

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**125522Orig1s000**

**PHARMACOLOGY REVIEW(S)**

## **Tertiary Pharmacology/Toxicology Review**

**Date:** August 20, 2015

**From:** Timothy J. McGovern, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO

**BLA:** 125522

**Agency receipt date:** August 27, 2014

**Drug:** REPATHA (evolocumab)

**Sponsor:** Amgen, Inc.

**Indication:** Adjunct therapy to diet for adult patients with primary hyperlipidemia or mixed dyslipidemia and for patients  $\geq 12$  years with homozygous familial hypercholesterolemia. Recommended dosing for the indications of primary hyperlipidemia or mixed dyslipidemia is 140 mg Q2W or 420 mg QM by subcutaneous injection; recommended dosing for the indication of homozygous familial hypercholesterolemia is 420 mg either QM or Q2W.

**Reviewing Division:** Division of Metabolism and Endocrinology Products

**Introductory Comments:** The pharmacology/toxicology reviewer and supervisor concluded that the nonclinical data support approval of REPATHA (evolocumab) for the indication listed above.

Evolocumab is a human IgG2 monoclonal antibody that binds to human PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9). The recommended Established Pharmacologic Class (EPC) for evolocumab is PCSK9 inhibitor antibody. One other product in this EPC, PRALUENT, was recently approved.

An appropriate nonclinical program was conducted by the sponsor to support approval of evolocumab. Evolocumab elicited expected pharmacological responses in hamsters and monkeys; evolocumab lowered total cholesterol and LDL-cholesterol in the species tested and decreased HDL-cholesterol in hamsters.

The primary nonclinical toxicity studies of evolocumab were conducted in hamsters for up to 3 months and monkeys for up to 6 months duration with once weekly subcutaneous dosing. No significant adverse findings were observed in a 3-month study in hamsters at doses that achieved exposure multiples of 112-, 48- and 20-fold compared to the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively. Evolocumab was also well-tolerated in a 6-month study in monkeys at doses that achieved exposure multiples of 744-, 300- and 134-fold compared to the recommended human doses. A combination toxicity study in monkeys was conducted with rosuvastatin for up to 3 months duration; no additive or synergistic effects on statin-induced toxicities were observed. The low production of neutralizing anti-drug antibodies did not compromise the toxicological assessment of evolocumab in any study.

Genetic toxicity studies were not applicable for this program. The sponsor conducted a single carcinogenicity study in hamsters; no drug-related tumors were identified at doses

that provided exposure margins of 38-, 15, and 6.6-fold compared to the recommended human doses.

Evolocumab did not produce any effect on fertility parameters evaluated as part of the 6-month toxicity study in monkeys and a fertility and mating study in hamsters at exposure multiples of 134-fold or greater in monkeys and 5-fold or greater in hamsters, compared to the maximum recommended human dose. In an expanded developmental study in monkeys in which offspring were followed into infancy for 6 months, no significant drug-related effects were observed. Of note, humoral immune suppression was observed in infant monkeys exposed in utero to another drug in this class (alirocumab); effects on humoral immunity were not evaluated in the developmental studies for the evolocumab program. Evolocumab was detected in serum samples from infants of monkeys treated with the drug.

**Conclusions:**

I agree with the division pharmacology/toxicology conclusion that this BLA can be approved from the pharmacology/toxicology perspective. I agree that it is appropriate to waive the genetic toxicology for this drug. The proposed EPC is appropriate and is consistent with that for PRALUENT. I have discussed the labeling revisions regarding the relevant nonclinical sections with the Division.

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/s/  
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TIMOTHY J MCGOVERN  
08/20/2015

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

**PHARMACOLOGY/TOXICOLOGY BLA REVIEW AND EVALUATION**

**Application number:** BLA 125522  
**Supporting document/s:** SDN1, SN0000 (eCTD)  
**Applicant's letter date:** 27 August 2014  
**CDER stamp date:** 27 August 2014  
**Product:** Evolocumab (Repatha®)  
**Indication:** Adults with primary hyperlipidemia (mixed dyslipidemia or heterozygous familial hypercholesterolemia) and adults and patients ≥12 years with homozygous familial hypercholesterolemia  
**Applicant:** Amgen, Inc.  
**Review Division:** Division of Metabolism and Endocrinology Products  
**Reviewer:** C. Lee Elmore, PhD  
**Supervisor/Team Leader:** Karen Davis-Bruno, PhD  
**Division Director:** Jean-Marc Guettier, MD  
**Project Manager:** Kati Johnson

**Definitions:**

HDL-C, high-density lipoprotein cholesterol; HeFH, heterozygous familial hypercholesterolemia; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; LDLR, low density lipoprotein receptor; MAb, monoclonal antibody; PCSK9, proprotein convertase subtilisin/kexin type 9; TDAR, T cell dependent antibody response (assay)

## TABLE OF CONTENTS

<b>1</b>	<b>EXECUTIVE SUMMARY .....</b>	<b>7</b>
1.1	INTRODUCTION .....	7
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS .....	7
1.3	RECOMMENDATIONS .....	9
<b>2</b>	<b>DRUG INFORMATION .....</b>	<b>14</b>
2.1	DRUG .....	14
2.2	RELEVANT INDS, NDAs, BLAs AND DMFs .....	17
2.3	DRUG FORMULATION .....	17
2.4	COMMENTS ON NOVEL EXCIPIENTS .....	18
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN .....	18
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN .....	18
12.7	REGULATORY BACKGROUND .....	18
<b>3</b>	<b>STUDIES SUBMITTED.....</b>	<b>20</b>
3.1	STUDIES REVIEWED.....	20
3.2	STUDIES NOT REVIEWED .....	21
3.3	PREVIOUS REVIEWS REFERENCED.....	21
<b>4</b>	<b>PHARMACOLOGY.....</b>	<b>21</b>
4.1	PRIMARY PHARMACOLOGY .....	21
4.2	SECONDARY PHARMACOLOGY .....	30
4.3	SAFETY PHARMACOLOGY .....	34
<b>5</b>	<b>PHARMACOKINETICS/ADME/TOXICOKINETICS .....</b>	<b>35</b>
5.1	PK/ADME.....	35
5.2	TOXICOKINETICS .....	39
<b>6</b>	<b>GENERAL TOXICOLOGY.....</b>	<b>39</b>
6.1	SINGLE-DOSE TOXICITY .....	39
6.2	REPEAT-DOSE TOXICITY .....	40
<b>7</b>	<b>GENETIC TOXICOLOGY .....</b>	<b>94</b>
<b>8</b>	<b>CARCINOGENICITY .....</b>	<b>94</b>
<b>9</b>	<b>REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY .....</b>	<b>110</b>
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT .....	110
9.2	ENHANCED PRE-/POSTNATAL DEVELOPMENT .....	116
<b>10</b>	<b>SPECIAL TOXICOLOGY STUDIES.....</b>	<b>134</b>
<b>11</b>	<b>INTEGRATED SUMMARY AND SAFETY EVALUATION.....</b>	<b>140</b>
<b>12</b>	<b>APPENDIX/ATTACHMENTS .....</b>	<b>150</b>
	APPENDIX 1: EXECUTIVE CARCINOGENICITY ASSESSMENT COMMITTEE MINUTES .....	150
	APPENDIX 2: TUMOR RATES IN A TWO-YEAR HAMSTER CARCINOGENICITY BIOASSAY .....	152

## Table of Tables

Table 1: Formulation of the to-be-marketed evolocumab solution for injection (140 mg/mL).....	18
Table 2: Dissociation constants (apparent binding affinity) for evolocumab binding to human ( <span style="background-color: #cccccc; padding: 0 20px;"> </span> <sup>(b) (4)</sup> ), monkey, hamster and mouse PCSK9 .....	26
Table 3: Summary evolocumab pharmacokinetics after single subcutaneous or intravenous doses in monkeys .....	36
Table 4: Study design for a 28-day toxicology study of evolocumab in hamsters .....	41
Table 5: Pharmacodynamics of evolocumab in a 28 day subcutaneous toxicity study in male hamsters.....	45
Table 6: Pharmacodynamics of evolocumab in a 28 day subcutaneous toxicity study in female hamsters.....	45
Table 7: Summary evolocumab toxicokinetics for a 28-day hamster toxicity study .....	46
Table 8: Study design for a 3-month subcutaneous toxicity study of evolocumab in hamsters .....	48
Table 9: Pharmacodynamics for evolocumab in a 3-month subcutaneous toxicity study in male hamsters.....	51
Table 10: Pharmacodynamics for evolocumab in a 3-month subcutaneous toxicity study in female hamsters.....	52
Table 11: Summary evolocumab toxicokinetics for a 3-month subcutaneous hamster toxicity study.....	54
Table 12: Study design for a 6 month toxicity study of evolocumab in monkeys.....	56
Table 13: Baseline lymphocyte counts in a 6 month toxicity study with evolocumab in monkeys.....	62
Table 14: Summary toxicokinetics for evolocumab in a 6-month monkey toxicity study* .....	66
Table 15: Trend analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (all animals) .....	69
Table 16: Trend analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)..	70
Table 17: ANOVA analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (all animals) .....	70
Table 18: ANOVA analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)..	71
Table 19: Study design for a 3 month combination toxicity study of evolocumab and rosuvastatin in monkeys.....	73
Table 20: Incidence of pre-existing anti-KLH antibodies in monkeys coadministered evolocumab and rosuvastatin in a 3-month toxicity study .....	81
Table 21: Summary of anti-evolocumab antibody data for a 3-month combination toxicity study of evolocumab with rosuvastatin.....	84
Table 22: Summary toxicokinetics for a 3-month combination toxicity study of evolocumab with rosuvastatin for ADA-negative monkeys.....	84

Table 23: Summary evolocumab toxicokinetics for a 6 month combination toxicity study of evolocumab +/- rosuvastatin in all monkeys.....	85
Table 24: Summary rosuvastatin toxicokinetics for a 6 month combination toxicity study of evolocumab +/- rosuvastatin in all monkeys.....	85
Table 25: Study design for a 6 week toxicity study of evolocumab in monkeys .....	87
Table 26: Summary toxicokinetics for evolocumab in a 6 weeks toxicity study in monkeys.....	90
Table 27: Study design for a local tolerance test of evolocumab in hamsters .....	91
Table 28: Incidence and severity of skin histopathology in a local subcutaneous tolerance study of evolocumab in hamsters .....	92
Table 29: Study design for a local tolerance test of evolocumab in rabbits .....	93
Table 30: Study design for a subcutaneous lifetime carcinogenicity study of evolocumab in hamsters.....	96
Table 31: Summary toxicokinetics for evolocumab upon subcutaneous administration to hamsters in a lifetime carcinogenicity study .....	109
Table 32: Study design for a fertility and early embryonic developmental toxicity study of evolocumab in hamsters .....	110
Table 33: Study design for an enhanced pre/postnatal development study of evolocumab in the monkeys.....	117
Table 34: Summary toxicokinetics for evolocumab in an enhanced pre/postnatal developmental assessment in monkeys.....	125
Table 35: Summary rates of anti-drug antibody positivity in female monkeys administered evolocumab during pregnancy.....	126
Table 36: Pregnancy outcome (percent and timing of fetal loss) in pregnant monkeys administered evolocumab during pregnancy.....	127
Table 37: Aborted fetuses in female monkeys administered evolocumab during pregnancy from GD20 to GD100.....	127
Table 38: Aborted fetuses in female monkeys administered evolocumab during pregnancy from GD100 to GD140.....	128
Table 39: Stillborn fetuses in female monkeys administered evolocumab during pregnancy from G 140 to parturition.....	128
Table 40: Infant Losses for female monkeys administered evolocumab during pregnancy .....	130
Table 41: Summary table of safety margins for nonclinical assessment of evolocumab .....	149

## Table of Figures

Figure 1: Cartoon structure of evolocumab .....	15
Figure 2: Amino acid sequence of evolocumab.....	16
Figure 3: PCSK9-mediated degradation of LDLR .....	22
Figure 4: Cellular regulation of cholesterol homeostasis .....	23
Figure 5: Antibody-mediated inhibition of PCSK9 increases LDLR .....	24
Figure 6: Antigen specificity of evolocumab for PCSK9 versus other subtilisin-like proprotein convertase family members .....	27

Figure 7: Effect of combined treatment of HepG2 cells with evolocumab and a statin on LDLR.....	28
Figure 8: Pharmacodynamic effect of evolocumab on non-HDL-C following a single-dose in hamsters.....	29
Figure 9: Pharmacodynamic effect of evolocumab on total cholesterol following a single dose in monkeys.....	29
Figure 10: Pharmacodynamic effect of evolocumab on LDL-C following a single dose in monkeys.....	30
Figure 11: Histopathology and creatine kinase levels in rats treated with cerivastatin and fed standard, cholesterol-enriched or mevalonate-enriched diets.....	33
Figure 12: Heart rate following a single intravenous dose of evolocumab in monkeys.....	34
Figure 13: Mean arterial pressure following a single intravenous dose of evolocumab in monkeys.....	35
Figure 14: Mean percent decrease from baseline for serum LDL-C concentrations following a single dose of evolocumab in monkeys.....	37
Figure 15: Mean fasting PCSK9 concentrations following a single dose of evolocumab in monkeys.....	38
Figure 16: Mean serum LDL-C and PCSK9 over time following a single dose of 3 and 30 mg/kg evolocumab in monkeys.....	39
Figure 17: Anti-KLH IgM responses in a 6 month toxicity study in monkeys (all animals).....	67
Figure 18: Anti-KLH IgM responses in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline).....	68
Figure 19: Anti-KLH IgG responses in a 6 month toxicity study in monkeys (all animals).....	68
Figure 20: Anti-KLH IgG responses in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline).....	69
Figure 21: AUC of interpolated anti-KLH IgG titer for selected* monkeys administered evolocumab for 6 months.....	71
Figure 22: Anti-KLH IgM responses in a 3 month combination toxicity study with evolocumab and rosuvastatin in monkeys.....	82
Figure 23: Anti-KLH IgG responses in a 3 month combination toxicity study with evolocumab and rosuvastatin in monkeys.....	83
Figure 24: Kaplan-Meier survival curve for male hamsters in a subcutaneous lifetime carcinogenicity study.....	97
Figure 25: Kaplan-Meier survival curves for female hamsters in a subcutaneous lifetime carcinogenicity study.....	98
Figure 26: Body weights of male hamsters in a subcutaneous lifetime carcinogenicity study.....	99
Figure 27: Body weights of female hamsters in a subcutaneous lifetime carcinogenicity study.....	100
Figure 28: Food consumption of male hamsters in a subcutaneous lifetime carcinogenicity study.....	101
Figure 29: Food consumption of female hamsters in a subcutaneous lifetime carcinogenicity study.....	102

Figure 30: Mean pharmacodynamic effect (LDL-C) of evolocumab administered to pregnant female monkeys ..... 125

Figure 31: Body weights for infant male monkeys exposed to evolocumab in utero ... 131

Figure 32: Body weights for infant female monkeys exposed to evolocumab in utero 131

Figure 33: Mean pharmacodynamic effect (LDL-C) of evolocumab for infant monkeys exposed to evolocumab in utero ..... 132

# 1 Executive Summary

## 1.1 Introduction

Amgen (“the Applicant”) is seeking approval of evolocumab (Repatha®) for the treatment of adults with primary hyperlipidemia, mixed dyslipidemia, heterozygous familial hypercholesterolemia (HEFH), and patients  $\geq 12$  years of age with homozygous familial hypercholesterolemia (HOFH).

## 1.2 Brief Discussion of Nonclinical Findings

### Background:

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a freely circulating proprotein convertase, which has the ability to bind LDL receptors (LDLR). PCSK9-binding to LDLR initiates LDLR/PCSK9 complex internalization and lysosomal degradation. Evolocumab is a human IgG2 monoclonal antibody that binds to human PCSK9 with high affinity (sub-nanomolar  $K_D$ ), and in doing so inhibits and removes PCSK9 from circulation. By inactivating PCSK9, evolocumab upregulates LDLR uptake of serum LDL-cholesterol (LDL-C), especially by the liver, with consequent lowering of the circulating level of LDL-C.

The Applicant identified the hamster and monkey as pharmacologically relevant species for toxicology testing with evolocumab; both species express PCSK9, to which evolocumab binds with high affinity. Evolocumab was subcutaneously administered to monkeys in a 6 month chronic toxicity study with once-weekly dosing. The tumorigenic potential of evolocumab was assessed in a lifetime hamster carcinogenicity assay with dosing once every other week. Fertility and early embryonic assessments were conducted in hamsters with dosing once every other week. Fertility assessments were also included in the 6 month monkey toxicity study. Evaluation of evolocumab administration during the periods of embryofetal and pre/postnatal development was conducted in monkeys with dosing once every other week. Overall, the toxicology program was appropriately designed to evaluate the clinical risks associated with chronic clinical administration of evolocumab per Agency guidance.

### Pharmacokinetics/Pharmacodynamics:

Increases in evolocumab doses in animals generally led to predictable, dose-proportional increases in evolocumab exposure across all dose ranges in toxicity studies. Low incidences of anti-drug antibody production, combined with robust pharmacodynamic reductions in mean plasma cholesterol and other lipid parameters indicate that anti-drug antibodies did not compromise interpretation of study results. Incidences of neutralizing antibodies were negligible in hamsters and low in monkeys. Evolocumab produced a profound lowering of total cholesterol and LDL-C in both hamsters and monkeys. Evolocumab was observed to have similar or greater LDL-C-lowering potency in hamsters and monkeys compared to humans. Due to physiologic differences between primates and rodents, evolocumab also caused decreases in high density lipoprotein (HDL-C) in the hamster. Hamsters have been shown to express

ApoE, which is capable of binding LDLR, on a substantial fraction of HDL particles, especially when systemic LDL-C is low.<sup>1,2</sup>

#### General toxicity:

Evolocumab was well tolerated by hamsters in a 3 month toxicology study with once-weekly subcutaneous dosing at up to 112-, 48- and 20-fold compared to the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively. Evolocumab was also well tolerated in monkeys in toxicology studies of up to 6 months duration with once weekly subcutaneous doses that provide exposure multiples up to 744-, 300- and 134-fold compared to the recommended human doses of 140 mg Q2W and 420 mg Q2W, respectively. The evolocumab injection site was identified as a potential target tissue (minimal to slight acute-chronic inflammation and slight fibrosis) in monkeys; the findings were low in incidence and of modest severity, which indicates that the toxicological significance of these lesions is limited. Local injection site reactions with administration of a human IgG in non-human primates are not unexpected, and are not necessarily predictive of a similar reaction in humans.

#### Combination with statins:

Evolocumab was coadministered to monkeys by the subcutaneous route once every other week for 3 months at up to 50-, 10- and 8.8-fold the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, with once daily oral rosuvastatin at 2-fold the maximum recommended human dose of 40 mg/day, based on plasma exposure. No additive or synergistic toxicity was observed; rosuvastatin was not administered at a dose that caused any statin-related toxicity in monkeys.

#### Mutagenicity/carcinogenicity:

Evolocumab is not expected to interact directly with DNA and mutagenicity studies were not conducted, per ICH-S6. Evolocumab did not cause any drug-related tumors when administered to hamsters for up to 2 years in a lifetime carcinogenicity assay at doses administered once every other week that provide a 38-, 15- and 6.6-fold safety margin for evolocumab at the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W administered subcutaneously.

#### Reproductive toxicology:

Effects of evolocumab on fertility and mating were assessed in hamsters. No effects of evolocumab (subcutaneous dosing once every two weeks) on mating, fertility, estrous cycling, or male reproduction were observed at exposure multiples up to 30-, 12- and 5.3-fold the plasma exposures measured in humans at the 140 mg Q2W, 420 mg QM and 420 mg Q2W evolocumab doses. Effects on fertility were also assessed in the 6 month chronic monkey toxicity study at exposure multiples of up to 744-, 300- and 134-fold compared to the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively. No effects on fertility endpoints were observed.

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<sup>1</sup> Goulinet S and Champan MJ "Plasma lipoproteins in the Golden Syrian hamster (*Mesocricetus auratus*): heterogeneity of apoB- and apoA-I-containing particles" *J Lipid Res* 1993; **34**:943-959.

<sup>2</sup> Evans GF, et al. "Inhibition of cholesteryl ester transfer protein in normocholesterolemic and hypercholesterolemic hamsters: effects on HDL subspecies, quantity, and apolipoprotein distribution" *J Lipid Res* 1994; **35**:1634-1645.



(b) (4)

(b) (4)

Data

*Animal Data*

In cynomolgus monkeys, no effects on embryo-fetal or postnatal development (up to 6 months of age) were observed when

evolocumab was dosed during organogenesis to parturition at 50 mg/kg once every two weeks by the subcutaneous route at 30-, 12-<sup>(b) (4)</sup> fold the recommended human doses of 140 mg Q2W, 420 mg QW<sup>(b) (4)</sup> respectively, based on plasma AUC.

No test of humoral immunity in infant monkeys was conducted with evolocumab.

**8.2 Lactation**

Risk Summary

(b) (4)

(b) (4)

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, and Mutagenesis, Impairment of Fertility

The carcinogenic potential of Repathaevolocumab was evaluated in a lifetime study conducted in the hamster at dose levels (b) (4) of 10, 30 and 100 mg/kg administered (b) (4) every 2 weeks (b) (4). There (b) (4) were no Repathaevolocumab-related tumors (b) (4) - at systemic exposures of 38-, 15- (b) (4) fold the (b) (4) -human doses of 140 mg Q2W, 420 mg QM (b) (4) respectively, based on plasma AUC. The mutagenic potential of Repathaevolocumab has not been evaluated; however, monoclonal antibodies are not expected to alter DNA or chromosomes.

(b) (4)

[Redacted] (b) (4)

Suggested labeling (changes accepted):

**INDICATIONS AND USAGE**

REPATHA is a PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) inhibitor antibody indicated as an adjunct (b) (4) to diet...

**8 USE IN SPECIFIC POPULATIONS**

**8.1 Pregnancy**

Risk Summary

There are no (b) (4) of REPATHA in pregnant women. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively. (b) (4)

[Redacted]

In animal studies, there were no effects on pregnancy or neonatal/infant development (b) (4)

[Redacted]

[Redacted] (b) (4)

## Data

### *Animal Data*

In cynomolgus monkeys, no effects on embryo-fetal or postnatal development (up to 6 months of age) were observed when evolocumab was dosed during organogenesis to parturition at 50 mg/kg once every two weeks by the subcutaneous route at 30-, 12- (b) (4) fold the recommended human doses of 140 mg Q2W, 420 mg QW (b) (4), respectively, based on plasma AUC. (b) (4)

(b) (4) No test of humoral immunity in infant monkeys was conducted with evolocumab.

## 8.2 Lactation

### Risk Summary

(b) (4)

(b) (4)

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis and Mutagenesis

The carcinogenic potential of evolocumab was evaluated in a lifetime study conducted in the hamster at dose levels of 10, 30 and 100 mg/kg administered (b) (4) every 2 weeks. There were no evolocumab-related tumors at systemic exposures of 38-, 15- (b) (4) fold the human doses of 140 mg Q2W, 420 mg QM (b) (4) respectively, based on plasma AUC. The mutagenic potential of evolocumab has not been evaluated; however, monoclonal antibodies are not expected to alter DNA or chromosomes.

## 2 Drug Information

### 2.1 Drug

#### CAS Registry Number

1256937-27-5

#### Generic Name

Evolocumab

#### Code Name

AMG 145

#### Chemical Name

Evolocumab is a human monoclonal antibody of the immunoglobulin G<sub>2</sub> (IgG2) subclass consisting of 2 heavy chains and 2 light chains of the lambda subclass. (b) (4)

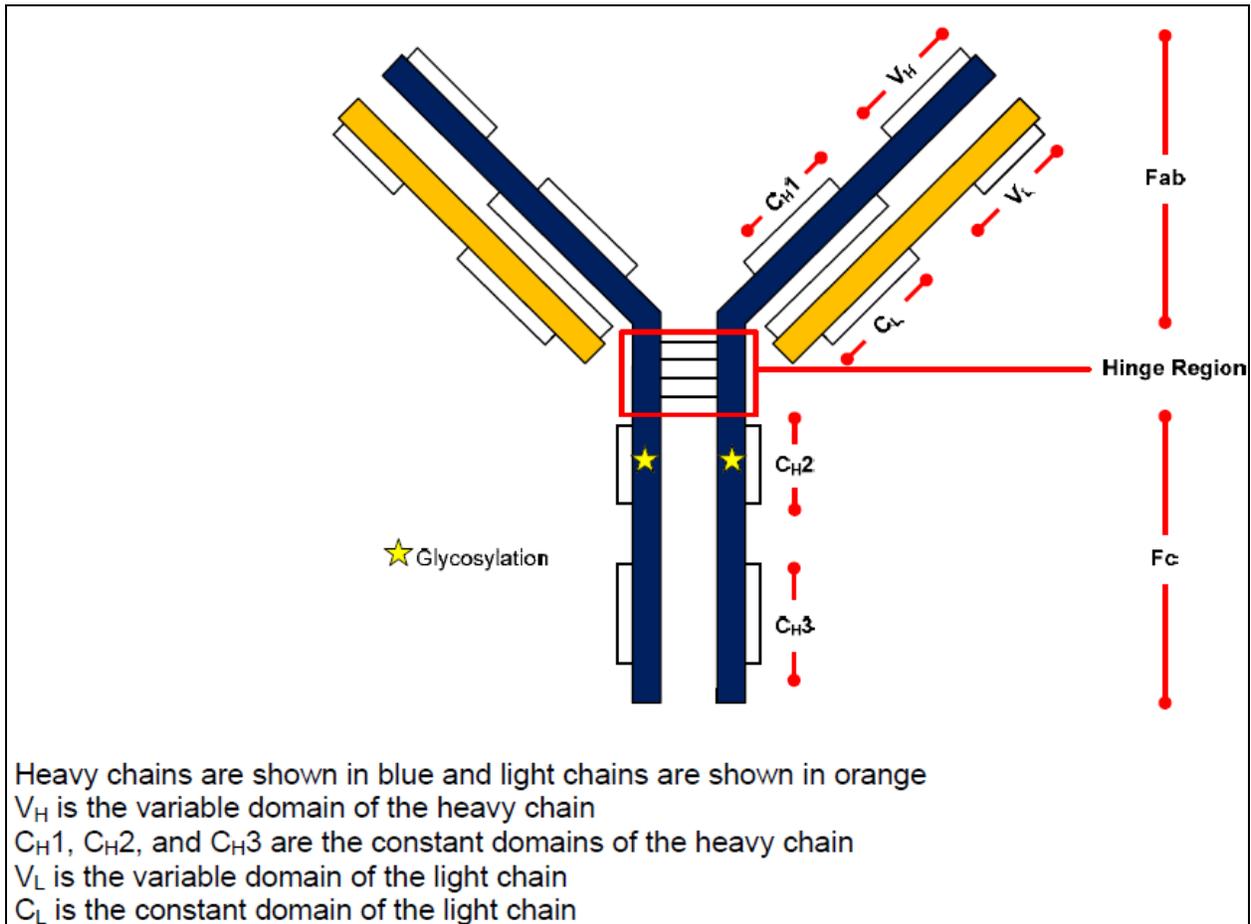
#### Molecular Formula/Molecular Weight

(b) (4)

The experimentally determined evolocumab mass is 144,428 Da.

**Structure or Biochemical Description of Evolocumab**

**Figure 1: Cartoon structure of evolocumab**



Heavy chains are shown in blue and light chains are shown in orange  
 V<sub>H</sub> is the variable domain of the heavy chain  
 C<sub>H</sub>1, C<sub>H</sub>2, and C<sub>H</sub>3 are the constant domains of the heavy chain  
 V<sub>L</sub> is the variable domain of the light chain  
 C<sub>L</sub> is the constant domain of the light chain

(Applicant)

**Figure 2: Amino acid sequence of evolocumab**



(Applicant)



(Applicant)

**Pharmacologic Class**

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor antibody

**2.2 Relevant INDs, NDAs, BLAs and DMFs**

IND 105188, Amgen, evolocumab, a proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitor monoclonal antibody

**2.3 Drug Formulation**

Toxicology formulation and Phase 1/2/3 (partial) formulation  (b) (4)

Phase 3 clinical formulation – 220 mM proline, 20 mM acetate, 0.01% (w/v) polysorbate 80 (pH 5.0)

**Table 1: Formulation of the to-be-marketed evolocumab solution for injection (140 mg/mL)**

Component	Grade	Function	Concentration	Quantity (per dose)
Evolocumab	In house <sup>a</sup>	Active ingredient	140 mg/mL	140 mg
Proline	USP, PhEur, JP	(b) (4)	220 mM	25 mg
Acetic acid, glacial	USP, PhEur, JP	(b) (4)	20 mM	1.2 mg
Polysorbate 80	NF, PhEur, JP	(b) (4)	0.01% (w/v)	0.10 mg
Sodium hydroxide <sup>b</sup>	NF, PhEur, JP	(b) (4)	Titrate to pH 5.0	Titrate
Water for injection	USP, PhEur, JP	(b) (4)	(b) (4)	(b) (4)

<sup>a</sup> Tested to internal specifications (3.2.S.4.1, Specification).  
<sup>b</sup> Sodium hydroxide may be used to adjust pH. The supplier tests sodium hydroxide (b) (4) to NF, PhEur, and JP standards.

(Applicant)

## 2.4 Comments on Novel Excipients

Evolocumab contains no novel excipients.

## 2.5 Comments on Impurities/Degradants of Concern

No impurities of concern were identified.

## 2.6 Proposed Clinical Population and Dosing Regimen

- 140 mg every 2 weeks (Q2W) or 420 mg once monthly (QM) administered subcutaneously for the treatment of adult patients with primary hyperlipidemia (heterozygous familial and nonfamilial) or mixed dyslipidemia
- 420 mg Q2W or 420 mg QM in adults and adolescents aged 12 years and over with homozygous familial hypercholesterolemia (HoFH).

## 12.7 Regulatory Background

The following is a concise summary of the regulatory history of the evolocumab product development program:

- The initial, IND-opening submission for IND 105188 was received on 15 May 2010.
- The Applicant proposed a lifetime hamster carcinogenicity study to address the FDA's concerns for carcinogenic potential of evolocumab. The protocol was submitted to the Agency on 11 August 2011 for Special Protocol Assessment. A meeting was held to discuss the Applicant's proposed doses. CDER's Executive Carcinogenicity Assessment Committee provided meeting minutes indicating concurrence with the proposed dosing regimen to the Applicant on 1 September 2011.

- A face-to-face end-of-phase 2 meeting with the Applicant was held at FDA on 10 July 2012. Pharm/Tox agreed that the Applicant had provided sufficient nonclinical data to support phase 3 clinical investigations.
- A face-to-face pre-BLA meeting was held at FDA on 10 April 2014. Pharm/Tox agreed that the Applicant's nonclinical safety database appeared appropriate in scope for filing the application.
- The BLA was submitted on 27 August 2014. The BLA was designated for a standard review.

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Study Title	Testing Facility
<p><b>Repeat-dose Toxicology Studies</b></p> <p>A 28-Day Subcutaneous Injection Toxicity Study of AMG 145 in Hamsters With a 16-Week Treatment-free Period</p> <p>3-Month Subcutaneous Injection Extended Pharmacology Study of AMG 145 in Hamsters</p> <p>6-Week Subcutaneous Toxicity Study of AMG 145 in Cynomolgus Monkeys, With a 21-Week Treatment-free Period</p> <p>6-Month Subcutaneous Injection Toxicity Study of AMG 145 in Cynomolgus Monkeys With a 25-Week Treatment-free Phase</p> <p>AMG 145 and Rosuvastatin: 3-Month Combination Toxicology Study in the Cynomolgus Monkey With a 4-Month Recovery Phase</p> <p><b>Carcinogenicity</b></p> <p>AMG 145: Subcutaneous Lifetime Pharmacology Study in Hamsters</p> <p><b>Reproductive and Developmental Toxicity</b></p> <p>AMG 145: Fertility and Early Embryonic Development Study in the Hamster</p> <p>AMG 145: Enhanced Pre-postnatal Development Study in the Cynomolgus Monkey With a 6-Month Postnatal Evaluation</p> <p><b>Local Tolerance</b></p> <p>AMG 145: Subcutaneous Local Tolerance Study in the Hamster</p>	<p>(b) (4)</p>
<p>Local Tolerance Test in Rabbits After Bolus Intravenous Administration of AMG 145</p>	

(Applicant)

Study Title	Testing Facility
<b>Other Toxicity Studies</b> Tissue Cross-Reactivity of Alexa Fluor 488-AMG 145 With Golden Syrian Hamster Tissues In Vitro  Cross-Reactivity Study of Alexa Fluor 488-AMG 145 With Normal Human and Cynomolgus Monkey Tissues	(b) (4)

(Applicant)

### 3.2 Studies Not Reviewed

None

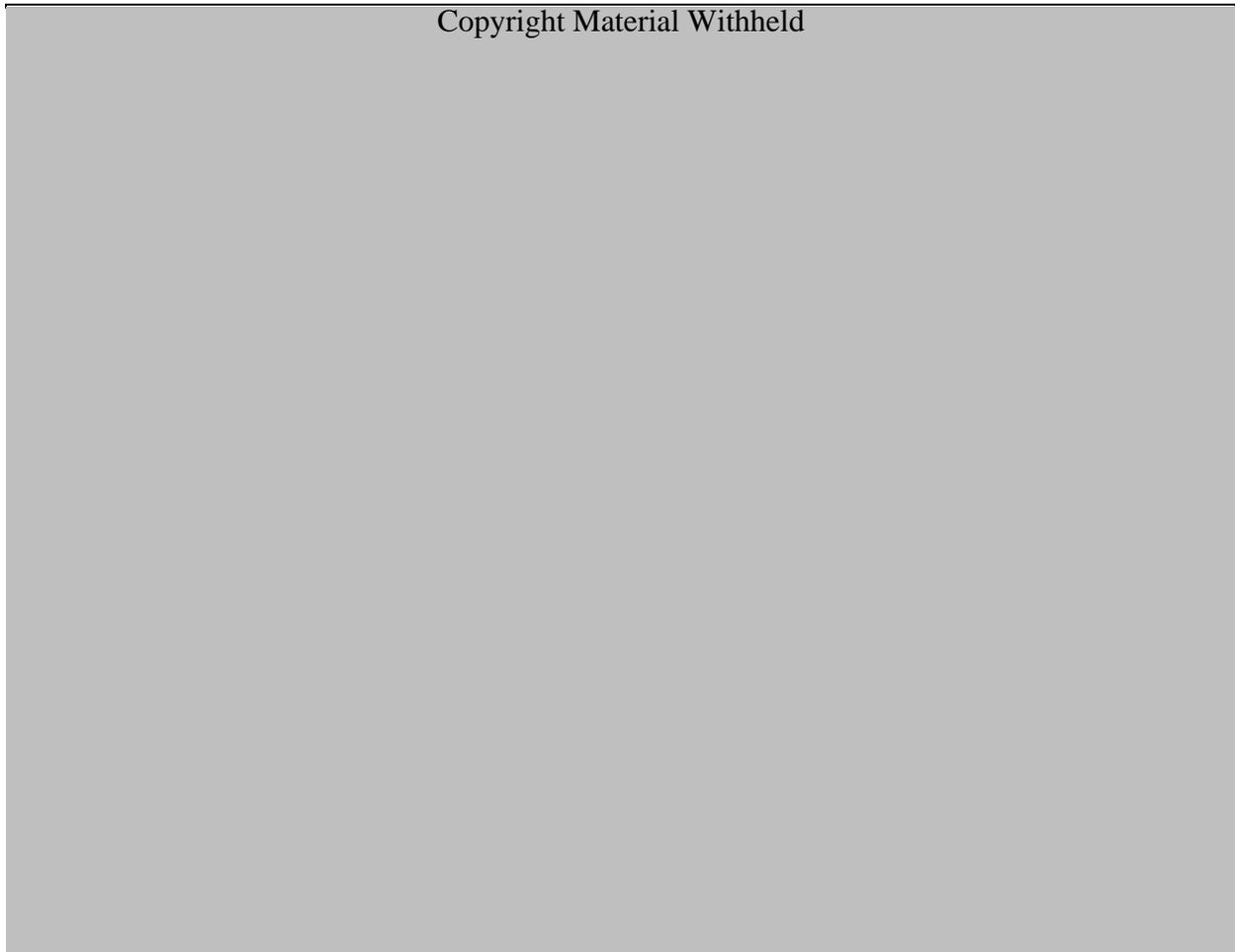
### 3.3 Previous Reviews Referenced

None

## 4 Pharmacology

### 4.1 Primary Pharmacology

Expression of hepatic LDL-receptor (LDLR) and its function in removing LDL-C from circulation is dependent on intracellular cholesterol levels and serum proprotein convertase subtilisin kexin type 9 (PCSK9) concentrations. Serum PCSK9 reduces hepatic LDLR protein levels by promoting its internalization and lysosomal degradation (see Figure 3). Any modulators that affect expression of PCSK9 will affect liver LDLR density and its capacity to remove LDL-C from circulation.

**Figure 3: PCSK9-mediated degradation of LDLR**

(Lambert G, et al.<sup>3</sup>)

Transcription of PCSK9 and LDLR genes share a common regulatory mechanism mediated by sterol regulatory element binding proteins (SREBPs), which are members of the basic helix-loop-helix leucine zipper family of transcription factors. Intracellular sterols inhibit LDLR and PCSK9 gene transcription by suppression of the processing and release of SREBP2.

Inactive SREBPs contain two transmembrane domains and remain bound to the endoplasmic reticulum after synthesis. In the endoplasmic reticulum, the C-terminal domains of SREBPs interact with another membrane protein SREBP-cleavage-activating protein (SCAP), which functions as a sterol sensor. In the sterol-depleted state, SCAP escorts the SREBPs from the endoplasmic reticulum to the Golgi where they are cleaved by proteases, which release the mature N-terminal transcription activation domain of SREBPs from the precursor proteins. The active SREBPs translocate to the nucleus where they bind to the promoters of SREBP target genes including those involved in the synthesis and metabolism of cholesterol (see Figure 4).

<sup>3</sup> Lambert G, et al. "The PCSK9 decade" *J Lipid Res* 2012; **53**:2515-2524.

Consequently, SREBP2 enters the nucleus and binds to the sterol regulatory element 1 (SRE-1) site of LDLR and PCSK9 promoters, leading to increased production of both proteins. Statins coordinately upregulate both the *PCSK9* and *LDLR* genes through decreased de novo production of cholesterol, which leads to SREBP2 activation and binding to the SRE-1 motifs present in the proximal promoter region of both genes.<sup>4</sup>

**Figure 4: Cellular regulation of cholesterol homeostasis**

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(Spann and Glass<sup>5</sup>)

Evolocumab is a fully human IgG2 monoclonal antibody with high affinity for human PCSK9. PCSK9 normally functions to down-regulate LDL receptor (LDLR) activity; PCSK9 binds LDLR and leads to internalization and lysosomal degradation of LDLR. PCSK9 is expressed in significant amounts in liver, neuronal tissues, adrenal glands, kidney mesenchymal cells, and intestinal epithelia. Evolocumab binds the EGF domain of PCSK9, disrupting its interaction with the extracellular domain of the LDLR. This allows increased LDLR presence on the surface of hepatocytes, increasing LDL-cholesterol (LDL-C) clearance, and lowering total cholesterol and LDL-C levels detected in the blood (see Figure 5). Statins increase PCSK9 production, and evolocumab overrides this compensatory effect of statins.

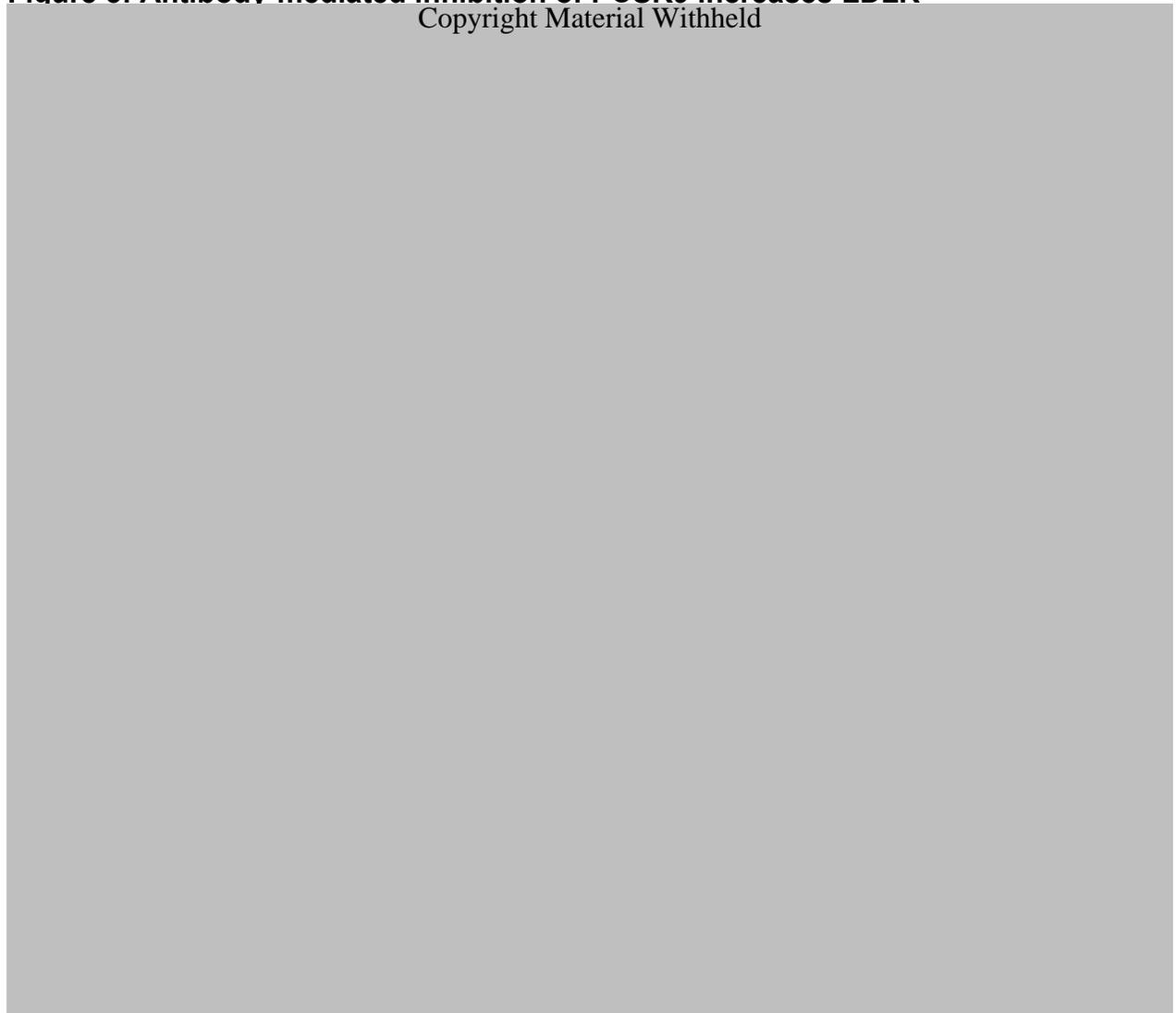
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<sup>4</sup> Dong B, et al. "CETP inhibitors downregulate hepatic LDL receptor and PCSK9 expression in vitro and in vivo through a SREBP2 dependent mechanism" *Atherosclerosis* 2014; **235**:449-462.

