REGN88 has been shown to be immunosuppressive in adult monkeys (e.g., decreased neutrophil counts, decreased effects in the TDAR assay in the 6-month study) as well as in adult humans. Lastly, REGN88 was not totally cleared from the circulation of F1 offspring at the time of necropsy (around postnatal day 30), and thus the drug may have uncharacterized effects beyond the one month of age in monkeys.

Methods

Doses:	0 (REGN88 Placebo), 5 (LD), 15 (MD) and 50 (HD) mg/kg/week
Frequency of dosing:	Once weekly
Dose volume:	5 mL/kg
Route of administration:	Intravenous infusion for ~ 30 minutes (±10 min)
Formulation/Vehicle:	Supplied placebo stock solution (sterile aqueous buffered vehicle, containing 6% sucrose, 0.13% polysorbate 20, and 10 mM histidine, pH 6.0) was diluted with sterile normal saline (0.9% NaCl) solution in the same ratio as test article administered to the HD group
Species/Strain:	Cynomolgus Monkeys (obtained through (b) (4)) Pregnant animals, a total of 48 assigned that weighed 2.050 kg to 3.334 kg and were 4.2 to 5.9 years old on GD 1.
Number/Sex/Group:	12 pregnant monkeys
Satellite groups:	None
Study design:	Dosing was initiated on GD 20 and maintained through the end of the gestation period (either natural delivery, approximately GD 160, or abortion/embryo-fetal death). All maternal animals were allowed to deliver naturally (except for two females that received emergency cesarean section) and were examined for 28 to 32 days after delivery. The middle day of the 3- or 5-day mating session, the day of natural

	delivery (pregnant monkeys), and the day of birth (F1 neonates) were designated GD 0 (Gestation Day 0), LD 0 (Lactation Day 0), and DB 0 (Day 0 after birth), respectively.
Deviation from study protocol:	There were no deviations that affected the integrity of the study.

Dosage Justification

The dosages used in this study were based on the results of a previously conducted 13-week IV study in cynomolgus monkeys. The high dose of 50 mg/kg/week was anticipated to show minimal adverse effects and provide an adequate multiple of the projected human therapeutic exposure. The middle and low doses were selected as 15 and 5 mg/kg/week, respectively, to assess dose-response.

Observations and Results

Mortality

Viability was checked twice daily.

There was no test article-related mortality.

One maternal animal each from the control (Animal No. 1), 5 mg/kg/week (Animal No. 31), and 50 mg/kg/week (Animal No. 81) groups was euthanized in moribund condition or died after delivery.

Clinical Signs

Clinical observations were performed twice daily, and pregnancy status via ultrasound was performed on GDs 25, 30, 37, 44, 51, 65, 79, 93, 107, 121, 135, and 149 ±1 day; and as needed.

There were no test article-related abnormal clinical observations in maternal animals.

Body Weight

Body weight was measured weekly during the gestation period and once between LDs 0 and 3 and on LDs 8, 15, and 22 (±1 day) and on the day of necropsy of their offspring.

There were no test article-related changes in mean body weights in maternal animals.

Feed Consumption

Food consumption was qualitatively measured weekly.

Hematology

Blood samples for hematology and coagulation evaluation were collected at predose on GD 20 and GD 153, once on LD 7, and once on LD 28-32 for maternal animals and once at necropsy for F1 neonates.

In maternal animals, there were decreased mean counts of lymphocytes, neutrophils, and white blood cells in all test article-dosed groups in a non-dose-related manner on GD 153 and/or LD 7. Slightly decreased mean levels of fibrinogen were also observed in all test article-dosed groups in a non-dose-related manner on GD 153, LD -7, and/or LD 30. These changes were consistent with what was observed in general toxicity studies in monkeys administered REGN88.

Table 47 Changes in the hematology parameters in maternal animals

Parameter \ Dose Group (mg/kg/wk)		Group 1	Group 2	Group 3	Group 4
		0	5	15	50
Abs. lymphocytes	GD 20	6.98 ± 3.0	5.63 ± 1.5 (-19%)	6.19 ± 2.2 (-11%)	5.94 ± 2.4 (-15%)
	GD 153	4.53 ± 1.6	3.02 ± 0.9 (-33%)	2.84 ± 0.5 (-37%)	3.01 ± 0.8 (-34%)
	LD 7	3.49 ±1.7	2.96 ± 1.1 (-15%)	2.66 ± 0.8 (-24%)	2.74 ± 0.5 (-21%)
	LD 30	3.8 ± 1.8	3.4 ± 1.7 (-11%)	3.12 ± 1.2 (-18%)	3.18 ± 0.7 (-16%)
Abs. neutrophils	GD 20	6.17 ± 1.7	7.09 ± 1.8 (+15%)	7.1 ± 2.6 (+15%)	6.93 ± 1.8 (+12%)
	GD 153	5.85 ± 1.2	4.77 ± 2.4 (-18%)	4.74 ± 2.7 (-19%)	4.52 ± 0.8 (-23%)
	LD 7	3.65 ± 1.4	3.17 ± 2.1 (-13%)	2.34 ± 1.1 (-36%)	3.14 ± 1.5 (-14%)
	LD 30	2.63 ± 1.2	2.72 ± 1.4 (+3%)	2.64 ± 2.2 (+0.4%)	4.15 ± 2.9 (+58%)
White blood cells	GD 20	14.15 ± 4.0	13.74 ± 2.6 (-3%)	14.12 ± 3.5 (-0.2%)	13.89 ± 3.3 (+2%)
	GD 153	11.6 ± 2.5	8.98 ± 3.2 (-23%)	8.51 ± 3 (-27%)	8.56 ± 1.3 (-26%)
	LD 7	7.65 ± 2.9	6.74 ± 1.8 (-12%)	5.49 ± 1.8 (-28%)	6.45 ± 1.3 (-16%)
	LD 30	7.06 ± 2.8	6.71 ± 3.0 (-5%)	6.19 ± 3.2 (-12%)	8.07 ± 3.2 (+14%)
Fibrinogen	GD 20	285 ± 35	282 ± 37 (-1%)	297 ± 56 (+4%)	298 ± 50 (+5%)
	GD 153	339 ± 32	329 ± 32 (-3%)	306 ± 70 (-10%)	273 ± 41 (-19%)
	LD 7	258 ± 28	182 ± 34	209 ± 24	210 ± 14

			(-29%)	(-19%)	(-19%)
	LD 30	235 ± 47	210 ± 54 (-11%)	204 ± 41 (-13%)	190 ± 13 (-19%)

In F1 neonates, decreased mean counts of lymphocytes (-34%) and white blood cells (-29%) were observed in the HD group on DB 30 (+/-2 days), compared to the control group (Table 48). However, the values exhibited large individual variations within the groups and thus, these changes were not considered test article-related. In addition, there were no test article-related changes in mean neutrophil counts and fibrinogen levels.

Table 48 Changes in the hematology parameters [mean ± SD] in F1 neonates on DB 30 [+/-2 days]

Dose Group (mg/kg) Parameter	Group 1 0	Group 2 5	Group 3 15	Group 4 50
	N= 6	N = 5	N = 4	N = 4
Abs. lymphocytes	4.8 ± 1.8	4.17 ± 1.9 (-13%)	5.05 ± 2.2 (+5%)	3.17 ± 0.9 (-34%)
Abs. neutrophils	0.99 ± 0.7	1.12 ± 0.7 (+13%)	0.89 ± 0.6 (-10%)	0.9 ± 0.4 (-9%)
White blood cells	6.33 ± 2.6	5.63 ± 2.8 (-11%)	6.34 ± 3.2 (+0.2%)	4.52 ± 1.2 (-29%)
Fibrinogen	163 ± 27	181 ± 10 (+11%)	162 ± 24 (-0.6%)	208 ± 50 (+28%)

Serum Chemistry

Blood samples for serum chemistry evaluation were collected at predose on GD 20 and GD 153, once on LD 7, and once on LD 28-32 for maternal animals, and once at necropsy for F1 neonates.

There were no apparent test article-related effects on serum chemistry parameters in maternal and F1 neonate animals.

Changes seen in this study were considered incidental because there was large individual variability.

Immunophenotyping by Flow Cytometry

Blood samples for immunophenotyping evaluation were collected at predose on GD 20 and GD 153, once on LD 7, and once on the day of each F1 neonate's necropsy for maternal animals and once at necropsy for F1 neonates. Immunophenotyping analysis was performed on all samples using antibodies against CD3, CD4, CD8, CD16, and CD20 to measure the relative percentages of lymphocytes.

There were no test article-related effects on immunophenotyping evaluation in maternal animals.

There were no test article-related effects on immunophenotyping evaluation in F1 neonates. B-cells counts (absolute and relative %) were apparently lower in the test article-dosed groups than in the control group (Table 49). However, the changes were not considered test article-related because there were large individual variations within the groups and the changes were not dose-related.

Table 49 Immunophenotyping analysis in F1 neonates on DB 30

Dose group (mg/kg) / Parameter	Group 1 0	Group 2 5	Group 3 15	Group 4 50
	N = 6	N = 7	N = 4	N = 4
% of CD3-CD20+ lymphocytes	31.81	17.96 (-44%)	21.45 (-33%)	19.13 (-40%)
Abs counts of CD3-CD20+ lymphocyte cells (10^3/µL)	1.49	0.75 (-50%)	1.15 (-23%)	0.64 (-57%)

Hormone Assay

Blood samples for hormone assays were collected at predose on GDs 20, 48, 76, 104, 132, and/or 160.

There are no apparent test article-related effects, but data showed huge individual variations.

Toxicokinetics

For evaluation of drug levels or toxicokinetic profiling, blood samples from maternal animals were collected at predose, at the end of infusion [within 5 minutes post-infusion], and 24 and 168 [prior to the next weekly dose] hrs postdose on GDs 20, 41, 97, and 146; once on LD 7 [±1 day], and once on the day of necropsy of each respective F1 neonate. For the anti-drug antibody (ADA) analyses, blood samples from maternal animals were collected at predose on GDs 20, 41, 97, and 146, once on LD 7 (±1 day), and once on the day of necropsy for each respective F1 neonate. Blood samples from F1 neonates were collected on DBs 7 and on the day of necropsy for REGN88 concentration and the presence of ADAs. Milk samples were also collected once between LDs 25 and 32, but not analyzed.

Total REGN88 concentrations in monkey serum were measured using a validated ELISA, which was capable of detecting both free and bound forms of REGN88. The lower limit of quantitation (LLOQ) was 3.13 ng/mL of REGN88 in the assay (2% monkey serum) and 156.5 ng/mL in neat serum.

Anti-drug antibodies (ADAs) in monkey serum were detected using a validated bridging

Immunoassay, which was a non-quantitative, titer-based assay that used a floating cutpoint to determine positive response levels in samples, based on a negative control. Three positive quality controls (PQCs) were included in each assay run to monitor the performance and acceptability of the assay. The PQC was REGN593, a mouse anti-human IgG, CH1 domain specific, monoclonal antibody. The lower limit of detection (LLOD) using the mouse anti-human IgG positive control was about 26.9 ng/mL in the absence of REGN88 and 123.8 ng/mL in the presence of 20 µg/mL of REGN88.

Maternal animals:
REGN88 was not detected in any of the predose samples (GD 20 predose) from maternal animals in test article-dosed groups, but REGN88 was detected in 3 animals in the control group. Two animals (Animal Nos. 3 and 7) had detectable levels of REGN88 at a single time point (148 µg/mL at 24 hr on GD 42 and 2.91 µg/mL on LD 30, respectively). The third animal (Animal No. 11) exhibited circulating levels of REGN88 at multiple time points starting from GD 97 (420 µg/mL prior to dosing on GD 97). The peak REGN88 concentration in this animal was GD 98 (426 µg/mL at 24 hours after dosing on GD 97) and decreased gradually, suggesting that this animal was dosed in error with REGN88 at least once between GDs 48 and 90. The study report stated that this animal was included in the control group because all data from this animal were comparable to those of other control group animals.

All animals in test article-dosed groups had detectable concentrations of REGN88 at all time points evaluated, with the exception of one animal in the 5 mg/kg group on GD 42. Because of one isolated case of non-detectable level in this animal, it was likely due to a sampling error.

Following the first dose administration of REGN88, mean $AUC_{0\text{-}168h}$ in maternal animals increased in a dose-proportional manner (Table 51). There was a trend of accumulation in all test article-dosed groups over the dosing period, but the magnitude of accumulation was slight.

Table 50 Mean serum concentration of REGN88 (µg/mL) in maternal animals

Dose Number	Group 2 5 mg/kg	Group 3 15 mg/kg	Group 4 50 mg/kg
GD 20, 5 min	167	495	1627
GD 146, 5 min	309	851	3040
LD 7	66.9	311	1389
LD 30	15.7	154	482

Table 51 Mean AUC_{0-168h} of REGN88 in maternal animals [taken directly from the study report, pp. 61]

Dose Number	Group 2 5 mg/kg/week (h*µg/mL)	Group 3 15 mg/kg/week (h*µg/mL)	Group 4 50 mg/kg/week (h*µg/mL)
1 (GD20)	14750 ±2882	42491 ± 11712	141864 ± 22721
4 (GD41)	22885 ±7775	81588 ± 19764	287524 ± 35363
12 (GD97)	35554 ±8699	110616 ± 17911	354389 ± 58057
19 (GD146)	37260 ±7643	124845 ± 30236	396455 ± 62297

ADAs were not detected among the control maternal animals, but were detected in four maternal animals (3 and 1 animals in the 5 and 15 mg/kg dose groups, respectively) prior to the first dosing of REGN88. For three animals, the positive ADA response was transient, as samples from these animals were negative at the other time points examined. Positive ADA response prior to REGN88 administration appeared to have no effect on serum REGN88 concentration after REGN88 infusion. One animal in the 5 mg/kg group (Animal No. 35) was ADA positive on GDs 20 (prior to dosing) and 38 (post-abortion sample). Serum REGN88 concentration in this individual was comparable to those in animals that were ADA negative in the 5 mg/kg group. Two other animals in the 5 mg/kg group (Animal Nos. 37 and 39) exhibited a positive ADA response following REGN88 administration. In Animal No. 37, a positive ADA response was observed on GDs 41 and 97, but not at remaining time points. Animal No. 39 had a positive ADA response on GD 27, the day that the animal was removed from the study due to abortion. The presence of ADAs in these two monkeys did not affect serum REGN88 concentrations.

F1 neonates:
All serum samples collected from the F1 neonates in the test article-dosed groups had measurable concentrations of REGN88 at the time points examined. Mean concentrations in neonates increased with dose, and particularly, the increase was greater than dose-proportional between 5 and 15 mg/kg groups. Similar concentrations were present in serum samples from both F1 neonates and their mothers on DBs 7 and 30.

Table 52 Mean serum concentration of REGN88 in F1 neonates [taken directly from the study report, pp. 62]

Dose Number	Group 2 5 mg/kg/week (µg/mL)	Group 3 15 mg/kg/week (µg/mL)	Group 4 50 mg/kg/week (µg/mL)
DB7	54.7 ±40.9	521 ±179	1435 ±630
DB30	9.18 ±7.58	129 ±32.0	339 ±215

ADAs were not detected in serum samples obtained from the F1 neonates, except one neonate in the control group. This neonate had a positive ADA response on DB 7, but not on DB 30. The maternal animal of this neonate did not exhibit a positive ADA response at any time points evaluated.

Dosing Solution Analysis

On the first day of dosing for the first animal in each group, duplicate 1 mL samples were collected from the top, middle, and bottom of each dose formulation container and used for nominal protein concentration and homogeneity analyses. Subsequently, samples for protein concentration were collected every 3 - 5 weeks from the middle portion of each container. In addition, triplicate samples were collected once prior to initiation of dosing. One set of the triplicate samples was analyzed immediately after preparation. The second and third sets of samples were analyzed after maintenance at ambient temperature for approximately 8 hours. Samples were analyzed by a validated UV spectrophotometric method.

REGN88 was not detected in any control samples. The analyzed concentrations were within 90% to 110% of the nominal concentration. Stability of the dosing formulations was demonstrated for 8 hours at ambient temperature.

Necropsy

Surviving maternal animals were not euthanized unless moribund. Pregnant monkeys and F1 neonates in declining clinical condition were euthanized for humane reasons and necropsied.