<sup>5</sup> Spann NJ and Glass CK "Sterols and oxysterols in immune cell function" *Nature Immunol* 2013; **14**(9):893-900.

**Figure 5: Antibody-mediated inhibition of PCSK9 increases LDLR**

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(Lambert G, et al.<sup>6</sup>)

High circulating levels of LDL-C are associated with atherosclerosis and are an independent risk factor for myocardial infarction, stroke, and death. PCSK9 is a novel target for LDL-C lowering therapy. Inhibition of PCSK9 function with evolocumab was shown by the Applicant to lower circulating plasma LDL-C levels in nonclinical species, including hamsters and monkeys.

**Brief summary of literature data supporting an important role for PCSK9 in modulating LDL-C:**

- Gain-of-function mutations in PCSK9 in humans are associated with elevated LDL-C levels (>300 mg/dL) and premature coronary heart disease<sup>7</sup>.

<sup>6</sup> Lambert G, et al. "The PCSK9 decade" *J Lipid Res*; 2012; **53**:2515-2524.

<sup>7</sup> Soutar AK and Naoumova RP "Mechanisms of disease: genetic causes of familial hypercholesterolemia" *Nat Clin Pract Cardiovasc Med* 2007; **4**(4):214-225.

- Loss-of-function (LOF) mutations in PCSK9 in humans are associated with low LDL-C levels (< 100 mg/dL)<sup>8</sup> and a reduction in the incidence of coronary heart disease over a 15-year period compared to patients lacking this mutation<sup>9</sup>.
- Patients with LOF mutations, including 2 subjects identified who apparently lack PCSK9 (due to compound heterozygous LOF mutations) appear to be healthy.<sup>10</sup>
- Overexpression of PCSK9 in mice is associated with reduced hepatic LDLR protein and increases in the level of total serum cholesterol.<sup>11</sup>
- PCSK9 knockout mice and mice treated with PCSK9 antisense oligonucleotide exhibit a 2- to 3-fold increase in hepatic LDLR protein level, accompanied by an approximately 50% reduction in serum total cholesterol.<sup>12</sup>

Brief summary of Applicant-submitted data:

- Evolocumab binds to human, cynomolgus monkey and hamster PCSK9 with high affinity ( $K_D = 16, 8$  and  $14$  pM, respectively; see Table 2).
- Evolocumab has lower affinity for mouse PCSK9 ( $17,000$  pM). (Note: The  $K_D$  for mouse PCSK9 binding to evolocumab was measured using a very different assay procedure than for human, monkey and hamster, and some of the difference in measured  $K_D$  may be artifactual. Evolocumab affinity for rat PCSK9 was not tested by the Applicant, but the rat and mouse PCSK9 are more similar than that of human/monkey/hamster.)

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<sup>8</sup> Cohen J, et al. "Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9" *Nat Genet* 2005; **37**(2):161-165.

<sup>9</sup> Cohen J, et al. "Sequence variations in PCSK9, low LDL, and protection against coronary heart disease" *N Engl J Med* 2006; **354**(12):1264-1272.

<sup>10</sup> Zhao Z, et al. "Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote" *Am J Hum Genet* 2006; **79**(3):514-523.

<sup>11</sup> Maxwell KN and Breslow JL "Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype" *PNAS* 2004; **101**(18):7100-7105.

<sup>12</sup> Rashid S. et al. "Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9" *PNAS* 2005; **102**(15):5374-5379.

**Table 2: Dissociation constants (apparent binding affinity) for evolocumab binding to human (b) (4) monkey, hamster and mouse PCSK9**

PCSK9	Lot #	K <sub>D</sub> (pM)	95% Confidence Interval
Human (b) (4)	011007	16	8 to 27 pM
Human (b) (4)	021507	7	5 to 9 pM
Cynomolgus Monkey	122106	8	5 to 11 pM
Hamster	84357-24	14	11 to 16 pM
Mouse	081006	17000	NA

Binding affinity of AMG 145 to human (b) (4), monkey, or hamster PCSK9 was determined in an equilibrium binding assay by (b) (4) as described in [Methods](#). (b) (4)

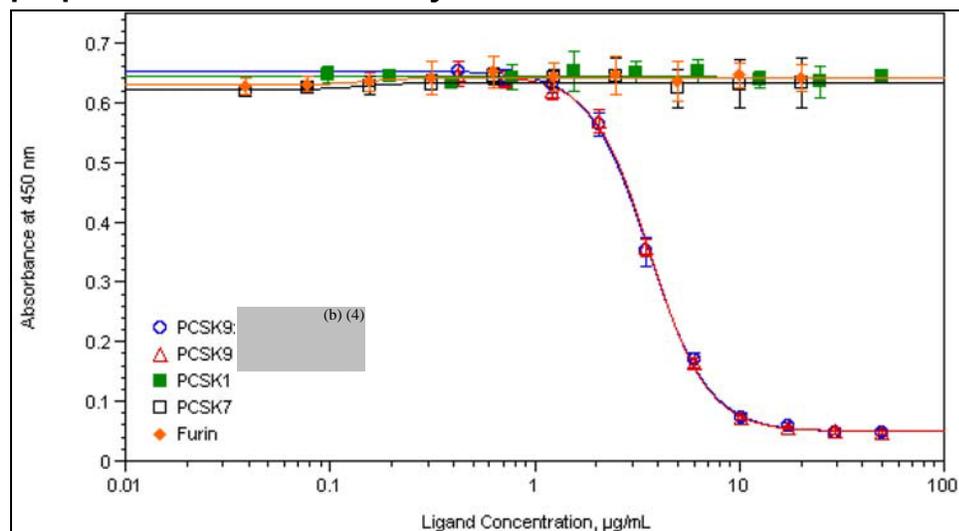
(b) (4)

(b) (4) The dissociation equilibrium constant (K<sub>D</sub>) was obtained from nonlinear regression of the competition curves using an n-curve one-site homogeneous binding model provided in the KinExA Pro software (Sapidyne Instruments Inc., Boise, ID). Binding affinity of AMG 145 to mouse PCSK9 was determined in a (b) (4) kinetic binding assay as described in [Methods](#). (b) (4)

(b) (4)

(Applicant)

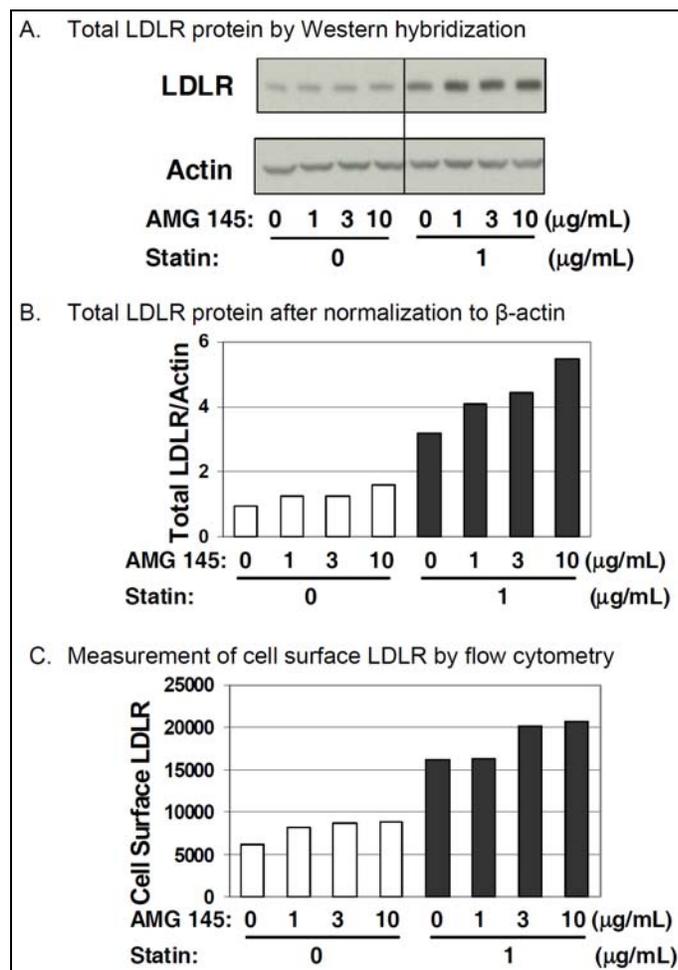
- By competitive ELISA, evolocumab was demonstrated to be specific for PCSK9, and did not bind to the subtilisin-like proprotein convertase family members PCSK1, PCSK7 or Furin (see Figure 6). Binding to other PCSK family members were not measured (i.e., there are currently 9 identified family members).

**Figure 6: Antigen specificity of evolocumab for PCSK9 versus other subtilisin-like proprotein convertase family members**

(Applicant)

- Evolocumab (AMG 145) caused a dose-dependent inhibition of PCSK9 binding to LDLR and the PCSK9-mediated reduction in LDL-C uptake in HepG2 cells (human hepatocellular carcinoma cell line) in culture.
- Combined treatment of HepG2 cells with evolocumab and a statin increased total and cell-surface LDLR protein levels to a greater extent than either treatment did alone (see Figure 7).

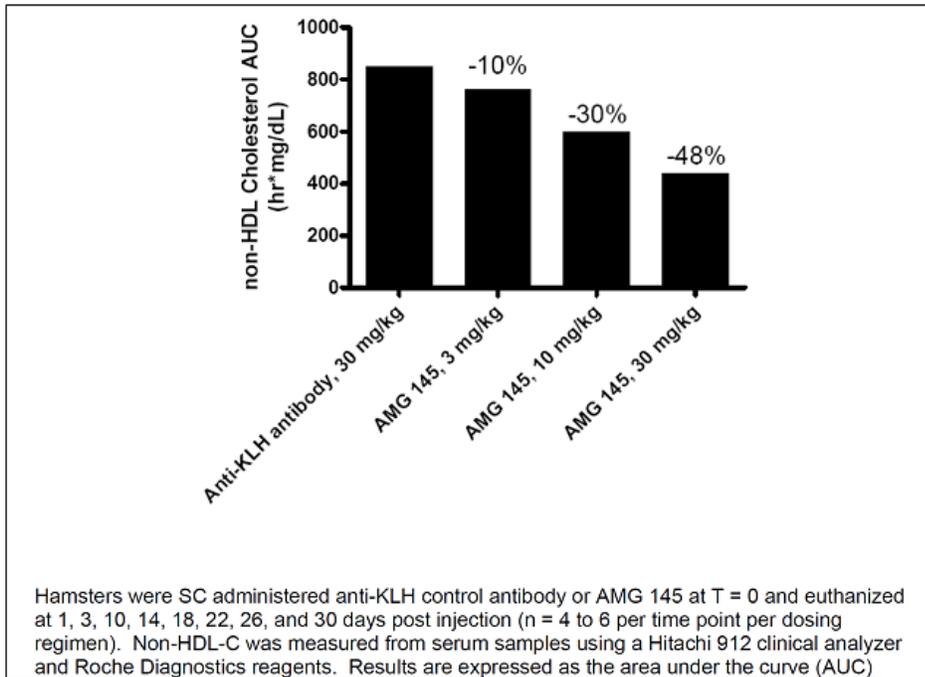
**Figure 7: Effect of combined treatment of HepG2 cells with evolocumab and a statin on LDLR**



(Applicant)

- In vivo administration of evolocumab in hamsters resulted in increased hepatic LDLR protein (data not shown), decreased serum non-HDL-C (see Figure 8), and total cholesterol (data not shown).

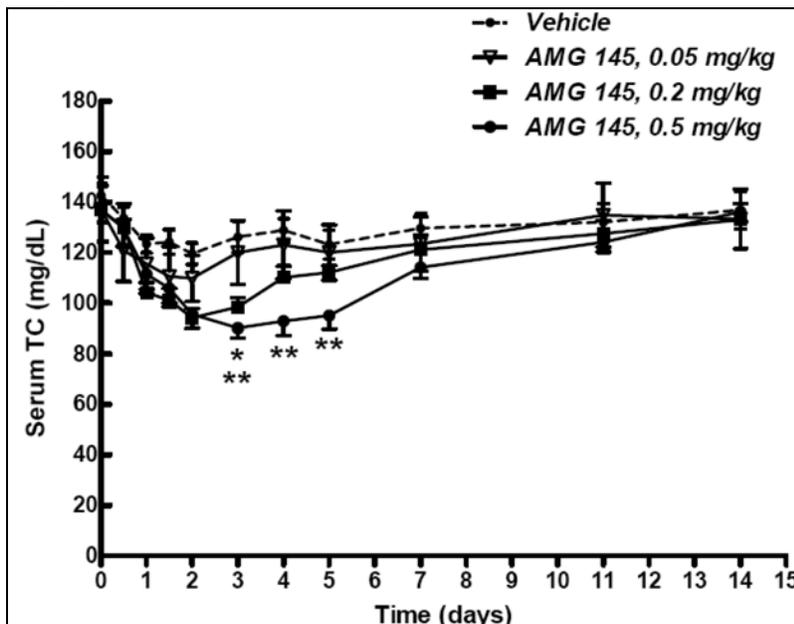
**Figure 8: Pharmacodynamic effect of evolocumab on non-HDL-C following a single-dose in hamsters**



(Applicant)

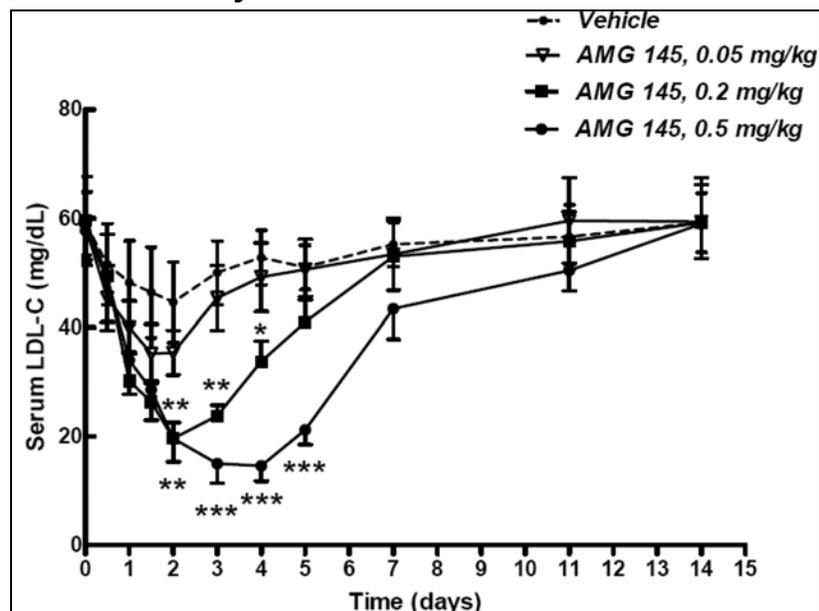
- In vivo administration of evolocumab in male cynomolgus monkeys resulted in decreased total cholesterol and serum LDL-C (see Figure 9 and Figure 10).

**Figure 9: Pharmacodynamic effect of evolocumab on total cholesterol following a single dose in monkeys**



(Applicant)

**Figure 10: Pharmacodynamic effect of evolocumab on LDL-C following a single dose in monkeys**



(Applicant)

## 4.2 Secondary Pharmacology

The likelihood that evolocumab may provoke toxicity through exaggerated pharmacology is addressed briefly, below. No formal secondary pharmacology studies were conducted.

### Brain and cognitive function:

- Evolocumab is too large (~144 kDa) to cross the intact blood-brain barrier (BBB).<sup>13</sup>
- Cholesterol in brain is understood to be primarily derived from de novo biosynthesis.<sup>14</sup>
- Under normal conditions with an intact BBB, evolocumab is considered unlikely to directly affect cholesterol levels in the brain.
- While not designed to rule out an effect with prolonged duration of exposure, evolocumab showed no adverse effects on neurological behavior in a single-dose safety pharmacology study with administration of 300 mg/kg to cynomolgus monkeys.
- A six month chronic monkey toxicity study did not show obvious adverse behavioral signs, but that study did not include a functional observational battery assessment.

<sup>13</sup> Pardridge WM "The blood-brain barrier: bottleneck in brain drug development." *NeuroRx* 2005; **2**(1):3-14.

<sup>14</sup> Bjorkhem I and Meaney S "Brain cholesterol: long secret life behind a barrier" *Arterioscler Thromb Vasc Boil* 2004; **24**(5):806-815.

PCSK9 interaction with other LDLR family members:

- The LDLR family of proteins consists of LDLR, VLDLR, ApoER2 and LRP6.
- These proteins share many features, including ligand binding domains, epidermal growth factor (EGF)-like domains and short cytoplasmic tails.
- Evolocumab disrupts PCSK9 binding to the EGF-A domain.
- Homology between the EGF-A domains of LDLR and those of other LDLR family members is high.<sup>15</sup>
- Available data are currently insufficient to determine the extent to which PCSK9, and therefore evolocumab, may impact regulation of other LDLR family member proteins.

Hepatitis C virus (HCV) infectivity:

- Based on the current consensus, HCV infection is dependent upon viral entry via a core viral “receptor” composed of CD81, SR-B1, claudin-1 and occludin.<sup>16</sup>
- The role of LDLR in HCV infectivity is less clear, but evidence indicates LDLR may also facilitate HCV entry into hepatocytes in the presence of the core viral receptor proteins<sup>17</sup>. It is well-established that PCSK9 regulates LDLR levels. PCSK9 has been implicated in regulation of CD81<sup>18</sup>. Therefore, evolocumab could theoretically increase HCV infectivity by multiple mechanisms. Available data are conflicting regarding whether PCSK9 inhibition can affect CD81 and/or hepatitis C infectivity/viral loads.

Insulin resistance and diabetes:

- Several studies have associated statin therapy with increased risk for development of type 2 diabetes mellitus.<sup>19</sup>
- The potential role of cholesterol metabolism, and in particular PCSK9 inhibition, in development of insulin resistance is unclear. No effect on plasma glucose was observed in hamsters or monkeys at doses up to 300 mg/kg/week for durations up to 3 months (hamster) and 6 months (monkey). No effects on pancreas that might indicate potential effects on insulin production or sensitivity were observed.

Statin-related myopathy:

- A clinically significant, dose-related toxicity of statins is myopathy, which led to the voluntary removal of cerivastatin from the market in 2001.
- There is the theoretical potential for evolocumab to pharmacodynamically interact with statins (e.g., decreased LDL-C) to exacerbate myopathy. The mechanism by which statins induce myopathy is not completely understood.

<sup>15</sup> Gu HM, et al. “Characterization of the role of EGF-A of low density lipoprotein receptor in PCSK9 binding” *J Lipid Res* 2013; **54**(12):3345-3357.

<sup>16</sup> Zeisel MB, et al. “Hepatitis C virus entry into hepatocytes: molecular mechanisms and targets for antiviral therapies” *J Hepatol* 2011; **54**(3):566-576.

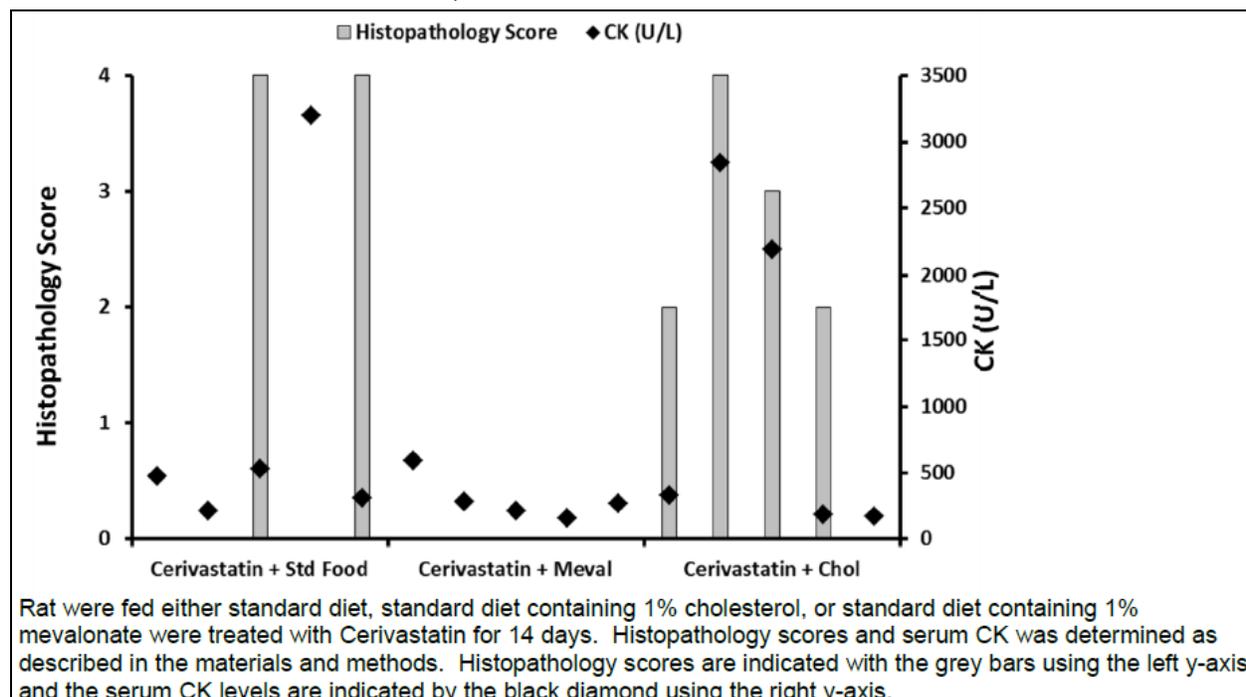
<sup>17</sup> Diedrich G “How does hepatitis C virus enter cells?” *FEBS J* 2006 **273**:3871-3885.

<sup>18</sup> Labonte, P. et al. “PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression” *Hepatology* 2009; **50**(1):17-24.

<sup>19</sup> Shah, R.V. and Goldfine, A.B. “Statins and risk of new-onset diabetes mellitus” *Circulation* 2012; **126**(18):e282-284.

- To address this concern, the Applicant performed a study where 1 mg/kg cerivastatin (a statin with a high potential for induction of myopathy) or vehicle, was administered to female Sprague-Dawley rats for 2 weeks.
- Rats were fed a standard chow or one fortified with mevalonate or cholesterol from Study Day -4 to Day 14. Mevalonate is a precursor of cholesterol downstream of the rate limiting step, HMG-CoA reductase.
  - Clinical chemistry and histopathology data showed evidence of liver and muscle toxicity (increased AST, creatine kinase, serum fatty acid binding protein 3 (FABP3), serum skeletal troponin I (sTnI), urinary myoglobin and myopathy) with administration of cerivastatin, which occurred with both standard and cholesterol-enriched diets.
  - Cerivastatin toxicity did not occur with supplementation of mevalonate.
  - A representative figure showing creatine kinase levels and histopathology scores for rats administered cerivastatin and fed one of three diets is shown in Figure 11. (Note: Data for AST, FABP3, sTnI and urinary myoglobin showed similar patterns when represented graphically.)
- Data indicate that toxicity with cerivastatin may be caused by inhibition of cholesterol biosynthesis upstream of mevalonate.
- While HMG-CoA reductase inhibitors would be expected to cause toxicity (the HMG-CoA reductase enzyme produces mevalonic acid), PCSK9 inhibitors would not (PCSK9 inhibitors are not expected to directly affect mevalonate levels).
- No direct comparison between cerivastatin and evolocumab was conducted.
- No creatine kinase elevation or increased muscle toxicity was observed in hamsters or monkeys administered evolocumab at doses up to 300 mg/kg/week for durations of 3 months (hamster) and up to 6 months (monkeys). No increase in statin-induced toxicity was observed in a 3 month combination toxicity study in monkeys.

**Figure 11: Histopathology and creatine kinase levels in rats treated with cerivastatin and fed standard, cholesterol-enriched or mevalonate-enriched diets**



N=20/group, standard chow; N=5/group, fortified mevalonate diet; N=5/group, fortified cholesterol diet (Applicant)

#### Bile acid load in the intestine:

- Bile acids are synthesized from cholesterol in the liver.<sup>20</sup>
- The presence of cholesterol up-regulates production of bile acids.<sup>21</sup>
- The increased influx of cholesterol into the liver initiated by PCSK9 inhibition by evolocumab could theoretically lead to increased bile acid load in the intestine.
- High intestinal levels of bile acids have been demonstrated to promote colon tumors in rodent studies, with implications for humans.<sup>22</sup>
- In rodent and non-rodent toxicity studies with evolocumab, no colon abnormalities were observed in hamsters and cynomolgus monkeys treated with evolocumab for 1 and up to 6 months, respectively, or when evolocumab was administered in combination with rosuvastatin in cynomolgus monkeys for 3 months. No increase in tumor incidence (including the gastrointestinal tract) was observed when evolocumab was administered to hamsters at dose levels up to 100 mg/kg every two weeks for 2 years (i.e., a lifetime). These data indicate increases in bile acid load in the intestine, if they occur, are unlikely to be physiologically relevant.

<sup>20</sup> Russell D.W. "The enzymes, regulation, and genetics of bile acid synthesis." *Annu Rev Biochem* 2003; **72**:137-174.

<sup>21</sup> Russell DW "Bile acid biosynthesis." *Biochemistry* 1992; **31**(20):4737-4749.

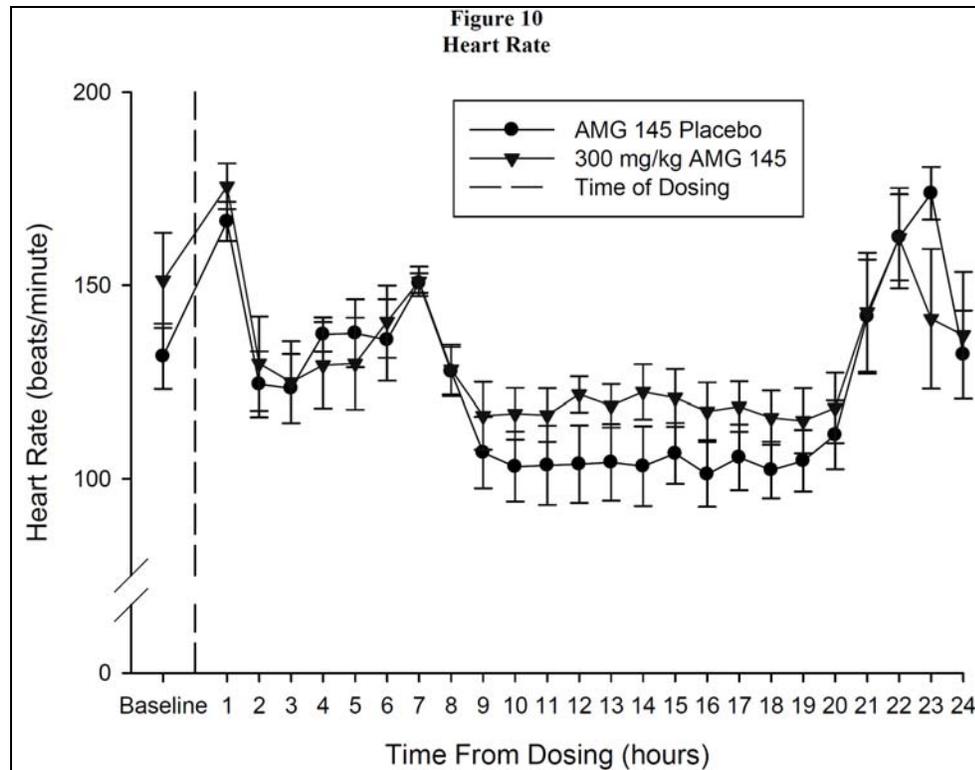
<sup>22</sup> Weisburger JH, et al. "Bile acids, but not neutral sterols, are tumor promoters in the colon in man and in rodents." *Env Health Perspect* 1983; **50**:101-107.

Immune system:

- Measures of immune function, including peripheral blood immunophenotyping, T cell dependent antibody response (TDAR), and natural killer cell (NKC) activity, as well as lymphoid organ histopathology and hematology were assessed in cynomolgus monkeys.
- No significant effects on immune system functional end-points, clinical chemistry, or histopathology were observed when evolocumab was administered alone for 6 months or in combination with rosuvastatin for 3 months.

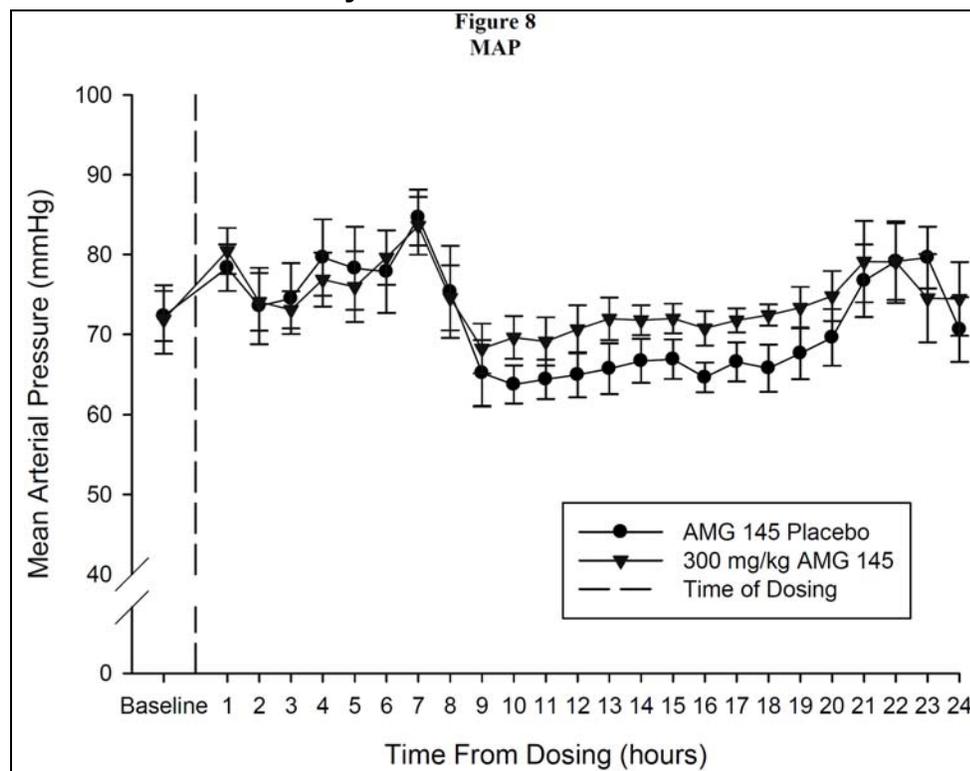
**4.3 Safety Pharmacology**

- When administered as a single intravenous dose of 300 mg/kg to conscious telemetry-instrumented cynomolgus monkeys, evolocumab (AMG 145) was well-tolerated and had no adverse effects on cardiovascular function, respiration rate, neurological behavior, or body temperature.
  - Slight increases in heart rate and blood pressure were observed between 9-20 hours post-dose (see Figure 12 and Figure 13). These slight effects are not considered to represent significant safety concerns, since the effects were not statistically significant and increased heart rate and blood pressure were not observed with longer durations of dosing at similar exposures.

**Figure 12: Heart rate following a single intravenous dose of evolocumab in monkeys**

(Applicant)

**Figure 13: Mean arterial pressure following a single intravenous dose of evolocumab in monkeys**



(Applicant)

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

Pharmacokinetics and pharmacodynamics of evolocumab were evaluated in monkeys (N = 4 to 5 males/group) following single intravenous (3 mg/kg) or single subcutaneous (3, 10, 30 mg/kg) evolocumab administration.

#### Single-dose pharmacokinetics

- The serum profile of unbound evolocumab following a single intravenous 3 mg/kg dose to cynomolgus monkeys was consistent with non-linear elimination (see Table 3).
  - Mean serum systemic clearance was 5.4 mL/day/kg.
  - Mean estimated volume of distribution at steady-state was 25.3 mL/kg (the estimated total blood volume of cynomolgus monkeys is ~55 mL/kg).
- The pharmacokinetic data from the single subcutaneous injections confirmed non-linear elimination kinetics.
  - Decreases in apparent clearance (CL/F) occurred with increasing dose.
  - Increases in AUC were greater than dose-proportional.
  - Increases in  $C_{max}$  were greater than dose-proportional.
  - Estimated bioavailability was 82%.

- Median  $T_{max}$  was 3 days for all subcutaneous doses.
- $T_{1/2}$  increased with increasing dose from 2.5 hours (3 mg/kg SC) to 6 hours (30 mg/kg SC).
- The data are consistent with saturation of clearance mechanisms for this monoclonal antibody (non-specific clearance by FcR $\gamma$  in monocytes, macrophages, neutrophils and eosinophils, and other cells of the immune system).<sup>23</sup>

**Table 3: Summary evolocumab pharmacokinetics after single subcutaneous or intravenous doses in monkeys**

Treatment Group	Subject	$T_{max}$ (day)	$C_{max}$ or $C_0$ (ng/ml)	$AUC_{0-inf}$ (day*ng/ml)	Extrap AUC (%)	$T_{1/2,z}$ (day)	CL or CL/F (ml/day/kg)	$V_z$ or $V_z/F$ (ml/kg)	$V_{ss}$ (ml/kg)
Group 1 3 mg/kg IV	N	4	4	4	4	4	4	4	4
	Mean	NC	10900 0	573000	5.5	2.53	5.4	19.4	25.3
	SD	NC	16100	109000	9.7	0.50	1.0	4.5	1.7
	Median	NC	10700 0	563000	0.8	2.62	5.5	18.0	25.1
	CV%	NC	14.8	19.0	177.0	19.9	18.7	23.0	6.5
Group 3 3 mg/kg SC	N	5	5	5	5	5	5	5	NC
	Mean	2.8	71100	469000	1.1	2.07	6.5	19.3	NC
	SD	0.45	7170	60100	0.6	0.17	0.8	2.2	NC
	Median	3.0	70700	500000	1.1	2.07	6.4	20.0	NC
	CV%	16.0	10.1	12.8	57.2	8.1	12.3	11.2	NC
Group 4 10 mg/kg SC	N	5	5	5	5	5	5	5	NC
	Mean	3.6	17200 0	2570000	0.1	4.13	4.1	23.2	NC
	SD	1.9	13900	751000	0.0	1.28	0.9	5.0	NC
	Median	3.0	17200 0	2000000	0.1	4.50	4.1	26.3	NC
	CV%	54.1	8.1	29.2	37.3	30.9	22.4	21.6	NC
Group 5 30 mg/kg SC	N	5	5	5	5	5	5	5	NC
	Mean	4.2	83300 0	13500000	0.0	5.92	2.4	19.9	NC
	SD	2.6	15700 0	4010000	0.1	1.47	0.8	5.2	NC
	Median	3.0	91700 0	20000000	0.0	5.51	1.9	21.9	NC
	CV%	61.6	18.9	29.7	136.9	24.8	33.3	26.3	NC

(Applicant)

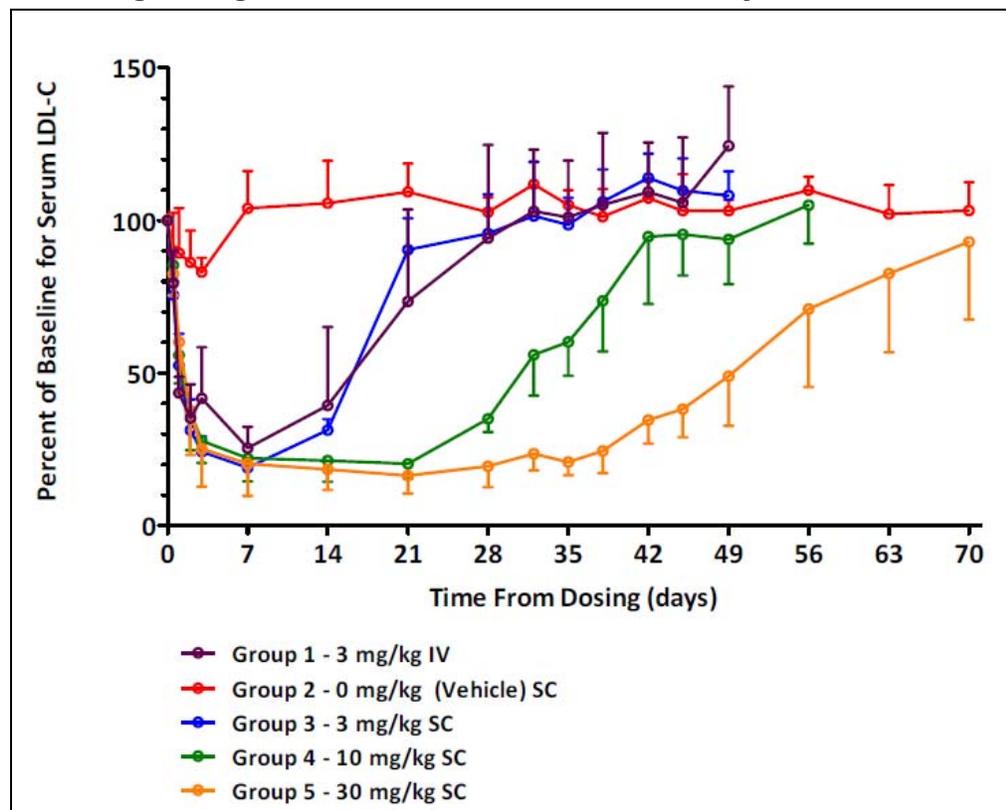
#### Single-dose pharmacodynamics

- A single administration of evolocumab at 3 to 30 mg/kg in monkeys by the subcutaneous route or 3 mg/kg by the intravenous route resulted in a statistically significant mean reduction in LDL-C of approximately 80% in all treatment groups within the first 7 to 14 days of treatment ( $p < 0.001$ ), see Figure 14.
- While the maximal LDL-C decrease was saturable under the conditions tested, the rate of return of LDL-C to baseline was dose-dependent, as follows:

<sup>23</sup> Tabrizi M, et al. "Biodistribution Mechanisms of Therapeutic Monoclonal Antibodies in Health and Disease" *AAPS Journal* 2010; **12**(1):33-43.

- 14 days in the 3 mg/kg subcutaneous dose group
- 38 days in the 10 mg/kg subcutaneous dose group
- 63 days in the 30 mg/kg subcutaneous dose group

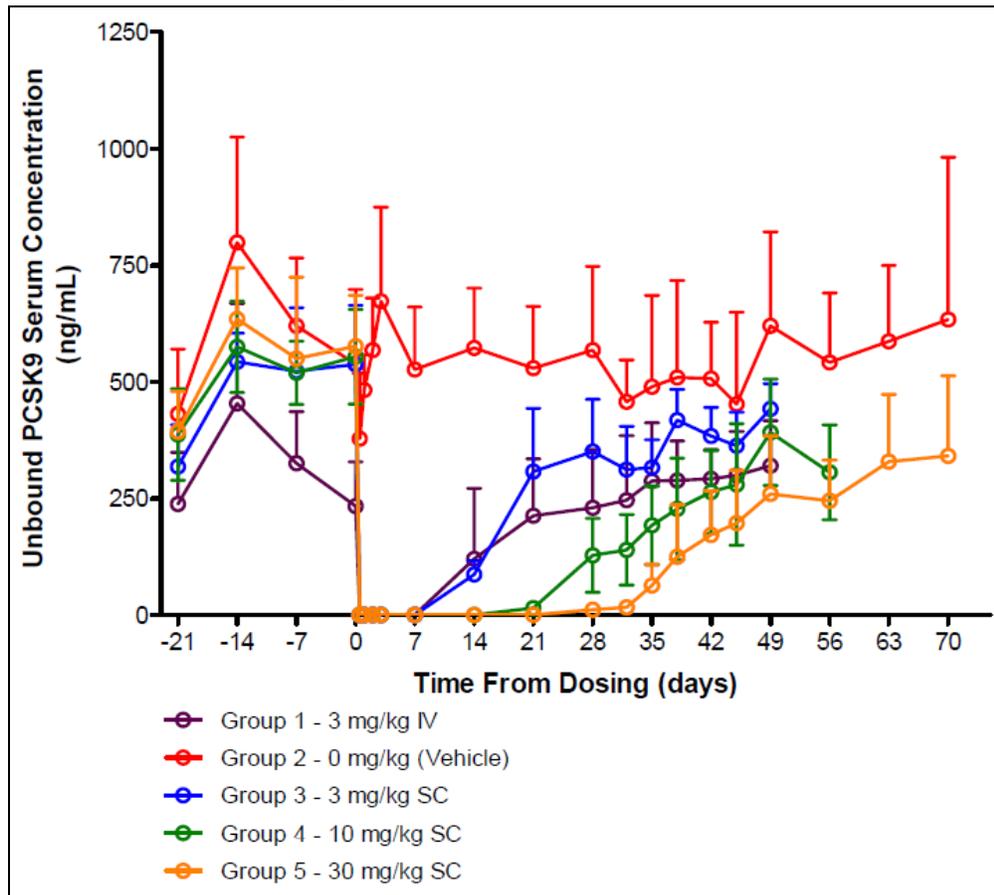
**Figure 14: Mean percent decrease from baseline for serum LDL-C concentrations following a single dose of evolocumab in monkeys**



(Applicant)

- Evolocumab treatment was not associated with changes in HDL-C and triglycerides, or ApoA1 (data not shown) that is expressed primarily on HDL particles. ApoB, which is expressed primarily on non-HDL particles, was well correlated with LDL-C (data not shown).
- Evolocumab treatment immediately decreased measurable PCSK9 levels, and resulted in unbound serum PCSK9 levels below the level of quantitation in all dose groups (see Figure 15).