A total of three maternal animals and five F1 neonates were found dead or were euthanized in moribund condition. Additionally there were eight stillborn neonates, eleven abortions, and two in utero fetal deaths. These unscheduled necropsies are summarized the following tables. None of these deaths was considered test article-related.

Table 53 Summary of unscheduled necropsy – maternal animals [taken directly from the study report, pp. 63]

Maternal Animals – Found Dead and Moribund					
Dose (mg/kg/week)	**SSAN**	**Status**	**Day of Necropsy**	**Summary of Major Gross Findings**	**Summary of Major Histopathology Findings**
0	1	Dead	LD0	Stomach and Intestines: red or red brown contents Uterus: enlargement, dark red discoloration of lumen Vagina: dark red discoloration of luminal surface Abdominal cavity: clear ascites	Not conducted
5	31	Dead	LD2	Lungs: pale discoloration Uterus: enlargement, dark red discoloration of lumen, bloody fibrous material in the luminal surface Vagina: perforation, dark red discoloration of luminal surface Abdominal cavity: blood covered omentum and abdominal organs	Not conducted
50	81	Moribund (received emergency cesarean section)	GD165	None (sutures in uterus - after cesarean section)	Not conducted

Table 54 Summary of unscheduled necropsy – F1 neonates [taken directly from the study report, pp. 64]

F1 Neonates – Found Dead and Moribund					
Dose (mg/kg/week)	**SSAN**	**Status**	**Day of Necropsy**	**Summary of Major Gross Findings**	**Summary of Major Histopathology Findings**
0	1N	Dead	DB1	None	Lungs (alveolus): hem and edema Liver: MNC infilt Spleen: congestion, ly-foll hypotrophy Stomach, Duodenum, Jejunum: hem Testes: hem Thymus: atrophy
5	31N	Dead	DB3	Brain: subarachnoid congestion Esophagus and Stomach: distention with milk Eyes: periocular hem / congestion	Brain: hem Lymph nodes: atrophy Lungs (alveolus): edema, NEUT infilt Liver: dec glycogen, vacuol Spleen: ly-foll hypotrophy Testes: hem
15	69N	Dead	DB2	Cerebellum: dark red discoloration Stomach and Intestine: small amount of contents Lungs: petechia	Brain: hem Lungs (alveolus): hem and edema Liver: EMH, dec glycogen, vacuol Kidney: vacuol (tubular epithelium) Spleen: congestion, ly-foll hypotrophy Pancreas: dec zymogen granule (acinar cell) Testes: hem
50	75N	Moribund	DB26	Stomach, jejunum and Ileum: black contents	Lymph nodes: atrophy Thymus: atrophy Liver: dec glycogen Spleen: ly-foll hypotrophy Pancreas: dec zymogen granule Testes: hem
	95N	Moribund	DB1	Cerebellum: hem Stomach and Intestines: watery contents	Brain: hem Lymph nodes: EMH Esophagus: hemorrhage (lamina propria) Liver: EMH, dec glycogen Kidney: dilation of distal tubule Spleen: EMH

Dec: decreased; EMH: extramedullary hematopoiesis; ly-foll: lymphoid follicle; infilt: infiltration; hem: hemorrhage; MNC: mononuclear cell; NEUT: neutrophils; vacuol: vacuolation

Table 55 Summary of unscheduled necropsy – F1 neonates, continued [taken directly from the study report, pp. 65]

F1 Neonates – Stillborn					
Dose (mg/kg/week)	**SSAN**	**Status**	**Day of Necropsy**	**Summary of Major Gross Findings**	**Summary of Major Histopathology Findings**
0	19N	Stillbirth	GD163	Placenta: discoloration (white/yellow) and nodular surface (findings were not recorded for several organs/tissues)	Thyroid: ectopic thymus Lungs (alveolus): edema Liver: EMH Spleen: EMH, ly-foll hypotrophy, congestion Lymph nodes: brown pigment Ovary: hem: oocyte mineralization Uterus: hem Vagina: hem
	21N	Stillbirth	Retrieved by emergency cesarean section (breech position) on GD151, and died on DB2	Adrenals: enlargement (left) Brain: dark red discoloration of leptomeninges Esophagus: food was found through the organ Lungs: multifocal red discoloration	Brain: hem Thymus: cyst Lungs (alveolus): hem, edema, NEUT infilt Liver: EMH, dec glycogen, vacuol Kidney: dilation of distal tubule Spleen: ly-foll hypotrophy Lymph nodes: brown pigment Testis: hem
5	45N	Stillbirth	GD167	None	Lymph nodes: EMH Thymus: hem Lungs (alveolus): edema, NEUT infilt Liver: EMH Spleen: ly-foll hypotrophy, congestion Rectum: hem Testis: hem Epididymis: hem
15	51N	Stillbirth	GD156	Lungs: atelectasis Nose: bleeding Abdomen: ascites (red/watery)	Lymph nodes: EMH, brown pigment Lungs (alveolus): hem, edema Liver: MNC infilt Spleen: EMH, congestion Testis: hem

Dec: decreased; EMH: extramedullary hematopoiesis; ly-foll: lymphoid follicle; infilt: infiltration; hem: hemorrhage; MNC: mononuclear cell; NEUT: neutrophils; vacuol: vacuolation

Table 56 Summary of unscheduled necropsy – F1 neonates, continued [taken directly from the study report, pp. 66]

F1 Neonates – Stillborn					
Dose (mg/kg/week)	**SSAN**	**Status**	**Day of Necropsy**	**Summary of Major Gross Findings**	**Summary of Major Histopathology Findings**
15 (cont.)	63N	Stillbirth	GD163	Lungs: atelectasis	Thyroid: cyst Thymus: atrophy, cyst Lungs (alveolus): edema Liver: EMH, vacuol Spleen: ly-foll hypotrophy, congestion Rectum: hem Testis: hem Epididymis: hem
50	77N	Stillbirth	GD161	None	Lymph nodes: EMH Thyroid: ectopic thymus Thymus: hem Lungs (alveolus): hem, foamy macrophage infilt Liver: dec glycogen Spleen: EMH, ly-foll hypotrophy Uterus: hem
	81N	Stillbirth (retrieved by emergency cesarean section, breech position)	GD165	Carcass: autolyzed Arm (left): trauma, light red discoloration, swollen Thorax (left): trauma, light red discoloration (fetus was stacked in the birth cannel at cesarean section)	Lymph nodes: hem Thyroid: ectopic thymus Lungs (alveolus): edema Liver: EMH Spleen: ly-foll hypotrophy, congestion Testis: hem
	83N	Stillbirth	GD162	Lungs: dark red firm mass attached by thin stalk to caudal lobes	Lungs (alveolus): hem, foamy macrophage infilt Spleen: EMH Testis: hem Prostate: hem

Dec: decreased; EMH: extramedullary hematopoiesis; ly-foll: lymphoid follicle; infilt: infiltration; hem: hemorrhage; MNC: mononuclear cell; NEUT: neutrophils; vacuol: vacuolation

Table 57 Summary of unscheduled necropsy – F1 neonates, continued [taken directly from the study report, pp. 67]

Fetuses –Abortion and Embryo/Fetal Death					
Dose (mg/kg/week)	SSAN (Dam)	Status	Day of Necropsy	Summary of Major Gross Findings	Summary of Major Histopathology Findings
0	5	Abortion	GD50	No embryo retrieved	Not applicable
	13	Abortion	GD30	No embryo retrieved	Not applicable
	17	*In utero* fetal death	GD51	Dead fetus retrieved by emergency cesarean section on GD52 Fetus and placenta were slightly autolyzed but there were no external abnormalities. No visceral examination was conducted.	Not applicable
5	35	Abortion	GD37	No embryo retrieved	Not applicable
	39	Abortion	GD26	No embryo retrieved	Not applicable
	47	Abortion	GD33	No embryo retrieved	Not applicable
15	49	Abortion	GD44	No embryo retrieved	Not applicable
	57	*In utero* embryo death	GD38	No embryo retrieved	Not applicable
	61	Abortion	GD30	No embryo retrieved	Not applicable
	65	Abortion	GD44	No embryo retrieved	Not applicable
50	85	Abortion	GD37	No embryo retrieved	Not applicable
	87	Abortion	GD37	No embryo retrieved	Not applicable
	93	Abortion	GD24	No embryo retrieved	Not applicable

Offspring (Malformations, Variations, etc.)