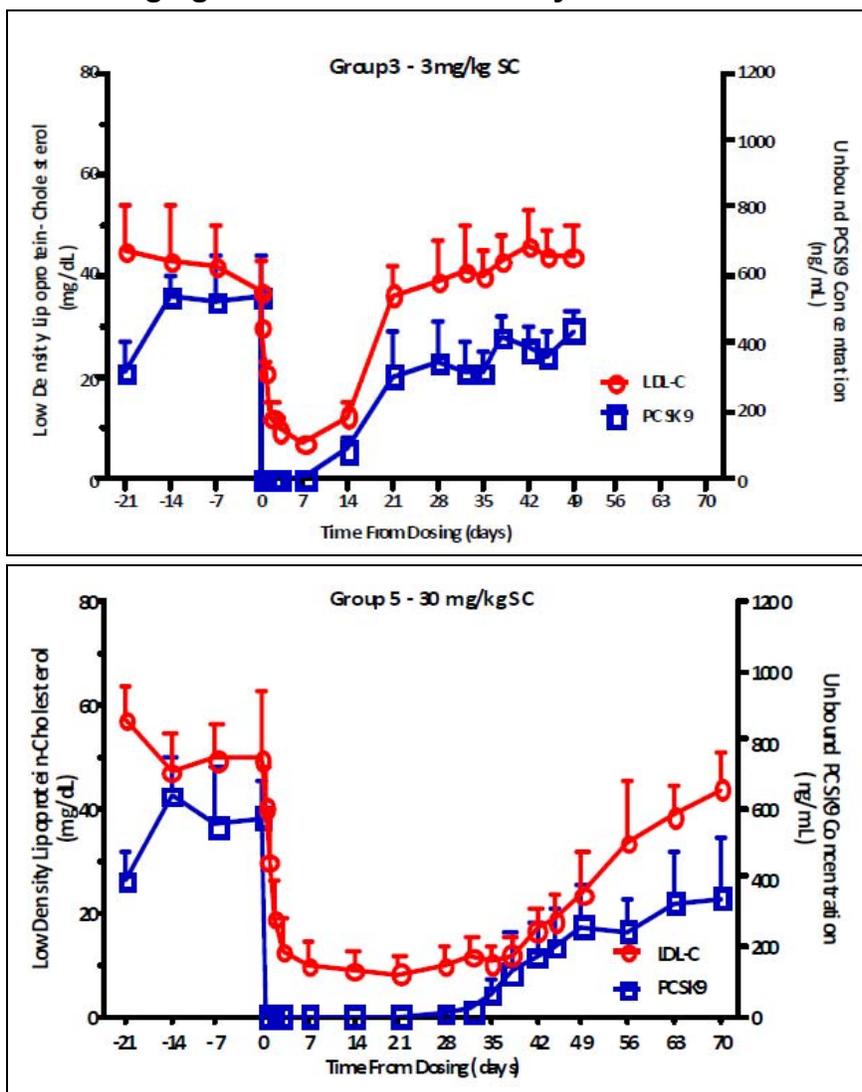
**Figure 15: Mean fasting PCSK9 concentrations following a single dose of evolocumab in monkeys**



(Applicant)

- The decrease in unbound PCSK9 preceded the nadir in LDL-C. The return of unbound PCSK9 levels to baseline paralleled the return of LDL-C to baseline (see Figure 16).

**Figure 16: Mean serum LDL-C and PCSK9 over time following a single dose of 3 and 30 mg/kg evolocumab in monkeys**



(Applicant)

## 5.2 Toxicokinetics

See individual toxicology study report reviews.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

No single-dose toxicity studies were conducted.

## 6.2 Repeat-Dose Toxicity

### 6.2.1 Repeat-dose rodent toxicity

#### A 28-Day Subcutaneous Injection Toxicity Study of [Evolocumab] AMG 145 in Hamsters with a 16-Week Treatment-Free Period

**Study no.:** 112699 (b) (4)  
**Study report location:** SDN1, SN0000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 8 September 2009  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Evolocumab, batch #0010008131, 98.9% purity (SE-HPLC)

#### Key Study Findings

- Hamsters (30/sex/dose) were administered 0, 30, 100 and 300 mg/kg/week evolocumab by once-weekly subcutaneous injection. There were no toxicologically significant findings at any dose in this study. The NOAEL is considered to be 300 mg/kg/week evolocumab.
- There were no drug-induced effects on mortality, clinical signs, body weights, food consumption, ophthalmoscopy, or hematology.
- Mean total cholesterol, LDL-C and HDL-C for males and females were decreased up to 49, 67 and 49%, respectively, by Study Day 28.
- Mean ALP was increased 13% in males, which could indicate slight hepatocyte portal blockage; there were no liver histopathologic correlates or liver weight changes. No changes in ALP were observed in females.
- No toxicologically significant effects on organ weights occurred. There was a non-statistically significant, dose-related increase in the weight of the epididymis; however, in the absence of any histopathology findings in this or related tissues this finding is not considered to be toxicologically significant.
- No organs or tissues showed drug-related histopathological lesions.
- All serum samples were negative for anti-drug antibodies. It cannot be ruled out that high circulating drug levels interfered with antidrug antibody (ADA) detection. No effects on pharmacokinetics or on the pharmacodynamic response to evolocumab were observed.
- This assessment in hamsters was performed to address concerns raised in monkeys, which are considered the most relevant species for human risk assessment, per ICH-S6.

**Table 4: Study design for a 28-day toxicology study of evolocumab in hamsters**

Group	No. of Animals <sup>a</sup>		Dose Level <sup>b</sup> (mg/kg/dose)	Dose Concentration (mg/mL)
	Male	Female		
<b>Toxicity Animals</b>				
1 (Control) <sup>c</sup>	30	30	0	0
2 (Control) <sup>c</sup>	30	30	0	0
3 (Low)	30	30	30	7.48
4 (Mid)	30	30	100	24.9
5 (High)	30	30	300	74.8
<b>Toxicokinetic Animals<sup>d</sup></b>				
6 (Control) <sup>c</sup>	8	8	0	0
7 (Low)	8	8	30	7.48
8 (Mid)	8	8	100	24.9
9 (High)	8	8	300	74.8
a 15 toxicity animals/sex/group (dependent on survival) were necropsied on Study Day 29; all remaining toxicity animals underwent a 16-week treatment-free phase following dose administration.				
b Animals were dosed at a volume of 4.01 mL/kg.				
c The control groups received the vehicle control article only.				
d Toxicokinetic animals were included solely for the purpose of blood sample collections.				

(Applicant)

**Methods**

**Doses:** 0, 30, 100 and 300 mg/kg/week

**Frequency of dosing:** Once-weekly

**Route of administration:** Subcutaneous injection

**Dose volume:** 4 mL/kg

**Formulation/Vehicle:** (b) (4)

**Species/Strain:** Crl:LVG(SYR) Golden Syrian hamsters

**Number/Sex/Group:** 15/sex/group, main study  
15/sex/group, recovery

**Age:** 9.7 to 10.6 weeks old

**Weight:** Males: 84 to 141 g; Females: 84 to 133 g

**Satellite groups:** 8/sex/group (toxicokinetics)

**Unique study design:** Anti-drug antibodies were measured.

**Deviation from study protocol:** No significant deviations were reported.

**Observations**

**Mortality:** Twice daily checks were conducted for mortality, pain/distress and abnormalities.

**Clinical signs:** Daily cage-side observations and post-dose observation (2 to 4 hours after dosing) were made during the dosing phase. Detailed observations were conducted once-weekly during the dosing phase.

**Body weights:** Assessed weekly during the dosing phase

- Feed consumption:** Assessed weekly during the dosing phase
- Ophthalmoscopy:** All animals, once prior to initiation of dosing and on Day 28. Examination by board-certified veterinary ophthalmologist using indirect ophthalmoscope and slit lamp biomicroscope.
- Hematology:** Blood was collected before the dosing and treatment-free phase necropsies (animals scheduled for necropsy only) by cardiac puncture. Blood was also collected (when possible) from animals euthanized at an unscheduled interval.
- |   |  |
|---|--|
| red blood cell (erythrocyte) count        | platelet count   |
| hemoglobin                                | mean platelet volume                                       |
| hematocrit                                | white blood cell (leukocyte) count                         |
| mean corpuscular volume                   | differential blood cell count (report only absolute count) |
| mean corpuscular hemoglobin               | blood smear <sup>a</sup>                                   |
| mean corpuscular hemoglobin concentration | reticulocyte count (absolute count)                        |
| red cell distribution width               |  |
- Clinical chemistry:** Blood was collected before the dosing and treatment-free phase necropsies (animals scheduled for necropsy only) by cardiac puncture. Blood was also collected (when possible) from animals euthanized at an unscheduled interval.
- |                                     |                                      |
|-------------------------------------|--------------------------------------|
| glucose                             | alanine aminotransferase             |
| urea nitrogen                       | alkaline phosphatase                 |
| creatinine                          | gamma glutamyltransferase            |
| total protein                       | aspartate aminotransferase           |
| albumin                             | calcium                              |
| globulin                            | inorganic phosphorus                 |
| albumin/globulin ratio              | sodium                               |
| triglycerides                       | potassium                            |
| total bilirubin                     | chloride                             |
| total cholesterol                   | high density lipoprotein cholesterol |
| low density lipoprotein cholesterol |                                      |
- Urinalysis:** Not conducted
- Gross pathology:** On Study Day 28, 15 animals/sex/group (dependent on survival) were fasted overnight and on Study Day 29, the animals were anesthetized with sodium pentobarbital, exsanguinated, and necropsied. Terminal body weights were recorded. After at least 28 days of treatment followed by a 16-week treatment-free phase all surviving animals were fasted overnight and anesthetized with sodium pentobarbital, exsanguinated, and necropsied on Treatment-Free Day 113 (Study Day 141). Terminal body weights were recorded.



the treatment-free phase on Treatment-Free Days 2, 28, 56, and 84 and at the treatment-free phase necropsy on Treatment-Free Day 113 (Study Day 141).

## Results

### Mortality

No test item-related deaths occurred.

### Clinical signs

No clear test item-related effects

### Body weights

No clear test item-related effects

### Food consumption

No clear test item-related effects

### Ophthalmoscopy

Unremarkable

### Hematology

Unremarkable

### Clinical chemistry

No toxicologically significant effects of the test item were measured. Treated males exhibited a statistically significant 13% increase in ALP ( $p < 0.05$ ). However, there was no dose-dependency and ALP was not affected in females. Moreover no effect was seen in liver weights or liver histology (e.g., cholestasis) or bone histology. Therefore, changes in ALP are not considered toxicologically meaningful. Minimal statistically significant increases (9%) in globulin in high-dose males were likely due directly to the test item, which is itself a gamma-globulin. This increase in globulin drove a corresponding decrease in the A:G ratio.

As expected from the established pharmacologic effect of the test item (see Table 5 and Table 6, below), total cholesterol and LDL-C cholesterol were decreased at the high-dose of 300 mg/kg/week evolocumab by up to 49% and 67%, respectively ( $p < 0.05$ ). HDL-C was also decreased up to 49% ( $p < 0.05$ ). The decrease in HDL-C is believed to be a consequence of a high degree of HDL-C binding to the LDLR in rodents, owing to the presence of ApoE in rodent HDL-C<sup>24</sup>.

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<sup>24</sup> Rashid S, et al. "Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *PCSK9*" *Biochemistry* 2005; **102**(15):5374-5379.

**Table 5: Pharmacodynamics of evolocumab in a 28 day subcutaneous toxicity study in male hamsters**

Test Article		Control		AMG 145		
Group		1	2	3	4	5
Level (mg/kg/dose)		0	0	30	100	300
Group/ Sex		CHOL mg/dL		HDL mg/dL		LDL mg/dL
1M	Mean	157		105		36
	SD	19.8		16.2		6.7
	N	30		30		30
2M	Mean	157		105		37
	SD	17.7		16.8		7.7
	N	30		30		30
3M	Mean	93 AB		64 AB		15 AB
	SD	9.0		6.3		4.2
	N	30		30		30
4M	Mean	93 AB		63 AB		16 AB
	SD	7.9		7.1		3.0
	N	30		30		30
5M	Mean	92 AB		62 AB		17 AB
	SD	9.5		6.5		4.5
	N	30		30		30

A Statistically significant from Group 1 at  $p \leq 0.05$ .  
B Statistically significant from Group 2 at  $p \leq 0.05$ .

(Applicant)

**Table 6: Pharmacodynamics of evolocumab in a 28 day subcutaneous toxicity study in female hamsters**

Test Article		Control		AMG 145		
Group		1	2	3	4	5
Level (mg/kg/dose)		0	0	30	100	300
Group/ Sex		CHOL mg/dL		HDL mg/dL		LDL mg/dL
1F	Mean	160		108		48
	SD	27.3		21.8		7.7
	N	30		30		30
2F	Mean	168		114		51
	SD	32.2		23.9		13.1
	N	30		30		30
3F	Mean	79 AB		55 AB		16 AB
	SD	9.3		6.5		4.2
	N	30		30		30
4F	Mean	80 AB		55 AB		16 AB
	SD	14.7		9.1		5.0
	N	30		30		30
5F	Mean	81 AB		55 AB		16 AB
	SD	11.6		9.1		3.0
	N	30		30		30

A Statistically significant from Group 1 at  $p < 0.05$ .  
B Statistically significant from Group 2 at  $p \leq 0.05$ .

(Applicant)

**Gross pathology**

Unremarkable

**Organ weights**

No toxicologically significant effect was observed. There was a non-statistically significant, dose-related increase in the weight of the epididymis; however in the absence of any histopathology findings in this or related tissues, this finding is not considered to be toxicologically significant.

**Histopathology**

Adequate Battery: Yes

Peer review: Yes (Amgen pathologist)

Unremarkable

## Toxicokinetics

Following once-weekly subcutaneous injections of evolocumab for 22 days at doses of 30, 100 and 300 mg/kg/dose, evolocumab exposure differences between male and female hamsters were minimal (generally less than 2-fold, with higher AUC in males). Serum evolocumab concentrations were not quantifiable in all samples collected from the control article-treated animals on Study Days 1 and 22. Exposure to evolocumab, as assessed by mean  $C_{max}$  and  $AUC_{last}$ , increased approximately dose proportionally on Days 1 and 22. Mean accumulation ratios (AUC at Day 1 vs. Day 22) were approximately 1.5-fold at all dose levels.

**Table 7: Summary evolocumab toxicokinetics for a 28-day hamster toxicity study**

Sex	Dose (mg/kg)	Day	$t_{max_1}$ (hr) <sup>1</sup>	$C_{max}$ (ug/mL)	$AUC_{0-t}$ (ug*hr/mL)	Acc ratio
Male	30	1	24.00	212	26800	NC
		22	24.00	325	41600	1.55
	100	1	24.00	780	95400	NC
		22	24.00	1090	149000	1.56
	300	1	8.00	1990	231000	NC
		22	24.00	2930	360000	1.56
Female	30	1	24.00	192	24100	NC
		22	24.00	286	35800	1.49
	100	1	8.00	623	78500	NC
		22	24.00	931	119000	1.52
	300	1	24.00	1780	219000	NC
		22	24.00	2330	302000	1.37
All	30	1	24.00	202	25400	NC
		22	24.00	305	38700	1.52
	100	1	16.00	702	86900	NC
		22	24.00	1010	134000	1.54
	300	1	16.00	1890	225000	NC
		22	24.00	2630	331000	1.47

<sup>1</sup> $t_{max}$  (hr) is Median  
 Source Data: 112699 - 112699-AMG 145\_28dHamster Description-"A 28-Day Subcutaneous Injection Toxicity Study of AMG 145 in Hamsters with a 16-Week Treatment-Free Period"  
 Scenario Source Data: Interim\_NCA final Version- 2 Description- Serum Base Scenario  
 Date Generated by PK Reporter S-PLUS: Tue Nov 24 10:09:03 PST 2009 User: (b) (6)  
 Acc ratio = Accumulation ratio (calculated as  $AUC_{0-168, Day 22} / AUC_{0-168, Day 1}$ )

(Applicant)

## Anti-drug Antibody Analysis (ADAs)

No anti-evolocumab antibodies were detected in any animal dosed with evolocumab (either in main study or toxicokinetic group animals). While it cannot be ruled out that high levels of circulating drug may have inhibited the assay's ability to detect ADAs, the persistent pharmacodynamic effect on cholesterol suggests that ADA, if present, did not affect drug activity.

## Dose Formulation Analysis

All dose preparations were within  $\pm 10\%$  of the listed concentrations. The mean concentration of all dose formulations on Study Days 1 and 22 ranged from 98.0 to 104% of the listed concentration. Stability of evolocumab was certified by the Applicant at 24 hours at room temperature and one week at 2 to 8 °C, and 18 months at -20 °C. The test article was not detected in any vehicle control article formulation.

**3-Month Subcutaneous Injection Extended Pharmacology Study of [Evolocumab] AMG-145 in Hamsters**

**Study no.:** 114375 (b) (4)  
**Study report location:** SDN1, SN0000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 22 March 2011  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Evolocumab, batch #0010037219, 98.9% purity (SE-HPLC)

**Key Study Findings**

- Hamsters were administered 0, 100, or 300 mg/kg/week evolocumab by subcutaneous injection for three months, which was performed by the Applicant to support dose selection for the 2-year carcinogenicity bioassay. This study is considered minimally adequate for a standard toxicology assessment, based on the low number of animals used (N = 6/sex/group). The NOAEL is considered to be 300 mg/kg evolocumab once-weekly.
- The data from this study confirms that evolocumab has persistent pharmacologic activity for at least 3 months in this model under the conditions of the study. The pharmacokinetic/pharmacodynamic data suggest that anti-evolocumab immunogenicity was unlikely to interfere with the Applicant's 2-year study. While anti-drug antibodies (ADAs) were not directly assessed in this study, pharmacodynamics is considered a valid surrogate.
- The pharmacodynamic data are consistent with results from the 1-month hamster study, in which ADA levels were negligible.
- This study establishes that a dose of 100 mg/kg once-weekly gives a maximal pharmacodynamic response, although the Applicant chose 100 mg/kg once every other week as the high-dose for the 2 year carcinogenicity study.
- No dose-limiting toxicity was identified.

**Table 8: Study design for a 3-month subcutaneous toxicity study of evolocumab in hamsters**

Group	No. of Animals		Dose Level <sup>a</sup> (mg/kg/dose)	Dose Concentration (mg/mL)
	Male	Female		
<b>Pharmacology Animals</b>				
1 (Control) <sup>b</sup>	6	6	0	0
2 (Low)	6	6	100	24.5
3 (High)	6	6	300	73.5
<b>Pharmacokinetic Animals<sup>c</sup></b>				
4 (Control) <sup>b</sup>	4	4	0	0
5 (Low)	4	4	100	24.5
6 (High)	4	4	300	73.5
a Animals received a subcutaneous injection of AMG 145 at a volume of 4.08 mL/kg.				
b The control groups received vehicle control article only.				
c Pharmacokinetic animals were included solely for the purpose of blood sample collections.				

(Applicant)

**Methods**

**Doses:** 0, 100, 300 mg/kg/week  
**Frequency of dosing:** Once-weekly  
**Route of administration:** Subcutaneous injection  
**Dose volume:** 4 mL/kg  
**Formulation/Vehicle:** (b) (4)  
**Species/Strain:** Crl:LVG(SYR) Golden Syrian hamsters  
**Number/Sex/Group:** 6/sex/group  
**Age:** ~10 weeks at initiation of dosing  
**Weight:** Males, 117 to 140 g; females, 110 to 134 g  
**Satellite groups:** 4/sex/group (toxicokinetics)  
**Deviation from study protocol:** Housing of control animals on separate racks from treated animals was not documented.

**Observations**

**Mortality:** Twice daily checks were conducted for mortality, pain/distress and abnormalities.  
**Clinical signs:** Daily cage-side observations and post-dose observation (2 to 4 hours after dosing) were made during the dosing phase. Detailed observations were conducted once-weekly during the dosing phase.  
**Body weights:** Assessed weekly during the dosing phase  
**Feed consumption:** Assessed weekly during the dosing phase  
**Ophthalmoscopy:** Not conducted  
**Hematology:** Overnight fasted blood samples were collected at the dosing phase

necropsy (Day 92).

red blood cell (erythrocyte) count  
hemoglobin  
hematocrit  
mean corpuscular volume  
mean corpuscular hemoglobin  
mean corpuscular hemoglobin  
concentration  
red cell distribution width  
prothrombin time  
fibrinogen

platelet count  
mean platelet volume  
white blood cell (leukocyte) count  
differential blood cell count (report only  
absolute count)  
blood smear<sup>a</sup>  
reticulocyte count (report only absolute  
count)  
activated partial thromboplastin time

**Clinical chemistry:** Overnight fasted blood samples were collected at the dosing phase necropsy (Study Day 92). Additional blood samples were collected for measuring cholesterol levels: blood samples (at least 0.4 mL) were collected in the early morning (within ~3 hours after the beginning of the light cycle) from all pharmacology animals on Study Days 29, 57 and 92 via a jugular vein.

glucose	alkaline phosphatase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
albumin/globulin ratio	potassium
triglycerides	chloride
total bilirubin	aldolase
alanine aminotransferase	
total cholesterol	high density lipoprotein cholesterol
low density lipoprotein cholesterol	

**Urinalysis:** Not conducted

**Gross pathology:** A necropsy was performed on animals that died at an unscheduled interval. On Study Day 92 after Week 13 of dosing, all remaining animals were euthanized and necropsied. Toxicokinetic animals were not necropsied.

**Organ weights:** Brain, heart, liver, kidney, spleen and testis

**Histopathology:** Protocol-specified tissues (see below) from animals in the control and high-dose groups (Groups 1 and 3) and from animals that died at an unscheduled interval were processed and examined microscopically. Potential target tissues identified from the high-dose group were to be evaluated in the low-dose group (Group 2). However, no potential target organs were identified. Macroscopic lesions were also examined microscopically for all animals.

adrenal (2)	mammary gland
brain	lung with large bronchi (inflated)
bone, femoral-tibial joint (knee) with bone marrow	lymph node (mesenteric)
colon	optic nerve (2) <sup>a</sup>
duodenum	ovary (2)
epididymis (2)	pancreas
esophagus	sciatic nerve
eyes (2) <sup>a</sup>	skeletal muscle (biceps femoris)
heart	spleen
ileum	stomach
injection site(s)	testis (2) <sup>a</sup>
jejunum	thymus
kidney (2)	thyroid (2 lobes) with parathyroid
lesions	trachea
liver (left lateral and left and right median lobes)	urinary bladder
	uterus
	vagina

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a Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

**Toxicokinetics:** Blood samples (>0.3 mL) were collected via jugular vein (except on the days of scheduled necropsy, when collection was via cardiac puncture) of non-fasted animals on Study Days 1 and 85 pre-dose and approximately 24, 72, 120 and 168 hours post-dose for pharmacokinetic animals only and at the dosing phase necropsy for pharmacology animals only. Concentrations of evolocumab in serum were quantitated using a validated ligand binding analytical method.

## Results

### Mortality and Clinical Signs

A toxicokinetic animal (a low-dose female) died on Day 90, which was attributed to blood collection. There were two deaths (a high-dose male, Day 57; a low-dose female, Day 61) attributed to the blood collection procedure in main study animals. There were no test article-related deaths.

### Clinical signs

There were no test item-related effects on clinical signs.

### Body Weights

No test item-related effect

### Feed Consumption

No test item-related effect

### Ophthalmoscopy

Ophthalmoscopy was not conducted.

### Hematology

Hematology data were unremarkable.

## Clinical Chemistry

Clinical chemistry data were unremarkable with the exception of the intended pharmacology (see Table 9 and Table 10); total cholesterol was decreased by up to 49%, HDL-C was decreased by up to 56% and LDL-C was decreased by up to 69%. The reductions plateaued at 100 mg/kg/week, indicating that the maximal pharmacodynamic effect is achieved at 100 mg/kg/week dose. The decrease in HDL-C is likely a consequence of a high degree of HDL-C binding to the LDL receptor in rodents, owing to the presence of ApoE in rodent HDL-C<sup>25</sup>. The group mean and individual pharmacodynamic effect was maintained throughout the study, which indicates the absence of an appreciable neutralizing anti-drug antibody response. There was no appreciable effect on triglycerides in hamsters at any dose.

**Table 9: Pharmacodynamics for evolocumab in a 3-month subcutaneous toxicity study in male hamsters**

Group/ Sex	Test Article Group Level (mg/kg/dose)	CHOL mg/dL			HDL mg/dL			LDL mg/dL		
		DSNG 29	DSNG 57	DSNG 92	DSNG 29	DSNG 57	DSNG 92	DSNG 29	DSNG 57	DSNG 92
1M	Mean	174	164	121	116	101	84	35	40	25
	SD	17.9	15.4	23.5	19.3	15.8	17.3	6.1	6.5	4.9
	N	6	6	6	6	6	6	6	6	6
2M	Mean	105*	97*	75*	66*	62*	54*	19*	18*	14*
	SD	8.6	10.8	10.2	9.7	4.8	9.0	6.3	5.4	3.1
	N	6	6	6	6	6	6	6	6	6
3M	Mean	104*	105*	77*	69*	68*	56*	20*	21*	12*
	SD	19.6	24.9	22.0	11.3	13.6	15.0	5.9	8.2	5.0
	N	6	5	5	6	5	5	6	5	5
Statistics		P	P	P	P	PK	P	P	P	P

\* P < or = 0.05

K = rank-transformed data  
P = ANOVA (and Dunnett's, if applicable)

(Applicant)

<sup>25</sup> Rashid S, et al. "Decreased plasma cholesterol and hypersensitivity to statins in mice lacking *PCSK9*" *Biochemistry* 2005; **102**(15):5374-5379.

**Table 10: Pharmacodynamics for evolocumab in a 3-month subcutaneous toxicity study in female hamsters**

Table 7 Mean Clinical Chemistry Data										
Test Article Group Level (mg/kg/dose)		Control 1 0		AMG 145 2 100		3 300				
Group/ Sex		DSNG 29	CHOL mg/dL DSNG 57	DSNG 92	DSNG 29	HDL mg/dL DSNG 57	DSNG 92	DSNG 29	LDL mg/dL DSNG 57	DSNG 92
1F	Mean	184	191	174	118	124	130	50	58	45
	SD	39.4	43.2	22.9	30.7	39.7	28.0	16.1	12.5	5.9
	N	6	6	6	6	6	6	6	6	6
2F	Mean	85*	90*	88*	56*	62*	65*	15*	22*	15*
	SD	17.4	7.2	7.7	8.5	5.6	4.8	3.3	5.6	3.4
	N	6	6	5	6	6	5	6	6	5
3F	Mean	107*	98*	89*	58*	66*	65*	17*	17*	14*
	SD	26.2	13.2	21.2	18.5	6.7	13.7	7.4	5.9	1.5
	N	6	6	6	6	6	6	6	6	6
Statistics		P	P	P	PK	PK	PK	PK	P	PK

\* P < or = 0.05  
K = rank-transformed data  
P = ANOVA (and Dunnett's, if applicable)

(Applicant)

**Urinalysis**

Not conducted

**Gross Pathology**

Unremarkable

**Organ Weights**

Unremarkable

**Histopathology**

Adequate Battery: Yes

Peer review: Yes (Amgen pathologist)

There were no clear test item-related effects observed upon histopathologic examinations. The following table captures findings with an imbalance in incidence between treatment groups, but that is unlikely to be attributable to the test item due to low incidence and/or being observed in one sex only.

Histopathology (Hamster)								
Tissue	Finding	Sev N	Male (mg/kg/week)			Female (mg/kg/week)		
			0	100	300	0	100	300
			6	0	6	6	1	6
Heart	infiltrates, lymphohistiocytic	min	0	-	0	0	0	1
Muscle, Biceps Femoris	infiltrates (focal), lymphohistiocytic	min	0	-	1	0	0	0
Kidney	basophilia, tubular, focal	min	0	-	0	0	0	1
	infiltrates, lymphocytes/ macrophages	min	0	-	0	0	0	1
Skin/ Subcutis	inflammation, lymphohistiocytic	slight	0	-	0	0	1	1
	hyperkeratosis/ parakeratosis	min slight	0 0	- -	0 0	0 0	1 -	- 1

### Toxicokinetics

Following weekly subcutaneous injections of evolocumab for 3 months at the doses of 100 and 300 mg/kg/dose, evolocumab exposure differences between male and female hamsters were generally less than 2-fold (see Table 11), with greater exposures generally observed in males. Serum evolocumab concentrations were not quantifiable in control article-treated animals on Study Days 1 and 85. Exposure to evolocumab, as assessed by mean  $C_{max}$  and  $AUC_{last}$ , increased approximately dose proportionally on Day 1. Increases in exposure with increasing dose were less than dose proportional on Day 85 (actual increase of ~2-fold for  $C_{max}$  and  $AUC$  compared to 3.33-fold expected). Mean accumulation ratios (Day 1 versus Day 85) were 2.1- and 1.3-fold for 3 months at the 100 and 300 mg/kg/dose levels, respectively.

**Table 11: Summary evolocumab toxicokinetics for a 3-month subcutaneous hamster toxicity study**

Dose (n: Study Day 1 / Study Day 85)	Study Day 1			Study Day 85			AR
	t <sub>max</sub> <sup>a</sup> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>last</sub> (µg·hr/mL)	t <sub>max</sub> <sup>a</sup> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>last</sub> (µg·hr/mL)	
<b>Male</b>							
100 mg/kg/dose (N = 3/3)	72 (24-72)	590 (45.5)	78600 (8000)	24 (24-72)	1160 (41.6)	161000 (8830)	2.05 (0.106) <sup>c</sup>
300 mg/kg/dose (N = 3/3)	24 (24-72)	1830 (210)	240000 (23900)	24 (24-72)	2140 (337)	281000 (61600)	1.20 (0.391)
<b>Female</b>							
100 mg/kg/dose (N = 3/2 <sup>b</sup> )	24 (24-24)	279 (234)	49000 NA	24 (24-72)	823 (118)	116000 (17200)	2.25 (NA)
300 mg/kg/dose (N = 3/3)	72 (24-72)	1390 (140)	177000 (32600)	24 (24-24)	2010 (576)	252000 (81600)	1.40 (0.232)
<b>Overall</b>							
100 mg/kg/dose, SC (N = 6/5 <sup>b</sup> )	24 (24-72)	434 (228)	66800 (18400)	24 (24-72)	993 (203)	138000 (27100)	2.13 (0.337)
300 mg/kg/dose, SC (N = 6/6)	48 (24-72)	1610 (289)	208000 (42800)	24 (24-72)	2080 (429)	266000 (66500)	1.30 (0.309)

C<sub>max</sub> and AUC<sub>last</sub> were reported as mean (SD) values.  
All values were rounded to 3 significant figures after calculations were performed, except t<sub>max</sub>, which is presented in 2 significant figures.  
C<sub>max</sub>: maximum observed concentration.  
<sup>a</sup>t<sub>max</sub>: time at observed maximum concentration, reported as a median (min-max) value.  
AUC<sub>last</sub>: area under the concentration-time curve from 0 to the last measurable concentration.  
AR: AUC<sub>last, Study Day 85</sub> / AUC<sub>last, Study Day 1</sub>  
<sup>b</sup>AUC<sub>last</sub> was not reportable for 1 female animal on Study Day 1.  
NA: Not applicable

(Applicant)

**Dosing Solution Analysis**

All dose preparations were within ±10% of the target concentrations. The mean concentration of dose formulations used for dosing on Study Days 1 and 85 ranged from 93.7 to 99.8% of the nominal concentration. Stability of evolocumab was certified by the Applicant as up to (b) (4) at (b) (4) °C or (b) (4) at (b) (4) °C. The test article was not detected in the vehicle control article formulation.

## 6.2.2 Repeat-dose non-rodent toxicity

### 6-Month Subcutaneous Injection Toxicity Study of [Evolocumab] AMG 145 in Cynomolgus Monkeys with a 25-Week Treatment-Free Period

**Study no.:** 110359 (b) (4)  
**Study report location:** SDN1, SN0000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 19 November 2009  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Evolocumab, batch #2000139, 98.9% purity (SE-HPLC)

#### Key Study Findings

- Evolocumab was administered to monkeys (6/sex/dose) at 0, 3, 30 and 300 mg/kg/week for 26 weeks via subcutaneous injection. Two monkeys/sex/group were allowed a 25-week treatment-free recovery period. The NOAEL is considered to be 300 mg/kg/week, the highest dose tested.
- The major observed effects of evolocumab were mean reductions in total cholesterol (up to 47%) and LDL-C (up to 83%). This is the intended pharmacology of evolocumab, and is not considered to be adverse.
- The injection site(s) was identified as a potential target tissue; minimal to slight acute-chronic inflammation and slight fibrosis at the injection site was observed in a few male monkeys at  $\geq 30$  mg/kg/week and at all doses in females.
- Evolocumab was weakly immunogenic in the monkey. One low-dose male was negative for binding ADA activity at baseline, and became strongly positive for binding and neutralizing activity by Day 57 (first time point assessed). This animal remained strongly positive for both activities at all additional time points assessed, and had greatly reduced exposures to evolocumab and was resistant to evolocumab-mediated LDL-C lowering. One mid-dose female who was negative for binding ADA activity at baseline and at every time point during the dosing period, was found to be positive for binding and neutralizing activity on Recovery Day 78. By Recovery Day 180 this animal was still positive for binding activity, but was negative for neutralizing activity. This animal was not resistant to evolocumab-mediated LDL-C lowering during the treatment phase. The anti-evolocumab immune response in only 2 animals is not considered to have compromised the interpretability of the study.
- The T cell dependent antibody response (TDAR) assay showed a mild dose-dependent decrease in anti-KLH IgG response. This effect was statistically significant for a dose-related trend in Applicant's original analysis. However, there were a disproportionate number of animals with pre-existing anti-KLH antibodies spread across the dose groups. This confounded the analysis and skewed the TDAR result. Upon reanalysis after exclusion of monkeys that were KLH-positive at baseline, the TDAR result still trended towards a positive, but was not statistically significant. The

residual concern was addressed by a second TDAR assay that was conducted in the 3 month combination toxicity study in monkeys at evolocumab doses up to 100 mg/kg administered every other week  $\pm$  5 mg/kg/day rosuvastatin. In that assay, animals expressing anti-KLH antibodies at baseline were excluded from the analysis. This second TDAR assay was negative.

- Reproductive endpoints, including menstrual cycling in females and sperm counts and sperm morphology in males were unremarkable.

**Table 12: Study design for a 6 month toxicity study of evolocumab in monkeys**

Group	No. of Animals <sup>a</sup>		Dose Level <sup>b</sup> (mg/kg/dose)	Dose Concentration (mg/mL)
	Male	Female		
1 (Control) <sup>c</sup>	6	6	0	0
2 (Low)	6	6	3	0.7
3 (Mid)	6	6	30	7
4 (High)	6	6	300	70

a Two animals/sex/group underwent 6 months of dosing, which was followed by a 25-week treatment-free phase. All animals received a subcutaneous injection of 1 mg KLH in 1 mL of vehicle on Study Days 92 and 106.

b Animals were dosed with AMG 145 or placebo at a volume of 4.3 mL/kg.

c The control group received placebo and received KLH as described in Footnote a.

(Applicant)

## Methods

<b>Doses:</b>	0, 3, 30, 300 mg/kg
<b>Frequency of dosing:</b>	Once-weekly
<b>Route of administration:</b>	Subcutaneous injection
<b>Dose volume:</b>	4.3 mL/kg
<b>Formulation/Vehicle:</b>	(b) (4)
<b>Species/Strain:</b>	Cynomolgus monkeys
<b>Number/Sex/Group:</b>	4/sex/group (main study); 2/sex/group (recovery)
<b>Age:</b>	Males, 5 to 6 years; Females, 4 to 6 years
<b>Weight:</b>	Males, 4.1 to 8.6 kg; Females, 2.4 to 3.8 kg
<b>Satellite groups:</b>	No
<b>Unique study design:</b>	- All animals received a subcutaneous injection of 1 mg keyhole limpet hemocyanin (KLH) on Study Days 92 and 106 for assessment of T cell-dependent antibody response (TDAR). - Menstrual cycle length was measured. - Sperm motility, density and morphology were assessed.
<b>Deviation from study protocol:</b>	No significant deviations were reported.

**Observations**

- Mortality:** Animals were checked twice daily for mortality, abnormalities and signs of pain or distress.
- Clinical signs:** Daily cage-side observations were conducted 2 to 4 hr post-dose. Detailed observations were conducted weekly. Physical examinations were conducted by a veterinarian on ketamine anesthetized animals once during the pre-dose phase, on Day 177 and on Recovery Day 174 (Study Day 356).
- Vital signs:** Body temperature and respiration rate were recorded once during the pre-dose phase, on Day 177 and on Recovery Day 174 (Study Day 356).
- Body weights:** Measured weekly during the dosing and recovery periods.
- Feed consumption:** Qualitatively assessed once daily.
- Ophthalmoscopy:** Exams were conducted once during the pre-dose phase, on Study Day 82 and on Study Day 177. Exams were conducted by a board-certified veterinary ophthalmologist using indirect ophthalmoscope and a slit lamp biomicroscope. Animals were anesthetized with ketamine and eyes dilated with a mydriatic agent.
- ECG:** Exams were conducted once during the pre-dose phase and on Day 180 on ketamine anesthetized animals. Eight lead ECGs were recorded. RR, PR, QRS, QT and QTc were determined. QTc was calculated according to Bazett.
- Blood sampling schedule:** Samples were collected from fasted animals twice during pre-dose phase (Days -16 and -10), on Days 29, 85, 141, 183 (dosing phase necropsy), on Recovery Days 22 and 78 (Study Days 204 and 260) and prior to recovery phase necropsy (Study Day 362).
- Hematology:**
- |   |  |
|---|--|
| red blood cell (erythrocyte) count        | platelet count   |
| hemoglobin                                | mean platelet volume                                       |
| hematocrit                                | white blood cell (leukocyte) count                         |
| mean corpuscular volume                   | differential blood cell count (report only absolute count) |
| mean corpuscular hemoglobin               | blood smear <sup>a</sup>                                   |
| mean corpuscular hemoglobin concentration | reticulocyte count (report only absolute count)            |
| red cell distribution width               | activated partial thromboplastin time                      |
| prothrombin time                          |  |
| fibrinogen                                |  |
- Clinical chemistry:** Samples collected from fasted animals twice during pre-dose phase (Study Days -16 and -10), on Study Days 29, 85, 141, 183 (dosing phase necropsy), on Recovery Days 22 and 78 (Study days 204 and 260) and prior to recovery phase necropsy (Study Day 362). Samples for cholesterol measurement were collected pre-dose on Study Days 57, 113 and 169 and Treatment-Free Days 51, 107, 134, 148 and 162 (Study Days 233, 289, 316, 330 and 344, respectively).

glucose	total bilirubin
urea nitrogen	alanine aminotransferase
creatinine	alkaline phosphatase
total protein	gamma glutamyltransferase
albumin	aspartate aminotransferase
globulin	calcium
albumin/globulin ratio	inorganic phosphorus
aldolase	sodium
cholesterol	potassium
high density lipoprotein cholesterol	chloride
low density lipoprotein cholesterol	creatine kinase
triglycerides	
cholesterol	high density lipoprotein cholesterol
triglycerides	low density lipoprotein cholesterol

**Urinalysis:** Urine was collected once during the pre-dose phase, and then prior to the dosing phase and recovery phase necropsies.

appearance/color	ketones
volume <sup>a</sup>	bilirubin
specific gravity	blood
pH	microscopic examination of sediment <sup>a</sup>
protein	urobilinogen
glucose	

**Gross pathology:** At dosing phase and recovery phase necropsy, assigned animals were anesthetized with sodium pentobarbital and exsanguinated. Examinations of the external features of the carcass; external body orifices; abdominal, thoracic and cranial cavities; organs; and tissues were conducted.

<b>Organ weights:</b>	adrenal (2)	pituitary gland
	brain	prostate
	epididymis (2)	spleen
	heart	testis (2)
	kidney (2)	thymus
	liver with gallbladder (drained)	thyroid (2 lobes) with parathyroid

**Histopathology:** Samples from all main study animals were analyzed. Samples from recovery animals were apparently not analyzed, presumably due to the Applicant's determination that there were no test item-related effects. Bone marrow smears were not analyzed.

adrenal (2)	ovary (2)
aorta	oviduct (2)
brain	pancreas
cecum	pituitary gland
cervix	prostate
colon	salivary gland [mandibular (2)]
duodenum	sciatic nerve (longitudinal)
epididymis (2)	sciatic nerve (transverse)
esophagus	seminal vesicle
eye (2) <sup>a</sup>	skeletal muscle (psoas, longitudinal)
femur with bone marrow (articular surface of the distal end)	skeletal muscle (psoas, transverse)
gallbladder	skin/subcutis
gut-associated lymphoid tissue <sup>b</sup>	spinal cord (cervical, thoracic, and lumbar)
heart	spleen <sup>b</sup>
injection site(s)	sternum with bone marrow
ileum	stomach
jejunum	testis (2) <sup>a</sup>
kidney (2)	thymus <sup>b</sup>
lesions	thyroid (2 lobes) with parathyroid
liver	tongue
lung with large bronchi	tonsil
lymph node (axillary) <sup>b</sup>	trachea
lymph node (mesenteric) <sup>b</sup>	ureter
mammary gland	urinary bladder
optic nerve (2) <sup>a</sup>	uterus
	vagina

a Preserved in modified Davidson's fixative.

b Collected in protocol-specified fixative for approximately 48 to 72 hours, then transferred to 70% ethyl alcohol. Tissues will then be processed to paraffin block within 9 days of transferring to 70% ethyl alcohol. Any potential immunohistochemistry will be added by amendment.

**Special evaluation:** Anti-drug antibody (ADA) analysis

Approximately 2 mL of blood was collected from non-fasted animals pre-dose on Days 1, 57 and 141, prior to necropsy on Day 183, and on Recovery Days 78 and 180 (Study Days 260 and 362) via a femoral vein. Samples were analyzed using a validated immunoassay. If positive for anti-evolocumab antibodies, samples were analyzed for antibody neutralization activity using a bioassay.

Immunophenotyping

Blood was collected for immunophenotyping from all surviving animals on Pre-dose Days 41 and 47 (Study Days -16 and -10), Study (Dosing) Days 85 and 183 and Recovery Day 180 (Study Day 362). The following lymphocyte subsets were quantitated by flow cytometry: Total T Cells (CD3+), Helper T Cells (CD3+CD4+CD8-), Cytotoxic T Cells (CD3+CD4-CD8+), B Cells (CD3-CD20+) and Natural Killer Cells (CD3-CD16+).

**Lymphocyte Subsets**

total T cells  
helper T cells  
cytotoxic T cells  
B cells  
natural killer cells

**Phenotype**

CD3+  
CD3+ CD4+ CD8-  
CD3+ CD4- CD8+  
CD3- CD20+  
CD3- CD16+

T cell dependent antibody response (TDAR) assay

KLH was administered by subcutaneous injection to all animals on Study Days 92 and 106 (i.e., after 3 months of once weekly dosing), and the levels of KLH-specific IgM and IgG antibodies were measured at various time points using a validated ELISA method. Blood samples were collected on Study Days -10 (pre-dose phase), 92, 99, 102, 106, 113, 117, 122, 127 and 134.

**Fertility assessments:** Menstrual cycling  
Vaginal swabbing was conducted once daily.

Male reproductive assessments

Sperm was collected at the time of necropsy from the right vas deferens/epididymis of all males. Sperm counts and sperm morphology were assessed.

**Toxicokinetics:** Approximately 0.5 mL of blood was collected from all animals on Days 1 and 176 pre-dose and approximately 24, 72, 120 and 168 hours post-dose. Blood samples were also collected from all animals pre-dose on Days 29, 57, 85, 113, 141 and 169 and on Recovery Days 22, 51, 78, 107, 134, 148, 162 and 180 (Study Days 204, 233, 260, 289, 316, 330, 344 and 362, respectively) approximately the same time of day as the last dose. Blood was collected via a femoral or saphenous vein from non-fasted animals. Evolocumab concentrations in serum were quantitated using a validated ELISA.

## Results

### Mortality

No unscheduled deaths occurred.

### Clinical Signs

There were no clear test item-related clinical signs.

One high-dose male (SSAN I00350) had limited use of the left arm on Study Days 82 and 85 and of the left hand on Study Days 92 and 99. Circling behavior was seen in another high-dose male (SSAN I00351) on Study Days 134 and 141. Given that these findings only occurred in single individuals and spontaneously resolved while the animals remained on drug, their relationship to test item is considered unlikely.

Two high-dose females that had apparently sound teeth when examined during the pre-dose period were found to have fractured teeth following the dosing period.

### Vital Signs

No test item-related effect

### Body Weights

No test item-related effect

**Feed Consumption**

No test item-related effect

**Ophthalmoscopy**

No test item-related effect

**ECG**

No test item-related effect

**Hematology**

No test item-related effects were observed.

It is worth noting some baseline differences in total lymphocyte count between the control and treated groups of males, since this has bearing on the interpretation of the immunophenotyping data discussed later. The absolute lymphocyte value at baseline (Day -10) of the high-dose group was only 83% of that in the control group. Throughout the dosing period, the high-dose group mean remained between 75% and 99% of the control group mean. Notably, the two high-dose animals (see boxed data in Table 13) that comprised the high-dose recovery group were among the animals with the lowest baseline lymphocyte values in the study, and these animals remained substantially lower than their dose cohort throughout the study.

**Table 13: Baseline lymphocyte counts in a 6 month toxicity study with evolocumab in monkeys**

Group/ Sex	Animal Number	LYM E3/uL	
		PRED 41	PRED 47
1M	I00328	9.29	9.55
1M	I00329	11.37	10.36
1M	I00330	10.40	8.78
1M	I00331	5.08	5.05
1M	I00332	6.02	6.79
1M	I00333	10.26	11.12
Mean		8.74	8.61
SD		2.572	2.292
N		6	6
2M	I00334	9.45	9.09
2M	I00335	7.28	6.56
2M	I00336	5.42	5.00
2M	I00337	11.45	9.25
2M	I00338	12.73	8.37
2M	I00339	8.38	4.85
Mean		9.12	7.19
SD		2.690	1.996
N		6	6
3M	I00340	4.48	4.28
3M	I00341	5.18	4.86
3M	I00342	6.99	5.38
3M	I00343	6.74	6.59
3M	I00344	9.39	12.81
3M	I00345	9.04	8.38
Mean		6.97	7.05
SD		1.980	3.175
N		6	6
4M	I00346	10.44	8.80
4M	I00347	13.15	9.84
4M	I00348	10.31	8.20
4M	I00349	9.31	6.84
4M	I00350	6.01	4.61
4M	I00351	6.65	4.64
Mean		9.31	7.16
SD		2.645	2.187
N		6	6

(Applicant data; box added by the FDA reviewer)

**Clinical Chemistry**

No toxicologically significant changes occurred.

Dose-related marked decreases in total cholesterol (up to 47%) and LDL-C (up to 83%) are due to the intended pharmacology of evolocumab. HDL-C and triglycerides were also modestly decreased compared to controls (up to 26% and 50%, respectively), without a dose dependence. Differences in HDL-C and triglycerides were much reduced or absent when compared to baseline data of the respective groups, while differences in total cholesterol and LDL-C remained robust. The apparent increase above baseline LDL-C (and total cholesterol) in the high-dose female recovery group is spurious. One of the two high-dose recovery females (SSAN I00374) had aberrantly high LDL-C values at baseline (163 mg/dL, compared to a mean of 61 mg/dL for the rest of the dose cohort). The baseline LDL-C levels of high-dose female SSAN I00374 responded well to evolocumab, returning to its aberrantly high baseline during the recovery period. Statistical analyses were not reported.

Total cholesterol (mg/dL) – monkeys						
Evolocumab dose group (mg/kg/week)	Male			Female		
	D29	D183	Recovery D180	D29	D183	Recovery D180
	N	6	6	2	6	6
0	137	115	114	156	151	115
3	91	90	158	83	83	119
30	86	77	148	86	94	138
300	77	71	110	82	90	165

Statistical analysis was not conducted by the CRO/Applicant.

Plasma LDL-C (mg/dL) – monkeys						
Evolocumab dose group (mg/kg/week)	Male			Female		
	D29	D183	Recovery D180	D29	D183	Recovery D180
	N	6	6	2	6	6
0	63	47	42	73	63	45
3	20	21	74	11	10	54
30	13	12	62	10	10	63
300	11	10	41	18	19	104

Statistical analysis was not conducted by the CRO/Applicant.

Plasma HDL-C (mg/dL) – monkeys						
Evolocumab dose group (mg/kg/week)	Male			Female		
	D29	D183	Recovery D180	D29	D183	Recovery D180
	N	6	6	2	6	6
0	72	64	68	73	71	68
3	66	65	80	64	63	80
30	66	58	77	65	70	77
300	59	55	65	54	59	65

Statistical analysis was not conducted by the CRO/Applicant.

Plasma triglycerides (mg/dL) – monkeys						
Evolocumab dose group (mg/kg/week)	Male			Female		
	D29	D183	Recovery D180	D29	D183	Recovery D180
	N	6	6	2	6	6
0	52	41	37	57	54	48
3	33	30	26	34	51	54
30	26	28	34	44	43	60
300	40	27	32	51	41	75

Statistical analysis was not conducted by the CRO/Applicant.

### Urinalysis

Unremarkable

### Gross Pathology

Unremarkable

### Organ Weights

Organ weight data were unremarkable.

### Histopathology

Adequate Battery: Yes

Peer Review: Yes (Amgen pathologist)

Evolocumab is associated with minimal-slight acute/chronic inflammation at the test article injection site. The high-dose was associated with minimal-slight pigment in the duodenum. One high-dose male had slight proteinaceous casts (unilateral) in kidney, moderate dilatation of tubules (unilateral) and minimal pigment (bilateral). The relationship of these renal findings to the test item is unlikely, since they were seen in only a single animal, were unilateral and are common spontaneous findings in macaques of this age.

There were no apparent test item-related effects on the histology of the primary or secondary immune tissues.

There was no clear evidence of test item-related hypertrophic or hyperplastic events, although one high-dose male did have slight hypertrophy of the adrenal cortex. However, this is not an uncommon spontaneous finding in the macaque.

Histopathological imbalances – Incidence (monkeys)										
Tissue	Finding	Sev N	Male (mg/kg/week)				Female (mg/kg/week)			
			0	3	30	300	0	3	30	300
			4	4	4	4	4	4	4	4
Injection site	acute inflammation	min slight	0 0	0 0	0 0	0 1	0 0	0 0	1 0	0 0
	chronic inflammation	min slight	0 0	0 0	1 0	2 0	0 0	2 0	0 1	0 1
	fibrosis	slight	0	0	0	0	0	0	0	1
Duodenum	pigment	min slight	0 0	0 0	0 0	0 2	0 0	0 0	0 0	1 0
Kidney	proteinaceous cast	slight	0	0	0	1 <sup>a</sup>	0	0	0	0
	tubular dilatation	mod	0	0	0	1 <sup>a</sup>	0	0	0	0
	pigment	min	0	0	0	1 <sup>a</sup>	0	0	0	0

<sup>a</sup> Male SSAN I00346

### Toxicokinetics

There were no significant sex-based differences in exposure to evolocumab (see Table 14). Evolocumab was not detected in control group samples at any time point. Exposure increased dose proportionally on Day 1 across all dose groups. Exposures on Day 176 showed a greater than proportional increase between 3 and 30 mg/kg and a less than proportional increase between 30 and 300 mg/kg. Mean accumulation ratios ranged between 2.7- to 4.2-fold after weekly subcutaneous doses between the first dose and the last dose at Week 26. One low-dose male (SSAN I00337) tested positive for anti-evolocumab binding and neutralizing antibodies during the dosing phase. This animal had decreased evolocumab exposure coincident with the development of antibodies; the data from this animal are excluded from the toxicokinetic data table below.

**Table 14: Summary toxicokinetics for evolocumab in a 6-month monkey toxicity study\***

Dose (n: Study Day 1 / Study Day 176)		Study Day 1			Study Day 176			AR
		t <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>168</sub> (ng•hr/mL)	t <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>168</sub> (ng•hr/mL)	
Male								
3 mg/kg/dose (n = 5/5)	Mean	72.0	37400	4790000	72.0	122000	18700000	3.91
	SD	(72.0 - 168)	7900	1180000	(24.0 - 72.0)	35400	5230000	0.588
30 mg/kg/dose (n = 6/6)	Mean	72.0	466000	61300000	48.0	1990000	289000000	4.72
	SD	(72.0 - 72.0)	44600	5160000	(24.0 - 72.0)	656000	72400000	1.19
300 mg/kg/dose (n = 6/6)	Mean	72.0	5380000	671000000	72.0	12700000	1920000000	2.87
	SD	(72.0 - 168)	1340000	74800000	(24.0 - 72.0)	1740000	263000000	0.391
Female								
3 mg/kg/dose (n = 6/6)	Mean	72.0	35800	4040000	48.0	61400	8460000	2.10
	SD	(72.0 - 72.0)	5080	745000	(24.0 - 72.0)	20500	2840000	0.629
30 mg/kg/dose (n = 6/6)	Mean	72.0	496000	67900000	72.0	1670000	251000000	3.73
	SD	(72.0 - 120)	51200	8820000	(24.0 - 120)	183000	22500000	0.519
300 mg/kg/dose (n = 6/6)	Mean	72.0	4530000	621000000	48.0	10700000	1540000000	2.50
	SD	(24.0 - 120)	296000	37500000	(24.0 - 72.0)	1720000	261000000	0.500
All								
3 mg/kg/dose (n = 11/11)	Mean	72.0	36500	4380000	72.0	88700	13100000	2.92
	SD	(72.0 - 168)	6210	994000	(24.0 - 72.0)	41200	6600000	1.11
30 mg/kg/dose (n = 12/12)	Mean	120	481000	64600000	72.0	1830000	270000000	4.23
	SD	(72.0 - 120)	48300	7710000	(24.0 - 120)	489000	55000000	1.01
300 mg/kg/dose (n = 12/12)	Mean	72.0	4950000	646000000	72.0	11700000	1730000000	2.69
	SD	(24.0 - 120)	1030000	62300000	(24.0 - 72.0)	1960000	317000000	0.470

All values were rounded to 3 significant figures after calculations were performed.

Only one male given 3 mg/kg/dose was found to be anti-AMG 145 antibody positive.

C<sub>max</sub>: maximum observed concentration.

t<sub>max</sub>: time at observed maximum concentration. t<sub>max</sub> is presented as median (min-max).

AUC<sub>168</sub>: area under the concentration-time curve from 0 to 168 hours postdose.

AR: AUC<sub>168</sub>, Study Day 176 / AUC<sub>168</sub>, Study Day 1.

\*Excluding ADA positive animals  
(Applicant)

## Special Evaluation

### Anti-drug antibody analysis

Evolocumab was weakly immunogenic in monkeys; however there were two monkeys that mounted a humoral immune response to evolocumab, and in one case ADAs inhibited the pharmacodynamic effect of the drug. These isolated ADA responses are not considered to have interfered with the interpretation of this study.

One low-dose male (SSAN I00334) was weakly positive to binding ADA activity at baseline, but was negative at all other time points, and had exposures comparable to the rest of the dose cohort.

One low-dose male (SSAN I00337), who was negative for binding ADA activity at baseline, became strongly positive for binding and neutralizing activity by Day 57 (first time point assessed). This animal remained strongly positive for both activities at all additional time points assessed. This animal had greatly reduced exposures to evolocumab and was resistant to evolocumab-mediated LDL-C lowering.

One mid-dose female (SSAN I00368), who was negative for binding ADA activity at baseline and at every time point during the dosing period, was found to be positive for binding and neutralizing activity on Recovery Day 78. By Recovery Day 180 this animal was still positive for binding activity, but was negative for neutralizing activity. This

animal was not resistant to evolocumab-mediated LDL-C lowering during the treatment phase.

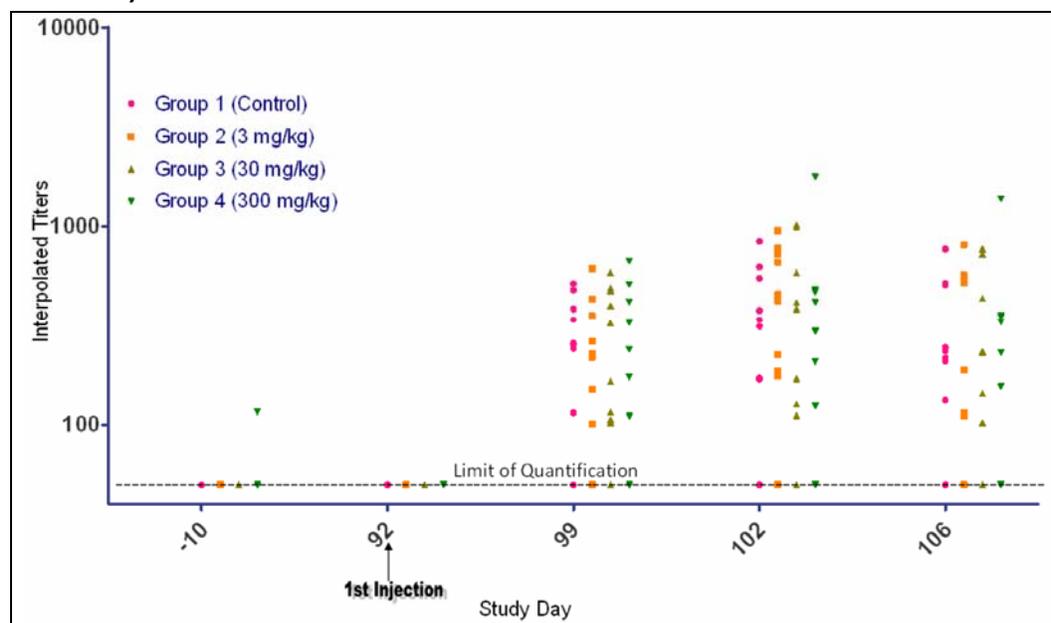
### Immunophenotyping

There was no effect of the test item on the proportion of the different lymphocyte subsets (Total T cells, helper T cells, cytotoxic T cells, B cells and natural killer cells). While high-dose males exhibited an apparent decrease in the absolute number of all lymphocyte subsets, especially during the recovery period; this finding is considered to be an artifact of non-uniform baseline lymphocyte numbers in the recovery animals. There was no effect on the number of cells in the lymphocyte subpopulations in females.

### T cell dependent antibody response (TDAR) assay

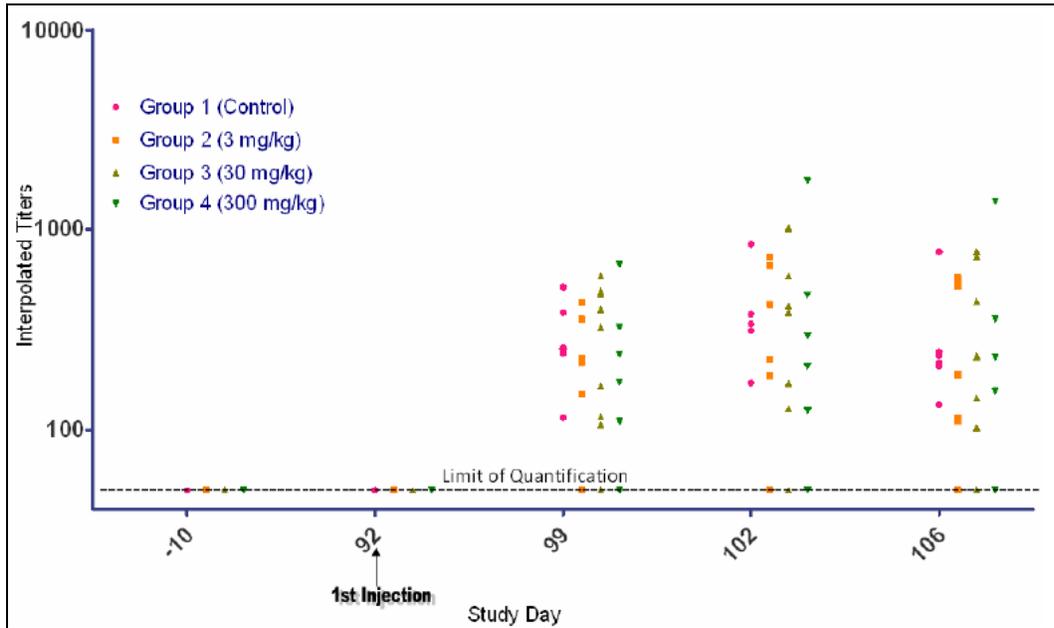
Evolocumab did not appreciably affect the initial (IgM) TDAR response in monkeys (see Figure 17). However, evolocumab appeared to dose-dependently decrease the IgG response to KLH (see Figure 19). While the magnitude of the response appears to be blunted by evolocumab, the kinetics of the response does not appear to be affected. When all animals were included in the Applicant's statistical analysis for trend (Jonckheere-Terpstra test), statistically significant differences were found on Study Days 99, 113, 117, 122, 127, 134 and for total AUC (see Table 15). When the Applicant reanalyzed the data, excluding animals that had positive titers for anti-KLH IgG at either baseline or Study Day 92 (day of 1<sup>st</sup> inoculation) (see Figure 18 and Figure 20), the trend test was no longer statistically significant (Table 16 and Table 18). A final scatter plot with mean AUC for IgG responses is provided in Figure 21). While downward trend for IgG responses remained after removing animals that were anti-KLH positive at baseline from the analysis, the lack of a statistically significant difference between controls and dosed animals indicates this trend may not be meaningful.

**Figure 17: Anti-KLH IgM responses in a 6 month toxicity study in monkeys (all animals)**

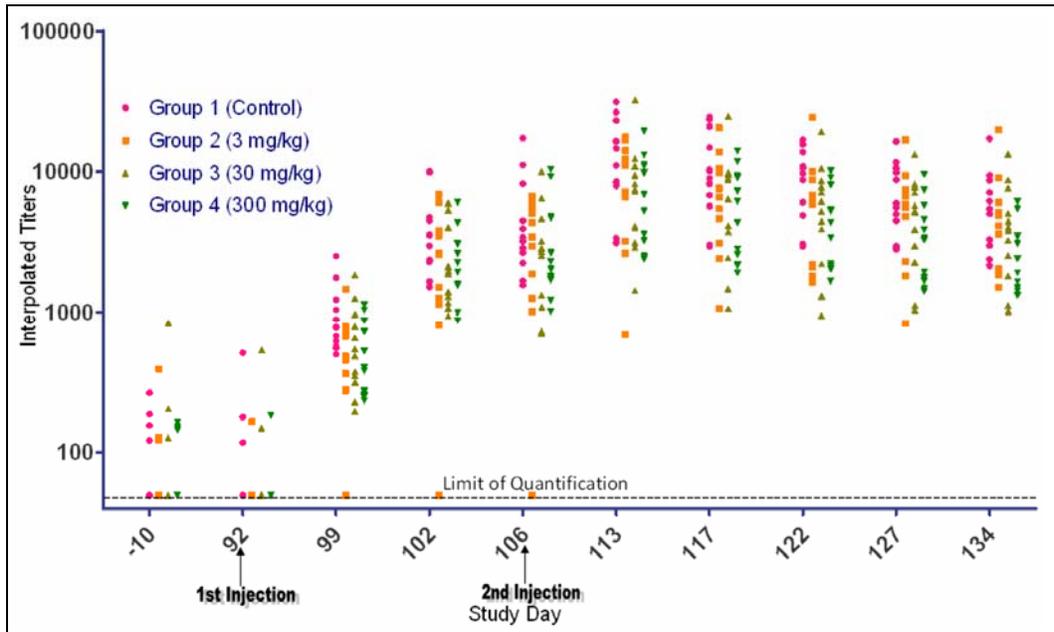


(Applicant)

**Figure 18: Anti-KLH IgM responses in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)**

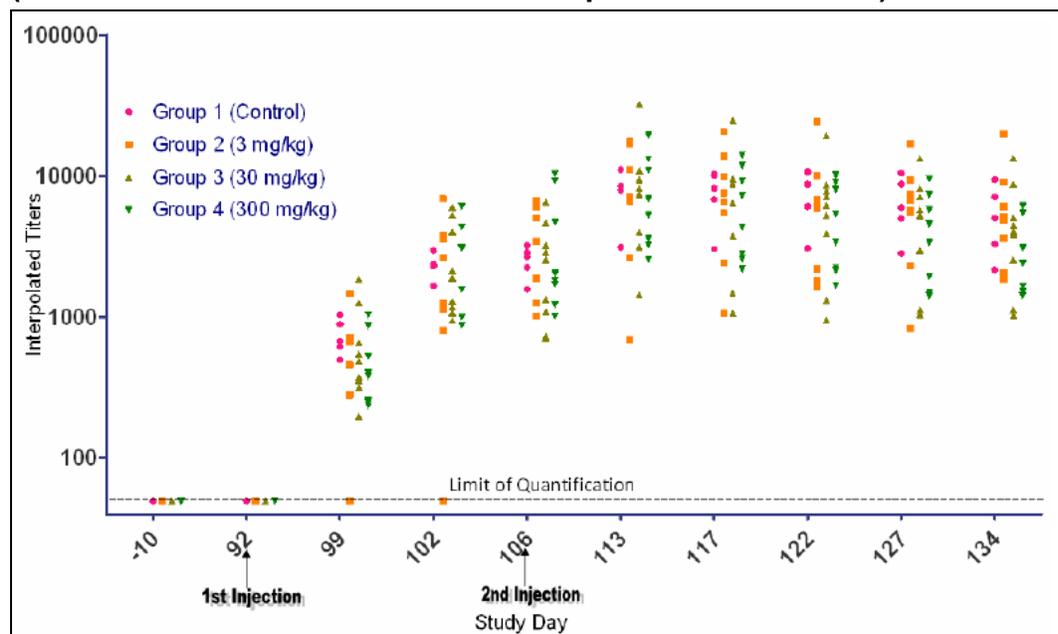


**Figure 19: Anti-KLH IgG responses in a 6 month toxicity study in monkeys (all animals)**



(Applicant)

**Figure 20: Anti-KLH IgG responses in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)**



**Table 15: Trend analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (all animals)**

Parameter	Value	Interval	Jonckheere-Terpstra Test for Trend	
Interpolated titers anti-KLH IgM	Titer	PRED 47	0.3702	
		DSNG 92	a	
		DSNG 99	0.4306	
		DSNG 102	0.2280	
		DSNG 106	0.1885	
		AUC	NA	0.2688
Interpolated titers anti-KLH IgG	Titer	PRED 47	0.3598	
		DSNG 92	0.2999	
		DSNG 99	0.0080*	
		DSNG 102	0.0895	
		DSNG 106	0.1037	
		DSNG 113	0.0250*	
		DSNG 117	0.0164*	
		DSNG 122	0.0055*	
		DSNG 127	0.0045*	
		DSNG 134	0.0035*	
		AUC	NA	0.0107*

\* = Significant at 5% level.  
 - = Effect in the decreased direction.  
 NA = Not applicable.  
 a All values were the same; due to lack of variation, the analysis could not be performed.

(Applicant)

**Table 16: Trend analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)**

Parameter	Value	Interval	Jonckheere-Terpstra Test for Trend	
Interpolated titers anti-KLH IgM	Titer	PRED 47	a	
		DSNG 92	a	
		DSNG 99	0.2122	
		DSNG 102	0.2114	
		DSNG 106	0.1235	
		NA	0.2182	
Interpolated titers anti-KLH IgG	Titer	PRED 47	a	
		DSNG 92	a	
		DSNG 99	0.0691	
		DSNG 102	0.3231	
		DSNG 106	0.4230	
		DSNG 113	0.4369	
		DSNG 117	0.3232	
		DSNG 122	0.2084	
		DSNG 127	0.1703	
		DSNG 134	0.1369	
		AUC	NA	0.3489

NA = Not applicable.  
a All values were the same; due to lack of variation, the analysis could not be performed.

**Table 17: ANOVA analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (all animals)**

Parameter	Value	Interval	ANOVA p-value	Treatment Comparisons	
Interpolated titers anti-KLH IgM	Titer	PRED 47	0.4018	NA	
		DSNG 92	a	NA	
		DSNG 99	0.9729	NA	
		DSNG 102	0.7995	NA	
		DSNG 106	0.8531	NA	
		NA	0.9248	NA	
Interpolated titers anti-KLH IgG	Titer	PRED 47	0.9465	NA	
		DSNG 92	0.5874	NA	
		DSNG 99	0.0442*	3 mg/kg/dose versus 0 mg/kg/dose: p = 0.0415* 300 mg/kg/dose versus 0 mg/kg/dose: p = 0.0341*	
		DSNG 102	0.2890	NA	
		DSNG 106	0.5196	NA	
		DSNG 113	0.2770	NA	
		DSNG 117	0.1920	NA	
		DSNG 122	0.0869	NA	
		DSNG 127	0.0689	NA	
		DSNG 134	0.0547	NA	
		AUC	NA	0.1574	NA

\* = Significant at 5% level.  
NA = Not applicable.  
a All values were the same; due to lack of variation, the analysis could not be performed.

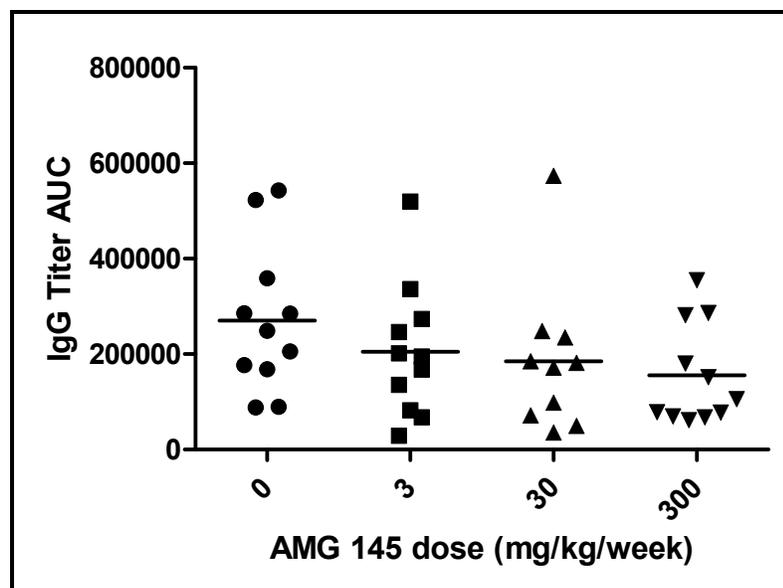
(Applicant)

**Table 18: ANOVA analysis of IgM and IgG anti-KLH antibody titer data in a 6 month toxicity study in monkeys (excludes animals that were anti-KLH-positive at baseline)**

Parameter	Interval	ANOVA p-value	Treatment Comparisons		
Interpolated titers anti-KLH IgM	Titer	PRED 47	a	NA	
		DSNG 92	a	NA	
		DSNG 99	0.3366	NA	
		DSNG 102	0.5402	NA	
		DSNG 106	0.6409	NA	
		NA	0.4613	NA	
Interpolated titers anti-KLH IgG	Titer	PRED 47	a	NA	
		DSNG 92	a	NA	
		DSNG 99	0.5243	NA	
		DSNG 102	0.6894	NA	
		DSNG 106	0.7859	NA	
		DSNG 113	0.9955	NA	
		DSNG 117	0.9861	NA	
		DSNG 122	0.8703	NA	
		DSNG 127	0.7587	NA	
		DSNG 134	0.5376	NA	
		NA	0.9826	NA	
		AUC	NA	0.9826	NA

NA = Not applicable.  
a All values were the same; due to lack of variation, the analysis could not be performed.

**Figure 21: AUC of interpolated anti-KLH IgG titer for selected\* monkeys administered evolocumab for 6 months**



\* Excludes animals that were positive for anti-KLH IgG at both baseline and Day 92 (date of 1<sup>st</sup> inoculation)

**Menstrual Cycling**

No effect on menstrual cycle length.

**Male Reproductive Assessment**

There were no test item-related effects on male reproduction. One low-dose male (SSAN I00337) and one high-dose male (SSAN I00347) had immature testes as defined by microscopic evaluation. Consistent with this, these animals had low sperm density and/or motility. These findings were not considered to be treatment-related, but rather due to sexual immaturity. Consistent with an explanation of sexual immaturity, both animals were among the youngest and lightest animals on the study. Additionally, the

affected low-dose male had neutralizing ADA at all measured time points and failed to exhibit a pharmacodynamic response to evolocumab.

### Dosing Solution Analysis

Mean values for the concentration verification analyses of the formulations used on Study Days 1, 85 and 176 ranged between 95.8 and 99.7% of nominal concentration, indicating they were accurately prepared. Certificates of stability indicated evolocumab was expected to retain nominal activity under the conditions of storage during the study. Evolocumab was not detected in control article formulations.

### [Evolocumab] AMG 145 and Rosuvastatin: 3-Month Combination Toxicology Study in the Cynomolgus Monkey with a 4-Month Recovery Phase

**Study no.:** 112311 (b) (4)

**Study report location:** SDN1, SN0000 (eCTD)

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 1 November 2011

**GLP compliance:** Yes

**QA statement:** Yes

**Drug, lot #, and % purity:** Evolocumab, batch #0010020373, 98.8% purity (SE-HPLC)  
Rosuvastatin, 5 mg (lot #115750), 10 mg (lots #115332, 116067), 20 mg (lots #115384, 115789), and 40 mg (lot #115503) tablets as manufactured by AstraZeneca

### Key Study Findings

- Evolocumab was administered at 0, 10 and 100 mg/kg every other week for 3 months by the subcutaneous route alone or in combination with 5 mg/kg/day rosuvastatin. The NOAEL in monkeys treated with up to 100 mg/kg evolocumab once every other week  $\pm$  5 mg/kg/day rosuvastatin was the high-dose based on the absence of toxicity.
- There is no evidence of combinatorial toxicity when 5 mg/kg/day rosuvastatin was combined with either 10 or 100 mg/kg evolocumab administered every other week in any of the standard toxicological assessments or in any of the immunotoxicological assessments made (immunophenotyping, T cell dependent antibody response (TDAR) assay and natural killer (NK) cell activity).
- A limitation of the study was that there was no direct evidence that the dose of rosuvastatin used had any effect on either HMG-CoA reductase or LDL-C. However, the expected level of rosuvastatin exposure was detected in these monkeys, and 5 mg/kg/day provides an approximate 10-fold clinical exposure margin for a Crestor® (rosuvastatin) dose of 10 mg/day and an approximate 2-fold margin for the maximum recommended human Crestor® (rosuvastatin) dose of 40 mg/day.
- Despite the caveats noted above, the study is acceptable as having adequately characterized the potential for combinatorial toxicity when a statin is used in

combination with evolocumab. This judgment is based on the following factors: 1) the dose of statin examined in the monkey study adequately covers likely clinical exposures, and 2) the literature suggests that LDL-C needs to fall below 0.5 mg/dL in order to impair human T cell proliferation (a level unlikely to be achieved clinically).<sup>26</sup>

**Table 19: Study design for a 3 month combination toxicity study of evolocumab and rosuvastatin in monkeys**

Group	No. of Animals <sup>a</sup>		Dosed with:	Dosing Frequency (Method)	Dose Level <sup>b</sup> (mg/kg)	Dose Concentration (mg/mL)
	Male	Female				
1 (Control) <sup>c</sup>	5	5	Vehicle Control Article	Biweekly (SC)	0	0
			Gelatin Capsule	Daily (Oral)	0	0
2	3	3	Rosuvastatin	Daily (Oral)	5	NA
3	3	3	Rosuvastatin	Daily (Oral)	5	NA
4	5	5	AMG 145	Biweekly (SC)	10	2.33
			Rosuvastatin	Daily (Oral)	5	NA
			AMG 145	Biweekly (SC)	100	23.26

NA = Not applicable; Oral = Tablet; SC = Subcutaneous injection.

a Two animals/sex in Groups 1 and 4 underwent 13 weeks of dosing and entered a 4-month recovery phase.

b For AMG 145 and vehicle control article, animals were dosed at a volume of 4.3 mL/kg. For rosuvastatin, the dose level of 5 mg/kg was approximate, since animals were dosed with the appropriate number of whole tablets (5, 10, 20 or 40 mg/tablet) to achieve as close as possible the required dose level.

c The control group received the vehicle control article biweekly and one gelatin capsule orally daily.

(Applicant)

<sup>26</sup> Cuthbert JA and Lipsky PE "Provision of cholesterol to lymphocytes by high density and low density lipoproteins: requirement for low density lipoprotein receptors" *J Biol Chem* 1987; **262**(16):7808-7818.

**Methods**

<b>Doses and frequencies of dosing:</b>	Rosuvastatin: 5 mg/kg QD Evolocumab: 10 or 100 mg/kg every other week
<b>Route of administration:</b>	Rosuvastatin: Tablets in oral gelatin capsules evolocumab: Subcutaneous injection
<b>Dose volume:</b>	Rosuvastatin: n/a Evolocumab: 4.3 mL/kg
<b>Formulation/Vehicle:</b>	Rosuvastatin: Crestor® (rosuvastatin calcium) tablets Evolocumab: <span style="background-color: #cccccc; padding: 2px;">(b) (4)</span>
<b>Species/Strain:</b>	Cynomolgus monkeys
<b>Number/Sex/Group:</b>	Main study: 3/sex/group Recovery: 2/sex/group (control and high-dose only)
<b>Age:</b>	4.6 to 6.2 years old at initiation of dosing
<b>Weight:</b>	Males, 5.0 to 6.9 kg; females, 2.9 to 4.4 kg
<b>Satellite groups:</b>	No
<b>Unique study design:</b>	Study includes immunophenotyping, T cell dependent antibody response (TDAR) assay and natural killer (NK) cell activity assessments
<b>Deviation from study protocol:</b>	No significant deviations were reported.

**Observations**

<b>Mortality:</b>	Animals were checked twice daily for mortality, abnormalities and signs of pain or distress.
<b>Clinical signs:</b>	Cage-side observations were conducted once daily and 2 to 4 hours post-dose. Detailed observations were conducted weekly.
<b>Body weights:</b>	Body weights were recorded weekly.
<b>Food consumption:</b>	Qualitative food consumption was assessed once daily.
<b>Vital signs:</b>	Body temperature and respiration rate were determined on anesthetized animals at Day -12, on Day 87 and on Recovery Day 113 (Study Day 204).
<b>Ophthalmoscopy:</b>	Ophthalmic examinations were conducted on Study Day -12 and 87)
<b>Blood sampling schedule:</b>	Samples for hematology, coagulation, and clinical chemistry were collected twice prior to initiation of dosing (pre-dose phase), prior to the terminal necropsy (on Study Day 92) and prior to recovery necropsy [on Recovery Day 113 (Study Day 204)].

Samples for immunophenotyping were collected twice prior to initiation of dosing (pre-dose phase) and prior to the terminal necropsy (on Study Day 92).

In addition, blood samples (approximately 0.5 mL) were collected prior to dosing for LDL-C and cholesterol on Study Days 15 (LDL-C only), 29 and 57 and monthly during the recovery phase [Recovery Days 28, 56, 84 and 113 (Study Days 119, 147, 175 and 204)].

<b>Hematology:</b>	red blood cell (erythrocyte) count	platelet count
	hemoglobin	mean platelet volume
	hematocrit	white blood cell (leukocyte) count
	mean corpuscular volume	differential blood cell count (report only absolute count)
	mean corpuscular hemoglobin	blood smear <sup>a</sup>
	mean corpuscular hemoglobin concentration	reticulocyte count (report only absolute count)
	red cell distribution width	activated partial thromboplastin time
	prothrombin time	
	fibrinogen	
<b>Clinical chemistry:</b>	glucose	alanine aminotransferase
	urea nitrogen	alkaline phosphatase
	creatinine	gamma glutamyltransferase
	total protein	aspartate aminotransferase
	albumin	calcium
	globulin	inorganic phosphorus
	albumin/globulin ratio	sodium
	cholesterol	potassium
	triglycerides	chloride
	total bilirubin	creatine kinase
	aldolase	high density lipoprotein cholesterol
		low density lipoprotein cholesterol
<b>Urinalysis:</b>	appearance/color	ketones
	volume <sup>a</sup>	bilirubin
	specific gravity	blood
	pH	microscopic examination of sediment
	protein	urobilinogen
	glucose	
<b>Gross pathology:</b>	All animals were necropsied after 13 weeks of treatment (main study) or after 13 weeks of treatment followed by 16 weeks of treatment-free recovery. Animals were fasted the night before necropsy.	
<b>Organ weights:</b>	adrenal (2)	pituitary gland
	brain	prostate
	epididymis (2)	spleen
	heart	testis (2)
	kidney (2)	thymus
	liver with gall bladder (drained)	thyroid (2 lobes) with parathyroid

<b>Histopathology:</b>	adrenal (2)	ovary (2)
	aorta	oviduct (2)
	brain	pancreas
	cecum	pituitary gland
	cervix	prostate
	colon	salivary gland [mandibular (2)]
	duodenum	sciatic nerve (longitudinal)
	epididymis (2)	sciatic nerve (transverse)
	esophagus	seminal vesicle (2)
	eye <sup>a</sup> (2)	skeletal muscle (psoas, longitudinal)
	femur with bone marrow (articular surface of the distal end)	skeletal muscle (psoas, transverse)
	gall bladder (drained)	skin/subcutis (collected with injection site; for Groups 1, 3, and 4)
	gut associated lymphoid tissue (GALT)	skin/subcutis (dorsal; for Group 2)
	heart	spinal cord (cervical, thoracic, and lumbar)
	injection site(s) – subcutaneous	spleen
	injection site(s) - KLH	sternum with bone marrow
	ileum	stomach
	jejunum	testis <sup>a</sup> (2)
	kidney [cross section of each (2)]	thymus
	lesions <sup>b,c</sup>	thyroid (2 lobes) with parathyroid
	liver	tongue
	lung with large bronchi	tonsil
	lymph node (axillary)	trachea
	lymph node (mesenteric)	ureter
	mammary gland	urinary bladder
	optic nerve <sup>a</sup> (2)	uterus
		vagina

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Note: if repeated epistaxis is observed or treatment for fecal abnormalities is required, additional tissues will be collected (to be added by amendment).

- a To be collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.
- b If lesions are collected from treated animals, corresponding tissues/sites/organs, unless already required per protocol, may be collected from select control animals (same sex, if possible; to be added by amendment).
- c If skin abnormalities are present (marked), the skin will be collected as follows:  
Collect the affected skin (to include normal adjacent skin, if feasible).  
Collect a synonymous section of normal skin contra lateral (if applicable) to the affected site.

**Toxicokinetics:** *Evolocumab*

Serum samples were collected for toxicokinetic analysis of evolocumab from each animal in Groups 1, 3 and 4 pre-dose and at approximately 24, 80, 120 and 168 hours post-dose on Study Days 1 and 85. Samples were also collected from two animals/sex/group at the 0 and 100 mg/kg dose levels on study days 119, 147, 175 and 204 during the recovery phase. The serum samples were analyzed for evolocumab concentrations using a validated ligand binding analytical method.

*Rosuvastatin*

Plasma samples were collected for toxicokinetic analysis of rosuvastatin from each animal in Groups 2, 3 and 4 pre-dose and at approximately 1, 2, 4, 6, 12 and 24 hours post-dose on Study Days 1 and 85. The plasma samples were analyzed for rosuvastatin concentrations using a validated LC/MS-MS method.

**Antidrug antibodies:** Blood samples (approximately 2 mL) were collected via a femoral vein predose on Study Day 1 and on Study Days 29, 57, and 85. Samples were collected once monthly during the recovery phase [Recovery Days 28, 56, 84 and 113 (Study Days 119, 147, 175, and 204)].

**Immunotoxicology endpoints:** Immunophenotyping  
Blood was collected for immunophenotyping analysis via a femoral vein from all surviving animals on Pre-dose Days 8 and 15 (Study Days -21 and -14) and on Study Day 92. The levels of circulating total lymphocytes and lymphocyte subsets (T cells [total, CD4+ and CD8+], B cells and natural killer cells) present in blood were assessed by flow cytometry.

TDAR

KLH was administered on Study Days 30 and 44 via subcutaneous injection in the interscapular region. Blood was collected for IgM on Pre-dose Day 15 (Study Day -14) and Study Days 30, 34, 36, 38, 40, 42 and 44 and for IgG on Pre-dose Day 15 (Study Day -14) and Study Days 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 64 and 74 from all surviving animals. On days when KLH injections were given, blood collection occurred prior to KLH administration.

Natural killer cell (NKC) activity

Blood was collected for NKC analysis on Pre-dose Days 8 and 15 (Study Days -21 and -14) and on Study Day 92. Effector (isolated peripheral blood mononuclear cells) and target cells (radiolabeled K562 tumor cells) were co-cultured in triplicate at three different effector:target ratios (100:1, 33:1 and 11:1). Following incubation, at approximately 37°C for approximately 4 hours, supernatant fluids were harvested, and the released radiolabel was quantified in a gamma counter. Assay controls were included to correct for spontaneous releasable radiolabel from target cells in the absence of effector cells and for the determination of total release of radiolabel. Dexamethasone sodium phosphate (a known NK cell inhibitor) was used as an assay control. This assay control was used at a 100:1 effector to target ratio (in triplicate) at a final concentration of 0.5 mM/well for each animal, when possible. Results of the NKC assay were reported as the percent cytotoxicity for each effector to target ratio and as lytic units (LU)/10<sup>7</sup> effector cells, with 1 LU set at 20% lysis.

## Results

**Mortality**

All animals survived to their scheduled necropsy.