For naturally delivered F1 neonates, birth examinations (viability, sex determination, and external morphological examinations [body form, digits and nails, ears, external genital organs/anus, eyes, forelimbs, head/face/mandibular/hair, hindlimbs, nipple formation, oral cavity/palate, vertebral column, tail] once from DBs 0 and 3), clinical observations (twice daily), body weight (once between DBs 0 and 3 and on DBs 8, 15, and 22 [±1 day]), functional (pupillary reflex, Preyer reflex, pain response, grip strength, once between DBs 25 and 32) and morphological development (head width, head circumference, distance between the eyes, crown-rump length, tail length, chest circumference, paw and foot length, and ano-genital distance, once between DBs 25 and 32) and skeletal examination by X-ray imaging once between DBs 25 and 32), were assessed, and blood samples were drawn once at necropsy for assessment of hematology, coagulation, serum chemistry, and flow cytometry (immunophenotyping of peripheral lymphocytes). Neonate serum chemistry samples were collected without separation from maternal animals for at least 4 hours prior to blood collection. Therefore, neonates were considered to not have been fasted for 4 hours. Blood samples were collected once on DB 7 (±1 day) and at necropsy for TK and ADA response.

All surviving F1 neonates were euthanized between DBs 28 and 32. F1 neonates in declining clinical condition were euthanized for humane reasons and necropsied. Necropsies were performed on F1 neonates when found dead (including stillborn).

Organ weights were measured for all F1 neonates at scheduled necropsy. Paired organs were weighed together. Organs and tissues were collected and preserved for all animals necropsied. Histopathology (hematoxylin and eosin staining) was performed for all naturally delivered F1 neonates, including stillborn neonates. Portions of the spleen and thymus, and right side of the axillary, inguinal and mandibular lymph nodes from all F1 neonates were collected for possible immunohistochemistry, but not performed. Similarly, carcasses of F1 neonates were fixed in ethyl alcohol for possible skeletal staining, but skeletal staining was not performed.

Organs weighed [taken directly from the study report, pp. 45]

Adrenals	Pituitary
Brain (cerebrum, cerebellum and brain stem)	Prostate/Seminal vesicles
Epididymides	Spleen
Heart	Thyroids (including parathyroids)
Kidneys	Testes
Liver	Thymus
Lungs (including bronchi)	Uterus (body and cervix)
Ovaries	

Tissues collected for histopathology [taken directly from the study report, pp. 46]

Adrenals[1]	Lymph nodes (mesenteric)
Aorta (thoracic)	Mammary glands (or area of mammary glands)
Bone (femurs)[1]	Optic nerves[1]
Bone (sternum)	Ovaries[1]
Bone marrow (sternum)	Pancreas
Brain	Pituitary
Cecum	Prostate
Colon	Rectum
Duodenum	Sciatic nerves[1]
Epididymides[1]	Seminal vesicles
Esophagus (thoracic)	Skeletal muscles (quadriceps femoris)
Eyes[1]	Skin mammary
Gallbladder	Spinal cord (thoracic)
Heart	Spleen[3]
Ileum	Stomach
Injection site(s) (maternal animal only)	Submandibular salivary glands
Jejunum	Testes[1]
Joints (knee)[1]	Thymus[3]
Kidneys[1]	Thyroids (with parathyroids if possible)[1]
Lacrimal glands[1]	Tongue
Liver	Trachea
Lungs (with bronchi)	Urinary bladder

[1] Paired organs

[3] Portion of the organs was frozen (OCT)

Tissues collected for histopathology, continued [taken directly from the study report, pp. 47]

Lymph nodes (axillary)[1, 2]	Uterus (including conceptus)
Lymph nodes (inguinal)[1, 2]	Vagina
Lymph nodes (mandibular)[1, 2]	Carcass (F1 neonate only)[4]

[1] Paired organs

[2] The left side was fixed in 10% neutral buffered formalin / right side will be frozen (OCT)

[4] Fixed in ethyl alcohol.

Although it was intended that statistical analysis was to be conducted separately for each sex for neonates, statistical analysis was conducted with both male and female neonate data combined except for gender specific data due to the small number of survived neonates (e.g., N = 4 in Group 4, and only one male infant in Group 2).

Findings:

Embryo-fetal losses (including abortion and *in utero* embryo-fetal death) occurred in 3/12 (25%), 3/12 (25%), 4/12 (33%), and 3/12 (25%) females in the control, 5, 15, and 50 mg/kg groups, respectively. Stillbirth occurred in 2/9 (22%), 1/9 (11%), 2/8 (25%), and 3/9 (33%) females in the control, 5, 15, and 50 mg/kg/week groups, respectively. Because the incidences of embryo-fetal loss and stillbirth in the test article-dosed groups were

comparable to those of the control group and the conducting laboratory's historical control data, there were no test article-related effects on either maintenance of pregnancy or natural delivery (Table 58).

Table 58 Embryo-fetal loss, stillbirth and neonate death [taken directly from the study report, pp.5]

	0 mg/kg/week	5 mg/kg/week	15 mg/kg/week	50 mg/kg/week	Historical Control Data
Embryo-fetal loss	3/12 (25.0%)	3/12 (25.0%)	4/12 (33.3%)	3/12 (25.0%)	13.9 ±9.3%* (0.0 – 33.3%)**
Stillbirth	2/9 (22.2%)	1/9 (11.1%)	2/8 (25.0%)	3/9 (33.3%)	13.6 ±7.9%* (0.0 – 33.3%)**
Neonate death or unscheduled sacrifice	1/7 (14.3%)	1/8 (12.5%)	1/7 (14.3%)	2/6 (33.3%)	8/91 (8.8%)

* Mean ±SD, ** Minimum to maximum.

Table 59 Number of neonates that were examined for morphological development between DB25 and DB32

Dose group mg/kg	Group 1, 0	Group 2, 5	Group 3, 15	Group 4, 50
Total Nos.	6	7	5	5
No. of males	3	1	2	3
No. of females	3	6	3	2

Findings in F1 neonates:
There were no test article-related effects on birth examinations, clinical observation, mean body weights, functional and morphological development, and skeletal examination. No test article-related effects were noted on serum chemistry and immunophenotyping of peripheral lymphocytes. Decreased mean counts of lymphocytes (-34%) and white blood cells (-29%) were observed in the HD group on DB 30 (+/-2 days), compared to the control group. However, the values exhibited large individual variations within the groups and thus, the toxicological significance of these changes was unknown. There were no test article-related changes in mean neutrophil counts and fibrinogen levels. TK data showed that mean concentrations in neonates increased with dose. See the above Toxicokinetic section of this study for detailed information on F1 neonate's TK data.

The absence of the 12th rib was observed in a single neonate from each of the LD and MD groups (Table 60). This finding was not test article-related as there was no occurrence in the HD group, and also this finding is not uncommon in cynomolgus monkeys (the study report stated about 1.6% prevalence).

Table 60 Number of neonates with skeletal findings [taken directly from the study report, pp. 117]

		Group			
		1 (0 mg/kg)	2 (5 mg/kg)	3 (15 mg/kg)	4 (50 mg/kg)
	# Animals	8	9	7	9
parameter	value	animals	animals	animals	animals
Abnormalities	Absence of the 12th ribs (bilateral)	_	1	1	_
	None	8	8	6	9
Variations	Lumber ribs (right side)	_	1	_	_
	None	8	8	7	9

Study title: SAR153191/REGN88: Exploratory Subcutaneous 4-Week Toxicity Study in the Juvenile Mouse with REGN844

Study no.:	8272790 [Sponsor Reference No. JUP0016]
Study report location:	EDR
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 17, 2012
GLP compliance:	No
QA statement:	No
Drug, lot #, and % purity:	REGN844, Lot # T09001D600X11A; 98.5% purity

Key Study Findings

- Juvenile mice received 0, once weekly dose of 50 mg/kg, twice weekly dose of 25 mg/kg, once weekly dose of 200 mg/kg, or twice weekly dose of 100 mg/kg of REGN844 (mouse surrogate mAb) from PND 14 to PND 35.
- TK data showed that systemic exposure was proportionally increased with dose. There was no gender difference. Accumulation was observed (1.9 to 2.8 from PND 14 to 35). Both the once-weekly and twice-weekly dose groups showed similar corresponding AUC value on PND 35.
- There were no test article-related effects on examined parameters (mortality, clinical signs, body weight, food consumption, and necropsy).