**Clinical Signs**

No test item-related effect

**Physical Examination and Vital Signs**

No test item-related effect

**Body Weights**

No test item-related effect

**Feed Consumption**

No test item-related effect

**Ophthalmoscopy**

No test item-related effect

**ECG**

Not performed

**Hematology**

No test item-related effect

**Clinical Chemistry**

Aside from the expected pharmacology of evolocumab, which at the mid- and high-dose caused significant decreases in mean total cholesterol (up to 49%), LDL-cholesterol (up to 86%), and triglycerides (up to 50%), there was no effect of either rosuvastatin or evolocumab on clinical chemistry. Notably, rosuvastatin alone had no detectable effect on mean total cholesterol or LDL-C. There was a decrease in triglycerides at 100 mg/kg evolocumab in combination with 5 mg/kg rosuvastatin in female monkeys only, which was unexpected. However, the triglyceride concentrations in those animals were similar to male triglyceride concentrations in controls. Therefore, the apparent decrease in high-dose females for triglycerides was not considered biologically significant. There was no effect on HDL-C (data not shown). No pharmacodynamic data (e.g., plasma LDL-C) were collected at the time of the TDAR analysis.

Total cholesterol (mg/dL) – monkeys							
Evolocumab dose group (mg/kg)	Rosuvastatin dose group (mg/kg)	Male			Female		
		Pre-dose D8	D92	Recovery D113	Pre-dose D8	D92	Recovery D113
0	0	139	125	147	142	142	178
0	5	113	113	-	159	158	-
10	5	98	50*	-	146	102	-
100	5	119	61*	149	141	79*	165

\*indicates p<0.05

Plasma LDL-C (mg/dL) – monkeys							
Evolocumab dose group (mg/kg)	Rosuvastatin dose group (mg/kg)	Male			Female		
		Pre-dose D8	D92	Recovery D113	Pre-dose D8	D92	Recovery D113
0	0	53	49	49	61	66	81
0	5	50	45	-	82	78	-
10	5	46	7*	-	79	15*	-
100	5	59	8*	85	74	20*	79

\*indicates p<0.05

Plasma triglycerides (mg/dL) – monkeys							
Evolocumab dose group (mg/kg)	Rosuvastatin dose group (mg/kg)	Male			Female		
		Pre-dose D8	D92	Recovery D113	Pre-dose D8	D92	Recovery D113
0	0	36	31	36	55	48	54
10	5	35	40	-	62	56	-
30	5	36	21	-	52	43	-
100	5	46	28	34	58	29*	33

\*indicates p<0.05

### Urinalysis

No test item-related effect

### Gross Pathology

No test item-related effect

### Organ Weights

No test item-related effect

### Histopathology

Adequate Battery: Yes

Peer Review: Yes (Amgen pathologist)

No test item-related effects were observed.

### Special Evaluation

#### Immunotoxicity

At FDA's request, the Applicant conducted a combination toxicity assay with coadministration of statin and evolocumab, which included immunotoxicity endpoints (e.g., TDAR and natural killer cell activity). The Agency was invited to comment on the design of the protocol after the study was already initiated, and therefore did not have input into the dose or type of statin, or the overall design of the study. The Applicant elected to use a 5 mg/kg dose of rosuvastatin. This statin was justified by the Applicant on the basis that rosuvastatin was the only statin with monkey dosing information within the public domain, avoiding the need for a dose range-finding study, and reducing

animal usage (consistent with the principle of 3Rs). In the monkey study cited by Amgen that appears in the summary basis of approval for Crestor® (NDA 21-366), cynomolgus monkeys received 10 and 30 mg/kg/day rosuvastatin for 26 weeks. Both doses were effective in lowering LDL-C in the monkey. Although Amgen's did not provide a rationale for this dose, it is plausible that 5 mg/kg/day was selected to be an attempt to avoid overt rosuvastatin-related toxicity (pancreatic acinar cell vacuolation at  $\geq 10$  mg/kg/day), to maintain a pharmacodynamic effect (that was not achieved) and cover likely clinical exposures (approximately 2-fold at the 40 mg/day clinical rosuvastatin dose based on a body surface area ( $\text{mg}/\text{m}^2$ ) extrapolation).

FDA raised concerns in a February 2012 letter about the selection of 5 mg/kg rosuvastatin, because rosuvastatin may poorly partition into lymphocytes due to low lipophilicity. The Applicant subsequently cited a publication by Avis, et al., (2011)<sup>27</sup>, which showed that patients treated with rosuvastatin (5, 10 or 20 mg/day) had reduced levels of coenzyme Q10 in their peripheral blood mononuclear cells (PBMCs), indicating reduced HMG-CoA reductase activity in these PBMCs, and by inference arguing that it is reasonable to expect that monkey PBMCs will experience HMG-CoA reductase inhibition at a dose of 5 mg/kg/day.

The Applicant cited two papers that support their contention that lymphocytes are able to scavenge sufficient cholesterol from the circulation to maintain their normal function even at extremely low LDL-C levels, on the order of 5  $\mu\text{g}/\text{mL}$  (0.5 mg/dL).<sup>28, 29</sup> This is substantially lower (1 to 2 orders of magnitude) than the LDL-C levels that have been achieved in the clinic or in the monkey study reviewed above.

### **Immunophenotyping**

No test item-related effect (data not shown).

### **T cell dependent antibody response (TDAR) assay**

A number of animals (see Table 20) were found to have pre-existing titers for anti-KLH antibodies prior to KLH exposure. Detection of pre-existing anti-KLH titers is not uncommon in cynomolgus macaques, and it has been postulated that these represent antibodies to a *Schistosoma mansoni* carbohydrate antigen that cross-reacts with KLH.<sup>30</sup> Since the interpretation of the TDAR relies on comparisons between groups of KLH-naïve animals, these animals have been excluded from the TDAR analysis. The TDAR was negative for suppression of anti-KLH antibody response (both IgM and IgG) with coadministration of evolocumab (100 mg/kg every other week) and rosuvastatin 5 mg/kg/day.

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<sup>27</sup> Avis HJ, et al. "Rosuvastatin lowers coenzyme Q10 levels, but not mitochondrial adenosine triphosphate synthesis, in children with familial hypercholesterolemia," *J Pediatrics* 2011; **158**(3):458-462.

<sup>28</sup> Cuthbert JA and Lipsky, PE "Modulation of human lymphocyte responses by low density lipoproteins (LDL): Enhancement but not immunosuppression is mediated by LDL receptors" *PNAS* 1984; **81**:4539-4544.

<sup>29</sup> Cuthbert JA and Lipsky PE "Provision of Cholesterol to Lymphocytes by High Density and Low Density Lipoproteins: Requirement for low density lipoprotein receptors" *J Biol Chem* 1987; **262**(16):7808-7818.

<sup>30</sup> Grzych JM, et al. "Schistosoma mansoni shares a protective carbohydrate epitope with keyhole limpet hemocyanin" *J Exp Med* 1987; **165**(3):865-878.

**Table 20: Incidence of pre-existing anti-KLH antibodies in monkeys coadministered evolocumab and rosuvastatin in a 3-month toxicity study**

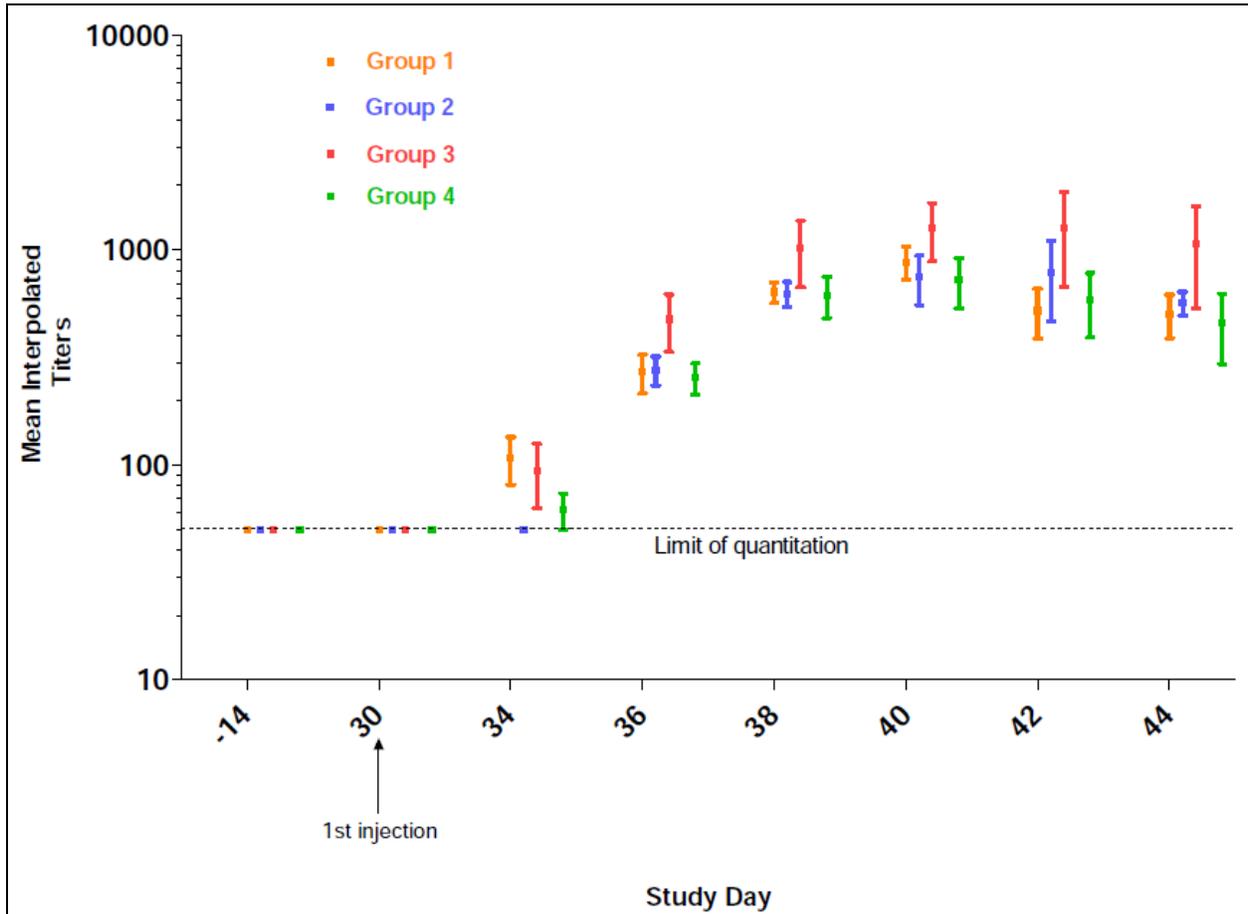
Group		IgM	IgG	Total
1	M	1/5	0/5	1/5
	F	1/5	0/5	1/5
	Total	2/10	0/10	2/10
2	M	1/3	0/3	1/3
	F	3/3	1/3	3/3
	Total	4/6	1/6	4/6
3	M	0/3	0/3	0/3
	F	1/3	0/3	1/3
	Total	1/6	0/6	1/6
4	M	0/5	0/5	0/5
	F	2/5	0/5	2/5
	Total	2/10	0/10	2/10
Overall Total		9/32	1/32	9/32

Group 1, vehicle control; Group 2, 10 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 3, 30 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 4, 100 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin

(Applicant)

There was no effect of rosuvastatin alone or rosuvastatin + evolocumab on the IgM or IgG anti-KLH TDAR response.

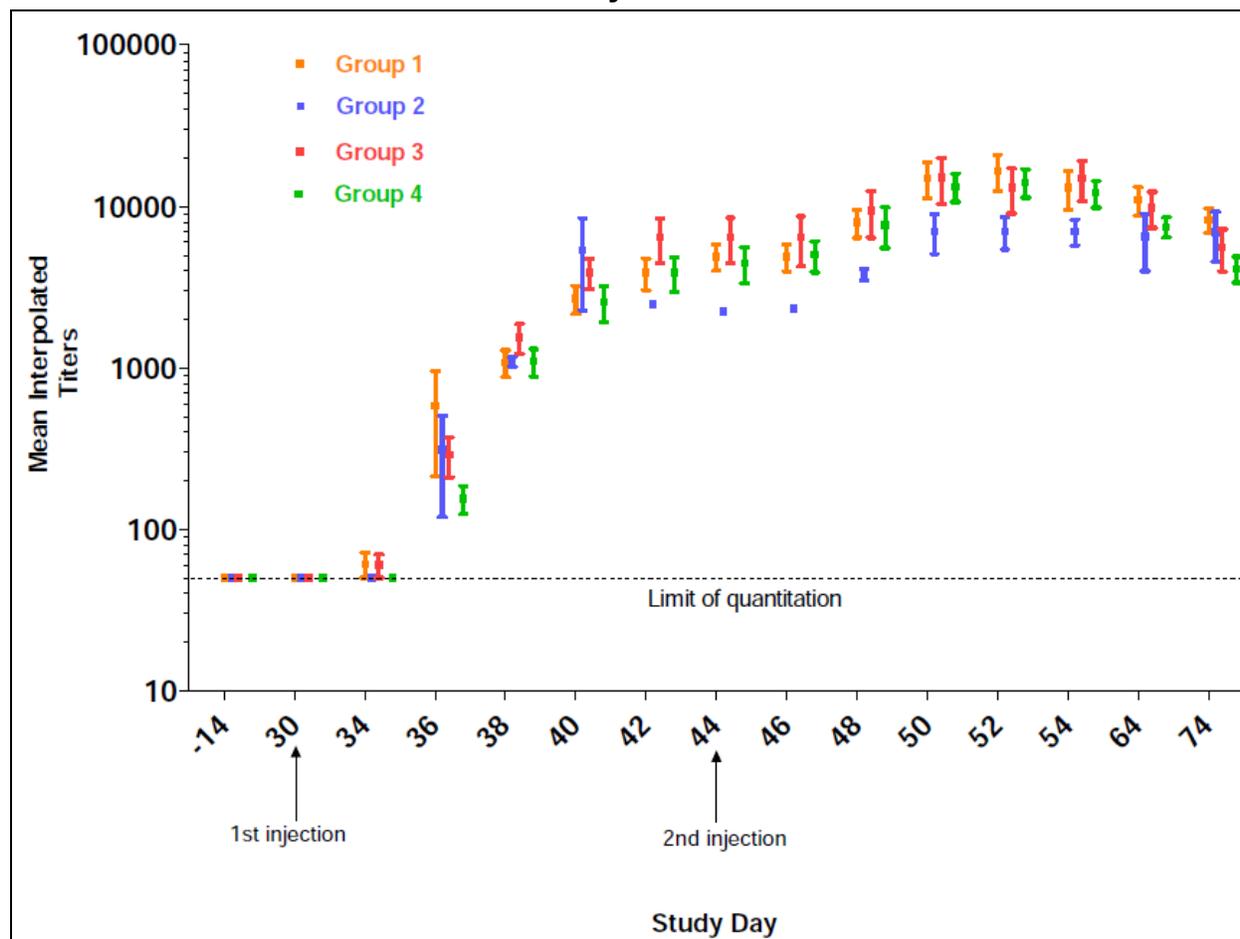
**Figure 22: Anti-KLH IgM responses in a 3 month combination toxicity study with evolocumab and rosuvastatin in monkeys**



Group 1, vehicle control; Group 2, 10 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 3, 30 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 4, 100 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin

(Applicant)

**Figure 23: Anti-KLH IgG responses in a 3 month combination toxicity study with evolocumab and rosuvastatin in monkeys**



Group 1, vehicle control; Group 2, 10 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 3, 30 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin; Group 4, 100 mg/kg Q2W evolocumab/5 mg/kg/day rosuvastatin

(Applicant)

### Natural Killer (NK) Cell activity

There was no effect of treatment with rosuvastatin  $\pm$  evolocumab on NK cell cytolytic activity (data not shown).

### Anti-Drug Antibodies (ADAs)

One Group 3 (10 mg/kg evolocumab/5 mg/kg rosuvastatin) monkey and one Group 4 (100 mg/kg evolocumab/5 mg/kg rosuvastatin) monkey tested positive for ADAs (see Table 21). The Group 4 animal had very high ADA activity, and was also found to be positive for neutralizing activity; the Group 3 animal was not positive for neutralizing ADA. It is possible that evolocumab interfered with the detection of ADA in other individuals; however, given the persistence of the pharmacodynamic effect of evolocumab, ADAs are not considered to have compromised interpretation of the study. One animal in the control group was positive for ADA at baseline, which questions the validity of the ADA assay.

**Table 21: Summary of anti-evolocumab antibody data for a 3-month combination toxicity study of evolocumab with rosuvastatin**

Group (Drug)	Dosing Phase Necropsy Animals		Recovery Phase Necropsy Animals	
	Binding Antibodies <sup>a</sup>	Neutralizing Antibodies <sup>a</sup>	Binding Antibodies <sup>a</sup>	Neutralizing Antibodies <sup>a</sup>
1 (Control)	17% (1 <sup>b</sup> /6)	0% (0/6)	0% (0/4)	0% (0/4)
2 (Rosuvastatin)	0% (0/6)	0% (0/6)	NA	NA
3 (Rosuvastatin/ AMG 145)	17% (1/6)	0% (0/6)	NA	NA
4 (Rosuvastatin/ AMG 145)	17% (1/6)	17% (1/6)	0% (0/4)	0% (0/4)
Total of all AMG 145-dosed animals	17% (2/12)	8% (1/12)	0% (0/4)	0% (0/4)

<sup>a</sup> Results are expressed as percentage and incidence. Incidence is the number positive/number evaluated  
<sup>b</sup> Animal I01884 was also predose positive.

(Applicant)

**Toxicokinetics****Evolocumab**

Exposures (AUC) increased dose-proportionally between 10 and 100 mg/kg, showed no significant gender differences, and minimal (<2-fold) accumulation between the first dose and Day 85, which is consistent with typical pharmacokinetics of monoclonal antibodies (see Table 22 and Table 23).

**Table 22: Summary toxicokinetics for a 3-month combination toxicity study of evolocumab with rosuvastatin for ADA-negative monkeys**

Sex	Dose Group	Dose (mg/kg/dose) Subcutaneous	N	Study Day 1			N	Study Day 85			AR
				t <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>1</sub> (µg·hr/mL)		t <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>1</sub> (µg·hr/mL)	
Male	3	10	3	80 (80)	119 (16.9)	15100 (2230)	3	24 (24-80)	168 (25.5)	23800 (4640)	1.59 (0.236)
	4	100	5	80 (80)	994 (160)	132000 (19500)	5	80 (80)	1820 (427)	262000 (55200)	1.97 (0.200)
Female	3	10	3	80 (80)	113 (14.0)	14700 (1410)	3	80 (80)	127 (17.1)	17200 (2840)	1.18 (0.238)
	4	100	5	80 (80)	1130 (174)	143000 (18000)	4	80 (24-80)	1640 (405)	231000 (49300)	1.62 (0.535)
All	3	10	6	80 (80)	116 (14.2)	14900 (1680)	6	80 (24-80)	148 (29.8)	20500 (4990)	1.38 (0.307)
	4	100	10	80 (80)	1060 (173)	137000 (18500)	9	80 (24-80)	1740 (402)	248000 (52000)	1.82 (0.403)

AR = Accumulation ratio; AUC<sub>1</sub> = Area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C<sub>max</sub> = Maximum observed drug concentration during a dosing interval; N = Number of animals; t<sub>max</sub> = Time to reach C<sub>max</sub>, reported as a median (range).  
Notes: All values were rounded to three significant figures after calculations were conducted, except t<sub>max</sub>, which was rounded to two significant figures.  
Rosuvastatin was administered once daily via oral administration at 5 mg/kg/dose to Groups 3 and 4.  
Only the one animal (Animal No. I01911) detected as neutralizing antibody positive was excluded from descriptive statistics.

(Applicant)

**Table 23: Summary evolocumab toxicokinetics for a 6 month combination toxicity study of evolocumab +/- rosuvastatin in all monkeys**

Sex	Dose Group	Dose (mg/kg/dose), Subcutaneous	N	Study Day 1			N	Study Day 85			AR
				t <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>t</sub> (µg·hr/mL)		t <sub>max</sub> (hr)	C <sub>max</sub> (µg/mL)	AUC <sub>t</sub> (µg·hr/mL)	
Male	3	10	3	80 (80)	119 (16.9)	15100 (2230)	3	24 (24-80)	168 (25.5)	23800 (4640)	1.59 (0.236)
	4	100	5	80 (80)	994 (160)	132000 (19500)	5	80 (80)	1820 (427)	262000 (55200)	1.97 (0.200)
Female	3	10	3	80 (80)	113 (14.0)	14700 (1410)	3	80 (80)	127 (17.1)	17200 (2840)	1.18 (0.238)
	4	100	5	80 (80)	1130 (174)	143000 (18000)	5	80 (24-80)	1410 (615)	197000 (86500)	1.39 (0.685)
All	3	10	6	80 (80)	116 (14.2)	14900 (1680)	6	80 (24-80)	148 (29.8)	20500 (4990)	1.38 (0.307)
	4	100	10	80 (80)	1060 (173)	137000 (18500)	10	80 (24-80)	1620 (544)	229000 (76400)	1.68 (0.565)

AR = Accumulation ratio; AUC<sub>t</sub> = Area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C<sub>max</sub> = Maximum observed drug concentration during a dosing interval; N = Number of animals; t<sub>max</sub> = Time to reach C<sub>max</sub>, reported as a median (range).  
Notes: All values were rounded to three significant figures after calculations were conducted, except t<sub>max</sub>, which was rounded to two significant figures.  
Rosuvastatin was administered once daily via oral administration at 5 mg/kg/dose to Groups 3 and 4.

(Applicant)

**Rosuvastatin**

No material effect of evolocumab on rosuvastatin pharmacokinetics was seen. There was no effect of sex, nor was there any accumulation (see Table 24).

**Table 24: Summary rosuvastatin toxicokinetics for a 6 month combination toxicity study of evolocumab +/- rosuvastatin in all monkeys**

Sex	Dose Group	Dose (mg/kg/dose), Oral	N	Study Day 1			N	Study Day 85			AR
				t <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>t</sub> (ng·hr/mL)		t <sub>max</sub> (hr)	C <sub>max</sub> (ng/mL)	AUC <sub>t</sub> (ng·hr/mL)	
Male	2	5	3	4.0 (2.0-6.0)	11.4 (5.00)	130 (26.5)	3	4.0 (2.0-6.0)	12.3 (8.24)	141 (95.9)	1.09 (0.679)
	3	5	3	4.0 (1.0-6.0)	15.4 (7.55)	129 (61.7)	3	4.0 (2.0-6.0)	24.9 (19.7)	192 (96.0)	1.49 (0.393)
	4	5	5	4.0 (1.-6.0)	22.8 (13.9)	201 (32.3)	5	4.0 (2.0-6.0)	15.0 (3.57)	189 (27.8)	0.973 (0.259)
Female	2	5	3	4.0 (4.0-6.0)	6.67 (2.61)	78.3 (18.7)	3	4.0 (4.0-12)	12.5 (4.93)	169 (23.5)	2.28 (0.82)
	3	5	3	6.0 (4.0-6.0)	14.0 (5.02)	168 (43.9)	3	6.0 (1.0-6.0)	10.2 (1.32)	162 (42.1)	1.04 (0.469)
	4	5	5	4.0 (2.0-6.0)	22.0 (11.3)	211 (106)	5	4.0 (2.0-12)	16.4 (9.91)	179 (84.4)	0.950 (0.407)
All	2	5	6	4.0 (2.0-6.0)	9.02 (4.41)	104 (35.1)	6	4.0 (2.0-12)	12.4 (6.07)	155 (64.2)	1.69 (0.938)
	3	5	6	5.0 (1.0-6.0)	14.7 (5.79)	148 (52.4)	6	5.0 (1.0-6.0)	17.6 (14.9)	177 (68.3)	1.27 (0.457)
	4	5	10	4.0 (1.0-6.0)	22.4 (11.9)	206 (73.9)	10	4.0 (2.0-12)	15.7 (7.06)	184 (59.5)	0.961 (0.322)

AR = Accumulation ratio; AUC<sub>t</sub> = Area under the concentration-time curve from time zero to the time of the last quantifiable concentration; C<sub>max</sub> = Maximum observed drug concentration during a dosing interval; N = Number of animals; t<sub>max</sub> = Time to reach C<sub>max</sub>, reported as a median (range).  
All values were rounded to three significant figures after calculations were conducted, except t<sub>max</sub>, which was rounded to two significant figures.

(Applicant)

**Dosing Solution Analysis**

The mean values for the concentration verification analyses of the evolocumab formulations used on Study Days 1, 15, 29, 43, 57, 71 and 85 ranged between 96.8 and 102.5% of nominal concentration, indicating they were accurately prepared. No evolocumab was detected in the vehicle control article formulations. Rosuvastatin consisted of marketed tablets.

**6-Week Subcutaneous Toxicity Study of [Evolocumab] AMG 145 in Cynomolgus Monkeys, with a 16-Week Treatment-Free Period**

**Study no.:** 110149 (b) (4)  
**Study report location:** SDN1, SN000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 24 August 2008  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot #, and % purity:** Evolocumab, batch #0010008131, 98.9% purity (SE-HPLC)

**Key Study Findings**

- Evolocumab was administered to monkeys (5/sex/dose) at 0, 3, 30 and 300 mg/kg/week by subcutaneous injection for 6 weeks. The NOAEL was considered to be 300 mg/kg/dose, in the absence of clear drug-related toxicity.
- One high-dose male (SSAN 4003) was euthanized on Day 30 following clinical signs of watery feces, hunched appearance and decreased skin turgor that began 3-5 days prior to euthanasia. This animal lost 1/3<sup>rd</sup> of his bodyweight between Week 2 of the study and Day 30 (unscheduled sacrifice). Findings for this animal also included histologic evidence of bacterial enterocolitis and lymphoid depletion and a constellation of clinical pathology changes correlating with inflammation, stress, dehydration, muscle injury, decreased red cell mass (caused by probable bleeding into the gastrointestinal tract) and iron deficiency. In the absence of these signs in any other evolocumab-treated animals in this study (and other studies of up to 6 months duration) and the presence of a similar finding in a control male, the findings leading to early sacrifice of this animal are not considered to be related to evolocumab administration. Analysis of immune-related findings at the end of the dosing period in all study animals showed:
  - Lymphoid hyperplasia in the spleen (mild-moderate): 1/6 low-dose, 1/6 mid-dose and 2/5 high-dose (correlated with non-statistically significant increase in spleen weight at the high-dose)
  - Lymphoid hyperplasia in the pylorus (mild): 1/5 high-dose
  - Thymic involution (mild-moderate): 1/6 mid-dose and 2/5 high-dose
  - Except for animal SSAN 4003 that was discovered moribund, there were no clear test item-related effects on clinical signs, body weights, food consumption and ophthalmoscopy.
  - The moribund state of animal SSAN 4003 appeared to be related to bacterial enterocolitis and is not considered test-article related.
- One control male (SSAN 1004) was euthanized on Day 182, also due to intestinal disease (i.e., bacterial enterocolitis). This animal also showed lymphoid depletion.

- All electrocardiograms were considered to be qualitatively within normal limits. One ventricular premature complex, considered by the veterinary cardiologist to be a normal variant was found on Day 39 in a high-dose monkey.
- There were no test item-related effects on hematology.
- Dose-related decreases in total cholesterol (up to 40%,  $p < 0.05$ ) and LDL-C (up to 81%,  $p < 0.05$ ) are considered to be due to the intended evolocumab pharmacology. HDL-C was also slightly decreased (up to 19%,  $p < 0.05$ ) at  $\geq 30$  mg/kg/week evolocumab, which was unexpected. Changes in HDL-C in monkeys administered evolocumab were not apparent with longer durations of dosing. There was no effect on triglycerides. The decreases in cholesterol, LDL-C and HDL-C are related to the intended pharmacology of the test item, and are not considered to be adverse.
- There were no drug-related effects on urinalysis or on gross pathology.
- No statistically significant effects of the test article on organ weights were seen. There was a trend for high-dose monkeys to exhibit elevated spleen weights (increased 2.3X in high-dose males and increased 1.6X in high-dose females) compared to controls, which correlated with lymphoid hyperplasia observed histologically. Males exhibited a dose-dependent trend towards increased pituitary weight (brain weight ratio was increased by 25%). Pituitary weight was not affected in females. Females (but not males) exhibited a dose-dependent trend towards increased thyroid weights (body weight ratio was increased by 73% in high-dose females compared to control females).
- One high-dose male (SSAN 4001) tested positive for anti-evolocumab antibodies. These antibodies were not neutralizing in a bioassay; however, the ADA-positive animal had greatly accelerated clearance of the drug compared to the rest of the drug cohort and to Day 1 pharmacokinetics for this animal. The pharmacodynamic effect of evolocumab (decreased LDL-C) was also blunted in the ADA-positive animal at later time points, which indicates the presence of neutralizing ADA.

**Table 25: Study design for a 6 week toxicity study of evolocumab in monkeys**

Group No.	Number of Males/Females	Dose Level (mg/kg)	Number Necropsied:	
			Week 7 (Day 43)	Week 26 (Day 186)
1	5/5	0 (control)	3/3	1 <sup>†</sup> /2
2	5/5	3	3/3	2/2
3	5/5	30	3/3	2/2
4	5/5	300	2/3*	2/2

\* Animal 4003 was euthanized on Day 30.

<sup>†</sup> Animal 1004 was euthanized on Day 182

(Applicant)

**Methods**

**Doses and frequencies of dosing:** 0, 3, 30 and 300 mg/kg/dose once-weekly

**Route of administration:** Subcutaneous injection

**Dose volume:** 4 mL/kg

**Formulation/Vehicle:** (b) (4)

**Species/Strain:** Cynomolgus monkeys

**Number/Sex/Group:** Main study: 3/sex/group  
Recovery: 2/sex/group

**Age:** Males, 2.6 to 3.2 years; females, 2.5 to 4.5 years

**Weight:** Males, 2.2 to 3.1 kg; females, 2.2 to 3.0 kg

**Deviation from study protocol:** Animals were not assigned to groups by a stratified randomization scheme designed to achieve similar group mean body weights and groups were not randomly assigned to dosing in accordance with the SOP and protocol, due to a failure to include the number of groups in the randomization criteria.

**Selected Results****Histopathology**

Adequate Battery: Yes

Peer review: Yes (Amgen pathologist)

Most findings appear to be incidental, and are common spontaneous lesions in cynomolgus monkeys. Findings that appear at an increased incidence and/or severity in the treatment groups are captured in the table below.

Minimal-mild glial nodules were present in the nervous tissue (brain or spinal cord) of 1/3 mid-dose male, 1/3 mid-dose female and 1/3 high dose female. The affected mid-dose male was also found to have minimal axonal degeneration in the thoracic spinal cord. These findings are unlikely to be related to evolocumab, as access to the central nervous system is limited and these findings were not observed in subsequent studies of longer duration. Furthermore, the Applicant notes that while the overall incidence of these aggregated findings is 1.5%, the incidence in control animals from individual studies can be as high as 40% (historical control range), which exceeds the observed incidence of 12.5% (3/24) observed in this study.

Evolocumab treatment was correlated with effects on immune tissues, in particular mild-moderate lymphoid hyperplasia of the spleen (1/6 low-dose, 1/6 mid-dose and 2/5 high-dose), mild lymphoid hyperplasia in the pyloric stomach (1/5 high-dose) and mild-moderate thymic involution (1/6 mid-dose and 2/5 high-dose). These findings were absent in longer duration studies, indicating recoverability and/or tolerance with continued dosing.

Minimal-mild mineralization of the ovaries was seen in 0/3 controls, 0/3 low-dose, 3/3 mid-dose and 2/3 high-dose females. Mononuclear cell infiltration (minimal to mild) of the pituitary (pars nervosa) was seen in mid (1/3) and high-dose males (2/2). These observations are not considered toxicologically significant.

Histopathology imbalances – monkeys										
Tissue	Finding	Sev N	Male (mg/kg/week)				Female (mg/kg/week)			
			0	3	30	300	0	3	30	300
			3	3	3	2	3	3	3	3
Brain	glial nodule <sup>A</sup>	min	0	0	0	0	0	0	1	0
		mild							0	1
Colon	congestion, submucosa, mucosa	min	0	0	0	1	0	0	0	0
Gallbladder	hypertrophy/hyperplasia	mild	0	0	0	0	0	0	0	1
Heart	multifocal mononuclear cell infiltrate	min	0	0	2	1	0	0	0	0
		mild	0	0	1	1	0	0	0	2
	multifocal mononuclear cell infiltrate	mild	1	0	0	0	0	0	0	0
Ileocecal Valve	acute hemorrhage	mild	0	0	0	0	0	0	0	1
Liver	multifocal fibrosis, capsule, arteritis	mild	0	0	0	0	0	0	0	1
		mild	0	0	0	1	0	0	0	0
		mild	0	0	0	0	0	0	0	1
Mandibular LN	lymphoid hyperplasia	mod	0	0	0	1	0	0	0	0
Ovary	mineralization	min					0	0	1	1
		mild					0	0	2	1
Parathyroid	cyst	p	0	0	1	1	0	0	0	0
		p	0	0	0	1	0	0	0	0
Pituitary	cyst	p	0	0	0	1	0	1	1	0
		p	0	0	0	1	0	0	0	0
	infiltrate, mononuclear cells, pars nervosa	min	0	0	1	1	0	0	0	0
		mild	0	0	0	1	0	0	0	0
	mineralization	min	0	0	0	0	0	1	0 <sup>B</sup>	0
mild	0	0	0	0	0	0	0	1	0	
	ossification, focal	p	0	0	0	0	0	0	0	1 <sup>C</sup>
Prostate	multifocal necrosis, gland	mild	0	0	0	1				
Spinal cord, Lumbar	glial nodule	min	0	0	1	0	0	0	0	0
Spinal cord, Thoracic	axonal degeneration	min	0	0	1	0	0	0	0	0
Spleen	lymphoid hyperplasia	mild	0	0	0	0	0	1	1	0
		mod	0	0	0	1	0	0	0	1
	decreased size/number, germinal center	mild	0	0	0	0	0	0	1	0
Stomach	lymphoid hyperplasia, pylorus	mild	0	0	0	1	0	0	0	0
Thymus	involution	mild	0	0	0	0	0	0	1	1
		mod	0	0	0	1	0	0	0	0

<sup>A</sup> Defined as foci of microglia about degenerating neurons

<sup>B</sup> Associated with minimal focus of cell degeneration.

<sup>C</sup> Pathologist considers that this could be an artifact from the ventral cranium.

Findings in the early decedent high-dose male (SSAN 4003) were generally consistent with bacterial enterocolitis and stress as having played a primary role in the morbid condition of this animal prior to early sacrifice.

## Toxicokinetics

Following weekly subcutaneous administration of evolocumab to cynomolgus monkeys for 6 weeks in the dose range of 3 to 300 mg/kg, the exposure to evolocumab, as measured by  $C_{max}$  and  $AUC_{0-168}$ , increased in an approximately dose-proportional manner across all dose groups, except for comparisons between the low- and mid-dose on Day 22 and Day 36 which were greater than dose proportional. Evolocumab exposures appeared to be similar (<2-fold difference) in males and females. Expected drug accumulation was observed from Day 1 to Day 36 in all dose groups. The mean accumulation ratio ranged from 1.8 to 3.5 in the combined groups.  $T_{max}$  was observed to occur at ~72 hrs. One high-dose male monkey (SSAN 4001) tested positive for anti-evolocumab antibodies which appeared to decrease exposure to evolocumab in this animal on Days 22 and 36 as compared to Day 1 and other dosing cohort members in this group (see Table 26).

**Table 26: Summary toxicokinetics for evolocumab in a 6 weeks toxicity study in monkeys**

Sex	Dose (mg/kg/)	Day	$T_{max}$ (hr)	$C_{max}$ (ng/mL)	$AUC_{0-168}$ (ng-hr/mL)	AR
Males (N=5)*	3	1	24.0 (24.0-24.0)	45700 (2950)	4740000 (890000)	NA
		22	72.0 (72.0-120.0)	78600 (13800)	10200000 (3250000)	NA
		36	72.0 (24.0-72.0)	78200 (14000)	11100000 (2800000)	2.33 (0.229)
	30	1	120.0 (72.0-120.0)	464000 (155000)	57800000 (13800000)	NA
		22	72.0 (72.0-72.0)	1150000 (154000)	181000000 (29100000)	NA
		36	24.0 (0.0-72.0)	1190000 (255000)	184000000 (46500000)	3.19 (0.631)
	300	1	72.0 (72.0-120.0)	5850000 (456000)	824000000 (95800000)	NA
		22	72.0 (24.0-120.0)	7930000 (2660000)	1130000000 (596000000)	NA
		36	72.0 (24.0-72.0)	9340000 (1350000)	1470000000 (193000000)	1.67 (0.0885)
Females (N=5)	3	1	72.0 (72.0-72.0)	47500 (4280)	4580000 (1850000)	NA
		22	72.0 (72.0-72.0)	57400 (11300)	6940000 (2690000)	NA
		36	72.0 (0.0-72.0)	52500 (10300)	6150000 (3730000)	1.52 (0.868)
	30	1	72.0 (72.0-120.0)	438000 (34200)	58500000 (5200000)	NA
		22	72.0 (72.0-120.0)	964000 (107000)	149000000 (18200000)	NA
		36	72.0 (24.0-72.0)	1550000 (1180000)	229000000 (154000000)	3.89 (2.56)
	300	1	72.0 (72.0-120.0)	5940000 (291000)	841000000 (52200000)	NA
		22	72.0 (24.0-72.0)	9190000 (951000)	1460000000 (139000000)	NA
		36	72.0 (24.0-168.0)	10300000 (837000)	1590000000 (117000000)	1.90 (0.156)
All (N=10)**	3	1	48.0 (24.0-72.0)	46600 (3600)	4660000 (1370000)	NA
		22	72.0 (72.0-120.0)	68000 (16300)	8580000 (3300000)	NA
		36	72.0 (0.0-72.0)	65300 (17800)	8630000 (4060000)	1.92 (0.736)
	30	1	120.0 (72.0-120.0)	451000 (107000)	58100000 (9850000)	NA
		22	72.0 (72.0-120.0)	1060000 (159000)	165000000 (28300000)	NA
		36	24.0 (0.0-72.0)	1370000 (830000)	206000000 (110000000)	3.54 (1.79)
	300	1	72.0 (72.0-120.0)	5900000 (364000)	833000000 (73200000)	NA
		22	72.0 (24.0-120.0)	8560000 (2000000)	1290000000 (444000000)	NA
		36	72.0 (24.0-168.0)	9940000 (1080000)	1550000000 (150000000)	1.81 (0.175)

All values were rounded to three significant figures after calculations were performed, except  $T_{max}$ , which is presented to one decimal place.  
 $T_{max}$  = time at which  $C_{max}$  was observed, presented as median and range (minimum-maximum).  
 $C_{max}$  = maximum observed serum concentration  
 $AUC_{0-168}$  = area under the concentration-time curve from time zero to 168 hours postdose  
AR = Accumulation ratio; calculated as  $AUC_{0-168, day 36} / AUC_{0-168, day 1}$ ; NA = Not applicable  
\* N = 3 for Males, Day 36, 300 mg/kg, \*\* N=8 for All, Day 36, 300 mg/kg; One male subject in group 4 tested positive for anti-AMG 145 antibody on day 43

(Applicant)

**Local tolerance****[Evolocumab] AMG 145: Subcutaneous Local Tolerance Study in the Hamster**

**Study no.:** 115815 (b) (4)  
**Study report location:** SDN1, SN000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 31 July 2012  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot #, and % purity:** Evolocumab, batch #0010114375, 98.4% purity (SE-HPLC)

**Key Study Findings**

- Administration of a single subcutaneous dose of evolocumab to the left lumbar region was associated with transient slight/very slight local edema and minimal microscopic skin changes in hamsters that were consistent with the subcutaneous injection and/or skin preparation procedures and not likely associated with drug. No vehicle control was employed.

**Table 27: Study design for a local tolerance test of evolocumab in hamsters**

Group	Route of Administration	Treatment		Dose Volume (mL/animal)	Number of Male Animals
		Left Lumbar Region (dose concentration)			
1	Subcutaneous	140 mg/mL		1.0	5

(Applicant)

**Methods**

**Doses:** 140 mg  
**Frequency of dosing:** Single  
**Route of administration:** Subcutaneous injection  
**Dose volume:** 1 mL  
**Formulation/Vehicle:** 140 mg/mL formulated in 220 mM proline, 20 mM acetate, 0.01% (w/v) polysorbate 80 (pH 5.0)  
**Species/Strain:** Crl:LVG(SYR) Golden Syrian hamsters  
**Number/Sex/Group:** 5 males  
**Age:** 9 to 10 weeks old  
**Weight:** 115 to 122 g  
**Deviation from study protocol:** No significant deviations were reported

**Methods**

The injection site (including the area immediately surrounding the point of insertion) was observed for redness and swelling before dosing, and approximately 1, 4, 24, 48 and 72

hours post-dose. Observations were scored according to the following scoring scale (Draize, 1959) along with any other special findings:

### The Draize Scoring Scale

Erythema (Redness)	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness to slight eschar formation, injuries in depth)	4
Edema (Swelling)	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area are well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond area of exposure)	4

(Applicant)

On Study Day 4, hamsters were sacrificed, the injection sites examined grossly and then prepared for histology. Injection sites were embedded in paraffin, sectioned and H&E stained. A peer review of the histopathology findings was conducted by the Applicant.

### Results

Both the test item and the vehicle were associated with very slight to slight local irritation. While there were no remarkable macroscopic observations, microscopic observations showed transient slight/very slight local edema and minimal microscopic skin changes that are reasonably explained as being a consequence of tissue trauma from injection.

**Table 28: Incidence and severity of skin histopathology in a local subcutaneous tolerance study of evolocumab in hamsters**

	AMG 145	Untreated
No. of Animals Examined	5	5
Total Skin Sections Examined	15	15
Subcutaneous Injection Site or Skin/Subcutis		
Inflammation, focal, dermal/epidermal		
Minimal (Grade 1)	1	1
Inflammation, subacute, focal		
Minimal (Grade 1)	2	2

(Applicant)

## Local Tolerance Test in Rabbits After Bolus Intravenous Administration of [Evolocumab] AMG 145

**Study no.:** 110151 (b) (4)  
**Study report location:** SDN1, SN000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 15 August 2008  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot #, and % purity:** Evolocumab, batch #0010008131, 98.9% purity (SE-HPLC)

### Key Study Findings

- Neither evolocumab nor vehicle was associated with local irritation of the skin or vasculature at the injection site.

**Table 29: Study design for a local tolerance test of evolocumab in rabbits**

Group	Control or Test Article <sup>a</sup>	Route of Administration <sup>b</sup>	Dose Level (mg/dose site)	Dose Volume	Number of Animals
1	Non Product Specific Placebo	IV	0	1 mL/site	3
	AMG 145	IV	74.8 <sup>c</sup>	1 mL/site	

<sup>a</sup> Each animal was dosed with the control article and the test article. Test article was administered at one intravenous (IV) location in the right ear. Control article was administered at one IV location in the left ear.  
<sup>b</sup> IV injections were administered to the marginal ear vein.  
<sup>c</sup> The dose concentration of the neat test article was 74.8 mg/mL.