Methods

Doses:	0 (G1), 50 (G2 & G3), 200 (G4 & G5) mg/kg/week
Frequency of dosing:	Groups 1, 2, and 4 were dosed once weekly on PND 14, 21, 28, and 35. Groups 3 and 5 were dosed twice weekly on PND 14, 17, 21, 24, 28, 31, 35, and 38.
Route of administration:	Subcutaneous injection
Dose volume:	5 mL/kg
Formulation/Vehicle:	REGN844 Placebo Product (10 mM histidine, 0.13% w/v polysorbate 20, 6.0% w/v sucrose, pH 6.0)
Species/Strain:	Crl:CD1(ICR) mouse
Number/Sex/Group:	10
Age:	Postnatal Day (PND) 14 at the initiation of dosing
Weight:	7.1-9.9 g for males; 7.0-10.1 g for females at the initiation of dosing
Satellite groups:	TK animals
Unique study design:	Offspring within each litter were randomly allocated to treatment groups and were weaned on PND 21. Pre-weaning animals were housed one litter with dam per cage and post-weaning animals in groups of up to five in cages.
Deviation from study protocol:	There were no deviations that affected the integrity of the study.

Observations and Results

Mortality

Viability was checked twice daily.

Clinical Signs

All animals were examined at least once daily for signs of ill health or overt toxicity. A detailed clinical observation was performed on the days of body weight measurement. On dosing days, main animals were observed right after dosing upon return to the cage, at 0.5, 1, 2, and 4 postdose.

There were no test article-related clinical signs.

Body Weights

Body weights were measured on PNDs 14, 17, 21, 24, 28, 35, 41, and 42 (terminal body weight).

There were no test article-related effects on mean body weight or body weight gain.

Feed Consumption

Food consumption was collected on a per cage basis on PNDs 24, 28, 31, 35, 38, and 41.

There were no test article-related effects on mean food consumption.

Hematology

Not performed.

Clinical Chemistry

Not performed.

Urinalysis

Not performed.

Gross Pathology

Parental females were euthanized after weaning of juvenile animals and discarded without further examination. Main study animals were euthanized on PND 42 and were subject to a full necropsy.

There were no test article-related necropsy findings.

Organ Weights

Not performed

Histopathology

Not performed

Toxicokinetics

Blood samples for TK analysis were collected from TK animals on PNDs 14 (9 animals/ time point) and 35 (3 animals/time point) at the following designated time points: pre-dose and at 24, 48, 72, 96, and 168 hours postdose for REGN844-dosed animals and at 24 hours postdose for control animals. The serum concentrations of REGN844 were measured by a validated ELISA method, and the lower limit of quantification was 0.078 µg/mL in neat mouse serum for the REGN844 assay.

Total REGN844 levels were not detectable from the samples from the control animals and PND 14 predose samples, except two samples (9.58 µg/mL in a Group3 male and 1.06 µg/mL in a Group 4 male).

TK data showed that there was no gender difference. The TK data from combined males and females are shown in Table 61. The systemic exposure was proportionally increased with dose. Accumulation was observed (1.9 to 2.8 from PND 14 to 35). Both the once-weekly and twice-weekly dose groups showed similar corresponding AUC values on PND 35.

Table 61 TK data of Total REGN844 in mouse serum following four weekly SC doses of 50 or 200 mg/kg or four twice-weekly SC doses of 25 or 100 mg/kg to juvenile mice [taken directly from the study report, pp. 6]

Parameter	Units	PND	50 mg/kg/week	25 mg/kg/twice weekly	200 mg/kg/week	100 mg/kg/twice weekly
Cmax	µg/mL	14 - 0HR inj	285	150	1170	614
Cmax	µg/mL	14 - 72HR inj	NA	237	NA	990
Cmax	µg/mL	35 - 0HR inj	435	357	2210	1490
Cmax	µg/mL	35 - 72HR inj	NA	376	NA	2150
AUC0-168	h•µg/mL	14	30700	24400	119000	104000
AUC0-168	h•µg/mL	35	59100	57400	283000	286000

Dosing Solution Analysis

REGN844 drug product was diluted with vehicle on each dosing day. Dosing solution was not analyzed.

10 Special Toxicology Studies

Title: Cross-Reactivity Study of Biotinylated REGN88 with Normal Human and Cynomolgus Monkey Tissues (Study Report: IM1436 [Sponsor Ref. No. REGN88-TX-06036]; GLP)

Method: To evaluate the potential cross-reactivity of biotinylated REGN88 (REGN88-bio) with cryosections of normal human and cynomolgus monkey tissues, REGN88-bio was applied to cryosections of normal human and cynomolgus monkey tissues (3 donors per tissue) at two concentrations (20 µg/mL and 0.5 µg/mL).

Tissues were obtained at autopsy or biopsy from humans. Cynomolgus monkey tissues were obtained at necropsy.

Controls included cryosections of recombinant hIL-6 receptor alpha Sepharose conjugated beads as Positive Control; ryosections of Crohn's disease colon (HT282 and HT257) as Ancillary Control; and ryosections of recombinant hIL-4 Sepharose conjugated beads as Negative Control.

Table 62 Normal Human and Cynomolgus Monkey Tissues from at Least Three Separate Donors [taken directly from the study report, pp.12]

• Adrenal	• Lung	• Spinal Cord
• Blood Cells[1]	• Lymph Node	• Spleen
• Blood Vessels (endothelium)	• Ovary	• Striated (skeletal) Muscle
• Bone Marrow	• Fallopian Tube (oviduct)	• Testis
• Brain – cerebrum (cortex)	• Pancreas	• Thymus
• Brain – cerebellum	• Parathyroid	• Thyroid
• Breast (mammary gland)	• Peripheral Nerve	• Tonsil
• Eye	• Pituitary	• Ureter
• Gastrointestinal Tract[2]	• Placenta	• Urinary Bladder
• Heart	• Prostate	• Uterus – body (endometrium)
• Kidney (glomerulus, tubule)[3]	• Salivary Gland	• Uterus – cervix
• Liver	• Skin	

Result: Controls yielded expected results. The negative control antibody, HuIgG-bio, did not react with either the positive, ancillary, or negative control materials. There also was no staining of the assay control slides. REGN88-bio did not react with the negative control material (cryosections of recombinant hIL-4 Sepharose conjugated beads). REGN88-bio produced strong to intense staining of the positive control material (cryosections of recombinant hIL-6Rα Sepharose conjugated beads). REGN88-bio also produced weak to moderate staining of cytoplasmic granules in very rare to occasional mononuclear cells and weak to strong staining of cytoplasmic granules in occasional to frequent surface mucosal epithelium within the ancillary control material (cryosections of Crohn's disease colon).