(Applicant)

### Methods

**Doses:** 0 and 75 mg in separate ears  
**Frequency of dosing:** Single dose  
**Route of administration:** Each animal was dosed with the control article and the test article by slow intravenous push. Test article was administered at one intravenous location in the right ear. Control article was administered at one intravenous location in the left ear.  
**Dose volume:** 1 mL/kg  
**Formulation/Vehicle:** (b) (4)  
**Species/Strain:** Hra:(NZW)SPF rabbits  
**Number/Sex/Group:** 3 males  
**Age:** 17 weeks  
**Weight:** 2.6 to 2.7 kg  
**Deviation from study protocol:** No deviations were reported.

## Observations

Each injection site (including the area immediately surrounding the point of insertion) was observed for redness and swelling before dosing, and approximately 4, 24, 48 and 72 hours post-dose. Any other abnormalities were recorded as they were observed. Observations were scored according to the following scoring scale (Draize, 1959) along with any other special findings:

Three days after dosing, rabbits were sacrificed, the injection sites examined grossly and then prepared for histology. Injection sites were embedded in paraffin, sectioned and H&E stained. A peer review of the histopathology findings was conducted.

## Results

Neither the test item nor the vehicle was associated with local irritation. There were no remarkable macroscopic observations. Microscopic observations did not indicate any test item or vehicle-related vascular irritation.

## 7 Genetic Toxicology

No genetic toxicology studies have been conducted, as evolocumab is a monoclonal antibody (per ICH-S6). As such, it is considered to have negligible capacity for directly interacting with genetic material.

## 8 Carcinogenicity

### [Evolocumab] AMG 145: Subcutaneous Lifetime Pharmacology Study in Hamsters

**Study no.:** 114976 (b) (4)

**Study report location:** SDN1, SN000 (eCTD)

**Conducting laboratory and location:** (b) (4)

**Date of study initiation:** 27 September 2011

**GLP compliance:** Yes

**QA statement:** Yes

**Drug, lot # and % purity:** Evolocumab, batch #0010008131, 98.8% purity (SE-HPLC)

**CAC concurrence:** ECAC meeting minutes noting concurrence with dose selection were faxed to the Applicant on 1 September 2011. On 3 May 2013, the Applicant requested to terminate all female groups when control and high-dose numbers reached 25/group. ECAC responded that it was premature to terminate the female groups, unless the female control group reached 20 animals or all dosed groups reached 15 animals. If any of the dosed groups reached 15 animals, the dose

group affected could be terminated. If approximately Week 100 was reached and a dosed group reached 15 animals, the entire female study could be terminated. The Applicant complied with the ECAC recommendations.

## Key Study Findings

### Adequacy of Carcinogenicity Study

- The carcinogenicity evaluation for evolocumab in Golden Syrian hamsters was carried out under a Special Protocol Assessment (SPA) agreement, consistent with CDER Executive Carcinogenicity Assessment Committee (Exec CAC) recommendations.
- Drug exposures were maintained throughout dosing in all male and female evolocumab dose groups.

### Appropriateness of Test Models

- Evolocumab produces a robust and sustained pharmacological response in Golden Syrian hamsters.

### Evaluation of Tumor Findings

- No drug-related tumors were observed in a lifetime carcinogenicity study with evolocumab administered at 0, 10, 30 and 100 mg/kg once every other week in (b) (4) for 105 weeks in male and 86 weeks in female Golden Syrian hamsters.
- The mean hamster exposure ( $AUC_{last}$ ) at the NOAEL of 100 mg/kg represents 37X, 15X and 6.6X the anticipated human exposures at 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively.

**Table 30: Study design for a subcutaneous lifetime carcinogenicity study of evolocumab in hamsters**

Group	Subgroup	No. of Animals		Dose Level (mg/kg)	Dose Concentration (mg/mL)
		Male	Female		
1 (Control) <sup>a</sup>	1 (Main Study)	60	60	0	0
	2 (Pharmacokinetic) <sup>b</sup>	15	15	0	0
2 (Low)	1 (Main Study)	60	60	10	2.5
	2 (Pharmacokinetic) <sup>b</sup>	15	15	10	2.5
3 (Mid)	1 (Main Study)	60	60	30	7.5
	2 (Pharmacokinetic) <sup>b</sup>	15	15	30	7.5
4 (High)	1 (Main Study)	60	60	100	25
	2 (Pharmacokinetic) <sup>b</sup>	15	15	100	25

a Group 1 received vehicle control article only (A52SuT Placebo).  
b In the study Protocol, Subgroup 2 satellite animals are referred to as “pharmacokinetic” animals. In the results and the study report, these same animals are described as “pharmacokinetic/pharmacodynamic” or “PKPD” satellite animals. This broader term more accurately describes their full purpose for study inclusion and is consistent with the evaluations conducted as per study Protocol.

(Applicant)

**Observations**

**Doses:** 0, 10, 30 and 100 mg/kg evolocumab. These doses were agreed upon by CDER’s Exec CAC under a Special Protocol Assessment agreement.

**Frequency of dosing:** Once every two weeks

**Dose volume:** 4 mL/kg body weight

**Route of administration:** Subcutaneous injection

**Formulation/Vehicle:** (b) (4)

**Basis of dose selection:** ≥10X AUC per ICH-S6 and S6(R1) and saturation of pharmacodynamics

**Species/Strain:** Crl:LVG(SYR) Golden Syrian hamsters

**Age:** 5 to 7 weeks at initiation of dosing

**Animal housing:** Animals were individually housed.

**Interim sacrifice:** No. Main study and toxicokinetic group males were sacrificed at Week 105 (scheduled), and females were sacrificed prematurely at Week 86.

**Satellite groups:** Fifteen animals/sex/group were used for toxicokinetic sampling. Three animals/sex/group/time point were sampled at pre-dose and at approximately 24, 72, 120, 216 and 336 hours post-dose during Weeks 5 and 27.

Trough serum samples were collected from up to 5 animals/sex/group at Months 4.5, 8, 12, 16, 20 (males only), and 24 (males only) and Day 601 (females only). Toxicokinetic animals were kept to the end of the study and were sacrificed along with main study animals.

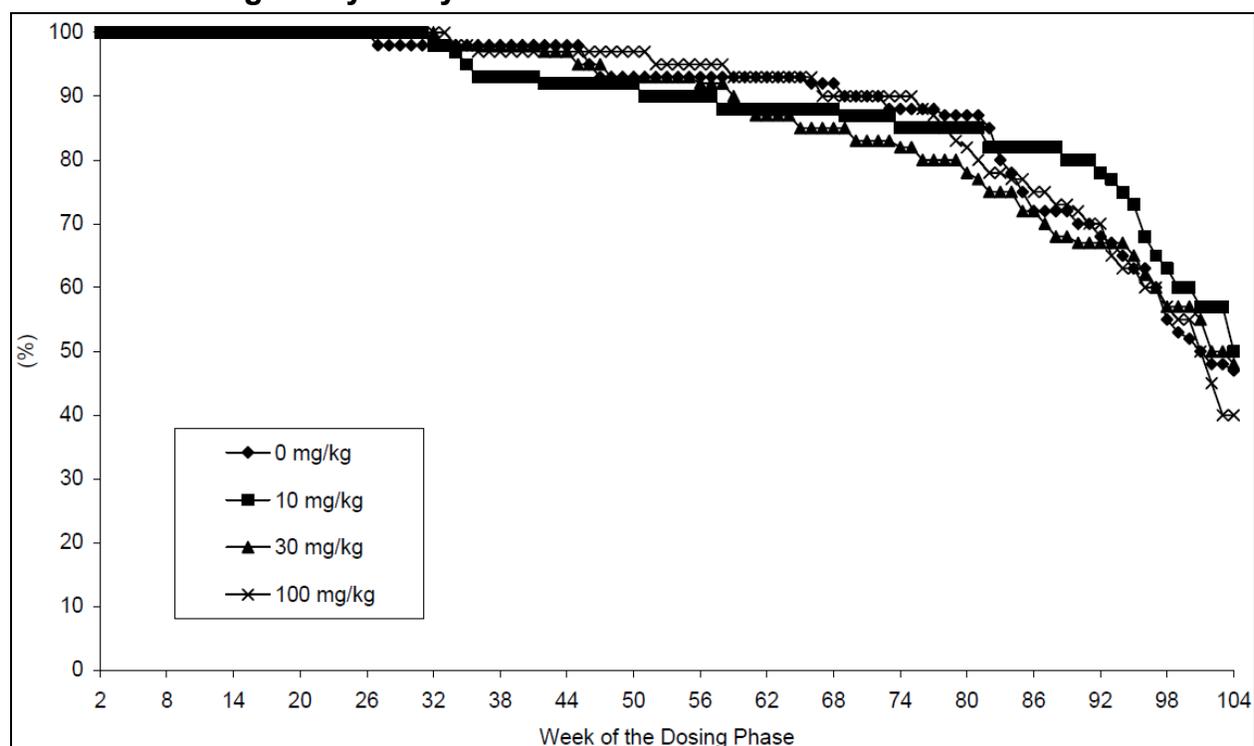
**Deviation from study protocol:** No significant deviations were reported.

**Results**

**Mortality**

There was no significant effect of evolocumab on male or female Golden Syrian hamster mortality in a lifetime study with subcutaneous dosing once every other week at doses up to 100 mg/kg (see Figure 24 and Figure 25). Male groups survived to the scheduled terminal time point at Week 105.

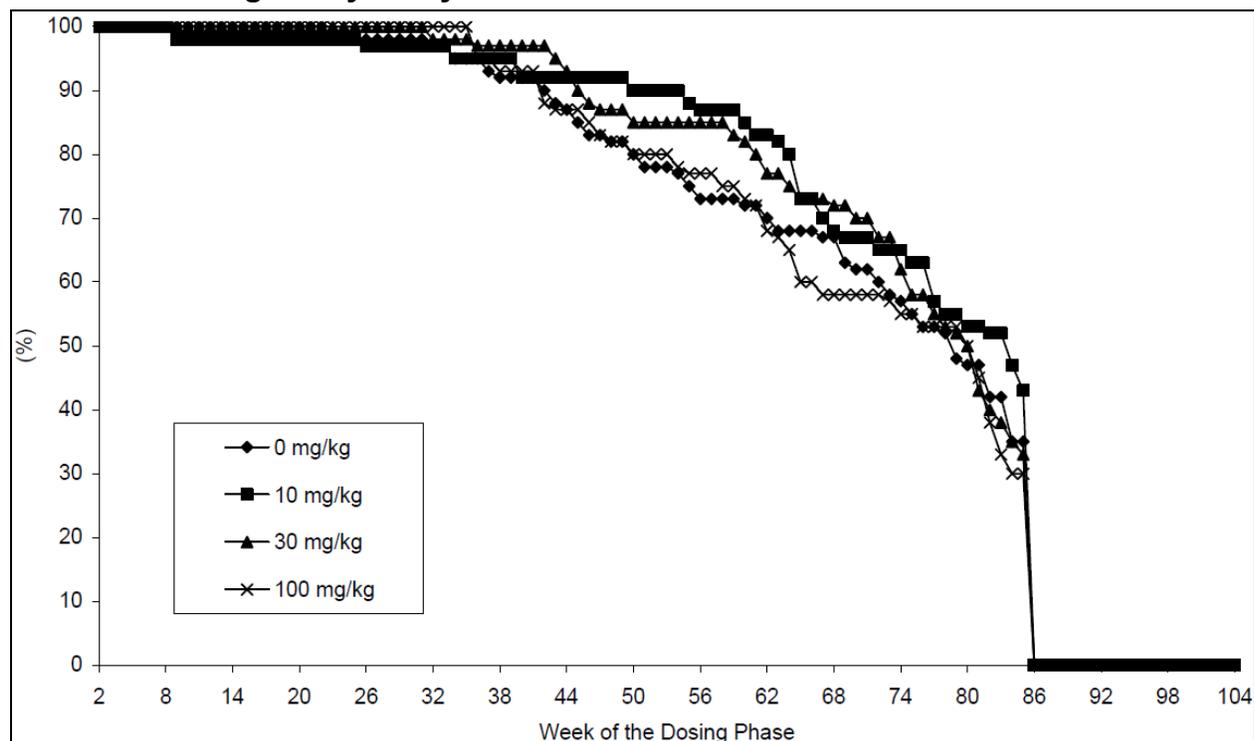
**Figure 24: Kaplan-Meier survival curve for male hamsters in a subcutaneous lifetime carcinogenicity study**



(Applicant)

Female hamster groups were terminated early at Week 86, based on control animal numbers falling to only 20 live females. Early termination of females was carried out with concurrence from CDER’s Exec CAC.

**Figure 25: Kaplan-Meier survival curves for female hamsters in a subcutaneous lifetime carcinogenicity study**



(Applicant)

### Clinical Signs

Pale appearance (entire body, feet, nose, digits, tail, etc.) was 4 times more common in high-dose males (total incidence) than in control males, and was twice the frequency in high-dose females (total incidence) compared to control females. Low-dose males also showed a high total incidence (>5-fold above control) of “pale appearance” (any site). Pale appearance (any site) was correlated with approximately 2-fold increases in “skin, cold to touch” (any site) and ataxic behavior between high-dose male and female hamsters and their respective controls. Pale appearance, ataxic behavior and skin, cold to touch, could indicate an immune response to this human IgG protein. The total incidence of nonformed feces at the high-dose showed a 5-fold increase in males and a 50% increase in females, compared to controls. These signs did not appear to impact survival.

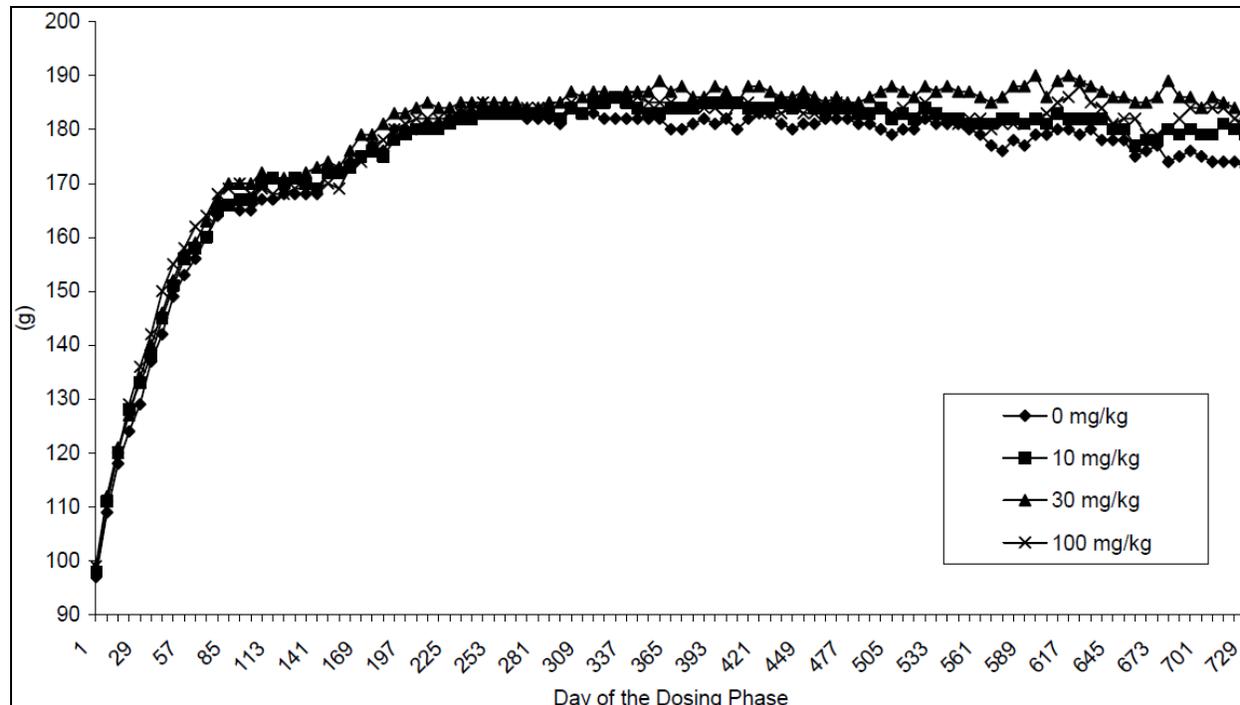
Clinical sign imbalances: Number of animals affected (total incidence) – hamsters, early deaths + terminal sacrifices									
Finding	dose	Male (mg/kg)				Female (mg/kg)			
		0	10	30	100	0	10	30	100
		N	60	60	60	60	60	60	60
Appearance, malocclusion		10 <i>(23)</i>	4 <i>(13)</i>	0 <i>(0)</i>	5 <i>(13)</i>	3 <i>(4)</i>	1 <i>(1)</i>	1 <i>(2)</i>	0 <i>(0)</i>
Appearance, pale (any site)		3 (4)	3 (23)	3 (3)	9 (16)	4 (4)	4 (4)	4 (5)	7 (8)
Behavior, ataxic		4 (4)	6 (6)	6 (6)	9 (9)	2 (2)	3 (4)	4 (4)	8 (8)
Excretion, nonformed feces		5 (9)	9 (64)	12 (29)	11 (45)	13 (50)	6 (15)	12 (22)	20 (75)
Skin and pelage, cold to touch (any site)		10 (13)	14 (14)	10 (12)	17 (22)	4 (4)	3 (3)	7 (7)	8 (8)

*Italics* indicates inverse drug-related trends

### Body Weights

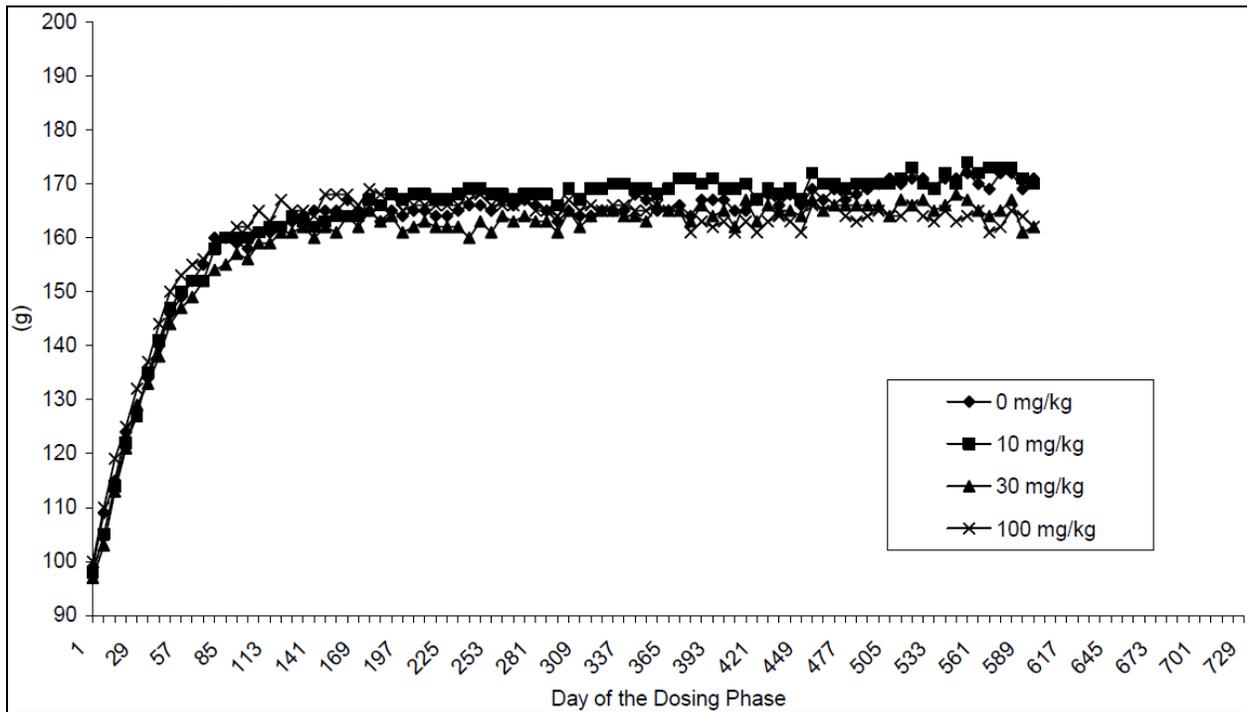
There were no consistent differences in body weights in hamsters administered up to 100 mg/kg evolocumab once every other week for 105 weeks in males for 86 for weeks in females (see Figure 26 and Figure 27). At Week 72, females administered  $\geq 30$  mg/kg once every other week showed a slight decrease in body weight compared to control and low-dose females; however, the absolute differences were  $\leq 5\%$  at every time point. These differences are considered incidental.

**Figure 26: Body weights of male hamsters in a subcutaneous lifetime carcinogenicity study**



(Applicant)

**Figure 27: Body weights of female hamsters in a subcutaneous lifetime carcinogenicity study**

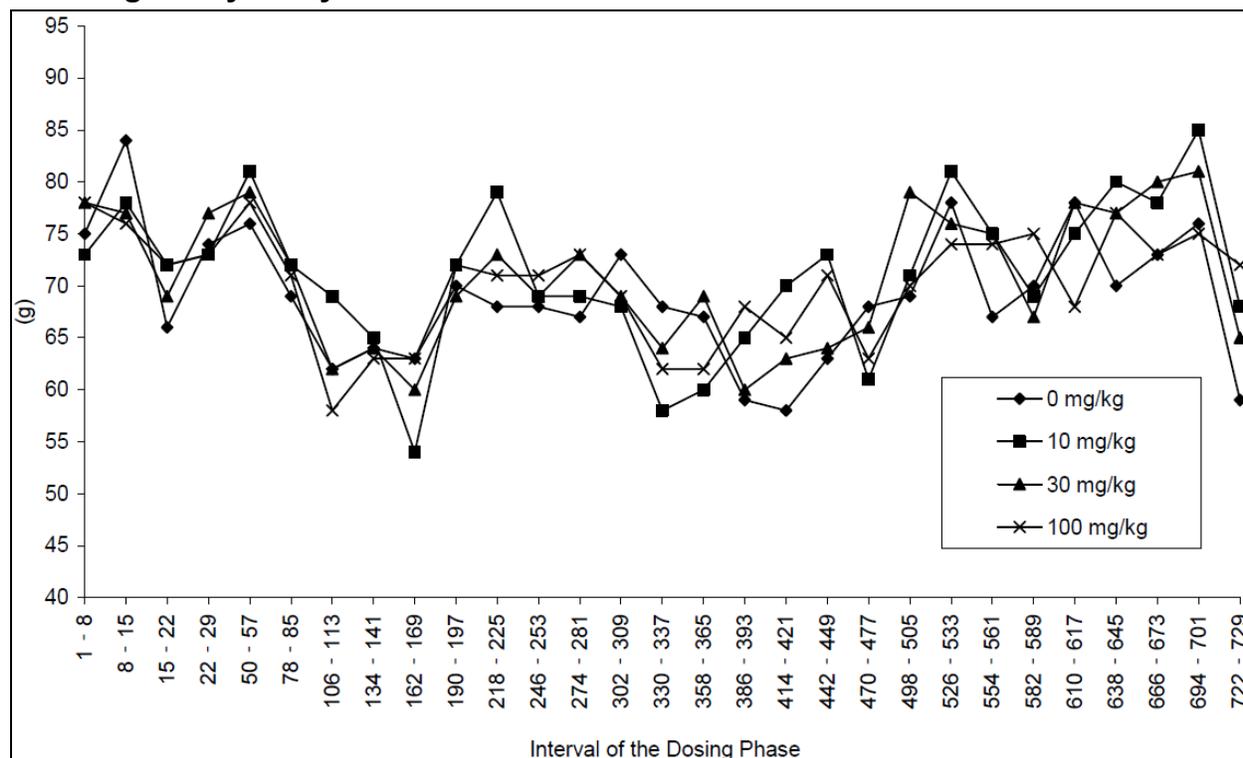


(Applicant)

**Feed Consumption**

In male hamsters, there were no test article-related effects on feed consumption during 2 years of administration of evolocumab once every other week by the subcutaneous route (see Figure 28).

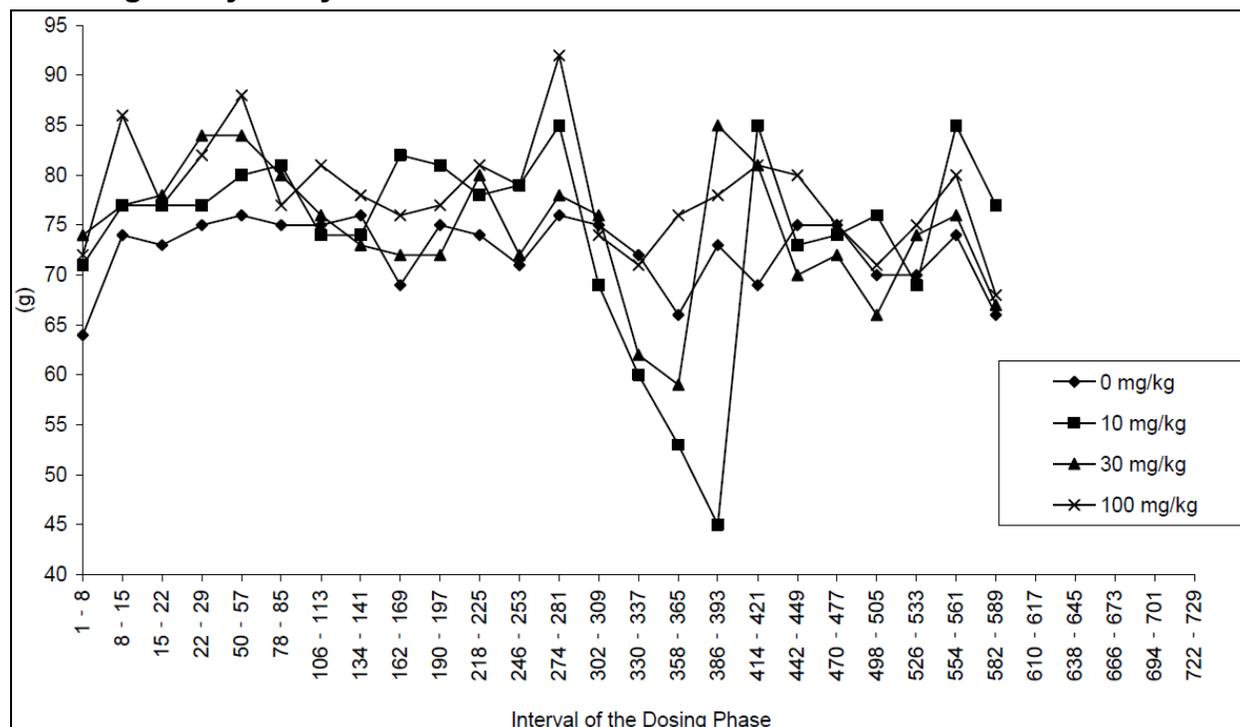
**Figure 28: Food consumption of male hamsters in a subcutaneous lifetime carcinogenicity study**



(Applicant)

Female hamsters administered 10 or 30 mg/kg evolocumab once every other week by the subcutaneous route showed decreased food consumption from approximately Week 44 to Week 59 (see Figure 29). However, this effect was transient, was not dose-related, and was not associated with decreased body weight during this period. Therefore, these differences are considered incidental.

**Figure 29: Food consumption of female hamsters in a subcutaneous lifetime carcinogenicity study**



(Applicant)

**Pharmacodynamic Monitoring to Assess Drug Exposure**

Mean plasma LDL-C levels were reduced consistently throughout the study, confirming adequate drug exposures in the vast majority of the animals for the duration of this lifetime carcinogenicity assessment in all dose groups. This is critical, because anti-drug antibodies were not directly assessed. HDL-C was not measured, but a significant reduction is presumed to have occurred, based on observations from the 1 and 3 month toxicity studies in the hamster.

Plasma LDL-C (mg/dL) – hamsters (N=3/sex/group at Day 29; N=4-6/sex/group at Day 183 and at study termination at Day 601)						
Evolocumab dose group (mg/kg)	Male			Female		
	D29	D183	Terminal D729	D29	D183	Terminal D601
0	45	31	21	69	43	30
10	18	7	<12	43	29	29
30	13	4	9	36	13	19
100	19	7	<3	22	16	18

**Gross Pathology**

There were no clear, treatment-related trends for gross pathological findings. Imbalances between treatment groups are provided in tables, below. Most imbalances were noted only in one sex or showed a low absolute difference between high-dose and

control groups. The large difference in total gelatinous injection site lesions between control and high-dose male animals is due, in part, to counting multiple lesions observed in a few individual animals. Correlations with histopathologic findings are discussed in that section. No imbalance for gelatinous injection sites was noted for females.

Gross pathology imbalances (incidence) – hamsters (Early deaths + terminal sacrifices)									
Tissue	Finding	Male (mg/kg)				Female (mg/kg)			
		0	10	30	100	0	10	30	100
	dose N	60	60	60	60	60	60	60	60
Cecum	<i>large</i>	0	0	0	0	3	1	0	0
Epididymis	discolored	0	0	0	2				
<b>Total for injection sites</b>	gelatinous	2	4	12	19	4	5	5	0
Injection site 1	gelatinous	0	1	2	5	0	1	0	0
Injection site 2	gelatinous	0	1	2	4	1	0	1	0
Injection site 3	gelatinous	1	1	4	5	1	4	4	0
Injection site 4	gelatinous	1	1	4	5	2	4	4	0
Kidney	<i>rough surface</i>	11	8	6	6	1	0	1	1
Liver	discolored	1	4	4	8	5	5	9	6
Mandibular LN	<i>large</i>	4	3	6	4	7	5	4	2
	discolored	1	1	1	5	3	2	2	2
Mesenteric LN	large	6	2	9	5	2	4	8	6
	discolored	0	0	2	2	0	0	0	0
Ovary	<i>cyst</i>					11	13	14	4
Seminal vesicle	large	2	0	2	6				
Skin/ subcutis	gelatinous	1	1	0	3	0	1	0	0
	thickened	0	1	1	2	0	0	0	0
Testis	<i>small</i>	3	2	1	1				
Thymus	cyst	0	0	0	2	0	0	0	0
Thyroid	<i>large</i>	0	0	1	0	4	0	0	0
Uterus	discolored					1	2	1	3

*Italics* indicates inverse drug-related trends

Gross pathology imbalances (incidence) – hamsters (Scheduled/terminal sacrifices-only)									
Tissue	Finding	Male (mg/kg)				Female (mg/kg)			
		0	10	30	100	0	10	30	100
	N	28	30	29	24	20	24	18	18
Ovary	<i>cyst</i>					9	8	5	3
Testis	<i>small</i>	2	1	1	0				
Thymus	cyst	0	0	0	2	0	0	0	0
Thyroid	<i>large</i>	0	0	0	0	3	0	0	0

*Italics* indicates inverse drug-related trends

Gross pathology imbalances (incidence) – hamsters (Unscheduled/early deaths-only)										
Tissue	Finding	N	Male (mg/kg)				Female (mg/kg)			
			0	10	30	100	0	10	30	100
			32	30	31	36	40	36	42	42
Adipose	mass		0	0	1	2	0	1	0	0
Cecum	<i>large</i>		<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>3</i>	<i>1</i>	<i>0</i>	<i>0</i>
Epididymis	discolored		0	0	0	2				
<b>Total for injection sites</b>	gelatinous		3	7	14	25	4	6	16	5
Injection site 1	gelatinous		0	1	2	5	0	0	1	0
Injection site 2	gelatinous		0	0	0	2	0	0	0	0
Injection site 3	gelatinous		1	1	4	5	1	2	4	0
Injection site 4	gelatinous		1	1	4	5	1	2	3	0
Kidney	<i>rough surface</i>		<i>5</i>	<i>2</i>	<i>2</i>	<i>2</i>	0	0	1	1
Liver	discolored		1	4	4	8	2	2	8	5
Mandibular LN	discolored		1	1	1	5	3	2	2	2
	large		3	2	4	4	<i>5</i>	<i>3</i>	<i>3</i>	<i>1</i>
Mesenteric LN	discolored		0	0	2	2	0	0	0	0
	large		4	2	6	3	2	2	5	5
Seminal vesicle	large		2	0	2	6				
Skin/ subcutis	gelatinous		1	1	0	3	0	0	0	0
	thickened		0	1	1	2	0	0	0	0
Uterus	discolored						0	1	1	3

*Italics* indicates inverse drug-related trends

## Histopathology

### Peer Review

A peer review was conducted by a pathologist employed by (b) (4). Selected slides were peer-reviewed, differences of opinion were discussed, and reported results were mutually agreed upon by study pathologist and peer reviewing pathologist. According to the study report, the tables reflected the consensus opinion of both pathologists.

### Neoplastic

There were no drug-related neoplastic lesions.

### Non-Neoplastic

**Adrenal:** No increases in preneoplastic lesions were reported. In fact, adrenal hyperplasia showed an inverse drug-related trend, with increasing doses resulting in less hyperplasia/hypertrophy. Increased cystic degeneration in the adrenals of males administered  $\geq 30$  mg/kg evolocumab every other week by the subcutaneous route was generally observed with adjacent hyperplasia and/or hypertrophy of neighboring tissues. The pathological significance of these lesions is uncertain; adrenal glands have been shown to be dependent on substantial amounts of cholesterol derived from the circulation.

**Cecum:** An imbalance for chronic-active inflammation in the cecum between males administered evolocumab and control males was observed. No imbalance was detected for other regions of the intestine and colon or in females. Therefore, this finding is considered incidental.

Liver: Hepatocyte degeneration/necrosis, hemorrhage, bile duct hyperplasia, mononuclear/granulocytic cell (comprised primarily of lymphocytes and varying populations of plasma cells and histiocytes, together with lymphoid follicle formation and often extramedullary hematopoiesis), nodular hyperplasia (females only) and tension lipidosis showed dose-related increasing trends in incidence and severity for both male and female hamsters. A dose-related increase in incidence of discoloration in livers was noted upon gross evaluation. Adverse liver pathologies were not associated with any increases in liver tumors.

Lymph node: Slight dose-related imbalances in sinus dilatation, plasma cells in sinus and lymphocyte hyperplasia were observed in the mandibular lymph node, primarily in males. Evolocumab is expected to be immunogenic in hamsters, and these findings are consistent with reactive lymph nodes. These lymph nodes were discolored and/or enlarged in these animals.

Seminal vesicle: Increased secretion of seminal vesicles in high-dose males (100 mg/kg evolocumab every other week) was noted. This finding correlates with enlarged seminal vesicles in the same animals. The significance of this finding is unknown.

Histopathology imbalances (incidence) – hamsters (early deaths + terminal sacrifices)										
Tissue	Finding	Sev N	Male (mg/kg)				Female (mg/kg)			
			0	10	30	100	0	10	30	100
			60	60	60	60	60	60	60	60
Adrenal, cortex	<i>congestion/ hemorrhage</i>	<i>slight</i>	0	0	0	0	2	0	1	0
		<i>mod</i>	0	0	0	0	2	0	0	0
	cystic degeneration	<i>slight</i>	0	1	5	0	0	0	0	0
		<i>mod</i>	0	0	0	2	0	0	0	0
		<i>mark</i>	0	0	1	0	0	0	0	0
		<i>hyperplasia</i>	<i>min</i>	4	2	1	1	2	1	1
	<i>slight</i>	2	2	1	1	1	1	3	0	
	<i>mark</i>	1	0	0	0	0	0	0	0	
Cecum	chronic-active inflammation	<i>min</i>	0	1	0	0	0	0	0	0
		<i>slight</i>	0	3	2	1	0	0	1	0
		<i>mod</i>	1	0	5	0	0	0	0	0
		<i>mark</i>	0	0	0	1	0	0	0	0
Cervix	<i>epithelial hyperplasia/ metaplasia</i>	<i>min</i>					2	4	2	2
		<i>slight</i>					11	15	5	4
		<i>mod</i>					2	3	1	0
		<i>mark</i>					0	1	0	0
Kidney	<i>infarct</i>	<i>min</i>	15	10	12	8	6	5	0	3
		<i>slight</i>	6	2	3	4	2	2	5	2
		<i>mod</i>	0	0	0	0	1	1	0	0
Liver	hepatocyte degeneration/ necrosis	<i>min</i>	3	4	1	3	3	6	2	3
		<i>slight</i>	2	5	2	5	5	2	5	1
		<i>mod</i>	3	2	3	7	1	3	4	5
		<i>mark</i>	0	0	0	0	0	0	0	2
	hemorrhage	<i>min</i>	1	0	1	0	0	0	0	2
		<i>slight</i>	1	3	0	6	1	0	1	3
		<i>mod</i>	1	2	3	4	2	4	4	2
		<i>mark</i>	0	1	0	0	0	0	1	0

Histopathology imbalances (incidence) – hamsters (early deaths + terminal sacrifices)										
Tissue	Finding	Sev N	Male (mg/kg)				Female (mg/kg)			
			0	10	30	100	0	10	30	100
			60	60	60	60	60	60	60	60
	hyperplasia, bile duct	min	1	0	1	0	2	1	1	0
		slight	0	1	1	1	1	1	1	2
		mod	0	0	0	0	0	0	1	1
		mark	0	0	0	1	0	0	0	0
	mononuclear/ granulocytic cell	min	17	12	22	21	25	21	16	24
		slight	6	11	10	10	5	8	14	8
		mod	0	3	0	2	0	0	0	1
	nodular hyperplasia	min	0	0	0	0	0	0	1	0
		slight	0	0	0	0	0	1	0	4
		mod	0	0	0	0	2	0	2	2
	tension lipidosis	min	0	0	0	0	0	1	1	2
		slight	0	1	0	1	0	0	0	1
mod		0	0	0	1	0	0	0	0	
LN, Mandibular	sinus dilatation	min	0	1	0	1	0	0	0	0
		slight	0	0	1	3	1	0	1	1
		mod	0	0	1	1	0	0	0	0
	plasma cells, sinus	slight	0	0	1	1	0	0	0	0
		mod	0	0	0	1	0	0	0	0
	lymphocyte hyperplasia	min	0	1	0	1	1	1	0	0
slight		0	0	1	0	1	5	1	0	
mod	0	0	0	0	0	0	0	1	1	
Marrow, femur	<i>hypocellular</i>	<i>slight</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>
		<i>mod</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>
Seminal vesicle	increased secretion	slight	1	0	2	5				
		mod	0	0	0	1				
Skin/ subcutis	<i>acanthosis/ hyperkeratosis</i>	<i>min</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>
		<i>slight</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>0</i>	<i>0</i>
		<i>mod</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>
Sternum	<i>fibro-osseous change</i>	<i>min</i>	<i>0</i>	<i>1</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>3</i>	<i>0</i>	<i>1</i>
		<i>slight</i>	<i>4</i>	<i>0</i>	<i>1</i>	<i>1</i>	<i>6</i>	<i>4</i>	<i>4</i>	<i>2</i>
		<i>mod</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>3</i>	<i>1</i>	<i>1</i>	<i>0</i>
Stomach, glandular	<i>ulcer</i>	<i>min</i>	<i>0</i>	<i>3</i>	<i>1</i>	<i>2</i>	<i>0</i>	<i>1</i>	<i>1</i>	<i>0</i>
		<i>slight</i>	<i>2</i>	<i>1</i>	<i>1</i>	<i>0</i>	<i>1</i>	<i>4</i>	<i>0</i>	<i>1</i>
		<i>mod</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>2</i>	<i>0</i>	<i>0</i>	<i>0</i>
		<i>mark</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>
Vagina	<i>epithelial hyperplasia/ metaplasia</i>	<i>min</i>					<i>4</i>	<i>4</i>	<i>5</i>	<i>1</i>
		<i>slight</i>					<i>11</i>	<i>14</i>	<i>8</i>	<i>3</i>
		<i>mod</i>					<i>5</i>	<i>5</i>	<i>3</i>	<i>1</i>
		<i>mark</i>					<i>1</i>	<i>2</i>	<i>0</i>	<i>0</i>

*Italics* indicates inverse drug-related trends

Histopathology imbalances (incidence) – hamsters (scheduled/terminal sacrifices-only)										
Tissue	Finding	Sev N	Male (mg/kg)				Female (mg/kg)			
			0	10	30	100	0	10	30	100
			28	30	29	24	20	24	18	18
Adrenal, cortex	cystic degeneration	slight	0	1	3	0	0	0	0	0
		mod	0	0	0	2	0	0	0	0
		mark	0	0	1	0	0	0	0	0
Cecum	chronic-active inflammation	slight	0	3	1	1	0	0	0	0
		mod	0	0	3	0	0	0	0	0
		mark	0	0	0	1	0	0	0	0
Cervix	<i>epithelial hyperplasia/metaplasia</i>	<i>min</i>					<i>2</i>	<i>4</i>	<i>2</i>	<i>1</i>
		<i>slight</i>					<i>10</i>	<i>14</i>	<i>5</i>	<i>2</i>
		<i>mod</i>					<i>1</i>	<i>2</i>	<i>0</i>	<i>0</i>
Kidney	<i>infarct</i>	<i>min</i>	<i>6</i>	<i>9</i>	<i>9</i>	<i>1</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>
		<i>slight</i>	<i>3</i>	<i>2</i>	<i>2</i>	<i>2</i>	<i>1</i>	<i>2</i>	<i>2</i>	<i>0</i>
		<i>mod</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>1</i>	<i>0</i>	<i>0</i>
Liver	hyperplasia, bile duct	min	0	0	1	0	1	0	0	0
		slight	0	1	0	0	1	0	0	2
		mod	0	0	0	0	0	0	0	1
	nodular hyperplasia	min	0	0	0	0	0	0	1	0
		slight	0	0	0	0	0	1	0	1
		mod	0	0	0	0	0	0	0	2
tension lipidosis	min	0	0	0	0	0	1	1	2	
	slight	0	1	0	1	0	0	0	0	
mod	0	0	0	1	0	0	0	0		
LN, mandibular	plasma cells, sinus	slight	0	0	1	0	0	0	0	0
		mod	0	0	0	1	0	0	0	0
Sternum	<i>fibro-osseous change</i>	<i>min</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>0</i>
		<i>slight</i>	<i>2</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>2</i>	<i>0</i>	<i>1</i>	<i>1</i>
		<i>mod</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>0</i>	<i>1</i>	<i>0</i>	<i>1</i>	<i>0</i>
Vagina	<i>epithelial hyperplasia/metaplasia</i>	<i>min</i>					<i>8</i>	<i>7</i>	<i>3</i>	<i>13</i>
		<i>slight</i>					<i>4</i>	<i>3</i>	<i>5</i>	<i>1</i>
		<i>mod</i>					<i>6</i>	<i>12</i>	<i>8</i>	<i>3</i>
		<i>mark</i>					<i>2</i>	<i>2</i>	<i>2</i>	<i>1</i>

*Italics* indicates inverse drug-related trends

Histopathology imbalances (Incidence) – hamsters (unscheduled/early deaths-only)										
Tissue	Finding	Sev N	Male (mg/kg)				Female (mg/kg)			
			0	10	30	100	0	10	30	100
			32	30	31	36	40	36	42	42
Adrenal, cortex	<i>congestion/ hemorrhage</i>	<i>slight</i>	0	0	0	0	2	0	1	0
		<i>mod</i>	0	0	0	0	2	0	0	0
	<i>hyperplasia</i>	<i>min</i>	2	0	1	0	2	0	1	0
		<i>slight</i>	1	1	0	0	1	0	3	0
		<i>mod</i>	1	0	0	0	0	0	0	0
Liver	hepatocyte degeneration/ necrosis	min	2	3	1	3	3	5	2	2
		slight	2	5	0	5	3	2	4	1
		mod	2	2	3	6	1	1	3	4
		mark	0	0	0	0	0	0	0	2
	hemorrhage	min	1	0	0	0	0	0	0	2
		slight	0	3	0	5	0	0	1	3
		mod	1	2	3	4	2	2	4	2
		mark	0	1	0	0	0	0	0	0
	hyperplasia, bile duct	min	1	0	0	0	1	1	1	0
		slight	0	0	1	1	0	1	1	0
		mod	0	0	0	0	0	0	1	0
		mark	0	0	0	1	0	0	0	0
mononuclear/ granulocytic cell	min	10	8	10	15	17	13	11	20	
	slight	4	5	4	8	4	8	11	6	
	mod	0	3	0	2	0	0	0	1	
LN, mandibular	sinus dilatation	min	0	1	0	1	0	0	0	0
		slight	0	0	0	3	0	0	0	0
		mod	0	0	1	0	0	0	0	0
lymphocyte hyperplasia	min	0	0	0	1	1	1	0	0	
	slight	0	0	0	0	1	3	0	0	
	mod	0	0	0	0	0	0	1	1	
Marrow, femur	<i>hypocellular</i>	<i>mod</i>	1	0	0	0	1	0	0	
Seminal vesicle	increased secretion	slight	1	0	2	5				
		mod	0	0	0	1				
Skin/ subcutis	<i>acanthosis/ hyperkeratosis</i>	<i>min</i>	1	0	0	0	0	0	0	0
		<i>slight</i>	0	0	0	0	1	1	0	0
		<i>mod</i>	0	0	0	0	1	0	0	0
Sternum	<i>fibro-osseous change</i>	<i>min</i>	0	1	2	1	2	2	0	1
		<i>slight</i>	2	0	1	1	4	4	3	1
		<i>mod</i>	0	1	0	0	2	1	0	0
Stomach, glandular	<i>ulcer</i>	<i>min</i>	0	1	0	1	0	0	1	0
		<i>slight</i>	1	0	1	0	1	3	0	0
		<i>mod</i>	0	0	0	0	1	0	0	0
		<i>mark</i>	1	0	0	0	0	0	0	0
Vagina	<i>epithelial hyperplasia/ metaplasia</i>	<i>min</i>					0	1	0	0
		<i>slight</i>					5	2	0	0
		<i>mod</i>					3	3	1	0
		<i>mark</i>					1	2	0	0

*Italics* indicates inverse drug-related trends

### Anti-drug Antibody Analysis

An analysis of anti-drug antibodies (ADA), which could theoretically cause a reduction in exposure to evolocumab, was not conducted. However, pharmacodynamics is a reliable surrogate for ensuring adequate drug exposure. Mean pharmacodynamic activity was retained at all doses throughout the study in all but a few animals. Therefore, the lack of anti-drug antibody analyses is not considered to have adversely affected study interpretation.