The sites of specific staining with REGN88-bio (20 µg/mL) of human tissues included:
• cytoplasm and/or cytoplasmic granules of various epithelia
• cytoplasmic granules of germinal (seminiferous) epithelium in the testis
• cytoplasm (perikaryon) and/or cytoplasmic granules of neurons in the cerebrum, cerebellum, spinal cord, colon, small intestine, and stomach
• cytoplasm and cytoplasmic granules in the glial cells of the cerebellum
• cytoplasm of the retinal neuroepithelium of the eye
• cytoplasm and cytoplasmic granules in mononuclear leukocytes within the esophagus, **prostate**, spleen, thymus, and tonsil
• cytoplasm and cytoplasmic granules in adipocytes of the esophagus
• cytoplasm and cytoplasmic striations of striated (cardiac) myofibers in the heart and **striated (skeletal) myofibers in the esophagus**
• cytoplasmic granules of axons in nerve fibers of the peripheral nerve

• neuropil in the neurohypophysis of the pituitary and **grey matter of the spinal cord**
• ductular, cytoplasmic granules in the mammary gland

The sites of specific staining with REGN88-bio (20 µg/mL) of cynomolgus monkey tissues included:
• cytoplasm and/or cytoplasmic granules of various epithelia
• cytoplasmic granules and/or cytoplasm in adipocytes of the adrenal, esophagus, **thymus**, **thyroid**, and **ureter**
• cytoplasmic granules in the glial cells of the cerebrum (cortex) and cerebellum
• cytoplasm and/or cytoplasmic granules in neurons of the cerebrum, cerebellum, spinal cord, colon, **esophagus**, small intestine, stomach, and **prostate**.
• cytoplasm of the retinal neuroepithelium of the eye
• cytoplasm and cytoplasmic striations of striated (cardiac) myofibers in the heart and **striated (skeletal) myofibers of the eye**
• cytoplasm and/or cytoplasmic granules in mononuclear leukocytes within the colon, esophagus, **small intestine**, and **stomach**, **lung**, **lymph node**, **peripheral nerve**, spleen, thymus, tonsil, and **urinary bladder**
• cytoplasm and cytoplasmic granules in **oocytes of the ovary**
• cytoplasmic granules of axons in nerve fibers of the peripheral nerve
• neuropil in the neurohypophysis of the pituitary
• cytoplasmic granules of **vascular smooth myofibers in striated (skeletal) muscle**
• cytoplasmic granules in germinal (seminiferous) epithelium of the testis
• cytoplasm in the **tail of spermatids in the testis**
• **glandular and ductular**, **membrane** and cytoplasmic granules in the mammary gland

Test article-specific staining was only of the cytoplasm and/or cytoplasmic granules in all human tissue elements. Membrane staining was not observed in any human tissues examined and was only observed in mammary gland epithelium in one monkey donor. The potential toxicologic consequences of cytoplasmic and cytoplasmic granular staining are uncertain as the accessibility of cytoplasmic sites is questionable in intact cells in vivo. Many of the tissue elements that specifically stained with REGN88-bio are known sites of IL-6 receptor expression. The staining pattern of REGN88-bio in human tissues appeared similar to that noted in cynomolgus monkey tissues as shown above. The tissues that were bolded and underlined above were the ones that were not common between humans and monkeys. REGN88-bio specifically stained cytoplasm and/or cytoplasmic granules in various epithelia, neurons, glial cells, retinal neuroepithelium, mononuclear leukocytes, cardiac and skeletal myofibers, adipocytes, axons of peripheral nerves, and germinal epithelium in the testis. There was also test article specific staining of neuropil in both the human and cynomolgus monkey pituitary gland. In addition, REGN88-bio also specifically stained vascular smooth myofibers, oocytes, and spermatids only in the cynomolgus monkey but not the human.

Title: Characterization of the Effects of Sarilumab (REGN88) on STAT3 Activation and Tumor Xenograft Growth [Study Report: REGN88-MX-11050-SR-01V1; non-GLP]

Method: REGN88 was suspended in 10 mM sodium phosphate, 0.0005% polysorbate 20, pH 6.2, whereas hFc was suspended in 5 mM sodium phosphate, 5 mM sodium citrate, 100 mM NaCl, 0.0005% polysorbate 20, pH 6.2.

Effects of REGN88 on the growth of human tumor xenografts were evaluated in immunocompromised mice as follows.

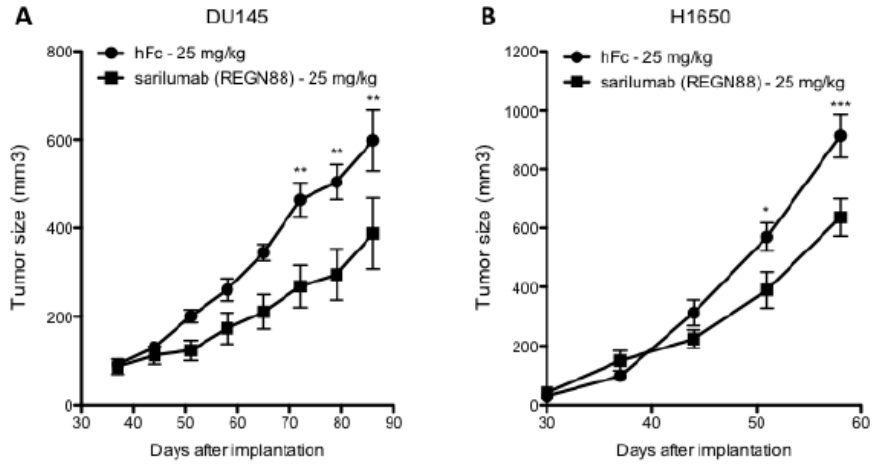
Effect of REGN88 on the growth of Du145 human prostate carcinoma tumor xenografts: 5 x 10^6 Du145 cells suspended in PBS with Matrigel were implanted sc into the right hind flank of male C.B.-17 SCID mice (7-8 weeks). When tumors reached an average volume of ~100 mm^3 (37 days after implantation), mice (n = 5/group) were once weekly administered a sc 25 mg/kg dose of either hFc or REGN88 for a total of 8 doses over 49 days and were sacrificed on Day 86.

Effect of REGN88 on the growth of NCI-H1650 human lung adenocarcinoma tumor xenografts: 2 x 10^6 NCI-H1650 cells were implanted S into the right hind flank of male C.B.-17 SCID mice (7-8 weeks). When tumors reached an average volume of ~125 mm^3 (37 days after implantation), mice (n = 5/group) were once weekly administered a sc 25 mg/kg dose of either hFc or REGN88 for a total of 4 doses over 21 days and were sacrificed on Day 58.

Tumor size of Du145 and NCI-H1650 human tumor xenografts in mice was measured *in vivo* once per week with calipers throughout the course of treatment. Immunohistochemistry was conducted with tumor sections with antibodies against cleaved caspase-3 (apoptosis), Ki67 (proliferation) or PECAM-1 (angiogenesis).

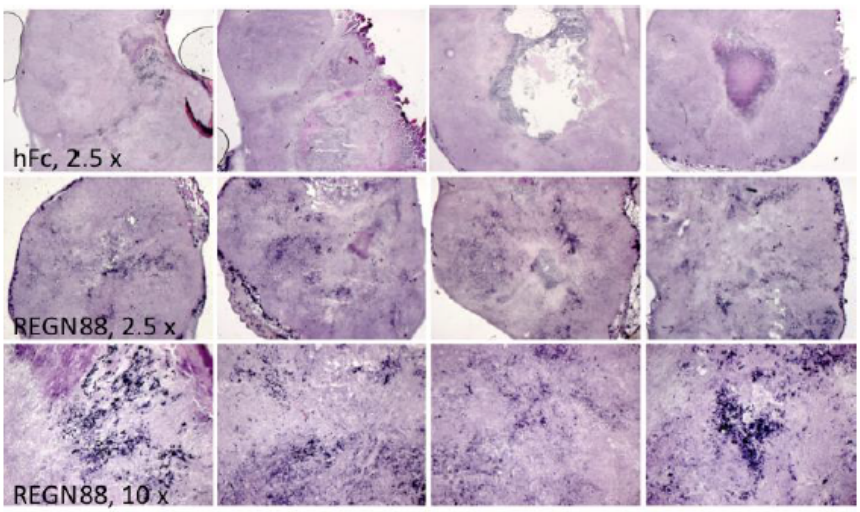
The effect of sarilumab (REGN88) on the activation of STAT3, one of the major signaling pathways stimulated by IL6, was tested in tumor cells treated with hFCc or REGN88 using western blot analysis using antibodies against phospho-STAT3.

Results: REGN88 inhibited the growth of Du145 (human prostate cancer) and NCI-H1650 (human lung carcinoma) tumor xenografts, compared to hFC-treated control mice (Figure 8). REGN88 caused increased apoptosis in Du145 xenograft mice as shown by immunohistochemistry for caspase-3 (Figure 9). REGN88 inhibited IL6-induced STAT3 activation in cultured Du145 and NCI-H1650 tumor cells (Figure 10). In addition, phospho-STAT3 levels were also reduced in Du145 tumor xenografts in mice treated with a single dose of REGN88, compared to mice treated with hFc (Figure 11). These studies indicate that REGN88 has anti-tumor effects and that these effects may be at least partly attributable to inhibition of the oncogenic STAT3 signaling pathway.