### Toxicokinetics

Exposures ( $AUC_{last}$ ) were predictable and dose-related in both sexes at study Weeks 5 and 27. Increases in exposures were slightly greater than a strict dose-dependent response would predict. Exposures ( $AUC$ ) were ~17 to 43% higher in male hamsters than in females.  $C_{max}$  was also increased in a greater than dose-proportional manner, but values were similar between the sexes at a given dose.

**Table 31: Summary toxicokinetics for evolocumab upon subcutaneous administration to hamsters in a lifetime carcinogenicity study**

Sex	Dose (mg/kg)	Week 5			Week 27			AR
		$t_{max}$ (hr)	$C_{max}$ ( $\mu\text{g/mL}$ )	$AUC_{last}$ ( $\mu\text{g}\cdot\text{hr/mL}$ )	$t_{max}$ (hr)	$C_{max}$ ( $\mu\text{g/mL}$ )	$AUC_{last}$ ( $\mu\text{g}\cdot\text{hr/mL}$ )	
Male	10	24	82.4	12900	72	69.5	12400	0.966
	30	24	317	56600	72	285	57300	1.01
	100	24	1110	220000	24	1040	187000	0.851
Female	10	24	69.0	8640	24	50.2	7130	0.825
	30	24	244	40400	24	221	32500	0.804
	100	24	851	169000	24	927	156000	0.925
All	10	24	75.7	10700	24	59.5	9770	0.909
	30	24	280	48500	24	247	44900	0.925
	100	24	982	195000	24	984	172000	0.883

AR = accumulation ratio =  $AUC_{last, \text{Week 27}}/AUC_{last, \text{Week 5}}$ ;  $AUC_{last}$  = area under the concentration-time curve from time zero to the time of the last quantifiable concentration;  $C_{max}$  = maximum observed drug concentration during a dosing interval;  $t_{max}$  = time to reach  $C_{max}$ .  
All values were rounded to 3 significant figures after calculations were performed, except  $t_{max}$  values, which were rounded to 2 significant figures.

(Applicant)

### Dosing Solution Analysis

Results showed that all evolocumab samples had mean concentrations ranging from 97.8 to 107.7% (within  $\pm 10\%$  of target concentrations). Evolocumab was not detected in any vehicle control article formulation. Stability of dosing formulations was confirmed.

## 9 Reproductive and Developmental Toxicology

### 9.1 Fertility and Early Embryonic Development

#### [Evolocumab] AMG 145: Fertility and Early Embryonic Development Study in the Hamster

**Study no.:** 114975 (b) (4)  
**Study report location:** SDN1, SN0000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 8 November 2011  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Evolocumab, batch #0010075910, 98.9% (SE-HPLC)

#### Key Study Findings

- Evolocumab was administered to hamsters (25/sex/group) at 0, 10, 30 and 100 mg/kg every other week by subcutaneous injection during the period of mating and early embryonic development. The NOAEL for this assessment of fertility and early embryonic development in hamsters was 100 mg/kg evolocumab administered every other week, based on the lack of toxicity for all endpoints.
- Evolocumab at doses up to 100 mg/kg had no effect on mating, fertility, estrous cycling, male reproductive assessments (reproductive organ weights and sperm parameters), or embryo-fetal survival.
- Although this study lacked an assessment of ADA status and pharmacodynamics, it is reasonable to assume that animals maintained exposure to active drug based on the results of the 3-month hamster toxicity study (study #114375), which showed persistent LDL-C lowering (a pharmacodynamic biomarker) throughout the study.

**Table 32: Study design for a fertility and early embryonic developmental toxicity study of evolocumab in hamsters**

Group No.	Test Material	Dose Level (mg/kg)	Concentration (mg/mL)	Dose Volume (mL/kg)	No. of Hamsters	
					Male	Female
1	Vehicle Control Article <sup>a</sup>	0	0	4	25	25
2	AMG 145	10	2.5	4	25	25
3	AMG 145	30	7.5	4	25	25
4	AMG 145	100	25	4	25	25

<sup>a</sup> Non-Product Specific Placebo (A52SuT Placebo).

(Applicant)

**Methods**

**Doses:** 0, 10, 30 and 100 mg/kg  
**Frequency of dosing:** Once every two weeks  
**Dose volume:** 4 mL/kg  
**Route of administration:** Subcutaneous injection  
**Formulation/Vehicle:** (b) (4)  
**Species/Strain:** Crl:LVG(SYR) Golden Syrian hamsters  
**Number/Sex/Group:** 25/sex/group  
**Satellite groups:** No  
**Study design:** Males: Male hamsters were administered evolocumab or the vehicle control once every other week via the subcutaneous route beginning 28 days before cohabitation, during cohabitation and continuing through the day before euthanasia. Based on a once every other week dosing schedule, males were administered 5 doses of evolocumab or the vehicle control. Males were euthanized during study Week 8.  
Females: Female hamsters were administered evolocumab or the vehicle control (subcutaneous, every other week) beginning 14 days before cohabitation, during cohabitation and continuing until Gestational Day (GD) 7. Based on a once every other week dosing schedule, females were administered 2 or 3 doses of evolocumab or the vehicle control. Females were euthanized on GD14.  
Parameters and endpoints evaluated: fetal viability, clinical signs, body weights, body weight changes, estrous cycling, mating performance, macroscopic observations, ovarian and uterine examination, male reproductive assessments (vas deferens sperm motility, cauda epididymal sperm density and reproductive organ weights) and bioanalytical assessment.  
**Deviation from study protocol:** No significant deviations were reported.

**Observations**

**Mortality:** Animals were checked twice daily for mortality, abnormalities and signs of pain or distress.  
**Clinical signs:** Clinical observations were recorded for female hamsters on GD0 and

once daily during the post-dose period. Post-dose observations were recorded between 1 and 2 hours of dose administration.

- Body weights:** Measured daily
- Feed consumption:** Monitored and replenished as needed, but not recorded
- Mating performance:** Evaluated daily during cohabitation
- Ovarian and uterine examinations:** On GD14, all females were euthanized and the reproductive tract was analyzed. The number and distribution of corpora lutea were recorded. The uterus of each hamster was opened and examined for implantation sites, placentae (gross evaluation), live and dead fetuses and early and late resorptions. Early versus late resorption was differentiated by whether organogenesis was or was not grossly evident. Live versus dead fetuses were differentiated by whether there was a response to stimuli or not. Dead fetuses and late resorptions were differentiated by the degree of autolysis present; marked to extreme autolysis indicated that the fetus was a late resorption. Placentae were examined for size, color and shape. Uteri of apparently non-pregnant females were examined while being pressed between glass plates to confirm the absence of implantation sites.
- Male fertility parameters:** Sperm motility and concentration were evaluated at necropsy.

**Organ weights and histopathology:**

Tissue	Weighed	Collected	Microscopic Evaluation	Comment
Cervix	-	X	-	Collected with uterus. All female hamsters.
Epididymides	X	X	-	Individually weighed. All male hamsters. The remaining portion of the left epididymis (corpus and caput) and right epididymis were fixed in neutral buffered 10% formalin for possible histopathological evaluation.
Epididymis, left cauda	X	X	-	-
Gland, prostate	X	X	-	-
Gland, seminal vesicle	X	X	-	Weighed with and without fluid.
Gross lesions/masses	-	X	-	All hamsters.
Heart	-	X	-	The male hamsters euthanized before scheduled termination.
Kidney	-	X	-	The male hamsters euthanized before scheduled termination.
Liver	-	X	-	The male hamsters euthanized before scheduled termination.
Lungs with Large Bronchi	-	X	-	Infused with 10% neutral buffered formalin. The male hamsters euthanized before scheduled termination.
Ovaries	-	X	-	All female hamsters.
Spleen	-	X	-	The male hamsters euthanized before scheduled termination.
Stomach	-	X	-	The male hamsters euthanized before scheduled termination.
Testes <sup>a</sup>	X	X	-	Individually weighed. All male hamsters.
Uterus	-	X	-	Collected with cervix. All female hamsters.
Vagina	-	X	-	All female hamsters.

X = Procedure conducted; - = Not applicable.

<sup>a</sup> Preserved in Bouin's solution for 48 to 96 hours and then retained in neutral buffered 10% formalin.

**Toxicokinetics:** On GD14 (females) or during study Week 8 (males), blood samples (0.8 mL) were collected from the vena cava following euthanasia from each male and female hamster on the day of scheduled euthanasia. Serum samples were analyzed for evolocumab by a validated ligand binding analytical procedure. No toxicokinetic parameters were estimated. Concentrations were used only to verify exposure.

**Results****Mortality**

High-dose male SSAN 6277 was euthanized on Study Day 36 because of an ocular injury sustained during cohabitation. All tissues appeared normal at necropsy. This male successfully impregnated its cohort female (SSAN 6377) and sired a normal litter (16 live fetuses and one early resorption). This death was not attributed to drug.

Two high-dose females (SSANs 6378 and 6380) were euthanized due to a mis-timed pregnancy. These mis-timed pregnancies were the result of missed evidence of mating following the first pairing during cohabitation.

**Clinical Signs**

Males: Two of 25 high-dose males had  $\geq 2$  incidents of red paws, limb(s) swollen, hunched posture, ungroomed coat, urine-stained abdominal fur. One high-dose male (SSAN 6289) had an ulcerated mass in the neck. These findings were not observed in

the control (or low- or mid-dose) group. All findings showed a low frequency of occurrence and are consistent with incidental lesions.

Females: No test item-related signs.

### Body Weight

No effect of test item was observed in either sex during any period of the study.

### Feed Consumption

Not measured

### Necropsy

No test item-related effects in males or females were observed at necropsy.

### Mating and Fertility Parameters

#### Reproductive Organ Weights (in Males):

No test item related effects were observed.

#### Vas Deferens Sperm Motility and Cauda Epididymal Sperm Density:

No statistically significant effects of the test item were observed. There is a slight, but non-statistically significant decrease in motility and density at the high-dose.

Male fertility – hamsters				
Parameter	Dose (mg/kg once every two weeks)			
	0	10	30	100
N	25	25	25	25
Percent motility <sup>a</sup> (%)	87	88	87	85
Sperm density <sup>b</sup> (sperm/g)	1385	1380	1450	1257

<sup>a</sup> vas deferens

<sup>b</sup> cauda epididymal

#### Male mating/fertility:

No effects of test item were observed.

Male mating and fertility – hamsters				
Parameter	Dose (mg/kg once every two weeks)			
	0	10	30	100
N	25	25	25	25
Pairs that mated (%)	100	100	100	100
Pregnant females (%)	88	96	92	96
Days to confirmed mating	2.8	2.3	2.7	2.6
Fertility index (%)	88	96	92	96
Mated with females (%) -				
Days 1-7	96	100	96	100
Days 8-14	4	0	4	0

Female mating/fertility: No effect of test item on cycling, mating or fertility parameters was detected.

Female estrous cycling – hamsters					
Parameter	N	Dose (mg/kg once every two weeks)			
		0	10	30	100
		25	25	25	25
<b>Pre-dose estrous cycling</b>					
estrous stages/14 days		2.7	3.2	2.9	2.6
females with 6 or more consecutive days of diestrus		0	1	1	0
females with 6 or more consecutive days of estrus		0	0	0	0
<b>Pre-cohabitation estrous cycling</b>					
estrous stages/14 days		3.3	3.2	2.5**	2.9
females with 6 or more consecutive days of diestrus		0	0	1	0
females with 6 or more consecutive days of estrus		0	0	0	0

Female mating and fertility – hamsters					
Parameter	N	Dose (mg/kg once every two weeks)			
		0	10	30	100
		25	25	25	25
Pairs that mated (%)		100	100	100	100
Pregnant females (%)		88	96	92	96
Days to confirmed mating		2.8	2.3	2.7	2.6
Fertility index (%)		88	96	92	96
<b>Mated with males (%) -</b>					
Days 1-7		96	100	96	100
Days 8-14		4	0	4	0

### C-section observations

No test article-related effects were observed.

Female C-section observations – hamsters				
Parameter	Dose (mg/kg once every two weeks)			
	0	10	30	100
	N	25	25	25
Pregnant females	22	24	23	22
Females C-sectioned	22	24	23	22
Corpora lutea	19.6	19.8	19.1	19.0
Implantations	19.2	19.1	18.8	18.7
Preimplantation loss (%)	2.2	2.9	1.2	1.7
Viable fetuses (mean)	18.1	18.1	18.0	17.4
Nonviable fetuses	1.0	1.0	0.9	1.3
Postimplantation loss (%)	5.7	5.3	5.1	7.5
Females with nonviable fetuses (%)	54.5	62.5	47.8	59.1
Females with all conceptuses nonviable (%)	0	0	0	0
Females with viable fetuses (%)	100	100	100	100
Placentae appeared normal (%)	100	100	100	100

### Toxicokinetics

No toxicokinetic parameters were estimated, but this is acceptable because toxicokinetics could be predicted based on previous assessments. Assessment of serum sample concentrations on Gestational Day 14 (females) or Study Week 8 (males) confirmed dose-dependent exposures to evolocumab.

### Dosing Solution Analysis

The mean evolocumab concentrations for all dose formulations were within the acceptable limits ( $\pm 15\%$  of theoretical concentration).

## 9.2 Enhanced Pre-/Postnatal Development

### [Evolocumab] AMG 145: Enhanced Pre-Postnatal Development Study in the Cynomolgus Monkey with a 6-Month Postnatal Evaluation

**Study no.:** 110370 (b) (4)  
**Study report location:** SDN1, SN000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 2 February 2012  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Evolocumab, batch #0010088852, 98.8% purity by SE-HPLC

### Key Study Findings

- Dosages of 0 and 50 mg/kg evolocumab were administered subcutaneously once every two weeks during the gestational period.
- The NOAEL for maternal toxicity and fetal toxicity was 50 mg/kg evolocumab administered once every other week during gestation, based on a lack of toxicity.

**Table 33: Study design for an enhanced pre/postnatal development study of evolocumab in the monkeys**

Group No.	No. of Pregnant Females	Test Material	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg/dose)
1	18 <sup>a</sup>	Control Article (placebo)	0	0	0.71
2	18	AMG 145	50	70	0.71

<sup>a</sup> One control female underwent unscheduled necropsy on GD146 due to accidental cage injury; therefore, it was not included in the calculation of overall percent fetal loss or third trimester loss. Calculation was based on 17 pregnancies that were allowed to go to final outcome for the control group.

(Applicant)

**Methods**

**Frequency of dosing:** Subcutaneous injections from Gestational Day (GD) 20 to GD22 (based on the day of pregnancy confirmation by ultrasound), on GD35, and then once every 2 weeks thereafter through gestation until parturition (for a maximum of 11 total doses)

**Route of administration:** Subcutaneous injection

**Formulation/Vehicle:**

(b) (4)

**Species/Strain:** Cynomolgus monkeys

**Study design:** Enhanced pre- and postnatal development study

**Observations:** Animals were observed for general health/mortality and moribundity at least once daily in the afternoon. Cage-side observations were performed once daily. Detailed clinical observations for adult females were conducted on all animals through at least GD60 and then were discontinued. The infants were removed from the cage, and a detailed clinical observation was performed once-weekly, beginning on Birth Day (BD) 7 until BD180 ±2 (the day of necropsy) to help minimize experimental intervention in the pregnant animals.

**Body weights:** Females were measured on the day of study enrollment (GD20-22), GD25 and weekly thereafter until delivery and Post-Parturition Day (PPD) 1 and weekly thereafter. Infants were weighed weekly.

**Feed consumption:** Maternal feed was weighed once daily, and instances of low food consumption were noted. Infant feeding was primarily by

- nursing, and was not analyzed.
- Viability of offspring in utero:** Embryo/fetal viability was assessed by measuring the embryo/fetal heart rate.
- Abortion/stillbirth:** In the event of an abortion (tissue was observed) or stillbirth ( $\geq$ GD140), or if the adult female was determined no longer pregnant by ultrasound, dosing of the female was stopped immediately. Blood samples for evaluation of toxicokinetics, anti-drug antibodies, and cholesterol panel were obtained from the adult female. The adult female was then released from the study. Aborted fetuses prior to GD100 were examined externally. Tissues were not collected from aborted fetuses prior to GD100. For aborted fetuses (GD100-GD139) and stillborn fetuses (GD140 or older), tissues were preserved in 10% neutral-buffered formalin. Aborted fetuses (GD100-GD139) and stillborn fetuses (GD140 or older) underwent teratologic evaluation, morphometric measurement and skeletal radiography.
- Postpartum and placental observations:** All infants were observed for evidence of nursing behavior. The infant may have been placed in an incubator if nursing was not observed within several hours of the first observation of the infant. The placenta, if present, was collected and gross examination was conducted.
- Birth Day (BD) 1 evaluations:** Infant body weight, a physical examination, and heart and respiration rate were evaluated on BD1. Adult females were checked for the availability of milk during the infant physical exam.
- Infant external assessments:** BD7, BD28, BD91 and at necropsy
- Morphometric measurements:** On BD7, BD28, BD91 and at necropsy, crown-rump length, femur length (bilateral), foot length (bilateral), horizontal head circumference, biparietal diameter, occipitofrontal diameter, chest circumference and anogenital distance were measured.
- Skeletal evaluations:** Infant radiographs were collected on BD28 and evaluated for skeletal bone count and malformations.

- Neurobehavioral assessments:** On BD7 and BD14, righting, palmar grasp, clasp-grasp, visual following, prone progression, lip smack orientation, oral reflexes (rooting, sucking and snout reflex), eye reflexes (pupil constriction, nystagmus and glabellar tap), Moro reflex, negative geotaxis and buildup (increasing arousal levels in response to manipulation) were studied.
- Skeletal evaluations:** Infant radiographs were collected on BD28 and evaluated for malformations and variations.
- Scheduled euthanasia:** The terminal infant body weight was recorded. All infants underwent teratologic evaluations (external and visceral including detailed heart evaluations), morphometric measurements, and were subjected to a complete necropsy examination. Organs were weighed.
- Adult female clinical pathology sampling schedule:** See table, below.

## Adult Females Sample Collection

Gestation Day or Postpartum Day	Time Points (Relative to Dosing)	Samples Collected <sup>a</sup>
GD20-GD22	Predose	ADA, Chol Panel, mCG
GD27-GD29	168 hours postdose	TK, Chol Panel
GD63	Predose	TK, ADA, Chol Panel
GD105	Predose	TK, ADA, Chol Panel
GD133	Predose	TK, ADA, Chol Panel
GD134	24 hours postdose	TK
GD135	48 hours postdose	TK
GD137	96 hours postdose	TK
GD140	168 hours postdose	TK, ADA, Chol Panel
PPD14	Not applicable	TK, ADA, Chol Panel
PPD28	Not applicable	TK, ADA, Chol Panel
PPD91	Not applicable	TK, ADA, Chol Panel
PPD180 ± 2 days	Not applicable	TK, ADA, Chol Panel Also blood and serum for potential pathogen identification (see <a href="#">Section 4.5.7</a> )
Day of abortion/pregnancy loss confirmation (including stillbirth and any C-sections)	Not applicable	TK, ADA, Chol Panel
Cross fosters (Birth Mother and Cross-Foster Mother)	Not applicable	TK, ADA, Chol Panel at intervals described above
Unscheduled necropsy of adult female	Not applicable	TK, ADA, Hem, Chem

<sup>a</sup> ADA = anti-drug antibodies; Chem = clinical chemistry; Chol Panel = (total cholesterol, LDL Cholesterol, HDL Cholesterol, and triglycerides); Hem = hematology; mCG = monkey chorionic gonadotropin; TK = toxicokinetics

**Infant clinical pathology  
sampling schedule:**

Infants Sample Collection

Birth Day	Time Points	Samples Collected <sup>a</sup>
BD14	Not applicable	TK, ADA, Chol Panel
BD28	Not applicable	TK, ADA, Chol Panel
BD91	Not applicable	TK, ADA, Chol Panel
BD180 ± 2 days (all infants)	Pre-necropsy	TK, ADA, Chol Panel Also blood and serum for potential pathogen identification (see <a href="#">Section 4.5.7</a> )
As applicable	Day of unscheduled necropsy of infant (including maternal neglect or health reasons)	TK, ADA, Hem, Chem

<sup>a</sup> ADA = anti-drug antibodies; Chem = clinical chemistry; Chol Panel (total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides); Hem = hematology; TK = toxicokinetics.

**Clinical pathology:**

Hematology Parameters

Red blood cell count Hemoglobin concentration Hematocrit Mean corpuscular volume Mean corpuscular hemoglobin concentration Mean corpuscular hemoglobin Reticulocyte count (absolute) Red cell distribution width Platelet count	White blood cell count Neutrophil count Lymphocyte count Monocyte count Eosinophil count Basophil count Large unstained cells Other cells (as appropriate) Mean Platelet Volume
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Clinical Chemistry Parameters

Alanine aminotransferase Aspartate aminotransferase Alkaline phosphatase Gamma-glutamyltransferase Creatinine Kinase Total bilirubin Urea nitrogen Creatinine Calcium Triglycerides	Phosphorus Total protein Albumin Globulin Albumin/globulin ratio Glucose Sodium Potassium Chloride Cholesterol
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Cholesterol Panel

Cholesterol – Total HDL Cholesterol	LDL Cholesterol Triglycerides
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**Bone marrow smears:** Smears were collected, but not analyzed.

**Toxicokinetics and ADA analyses:** Samples were obtained as described above.

**Terminal procedures:** Infants were processed as shown, below.

Terminal Procedures

Group No./ Animals	No. of Animals	Scheduled Euthanasia Day	Procedures				Histology <sup>a</sup>	Histopathology
			External Eval.	Visceral Eval. & Necropsy	Tissue Coll. <sup>a</sup>	Organ Wts		
Aborted fetuses (prior to GD100)			X	-	-	-	-	-
Aborted fetuses (GD100 or later) and stillbirths			X	X	X	-	b	b
1 Infants	12	BD180 ± 2	X	X	X	X	X	c
2 Infants	11	BD180 ± 2	X	X	X	X	X	c
Unscheduled infant necropsy			X	X	X	-	X	X
Adult Female Procedures								
			Necropsy	Tissue Coll.	Organ Wts	Histology	Histopathology	
Unscheduled Adult Female Necropsy			X	X	-	X	X	

AF = adult females; X = procedure conducted; - = not applicable

<sup>a</sup> See [Tissue Collection and Preservation table](#) for listing of tissues

<sup>b</sup> Abnormalities (gross lesions); those evaluated by histopathology determined by Study Pathologist in consultation with Sponsor.

<sup>c</sup> No microscopic examination of infant tissues from the BD180 ± 2 necropsy was conducted.

Organs Weighed at Necropsy

Brain	Kidney <sup>a</sup>
Epididymis <sup>a</sup>	Liver
Gallbladder	Ovary <sup>a</sup>
Gland, adrenal <sup>a</sup>	Spleen
Gland, pituitary	Testis <sup>a</sup>
Gland, prostate	Thymus <sup>a</sup>
Gland, thyroid	Uterus

<sup>a</sup> Paired organ weight

Tissue Collection and Preservation

Animal identification	Large intestine, rectum
Artery, aorta	Liver
Bone marrow, femur	Lung
Bone marrow smear <sup>a</sup>	Lymph node, mandibular
Bone marrow, sternum	Lymph node, mesenteric
Bone, femur	Muscle, skeletal psoas and diaphragm
Bone, sternum	Nerve, optic <sup>b</sup>
Brain	Nerve, sciatic
Cervix	Ovary
Epididymis	Oviduct
Esophagus	Pancreas
Eye <sup>b</sup>	Sites of potential infection
Gallbladder	Skin
Gland, adrenal	Small intestine, duodenum
Gland, lacrimal	Small intestine, ileum
Gland, mammary	Small intestine, jejunum
Gland, parathyroid	Spinal cord
Gland, pituitary	Spleen
Gland, prostate	Stomach
Gland, salivary mandibular	Testis <sup>c</sup>
Gland, seminal vesicle	Thymus
Gland, thyroid	Tongue
Gross lesions/masses	Trachea
Gut-associated lymphoid tissue	Ureter
Heart	Urinary bladder
Kidney	Uterus
Large intestine, cecum	Vagina
Large intestine, colon	

<sup>a</sup> Allowed to air dry, not fixed in formalin

<sup>b</sup> Preserved in Davidson's fixative

<sup>c</sup> Preserved in Modified Davidson's fixative

**Deviation from study protocol:** Effective 22 Jun 2012 (Protocol Amendment 7), the test article was thawed in the refrigerator overnight prior to dose preparation. Based on Applicant's request to perform this procedural change as soon as possible, refrigerated thawing was initiated on 15 Jun 2012, prior to the amendment being

finalized.

### Dosage Justification

Up to 300 mg/kg/week evolocumab was administered to monkeys for 6 months without significant toxicity. A once every two week dose of 50 mg/kg for the current study is consistent with the high dose selection criteria articulated in ICH-S6 and S6(R1), as it provides a >10-fold greater exposure over the maximum clinical dose (i.e., 15-fold) and the dose elicits a maximum pharmacological effect and saturated PCSK9 binding, albeit slightly delayed by comparison (based on previous studies conducted with evolocumab in the cynomolgus monkey at 30 mg/kg dosed once-weekly), as shown below.

Justification of dosage – pharmacodynamics in female monkeys			
Subchronic study evolocumab dose group* (mg/kg/week)	LDL-C (mg/dL)		
	Day 1 pre-dose	Day 8	Day 43
0	53	54	57
3	46	16	17
30	47	7	11
300	53	9	10
Current study evolocumab dose group (mg/kg/2Weeks)	GD20 pre-dose	GD27 (Day+7)	GD63 (Day +43)
50	55	19	9

\*Data from study #110149

## Results

### F<sub>0</sub> Adult Female Monkeys

#### Survival

One control group female (SSAN 1501) was injured in the cage and both the adult female and fetus were necropsied. One control group female (SSAN 1514) partially delivered prematurely on GD131 with the infant in the breech position. Both the adult female and infant were necropsied.

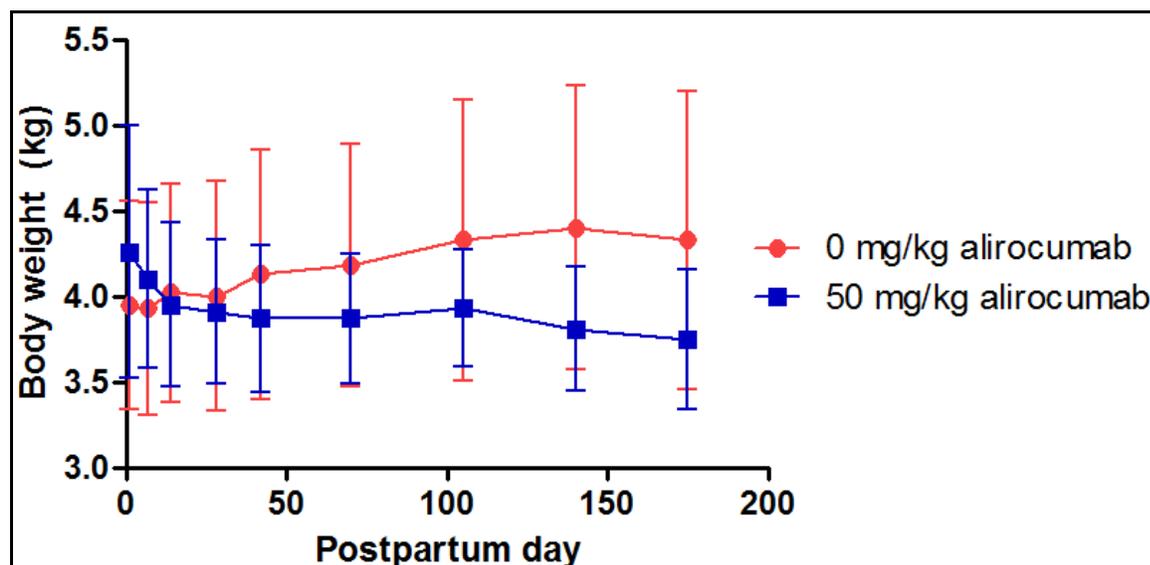
#### Clinical signs

There were no remarkable differences between clinical signs observed between controls and dosed female monkeys during the gestational or postpartum phases.

#### Body weight

There were no remarkable effects of evolocumab on body weights of female monkeys during the gestational phase. During the postpartum phase, females administered 50 mg/kg evolocumab once every other week lost 0.51 kg compared to control females that gained 0.38 kg during the same period (234% body weight decrement; 13% absolute body weight decrease) from PPD1/2 to PPD175. Changes were only statistically

significantly different at one time point, which indicates the effects may not be drug-related; values equal the mean body weight  $\pm$  SD.



(Reviewer)

### Feed consumption

Qualitative food consumption data were unremarkable during both the gestational and postpartum phases.

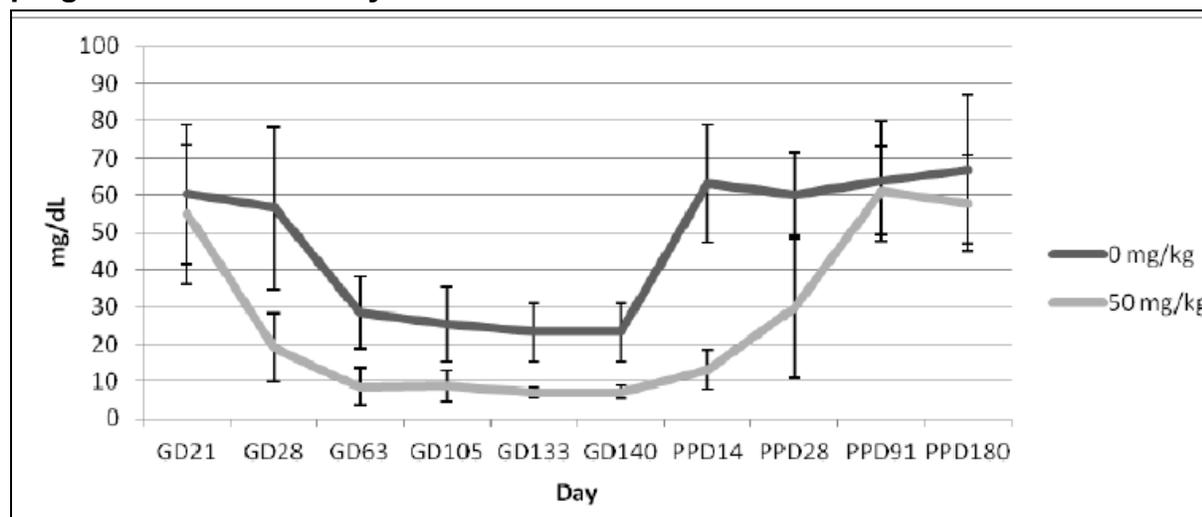
### Clinical pathology

Hematology, coagulation and clinical chemistry analyses were only performed for unscheduled necropsies. The only (two) unscheduled necropsies were in the control group.

### Pharmacodynamics

Mean LDL-C in pregnant female monkeys administered 50 mg/kg evolocumab every 2 weeks were below 10 mg/dL (approximately 80% decrease from baseline, see Figure 30). However, female monkeys administered the vehicle also experienced a steep decline in LDL-C during pregnancy (approximately 60% decrease from baseline), which is attributed to increased cholesterol demand during the period of fetal gestation. LDL-C returned to baseline in both groups with cessation of dosing coinciding with parturition. LDL-C remained below controls in female monkeys administered 50 mg/kg evolocumab, which is due to the prolonged half-life of elimination of monoclonal antibody drugs.

**Figure 30: Mean pharmacodynamic effect (LDL-C) of evolocumab administered to pregnant female monkeys**



(Applicant)

### Necropsy observation

Two adult females in the control group were euthanized (forearm injury and partial deliver of breech fetus). No adult females in the treatment group that survived to the end of the study were euthanized or necropsied.

### Toxicokinetics

Toxicokinetic data confirmed adequate drug exposures, which were consistent with data from other studies (see Table 34).

**Table 34: Summary toxicokinetics for evolocumab in an enhanced pre/postnatal developmental assessment in monkeys**

		GD133				
Sex	Dose (mg/kg), Subcutaneous	N	t <sub>max</sub> (day)	C <sub>max</sub> (µg/mL)	AUC <sub>168</sub> (µg·day/mL)	AUC <sub>336</sub> (µg·day/mL)
Female	50	12	3.5 (1.0-4.0)	598 (136)	3500 (789)	5600 (1220)

AUC<sub>168</sub>= area under the concentration-time curve from time zero to the time 168 hours postdose; AUC<sub>336</sub>= derived area under the concentration-time curve from time zero to the time 336 hours postdose using the predose concentration values as the 336 hour concentration assuming steady-state was achieved; C<sub>max</sub> = maximum observed drug concentration during a dosing interval; N = number of animals; t<sub>max</sub> = time to reach C<sub>max</sub>, reported as a median (range)

(Applicant)

### Anti-drug antibody (ADA) analyses

One control female and three evolocumab-treated females tested positive for anti-drug antibodies (see Table 35), using a validated method. It is considered unlikely that the control group female's positive responses represent an actual positive response, since the antibodies were detected in the initial blood draw (at start of dosing). The three evolocumab dose group positives may represent detection of ADA, because results were obtained after multiple doses of evolocumab (SSAN 2506, GD63; SSAN 2511, PPDs 91 and 180; SSAN 2515, PPDs 18, 28, 91 and 180). One dose-group female

(SSAN 2511) was positive for neutralizing ADA (PPDs 91 and 180). Overall, the effects of ADAs are not considered to have affected the reliability of this study.

**Table 35: Summary rates of anti-drug antibody positivity in female monkeys administered evolocumab during pregnancy**

Group (Dose)	Binding Antibodies <sup>a</sup>	Neutralizing Antibodies <sup>a</sup>
1 control group (adults)	6% (1/18)	0% (0/18)
1 control group (infants)	0% (0/12)	0% (0/12)
2 50 mg/kg (adults)	17% (3/18)	6% (1/18)
2 50 mg/kg (infants)	0% (0/11)	0% (0/11)

<sup>a</sup> Results are expressed as percentage and incidence. Incidence is the number positive/number evaluated. (Applicant)

### Dosing Solution Analysis

The test and control articles were not analyzed at the testing facility. All materials were utilized as provided by the Applicant. Certificates of stability were provided by the Applicant. The certificates provided by the Applicant indicate evolocumab is stable for up to (b) (4) months at (b) (4) °C or below, and for up to (b) (4) hours at (b) (4) °C. Control article is certified as stable for up to (b) (4) years at (b) (4) °C.

### F1 Generation

#### Pregnancy outcome

Abortions (≤GD100): Four of 18 fetuses (22%) were aborted in the 50 mg/kg evolocumab group, versus none in controls (see Table 36). Two fetuses (2/18; 11%) were aborted between GD20 and GD50, which is a slightly higher incidence than observed in the same laboratory for the same species of monkey (mean of 8.1%), but was within the historical control range (6.7 to 39%). Two fetuses were aborted between GD51 and GD99 (11%), which is slightly outside of the historical range (0 to 10%). The rates of third trimester abortion were within the historical range (0 to 28.6%) for control females (3/17, 17.6%) and for treatment group females (1/18, 5.6%).

**Table 36: Pregnancy outcome (percent and timing of fetal loss) in pregnant monkeys administered evolocumab during pregnancy**

Group (mg/kg)	No. Pregnant Enrolled	Gestation Day of Fetal Loss (Adult Female No. /Fetus No.)	Overall Percent Loss	1st TM GD20-50	2nd TM GD51-99	3rd TM ≥GD100
1 (0)	18 <sup>a</sup>	GD111 (1502/1021) GD151 (1518/1181) GD159 (1503/1031)	17.6% (3/17)	0% (0/18)	0% (0/18)	17.6% (3/17)
2 (50)	18	GD32 (2502/U) GD32 (2509/U) GD60 (2507/U) GD79 (2512/U) GD112 (2513/2131)	27.8% (5/18)	11.1% (2/18)	11.1% (2/18)	5.6% (1/18)
Historical Control Data <sup>b</sup>			23.9% (47/197)	8.1% (16/197)	1.4% (3/217)	14.3% (31/217)
Range			6.7 to 38.9%	0 to 15.0%	0 to 10.0%	0-28.6%

GD = gestation day; U = unable to determine; TM = trimester

<sup>a</sup> Adult Female No. 1501 underwent unscheduled necropsy on GD146 due to accidental cage injury on GD144, therefore was not included in the calculation of overall percent fetal loss or third trimester loss. Calculation was based on 17 pregnancies that were allowed to go to final outcome for the control group.

<sup>b</sup> Based on 12 ePPND studies conducted at the Testing Facility from 2008 to 2012.

(Applicant)

Aborted fetuses (<GD100) from adult females SSAN 2502, SSAN 2509 and SSAN 2507 were detected by ultrasound; no tissues were expelled (see Table 37). The aborted fetus (<GD100) from adult female SSAN 2512 was detected upon expulsion of tissues.

**Table 37: Aborted fetuses in female monkeys administered evolocumab during pregnancy from GD20 to GD100**

Group No.	Dose Level (mg/kg)	Fetus No.	Adult Female No.	Gestation Day of Abortion
2	50	N/A	2502	32
			2509	32
			2507	60
			2512	79

(Reviewer)

**Abortions at ≥GD100:** The abortion of the control group female SSAN 1021 was detected upon tissue expulsion. The aborted fetus of female SSAN 2131 was significantly autolyzed, indicating it had been dead in utero for some time; the fetus had been viable on GD102 (by ultrasound). There was a full thickness abdominal defect, and part of the small intestines and all of the cecum were not present (considered by the pathologist to be due to autolysis and post mortem condition, and was not considered an abnormal fetus). The placenta was likely the source of the fetal loss, because there was a large maternal retroplacental hematoma that was likely responsible for the placental disruption. Retroplacental hematoma may be an incidental finding in placentae from uncomplicated pregnancies of rhesus macaques in the third trimester, which has been reported at a 4.8% incidence (1 incidence out of 21

pregnancies)<sup>31</sup>, but in that case it involved “only a minor portion of one disc”. In this case, the placental disruption was significant enough to cause fetal death. Therefore, a relationship to drug is considered possible. Adult Female No. 2513 had cholesterol and LDL-cholesterol values during gestation consistent with the group average and with other females in the group that carried to full term.

**Table 38: Aborted fetuses in female monkeys administered evolocumab during pregnancy from GD100 to GD140**

Group No.	Dose Level (mg/kg)	Fetus No.	Fetus to Adult Female No.	Gestation Day of Abortion
1	0	1021	1502	111
2	50	2131	2513	112

(Applicant)

Stillbirths: Control female SSAN 1031 whose infant was stillborn had increased BUN and CK and decreased albumin. For the stillborn fetus meconium was still present in the colon, and the lungs were not inflated. There were large hematomas along the calvarium suture lines, consistent with delayed passage in the birth canal (dystocia). Otherwise the infant was grossly normal. The mother had consumed part of the placenta, but the remaining portion and the umbilicus were within normal range of appearance. Tissues from the stillborn control fetus from female SSAN 1181 were expelled.