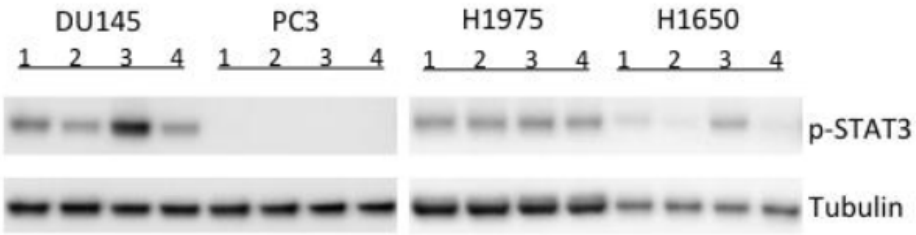
Mice bearing established Du145 **(Fig. 1A)** or NCI-H1650 **(Fig. 1B)** tumors (n = 5 mice per treatment group) were treated with hFc control protein or sarilumab (REGN88) at 25 mg/kg, once per week. The line graphs depict the average tumor volume +/- SD over the course of treatment. Individual tumor weight and mouse body weight data are shown in Tables 3, 4, 5 and 6. Tumor sizes were analyzed using Two-way ANOVA with Bonferroni's multiple comparison test (* p<0,05, ** p<0.01, *** p<0.005, **** p<0.001).

Figure 7 Effect of REGN88 on growth of Du145 and NCI-H1650 xenografts [taken directly from the study report]



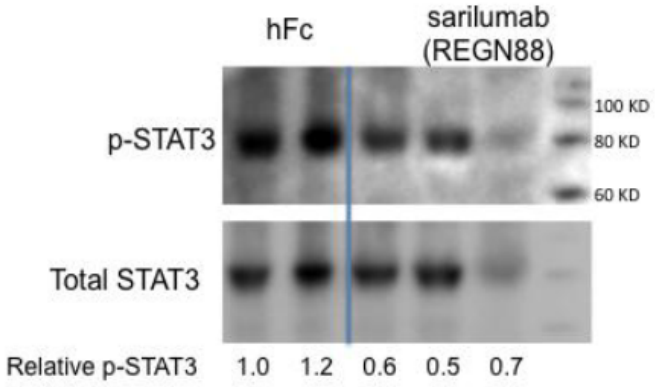
Human Fc or sarilumab (REGN88) treated Du145 tumors from Figure 1 were harvested at 24 hours after the last treatment and embedded in OCT. Immunohistochemistry staining for cleaved caspase-3 was performed on frozen sections. Images were taken at low (2.5X) magnification to show a more complete view of whole tumor sections, and at high (10X) magnification to show the areas of positive staining in sarilumab (REGN88) treated tumors.

Figure 8 Immunohistochemistry Analysis of Cleaved Caspase-3 in Du145 Tumor Xenografts Treated with REGN88 [taken directly from the study report]



Tumor cells were seeded and maintained under normal growth conditions. Cells were treated with hFc or sarilumab (REGN88) overnight, and then with or without IL6 for 30 minutes before cell lysates were collected for Western blot analysis on phospho-STAT3 and tubulin. Lane 1) hFc (10 ug/ml), lane 2) sarilumab (REGN88) (10 ug/ml), lane 3) hFc (10 ug/ml) + IL6 (10 ng/ml) and lane 4) sarilumab (REGN88) (10 ug/ml) + IL6 (10 ng/ml).

Figure 9 Effect of REGN88 on STAT3 Activation in Cultured Tumor Cells [taken directly from the study report]



Du145 tumor-bearing mice were treated with a single dose of hFc or sarilumab (REGN88) at 25 mg/kg. Tumors were harvested at 48 hours after treatment. Tumor lysates were prepared from two hFc-treated tumors (lanes 1 and 2) and three sarilumab (REGN88)-treated tumors (lanes 3-5). Phospho-STAT3 and total STAT3 levels were determined by Western blot. The p-STAT3 and total STAT3 signals were quantitated and the p-STAT3/total STAT3 ratio is shown below each lane.

Figure 10 Effect of REGN88 on STAT3 Activation in Du145 Tumor Xenografts in Mice [taken directly from the study report]

11 Integrated Summary and Safety Evaluation

Sarilumab (SAR153191 or REGN88) is a monoclonal antibody produced in CHO cells and it is composed of fully human Immunoglobulin G1 (IgG1) heavy chains and fully human kappa light chains. Sarilumab is directed against the alpha subunit of the IL-6 receptor complex (IL-6Rα), and it is being developed for the treatment of patients with rheumatoid arthritis (RA) (b) (4). Several clinical studies (Phase 1 and 2) have been conducted in healthy volunteers, RA patients, (b) (4).

Sarilumab binds human and cynomolgus monkey IL-6Rα with an equilibrium dissociation constant (Kd) of 54 pM and 123 pM, respectively, but no binding of sarilumab to the murine IL-6Rα was observed. Two *in vitro* functional bioassays conducted in human HepG2 cells expressing IL-6R transfected with a Stat3-luciferease reporter plasmid and DS-1 cells, which proliferated in response to exogenous human IL-6, showed that sarilumab inhibited IL-6-mediated luciferase activity and IL-6-stimulated proliferation of DS-1 cells, respectively.

In a tissue cross-reactivity study using biotinylated REGN88 (REGN88-bio) with cryosections of normal human and cynomolgus monkey tissues, REGN88-bio-specific staining was observed in the cytoplasm and/or cytoplasmic granules in all human tissue elements. Membrane staining was not observed in any human tissues examined and was only observed in mammary gland epithelium in one monkey donor. In general, the staining pattern of REGN88-bio in human tissues appeared similar to that noted in cynomolgus monkey tissues with a few exceptions. Many of the tissue elements that specifically stained with REGN88-bio are known sites of IL-6 receptor expression. However, the potential toxicologic consequences of cytoplasmic and cytoplasmic granular staining are uncertain as the accessibility of cytoplasmic sites is questionable in intact cells in vivo.

Five repeat-dose studies were conducted in monkeys using sarilumab (one 5-week IV, one 13-week IV, two 13-week SC, and one 6-month IV). In these studies, a total of 54 male and female monkeys were dosed with placebo and a total of 186 male and female monkeys were dosed with REGN88 at doses ranging from 0.5 to 100 mg/kg/week. Exposure to REGN88 increased with dose. There was no test article-related mortality in any of these studies. The test article-related microscopic findings were limited to injection site findings in a few studies. The most consistent findings in these studies were decreased levels of neutrophil and fibrinogen in males and females in REGN88-dosed groups during the dosing period. These findings were not generally dose-dependent, and the findings tended to be reversible during the recovery period. In addition, decreased levels of CRP were observed during the first several weeks of the dosing period in most of monkey studies.

The 6-month IV monkey study also included evaluation of serum levels of IL-6, immunophenotyping by flow cytometery, and T-cell dependent antigen response. Markedly increased levels of IL-6 were seen in all test article-dosed groups, however, the levels declined to the baseline during the recovery period. No apparent test article-related changes were observed in the immunophenotyping analysis. In the TDAR assay, slightly, but statistically significant decreases in primary and secondary IgG responses to KLH administration were noted in males and females at ≥5 mg/kg/week. The decreases were not generally dose-dependent. Overall, the findings in the general toxicity studies indicate that sarilumab is immunosuppressive in monkeys.

As these repeat-dose toxicity studies were conducted with sexually immature monkeys, potential fertility effects of REGN88 could not be evaluated from these studies. The sponsor conducted a fertility study in mice using the murine surrogate monoclonal antibody REGN844 as described below.

Changes in weights in the adrenal gland and thymus were frequently observed in these general toxicity monkey studies. However, there was inconsistency in the direction of changes between genders and/or across studies, and correlating macro- or microscopic findings were not observed. Thus, these changes were not considered test article-related.

In these nonclinical studies, there were 4 animals (3 monkeys and 1 mouse) with tumors. The tumor findings included adrenal cortical adenoma in one male monkey in the 100 mg/kg/week group in the 3-month SC bridging study, cervical papilloma (benign neoplasm) in one female monkey in the 10 mg/kg/week group in the 5-week IV study, squamous metaplasia in the cervix and uterus of one female monkey in the 10 mg/kg/week group in the 13-week SC bridging study, and cervical squamous cell metaplasia along with squamous cell hyperplasia in the vagina in one female mouse in the 200 mg/kg/week group in the fertility study using REGN844, a mouse surrogate antibody (IgG2a) against IL-6. In addition, there were a number of incidences of hyperplasia in various organs/tissues, however, some occurred in the control animals. Overall, all tumor and hyperplasia findings were considered not test article-related. For more detailed explanations of these findings, see the carcinogenicity risk assessment under the Carcinogenicity section.

The sponsor conducted a human tumor xenograft assay to assess effects of sarilumab on the growth of human tumor xenografts in immune-compromised mice and the activation of STAT3 in tumor cells. The effects of sarilumab on tumor growth were evaluated in Du145 (human prostate cancer) or NCI-H1650 (human lung carcinoma)-tumor bearing mice by measuring tumor sizes. The results showed that treatment with REGN88 inhibited the growth of Du145 and NCI-H1650 tumor xenografts, compared to control mice. Inhibition of tumors by REGN88 in the Du145 xenografts was associated with increased apoptosis. Furthermore, REGN88 inhibited IL-6-induced STAT3 activation in cultured Du145 and NCI-H1650 tumor cells. Phospho-STAT3 levels were also reduced in the Du145 tumor xenografts in mice treated with a single dose of REGN88, compared to control mice treated with hFc protein.

The sponsor submitted a carcinogenic risk assessment associated with sarilumab. Based on the weight of evidence for the available *in vitro* and *in vivo* data related to inhibition of IL-6 in tumor promotion in the literature, and the anti-tumor effects specifically observed with sarilumab in a tumor xenograft assay, the sponsor believed no additional nonclinical studies (e.g., 2-year carcinogenicity study in mice) are needed to evaluate the carcinogenic potential of sarilumab in patients. The Reviewer and Exec CAC members agreed that no additional nonclinical studies are needed to address the carcinogenic potential of sarilumab. Any potential risks in patients for immune suppression mediated tumor initiation and/or tumor promotion are better managed by appropriate labeling, clinical monitoring, and post-marketing surveillance approaches. The carcinogenic risk to humans for the chronic use of sarilumab in product label should include a balanced description of the literature information available to address the carcinogenic potential of sarilumab. For more detailed information, see the carcinogenicity risk assessment under the Carcinogenicity section.

The reproductive and developmental toxicity of sarilumab was evaluated in a fertility and early embryonic developmental toxicity study in mice using REGN844 and an enhanced

pre- and postnatal toxicity study in cynomolgus monkeys using REGN88. In the subcutaneous study of fertility and early embryonic development to implantation in mice, male and female mice (24/sex/group) were administered REGN844 twice weekly at doses of 0 (placebo control), 10 (LD), 25 (MD), and 100 (HD) mg/kg/dose. The dosing period encompassed 4 weeks prior to mating and continuously until each animal's necropsy for males and 2 weeks prior to mating and through Gestation Day 7 for females. REGN844 concentrations increased with dose in males and females. There was no test article-related parental toxicity. There were no test article-related effects on any reproductive and fertility parameters examined in the study, including number of corpora lutea, implantation sites, resorption and viable fetuses. However, estrous cycle observation was not included in the study.

In the enhanced PPND study, groups of 12 pregnant monkeys were intravenously administered REGN88 once weekly at doses of 0 (placebo control), 5 (LD), 15 (MD), or 50 (HD) mg/kg/week from gestation Day (GD) 20 through natural delivery (approximately GD 165), with subsequent maternal and offspring monitoring to approximately 28 days after delivery. There was no test article-related mortality. In maternal animals, there were decreased mean counts of lymphocytes, neutrophils, and white blood cells in all test article-dosed groups in a non-dose-related manner on GD 153 and/or LD 7. Slightly decreased mean levels of fibrinogen were also observed in all test article-dosed groups in a non-dose-related manner on GD 153, LD 7, and/or LD 30. These changes were similar to what was observed in general toxicity studies in monkeys. Decreases in neutrophil counts and fibrinogen levels were consistently observed in general toxicity studies as described above, but changes in lymphocyte and white blood cell counts were not consistent across the general toxicity studies. Thus, the toxicological significance of decreased counts of lymphocytes and white blood cells in maternal animals in this study is unclear. In F1 neonates, there were no test article-related effects on birth examinations, clinical observation, mean body weights, functional and morphological development, and skeletal examination. No test article-related effects were noted on serum chemistry and immunophenotyping of peripheral lymphocytes. Decreased mean counts of lymphocyte (-34%) and white blood cell (-29%) were observed in the F1 neonates in the HD group on DB 30 (+/-2 days), compared to the control group. However, the values exhibited large individual variations within the groups and thus, the toxicological significance of these changes is unknown. There were no test article-related changes in mean neutrophil counts and fibrinogen levels. Exposure to REGN88 increased with dose in maternal and F1 animals. REGN88 concentrations were still present in the F1 neonate animals on the day of their scheduled sacrifice.

There were some concerns about the adequacy of this ePPND study. First, numbers of neonates in each group at scheduled necropsy were relatively small (3 males and 3 females at the control; 1 male and 6 females at LD; 2 males and 3 females at MD; 2 males and 2 females at HD). Second, immune function testing in the offspring during the postnatal phase was not conducted (e.g., TDAR) although REGN88 has been shown to be immunosuppressive in adult monkeys (e.g., decreased neutrophil counts in almost all general toxicity studies, decreased effects in the TDAR assay in the 6-month study) as well as in adult humans. Lastly, REGN88 was not totally cleared from the circulation of F1

offspring at the time of necropsy (around postnatal day 30) and thus the drug may have uncharacterized effects beyond the one month of age in monkeys.

At the EOP2 meeting held on September 15, 2011, the concerns in the enhanced PPND study (as described above) were communicated to the sponsor, and the division also communicated that the investigator's brochure (IB), informed consent (IC), and potential product labeling need to use existing knowledge on sarilumab and the IL-6 pathway to report the potential immunosuppressive effects of sarilumab in infants of mothers exposed to sarilumab. During the meeting, the sponsor asked whether a GLP subcutaneous pre- and postnatal developmental toxicity study in mice using REGN844 (the murine surrogate against IL-6R) can be conducted to address the Division's concerns. The Division stated that additional developmental toxicity studies are not necessarily required if the sponsor agrees to incorporate information regarding the potential immunosuppressive effects of the product in infants in the IB, IC, and the potential product label as follows: "Infant monkeys were evaluated up to 1 month of age in the enhanced PPND study with monkeys and immune functional testing was not performed. Based on the findings from the 6-month toxicity study in adult monkeys and available adult human clinical data, sarilumab possesses the potential to cause immunosuppressive effects in the infants of mothers treated with sarilumab". The sponsor asked if it would be acceptable to include this information in the documents noted above (b) (4) . The Division responded that it is the sponsor's choice to take this approach. However, (b) (4) Then the sponsor asked whether the statement can be (b) (4) The Division did not recommend (b) (4) . See the meeting minutes (DARRTS dated September 26, 2011).

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/s/

MARCIE L WOOD
05/12/2016
Review finalized for Grace S. Lee. I concur with Dr. Lee's assessment.

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/s/

ELENI M SALICRU
08/24/2016

TIMOTHY W ROBISON
08/24/2016
I concur

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

BLA Number: 761037 **Applicant: Sanofi-Aventis U.S. LLC** **Stamp Date: 10/30/2015**

Drug Name: SAR153191/REGN88 (Sarilumab) Subcutaneous injection **BLA Type: Original**

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	x		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	x		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	x		
4	Are all required and requested IND studies (in accord with 505 (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).			The formulation used in the toxicology studies is the same as the clinical formulation.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	x		A bridging study between the intravenous and subcutaneous routes of administration has been provided.
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	x		

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	x		
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		The proposed labeling sections relative to pharmacology/toxicology are in the new PLLR format.
10	Have any impurity, degradant, extractable/leachable, etc. issues been addressed? (New toxicity studies may not be needed.)			No issues were identified during the IND review. Any issues that arise during the BLA review will be addressed in consultation with the chemist.
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.

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.

There are no filing issues from the nonclinical perspective.

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

None.

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/s/

ELENI M SALICRU
12/16/2015

TIMOTHY W ROBISON
12/16/2015
I concur