**Table 39: Stillborn fetuses in female monkeys administered evolocumab during pregnancy from G 140 to parturition**

Group No.	Dose Level (mg/kg)	Fetus No.	Fetus to Adult Female No.	Gestation Day of Stillbirth
1	0	1031	1503	159
		1181	1518	151

(Applicant)

Two infants from the control group (2/14) and two from the 50 mg/kg every other week evolocumab treatment group (2/13) were born dead (see Table 40). Both incidences were close to the historical incidence of 11.2% and within the historical range of 0% to 20%.

The infant (male SSAN 1101) from control female SSAN 1510 was born prematurely at GD131 with a birth weight of 227.6 g, well below the group average of 338.6 g for control male infants. The infant was robust at birth, had a good grasp response, and no abnormalities were present at BD1 evaluation. Milk could be expressed from both nipples of the mother. The mother was reluctant to care for its infant after the BD1 evaluation. The infant was not observed nursing, became weak/moribund and had to undergo unscheduled necropsy. Hematology and clinical chemistry parameters were unremarkable in blood collected from the infant at necropsy.

For the second control female (SSAN 1514), the male offspring's (SSAN 1141) tail was found protruding from the mother's vagina, suggesting a possible breech birth. A decision was reached for unscheduled necropsy of the adult female and its offspring.

<sup>31</sup> Bunton TE “Incidental Lesions in Nonhuman Primate Placentae” *Vet Pathol* 1986; **23**:431-438.

Maternal blood samples for the TK-ADA-Cholesterol Panel were collected consistent with pregnancy loss; observations were unremarkable. At necropsy, the offspring was determined to be alive; both the adult female and the infant were euthanized. The partially-delivered infant was in the normal in utero presentation (not breech) but with tail curled around the body and passing out beyond the head, through the cervix and vaginal canal and continuing to the exterior. Placental and infant evaluations (including x-ray for skeletal evaluation) were performed per protocol. External and visceral evaluations of the infant were normal. Placenta and umbilical cord were within normal limits. Maternal and infant tissues were evaluated by histopathology; microscopic observations were incidental.

The male infant (SSAN 2011) born to treatment group female SSAN 2501 was delivered on GD152. It was held in normal position by its mother and observed to be nursing, and BD1 evaluation of the infant was normal. On BD7 the neurobehavioral evaluation of the infant was normal. The infant had lost 9.5 g from BD1 (attributed to normal transitory postpartum body weight decline) and appeared slightly dehydrated. The mother expressed milk, and the infant nursed normally. A veterinary check of the infant was requested for precaution; normal monitoring of mother/infant pair was deemed adequate to bridge to PPD/BD14. On BD11 the infant was found in weakened and inactive condition, and was separated from its mother for evaluation. The mother had adequate milk (expressed from both nipples), but infant was not thriving for unknown reason(s). It was dehydrated, with low body temp of 87.6F, and body weight loss of approximately 33 g (12%) from BD7 (272 g) to BD11 (240 g). The infant was warmed on a Bair Hugger heated pad and/or in an infant incubator, was given Lactated Ringers subcutaneous to rehydrate, and was given numerous small amounts of Nutri-Cal PO over several hours. It became only slightly more responsive, was not able to hold its head up, and had a weak grasp response. The infant did not regain sufficient strength to be given PO Enfamil for nutrition. Unscheduled necropsy was performed on BD11. Blood samples collected prior to necropsy were analyzed for toxicokinetic, hematology and cholesterol panel. Toxicokinetic, total cholesterol and LDL-C values were consistent with other infants in the group. Similarly, toxicokinetic, total cholesterol and LDL-C values in the adult female were similar to other females administered 50 mg/kg evolocumab through GD140. No abnormalities were detected at necropsy upon external or skeletal examination of the infant. Infant white blood cell and neutrophil counts were elevated, consistent with histopathology observations of intestinal inflammation consistent with bacterial infection associated with neonatal weakness. Similar observations have been reported as a cause of mortality in untreated neonatal monkeys<sup>32</sup>.

The female infant (SSAN 2186) born to treatment group female SSAN 2518 was found moribund after premature delivery (GD130). The infant was necropsied. The placenta and umbilicus were within normal limits. There was approximately 2 mL of blood in the thoracic cavity and multiple blood clots were present within the pericardium. The lungs had extensive hemorrhages, with the entire left lobes affected and approximately half of the right lobes affected. There were smaller hemorrhages in the liver adjacent to the diaphragm. It appeared that the infant had severe chest trauma, the organs were

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<sup>32</sup> Anver MR, et al. "Simian neonatology: II. Neonatal pathology" *Vet Pathol* 1973; **10**(1):16-36.

otherwise within normal limits. The chest trauma was of unknown origin; it could have occurred postpartum, but no signs of maternal neglect were evident the morning of BD1. Microscopic observations in the lungs and heart were perimortem and were attributed to intracardiac euthanasia.

**Table 40: Infant Losses for female monkeys administered evolocumab during pregnancy**

Group No.	Dose Level (mg/kg)	Total No. Infants	Infant No./ Adult Female No.	Birth Day of Loss	Comment <sup>a</sup>
1	0	14	1510/1101	BD1	Premature (GD131) birth; maternal neglect; UNEC of infant on BD1
			1514/1141	BD1	Premature (GD131) partially delivered infant; UNEC of adult female and infant
2	50	13	2501/2011	BD11	UNEC of infant (born at GD152)
			2518/2186	BD1	Premature (GD130) birth; infant moribund and UNEC

UNEC = unscheduled necropsy

<sup>a</sup> Deliveries that occurred prior to GD140 were considered premature and those which occurred prior to GD159 ± 7 (based on historical control data for gestation length for 12 ePPND studies conducted at the Testing Facility from 2008 to 2012) were considered preterm.

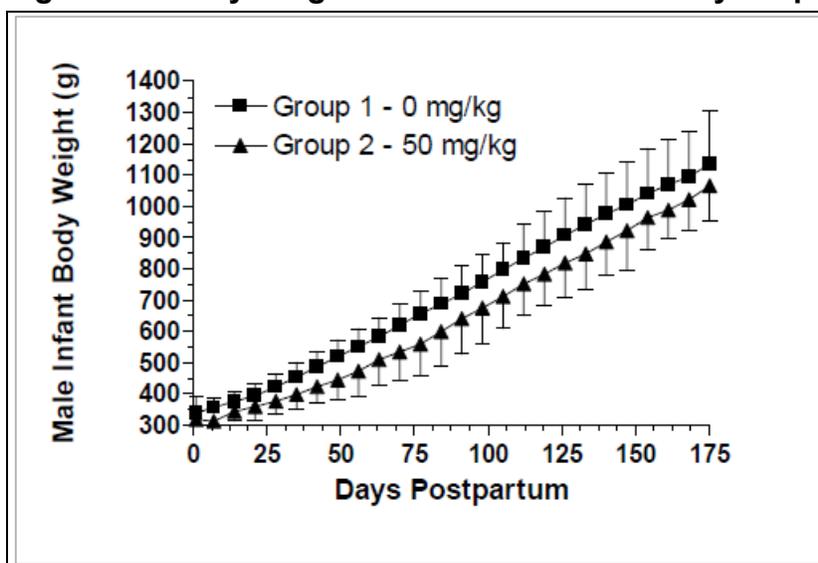
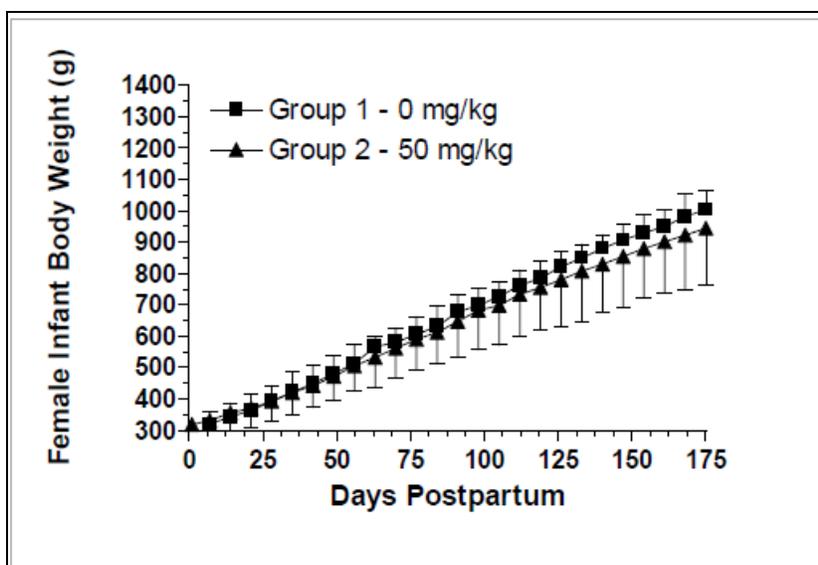
(Applicant)

### Clinical signs

Abrasions, bruises and other signs of minor injury were relatively common in both the control and the treatment group of offspring. There was no observable difference in incidence between the groups.

### Body weights

Infants born to female monkeys administered 50 mg/kg evolocumab by the subcutaneous route once every other week during the gestational period weighed on average 10.5% less than infants born to control females (n.s.s.) (see Figure 31 and Figure 32). Body weight gains were statistically significantly lower ( $p < 0.05$ ) in offspring of evolocumab treated females on BD21-28, BD35-42 and BD98-105, and were generally lower than control offspring at all other time points (n.s.s.).

**Figure 31: Body weights for infant male monkeys exposed to evolocumab in utero****Figure 32: Body weights for infant female monkeys exposed to evolocumab in utero**

(Applicant)

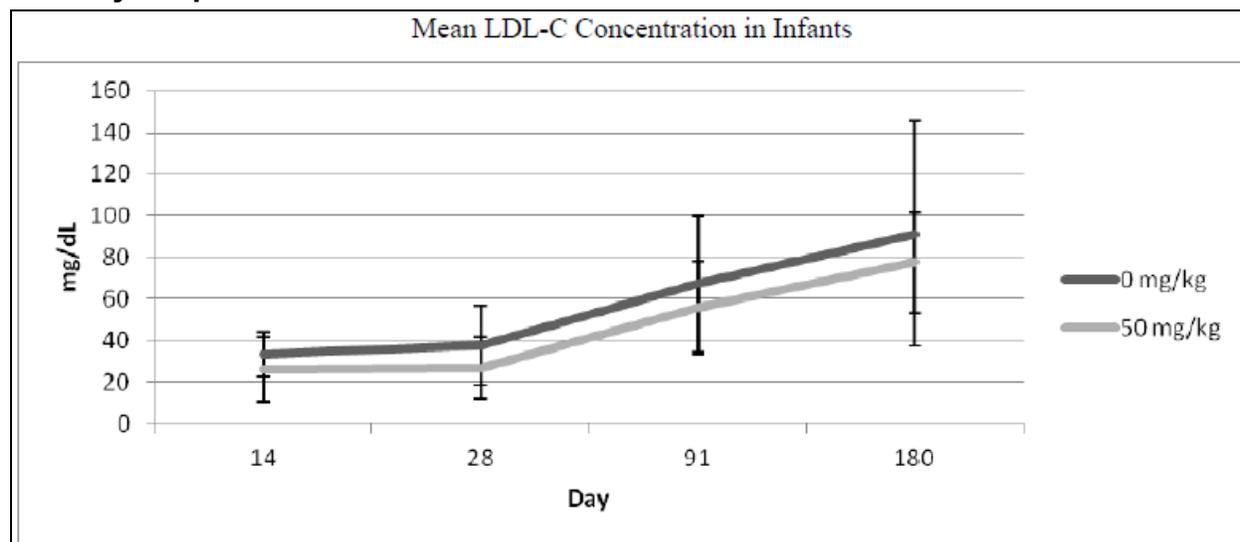
**Feed consumption**

Not assessed in infants, since the primary source of infant nutrition was breastfeeding.

**Clinical pathology**

Only cholesterol, LDL-C, HDL-C and triglycerides were assessed. Other clinical pathology parameters were not assessed in infants. Total cholesterol was reduced by 8 to 17% and LDL-C was reduced by 15 to 29% from the first measurement after birth (DB14) to DB180. HDL-C and triglycerides were unaffected by exposure in utero (see Figure 33).

**Figure 33: Mean pharmacodynamic effect (LDL-C) of evolocumab for infant monkeys exposed to evolocumab in utero**



(Applicant)

### Physical development

Morphometric measurements at BD180 in offspring of adult females administered 50 mg/kg evolocumab by the subcutaneous route once every other week during the gestational phase showed reductions in chest circumference, foot length, head circumference ( $p < 0.05$ ) and other parameters (n.s.s.) compared to measurements in offspring of control females. The Applicant cited fewer gestational days for evolocumab dose group infants versus control infants as a potential confounder. Indeed, birth weights were very similar between dose groups at birth, but diverted slightly (n.s.s.) thereafter in both sexes of infants, with dose group infant body weights trending lower than those of control infants. However, the mean gestational days of delivery for control and evolocumab dose groups were 154.3 and 159.4, respectively, which contradicts the argument that the number of days of gestation adversely impacted dose group infants. However, this reviewer noted a numerical sex imbalance between control infants (71% male: 29% female) and dose group (31% male: 69% female) infants. Sexual dimorphism likely accounts for several of the differences noted. The differences in chest circumference (-6%,  $p < 0.05$ ) and horizontal head circumference (-7%,  $p < 0.05$ ) were the only statistically significant differences between dose group and control animals after accounting for sexual dimorphism. While statistically significant, the magnitudes of these differences are low and considered of limited biological relevance. In the absence of other findings, 6 to 7 percent reductions in chest and head circumferences are not considered adverse.

<b>Morphological assessments – infant monkeys (Percent difference compared to control infants at BD180)</b>	
<b>Parameter</b>	<b>Δ length (%)</b>
Crown-rump length	-4
Chest circumference	-6*
Left femur length	-3
Right femur length	-3
Left foot length	-5*
Right foot length	-5*
Biparietal diameter	-1
Occipitofrontal diameter	-2
Horizontal head circumference	-7*
Anogenital distance♂	+4
Anogenital distance♀	-11

\* indicates  $p < 0.05$

### **Neonatal behavior assessments**

Unremarkable

### **Skeletal evaluation**

Unremarkable

### **Infant toxicokinetics**

Evolocumab was not detected in control females or their infants. Infants born to females administered 50 mg/kg evolocumab every other week showed plasma concentrations roughly equivalent to maternal drug levels. The rate of evolocumab clearance from infant plasma appeared to be reduced compared to that observed in maternal animals.

Appendix Table 9.2.4. Individual Infant and Maternal Serum AMG 145 Concentration–Time Data (ng/mL) After Biweekly Subcutaneous Administration of 50 mg/kg Group 2

Adult Female Animal Number	Maternal Serum				Infant Animal Number	Infant Serum			
	PPD14	PPD28	PPD91	PPD180±2		BD14	BD28	BD91	BD180±2
2503-MF33569F	19200	1460	<400	<400	2031-MF91076M	66900	40300	448	<400
2504-MF20784F	146000	38400	<400	<400	2046-MF91078F	171000	77000	440	<400
2505-MF46397F	46400	1820	<400	<400	2051-MF91080M	113000	66100	894	<400
2506-MF27745F	14800	572	<400	<400	2066-MF91082F	70300	11900	<400	<400
2508-MF43522F	22900	1100	<400	<400	2086-MF91084F	162000	89200	795	<400
2510-MF28782F	63100	26500	<400	<400	2106-MF91088F	119000	64000	428	<400
2511-MF45733F	18100	<400	<400	<400	2116-MF91089F	113000	61100	505	<400
2514-MF20756F	2790	<400	<400	<400	2146-MF91095F	13600	2150	<400	<400
2515-MF20759F	1860	<400	<400	<400	2156-MF91097F	9660	1580	<400	<400
2516-MF43527F	99300	31900	<400	<400	2166-MF91099F	165000	76700	1100	<400
2517-MF15701F	24200	918	<400	<400	2171-MF91102M	55400	7850	<400	<400
N	11	11	11	11	N	11	11	11	11
Mean	41700	9330	0	0	Mean	96300	45300	419	0
Median	22900	1100	0	0	Median	113000	61100	440	0
SD	45000	15000	0	0	SD	57700	33600	393	0
CV%	108	160	NA	NA	CV%	59.9	74.2	93.7	NA

## 10 Special Toxicology Studies

### Cross-reactivity study of Alexa Fluor 488-[ Evolocumab] AMG 145 with normal human and cynomolgus monkey tissues

Study no.: 110148 (b) (4)

Study report location: SDN1, SN000 (eCTD)

Conducting laboratory and location: (b) (4)

Date of study initiation: 5 November 2008

GLP compliance: Yes

QA statement: Yes

Drug, lot # and % purity: Drug: Evolocumab-Alexa 488, lot #89238-53, >95% (SDS-PAGE)

Control:  $\alpha$ KLH-IgG2-Alexa 488, lot #95740-40, >98% (SDS-PAGE)

#### Key study findings

- Human and cynomolgus tissue panels gave generally comparable patterns of staining with Alexa Fluor 488-labeled evolocumab.
- Hepatocytes, which are known to express high levels of PCSK9, the target of evolocumab, were negative for staining in both species in this assay. The reasons for this are unknown.
- Unexpectedly, there were relatively high levels of staining of myocytes (surface and cytoplasm) and spindle cells (cytoplasmic) from many tissues.

## Methods

**Doses:** 5 µg/mL and 20 µg/mL, Rationale, excerpted from the study report, appears below:

### 8.3.1. Method Development and Rationale for Concentration Selection

The methods were based on the qualification staining run results from (b) (4) Amgen Study No. 110147. These methods were validated in a qualification staining run for this study conducted on 12-Nov-2008. In the qualification staining run, AMG 145-Alexa 488 stained the positive control material (cryosections of PCSK9 beads) at all concentrations examined with a noticeable reduction in staining at concentrations below 5 µg/mL. Therefore, the ideal concentration of test article determined for the study (i.e., the greatest dilution of test article before staining of the positive control material was noticeably decreased) was 5 µg/mL. There was no specific staining of the positive control PCSK9 beads with the control article (αKLH-IgG2-Alexa 488) and no staining of the negative control material (cryosections of HSA beads) with the test article or control article. The higher concentration of test article was selected as 20 µg/mL as it was the greatest concentration examined during the preliminary staining runs that yielded specific staining of positive control material and human and cynomolgus monkey tissue sections with minimal nonspecific staining of the selected tissue sections. Thus, the staining concentrations for the test article and control article selected for the study were 5 µg/mL and 20 µg/mL.

**Formulation/Vehicle:** Alexa 488-Evolocumab in 2.96 mg/mL phosphate buffered saline

**Study design:** Sections of frozen normal human and cynomolgus monkey tissues were assessed for their capacity to specifically bind evolocumab, mAb directed against PCSK9. An isotype matched control antibody to KLH was used to assess specificity of evolocumab binding. Cryosections of PCSK9 beads (lot #T08XX3134-A) were used as a positive control for evolocumab binding under the conditions of the tissue cross-reactivity assay, and Cryosections of HSA (human serum albumin) beads (lot #T08XX3134-B) was used as a negative control. The following tissues were screened from at least three separate donors:

#### 8.2.2.2. Normal Human and Cynomolgus Tissues from at Least Three Separate Donors

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

<sup>a</sup> Blood cells included granulocytes, lymphocytes, monocytes and platelets.

<sup>b</sup> Examined in all tissues.

<sup>c</sup> Both longitudinal and cross-sections of peripheral nerve were evaluated in samples submitted from human and cynomolgus monkey donors.

<sup>d</sup> Gastrointestinal Tract included the colon (large intestine), esophagus, small intestine, and stomach.

Exceptions: only 2 donors evaluated for human placenta, only 2 donors evaluated for cynomolgus mammary gland, and no donors evaluated for cynomolgus parathyroid.

**Deviation from study protocol:**

No significant deviations were reported.

## Results

Alexa 488-Evolocumab labeling of the positive control (cryosections of PCSK9 beads) was variable (weak-strong) at both concentrations examined, but was deemed sufficient to perform the assay. For the negative control material (cryosections of HSA beads), weak staining was observed at the higher concentration of Alexa 488-Evolocumab. The control article,  $\alpha$ KLH-IgG2-Alexa 488 equivocally stained both positive and negative control materials at the higher concentration examined. There was no staining of either positive or negative control materials with the lower concentration of  $\alpha$ KLH-IgG2-Alexa 488. These results indicate that the assay was adequate.

Alexa 488-Evolocumab staining was observed in the human tissue panel examined as summarized below (incidence of binding is 3/3 donors, unless otherwise specified):

- Surface and extracellular granules in the following tissue elements:
  - striated skeletal muscle
  - smooth muscle myocytes in skin (arrector pili)
  - cardiomyocytes in heart
- Cytoplasm, cytoplasmic granules, cytoplasmic filaments and/or cellular processes (spindle cells only) in the following tissue elements:
  - endothelium in adrenal, cerebellum, cerebrum, mammary gland, eye, colon, esophagus, small intestine, heart, lung, parathyroid, peripheral nerve, spinal cord, testis, thyroid, urinary bladder and cervix
  - spindle cells in adrenal, mammary gland, colon, esophagus, small intestine, stomach, heart, kidney, liver, lung, lymph node, ovary, fallopian tube, pancreas, peripheral nerve, pituitary, placenta, prostate, salivary gland, spinal cord, spleen, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder and cervix
  - smooth myocytes in colon, esophagus, small intestine, stomach, fallopian tube, pancreas, prostate, skin, ureter, urinary bladder, uterus and cervix
  - decidual cells in placenta (1/3 donors)
  - ganglion cells of myenteric plexus in colon and stomach (1/3 donors)
  - adrenal cortical cells (1/3 donors)

Alexa 488-Evolocumab staining was observed in the cynomolgus monkey tissue panel examined as summarized below:

- Surface and/or extracellular granules in the following tissue elements:
  - skeletal muscle fibers
  - smooth myocytes in eye (iris) and skin (arrector pili)
  - cardiomyocytes
- Granules in adenohypophysis in pituitary
- Cytoplasm, cytoplasmic granules, cytoplasmic filaments and/or cellular processes (spindle cells only) in the following tissue elements:

- endothelium in adrenal, cerebellum, cerebrum, eye, esophagus, stomach, heart, liver, peripheral nerve, salivary gland, skin, testis, ureter and cervix
- spindle cells in adrenal, cerebellum, cerebrum, mammary gland, eye, colon, esophagus, small intestine, stomach, heart, kidney, liver, lung, lymph node, ovary, fallopian tube, pancreas, peripheral nerve, placenta, prostate, salivary gland, skin, spinal cord, spleen, striated muscle, testis, thymus, thyroid, tonsil, ureter, urinary bladder, uterus and cervix
- ganglion cells of myenteric plexus in colon, esophagus, small intestine, stomach, heart, spinal cord and cervix
- fibroblastic reticular cells in colon (submucosal lymphoid nodule), lymph node adjacent to small intestine and stomach, spleen, thymus and tonsil
- smooth muscle myocytes in eye, colon, esophagus, small intestine, stomach, fallopian tube, lung, lymph node, prostate, salivary gland, skin, thymus, ureter, urinary bladder, uterus and cervix
- epithelium in kidney (tubular, 1/3 donors)
- decidual cells in placenta (1/3 donors)
- mesothelium in spleen (1/3 donors)

Overall, the staining patterns (cell types stained, subcellular localization, and staining frequency and intensity) were similar between the human and cynomolgus monkey tissue panels examined. Staining of tissue elements observed in the human tissue panel that was not observed in the cynomolgus monkey tissue panel included adrenal cortical cells (1/3 donors). Staining of tissue elements observed in the cynomolgus monkey tissue panel that was absent in the human tissue panel included tubular epithelium in kidney, granules in adenohypophysis in pituitary, fibroblastic reticular cells in lymphoid tissues and mesothelium in spleen. The reasons for the observed differences in staining between the human and cynomolgus monkey tissues were unclear but might include differences in test article binding affinity to the reactive epitope in cynomolgus monkey tissues, differences in donor age and/or disease status and/or other unidentified differences.

It is notable that hepatocytes, the only cell type in which PCSK9 expression was explicitly expected, were negative for Alexa 488-Evolocumab staining. The reasons for the discrepancy between the expected tissue distribution and localization and the staining observed in this study are unclear but might include PCSK9 expression in these tissues being below the level of detection of the staining assay used or lack of availability of the epitope.

### Tissue cross-reactivity of Alexa Fluor 488-[Evolocumab] AMG 145 with Golden Syrian hamster tissues in vitro

**Study no.:** 112876 (b) (4)  
**Study report location:** SDN1, SN000 (eCTD)  
**Conducting laboratory and location:** (b) (4)  
**Date of study initiation:** 10 November 2009  
**GLP compliance:** Yes  
**QA statement:** Yes  
**Drug, lot # and % purity:** Drug: Alexa 488-Evolocumab, lot #89238-53, >95% (SDS-PAGE)  
 Control:  $\alpha$ KLH-IgG2-Alexa 488, lot #95740-40, (SDS-PAGE)

#### Key study findings

- The staining pattern observed with Alexa Fluor 488-labeled evolocumab in the panel of hamster tissues was generally comparable to that previously seen with this agent in human and cynomolgus tissue panels (study #110148). However, staining of mononuclear cells, Purkinje cells, neurons and islets cells was observed only in the Golden Syrian hamster tissue panel, and not in the human and cynomolgus monkey tissue panels.
- Hepatocytes, which are known to express high levels of PCSK9, the target of evolocumab, were negative for staining in this assay. The reasons for this are unknown, but it is notable that hepatocyte staining was also absent in the previously reviewed human and cynomolgus tissue cross-reactivity assay.

#### Methods

**Doses:** 5  $\mu$ g/mL and 20  $\mu$ g/mL, Rationale, excerpted from the study report, appears below:

##### 8.3.1. Method Development and Rationale for Concentration Selection

The methods were based on the qualification staining run results from (b) (4) (b) (4) Amgen Study No. 110147. These methods were validated in a qualification staining run for this study conducted on 12-Nov-2008. In the qualification staining run, AMG 145-Alexa 488 stained the positive control material (cryosections of PCSK9 beads) at all concentrations examined with a noticeable reduction in staining at concentrations below 5  $\mu$ g/mL. Therefore, the ideal concentration of test article determined for the study (i.e., the greatest dilution of test article before staining of the positive control material was noticeably decreased) was 5  $\mu$ g/mL. There was no specific staining of the positive control PCSK9 beads with the control article ( $\alpha$ KLH-IgG2-Alexa 488) and no staining of the negative control material (cryosections of HSA beads) with the test article or control article. The higher concentration of test article was selected as 20  $\mu$ g/mL as it was the greatest concentration examined during the preliminary staining runs that yielded specific staining of positive control material and human and cynomolgus monkey tissue sections with minimal nonspecific staining of the selected tissue sections. Thus, the staining concentrations for the test article and control article selected for the study were 5  $\mu$ g/mL and 20  $\mu$ g/mL.

**Formulation/Vehicle:** Evolocumab (b) (4)  
 Alexa 488-Evolocumab in 2.96 mg/mL phosphate buffered

saline

**Study design:** Study design:

Sections of frozen normal hamster tissues were assessed for their capacity to specifically bind evolocumab, mAb directed against PCSK9. An isotype matched control antibody to KLH was used to assess specificity of evolocumab binding. Cryosections of PCSK9 beads (lot #T08XX3134-A) were used as a positive control for evolocumab binding under the conditions of the tissue cross-reactivity assay, and Cryosections of HSA (human serum albumin) beads (lot #T08XX3134-B) was used as a negative control. The following tissues were screened from at least three unique animals:

**8.2.2.2. Normal Golden Syrian Hamster Tissue from at Least Three Unique Animals**

Adrenal	Liver	Skin
Blood Cells <sup>1</sup>	Lung	Spinal Cord
Blood vessels (endothelium) <sup>2</sup>	Lymph Node	Spleen
Bone Marrow	Ovary	Striated (Skeletal) Muscle
Brain – cerebrum (cortex)	Fallopian Tube (oviduct)	Testis
Brain – cerebellum	Pancreas	Thymus
Breast (mammary gland)	Parathyroid	Thyroid
Eye	Peripheral Nerve <sup>4</sup>	Ureter
Gastrointestinal Tract <sup>3</sup>	Pituitary	Urinary Bladder
Heart	Prostate	Uterus – body (endometrium)
Kidney (glomerulus, tubule)	Salivary Gland	Uterus – cervix

<sup>1</sup> Blood was evaluated by examination of blood within the vessels and recorded only if there was staining.

<sup>2</sup> Endothelium was evaluated in all tissues and recorded only if there was staining.

<sup>3</sup> Gastrointestinal Tract was evaluated in a sample of colon (large intestine), esophagus, small intestine, and stomach.

<sup>4</sup> Both longitudinal and cross-sections of peripheral nerve were evaluated.

**Deviation from study protocol:** No significant deviations were reported.

**Results**

In the Golden Syrian hamster tissue panel examined, Alexa 488-Evolocumab staining was observed as summarized below (incidence of binding is 3/3 donors, unless otherwise specified):

- Surface and extracellular granules in the following tissue elements:
  - striated myocytes of extra-ocular muscles of the eye, esophagus, skin and striated skeletal muscle
  - cardiomyocytes in heart
  - smooth muscle myocytes in skin (arrector pili, 2/3 donors)
- Cytoplasm, cytoplasmic granules and/or cytoplasmic filaments in the following tissue elements:
  - endothelium in bladder (2/3 donors), breast – mammary gland, cerebellum, cerebral cortex, colon (2/3 donors), fallopian tube (1/3 donors), small intestine (1/3 donors), kidney, lung (2/3 donors), pancreas, parathyroid (1/1 donor), peripheral nerve (2/3 donors), salivary gland (1/3 donors), skin, spinal cord,

- testis, thymus (1/3 donors), thyroid, ureter (1/3 donors) and uterus (cervix, 2/3 donors and endometrium, 2/3 donors)
- spindle cells in adrenal (2/3 donors), cerebellum, cerebral cortex, colon (2/3 donors), small intestine (2/3 donors), stomach, kidney, liver, lung, lymph node (1/3 donors), ovary (1/3 donors), peripheral nerve, salivary gland, skin (2/3 donors), spinal cord, spleen (1/3 donors), testis, thymus (2/3 donors), thyroid (1/3 donors), ureter (1/3 donors) and uterus (cervix and endometrium)
  - smooth myocytes (vascular and/or intrinsic) in adrenal, bladder, bone marrow (1/3 donors), cerebellum, cerebral cortex, colon, eye (2/3 donors), fallopian tube, esophagus, small intestine, stomach, kidney, liver (2/3 donors), lung, lymph node (2/3 donors), ovary (2/2 donors), pancreas, parathyroid (1/1 donor), peripheral nerve, pituitary (1/3 donors), prostate, salivary gland, spinal cord, spleen, striated muscle (1/3 donors), testis, thymus (1/3 donors), thyroid (2/3 donors), ureter and uterus (cervix and endometrium)
  - epithelium in breast – mammary gland (glandular, 1/3 donors), cerebral cortex (choroid plexus, 2/3 donors) and spinal cord (ependymal cells, 2/3 donors)
  - mononuclear cells in salivary gland (1/3 donors)
  - Purkinje cells in cerebellum (2/3 donors)
  - neurons in spinal cord (1/3 donors)
  - islet cells in pancreas (2/3 donors)
  - fibroblastic reticular cells in lymph node (1/3 donors), ovary (1/2 donors), spleen and thymus

## 11 Integrated Summary and Safety Evaluation

### Indications Under Consideration for Approval:

The Applicant is seeking approval of evolocumab (Repatha®) for the treatment of adults with primary hyperlipidemia (mixed dyslipidemia or heterozygous familial hypercholesterolemia) and adults and patients  $\geq 12$  years with homozygous familial hypercholesterolemia.

### Mechanism of Action:

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a negative regulator of the LDL-receptor (LDLR). When PCSK9 binds to cell surface LDLR, the complex is internalized and undergoes lysosomal degradation. Evolocumab is a fully human IgG2 monoclonal antibody that binds to PCSK9, and promotes complex internalization and lysosomal degradation, and thereby removes functional PCSK9 from circulation. Inactivation of PCSK9 with evolocumab results in increased LDLR cell-surface expression and increased uptake of low-density lipoprotein cholesterol (LDL-C), especially by the liver, with consequent lowering of circulating levels of LDL-C.

### Pharmacology and Pharmacodynamics:

Expression of hepatic LDLR and its function in removing LDL-C from circulation is dependent on two known factors: intracellular cholesterol levels and serum PCSK9 concentrations. Therefore, any modulators that affect expression of PCSK9 will affect liver LDLR density and its capacity to remove LDL-C from circulation. Transcription of

*PCSK9* and *LDLR* genes share a common regulatory mechanism mediated by sterol regulatory element binding protein 2 (SREBP2). SREBP2 interacts with another membrane protein SREBP-cleavage-activating protein (SCAP), which functions as a sterol sensor. Intracellular sterols inhibit *LDLR* and *PCSK9* gene transcription by suppressing the processing and release of SREBP2. In the presence of sterols, inactive SREBP2 remains bound to the endoplasmic reticulum after synthesis.<sup>33</sup>

In the sterol-depleted state, SCAP escorts the SREBP2 to the Golgi where it is proteolytically cleaved, releasing the mature SREBP2 capable of transcriptional activation. Mature SREBP2 enters the nucleus and binds to the sterol regulatory element 1 (SRE-1) site of *LDLR* and *PCSK9* promoters, leading to increased transcription and translation of both proteins. Statins lower intracellular cholesterol by decreasing de novo biosynthesis through inhibition of HMG-CoA reductase, thereby upregulating both the expression of *LDLR* and its negative regulator *PCSK9*.<sup>33</sup> Evolocumab overcomes this compensatory effect of statins.

Evidence of the intended pharmacology included marked dose-related reductions in total cholesterol and LDL-C in hamsters and monkeys, and reductions in high-density lipoprotein cholesterol (HDL-C) primarily in hamsters. Reductions in HDL-C observed in the hamster are consistent with observations that hamsters express ApoE, which is capable of binding *LDLR*, on a substantial fraction of HDL particles when LDL-C is low.<sup>34,35</sup> HDL containing ApoE in humans is negligible<sup>36</sup>, which is consistent with clinical trial data showing a lack of an effect of evolocumab on HDL-C levels. HDL particles are a heterogeneous population postulated to have a variety of functions outside of lipid transport, including anti-inflammatory, anti-oxidative, cytoprotective, antithrombotic, anti-infectious, and vasodilatory activities.<sup>37</sup> These data indicate that the monkey may be the more pharmacologically relevant model for humans, because human and monkey HDL-C levels are largely resistant to evolocumab, while hamster HDL-C was substantially reduced.

#### Differences in Tested and To-Be-Marketed Formulations:

The vehicle for the toxicology and clinical phase 1/2/3 studies was composed of (b) (4) [redacted]. The “pivotal” phase 3 clinical formulation contained 220 mM (b) (4) [redacted] proline, 20 mM acetate, 0.01% (w/v) polysorbate 80 (pH 5.0). L-Proline (115.13 g/mol) is the stabilizer of the active ingredient in Privigen (BLA 125201) at a concentration of 250 mmol/L in the 50 mL product. Privigen contains the warning, “Privigen is contraindicated in patients with hyperprolinemia because it contains the stabilizer L-proline”. For primary

<sup>33</sup> Dong B, et al. “CETP inhibitors downregulate hepatic LDL receptor and PCSK9 expression in vitro and in vivo through a SREBP2 dependent mechanism” *Atherosclerosis* 2014; **235**:449-462.

<sup>34</sup> Goulinet S and Champan MJ “Plasma lipoproteins in the Golden Syrian hamster (*Mesocricetus auratus*): heterogeneity of apoB- and apoA-I-containing particles” *J Lipid Res* 1993; **34**:943-959.

<sup>35</sup> Evans GF, et al. “Inhibition of cholesteryl ester transfer protein in normocholesterolemic and hypercholesterolemic hamsters: effects on HDL subspecies, quantity, and apolipoprotein distribution” *J Lipid Res* 1994; **35**:1634-1645.

<sup>36</sup> Weisgraber KH and Mahley RW “Subfraction of human high density lipoproteins by heparin-Sepharose affinity chromatography” *J Lipid Res* 1980; **21**:316-325.

<sup>37</sup> Camont L, et al. “Biological activities of HDL subpopulations and their relevance to cardiovascular disease” *Trends Mol Med* 2011; **17**(10):594-603.

immunodeficiency, Privigen is dosed at 200 to 800 mg/kg (2 to 8 mL/kg) every 3 to 4 weeks (480 mL total volume). Thus, the amount of <sup>(b)</sup><sub>(4)</sub>proline (~50.7 mg/month) in evolocumab is covered by the established safety of the L-proline-containing product Privigen (~13,800 mg L-proline/month). No other excipients of concern were identified.

#### Toxicological Assessment of Evolocumab:

There are three areas of particular toxicological concern for the evaluation of evolocumab in animals: 1) direct induction of toxicity due to PCSK9-inhibition and/or reductions of plasma cholesterol that were previously unattainable with existing pharmaceuticals, 2) immunogenicity upon administration of a human immunoglobulin to hamsters and monkeys that could cause production of neutralizing anti-drug antibodies (ADA) that might prevent adequate drug exposures in animals, and 3) other largely theoretical concerns for extremely low plasma cholesterol. Section I, below, describes the outcomes and implications of general toxicity studies conducted with evolocumab, which are then qualified based on the results of immunogenicity evaluations in the test species. Section II describes the theoretical mechanisms by which extreme cholesterol lowering might be secondarily detrimental, along with this reviewer's assessment of these hypothetical risks.

#### Section I – Toxicity with evolocumab in hamsters and monkeys

Overall, evolocumab was very well tolerated in hamsters at up to 300 mg/kg/week, which represents 112, 48 and 20X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on plasma exposures (AUC). Evolocumab was also well tolerated in monkeys at up to 300 mg/kg/week, which represents 744, 300 and 134X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively.

Evolocumab was administered to hamsters at 300 mg/kg/week for 3 months. Significant decreases in mean total cholesterol, LDL-C and HDL-C (up to 49, 67 and 49%, respectively) occurred in male and female hamsters by Study Day 28. No remarkable toxicity occurred at up to 112, 48 and 20X the recommended human doses of 140 mg Q2W, 420 mg QW and 420 mg Q2W, respectively.

Evolocumab was administered in monkeys at up to 300 mg/kg/week for 6 months. The major observed effects of evolocumab were mean reductions in total cholesterol (up to 47%) and LDL-C (up to 83%). No significant effect on HDL-C was detected in the 6 month study. The site of injection emerged as a possible target tissue. Evolocumab was associated with minimal to slight acute/chronic inflammation and slight fibrosis at the test item injection site in the 6-month subcutaneous toxicity study. These findings were not considered to be toxicologically significant and were not unexpected for administration of a human IgG monoclonal antibody in non-human primates. No significant toxicity occurred at up to 744, 300 and 134X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively.

#### Combination with statins:

Evolocumab (up to 100 mg/kg; 50, 10 and 8.8X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on AUC) was coadministered with rosuvastatin (5 mg/kg/day; 2X the recommended human doses, based on AUC) to monkeys for 3 months. No additive or synergistic toxicity was

observed when 5 mg/kg/day rosuvastatin was combined with up to 100 mg/kg evolocumab administered every other week. There were no effects on any of the immunotoxicological assessments made (immunophenotyping, T cell dependent antibody response assay and natural killer cell activity) at the end of dosing. A limitation of the study was that there was no direct evidence that the dose of rosuvastatin used had any effect on either HMG-CoA reductase or LDL-C. However, the expected level of rosuvastatin exposure was detected in these monkeys.

#### Immune Modulation:

Monkeys administered evolocumab at up to 300 mg/kg/week for six months initially showed a statistically significant trend towards immune suppression in a T cell dependent antibody response (TDAR) assay, a test of adaptive immune system function ( $p < 0.05$ ). However, a number of animals were found to have pre-existing titers for anti-KLH antibodies prior to KLH exposure. The presence of pre-existing anti-KLH titers is not uncommon in cynomolgus macaques, and it has been postulated that these represent antibodies to a *Schistosoma mansoni* carbohydrate antigen that cross-reacts with KLH.<sup>38</sup> Since the interpretation of the TDAR relies on comparisons between groups of KLH-naïve animals, these animals were excluded from the TDAR analysis; the results were no longer statistically significant. Immunophenotyping and assessment of natural killer cell activity showed no statistically significant effects of evolocumab in that study. A second TDAR assay was carried out in monkeys administered up to 100 mg/kg evolocumab every other week with 5 mg/kg/day rosuvastatin. No effect on antibody response to KLH was observed in this study for either IgM or IgG. This NOAEL in the combination toxicity study with rosuvastatin provides a 50X safety margin for 140 mg Q2W, a 10X safety margin for the 420 mg QM dose, and an 8.8X safety margin for the 420 mg Q2W dose. Rosuvastatin exposures were approximately 2X higher than the maximum recommended human dose of 40 mg/day, based on plasma exposure (AUC). The high-dose in the 6 month study of 300 mg/kg/day, which was equivocally linked to the most significant impairment of TDAR, represents 134, 300 and 744X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W evolocumab. Overall, the weight of evidence suggests a limited possibility that evolocumab could affect the adaptive immune response at very high exposures.

#### Genetic toxicity:

Under ICH-S6, genotoxic evaluation of evolocumab, a monoclonal antibody with no reasonable expectation of interacting with DNA, is not recommended.

#### Carcinogenicity:

Theoretical concerns for how evolocumab may increase the risk of cancer include the potential for increased bile acid production due to increased flux of cholesterol into the liver, immune suppression due to inadequate supplies of cholesterol for clonal expansion and/or signaling of immune cells and increased risk of hepatitis C infection and or increased titer, owing to increased “viral receptor” and resultant infection and liver tumors. To address these concerns, the Applicant conducted a two-year (i.e.,

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<sup>38</sup> Grzych et al., (1987), “*Schistosoma mansoni* shares a protective carbohydrate epitope with keyhole limpet hemocyanin” *J. Exp. Med.* **165**(3): 865-878.

lifetime) carcinogenicity bioassay in hamsters. Evolocumab was administered once every other week at up to 100 mg/kg via the subcutaneous route. Substantial reductions in total cholesterol, LDL-C were maintained throughout the study. No drug-related tumors were observed at exposure multiples of up to 38, 15 and 6.6X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W.

#### Reproductive Risks:

A perceived concern for the chronic treatment of patients with evolocumab is the potential risk during pregnancy to the rapidly developing fetus. A fertility and early embryonic development study was conducted in hamsters administered evolocumab at doses up to 100 mg/kg administered every other week. These doses had no effect on mating, fertility, estrous cycling, male reproductive assessments (reproductive organ weights and sperm parameters), or embryo-fetal survival. Substantial reductions in total cholesterol, LDL-C, and HDL-C were observed during the study. The NOAEL for this assessment of fertility and early embryonic development in hamsters therefore provides a 30X safety margin to the 140 mg Q2W dose, a 12X safety margin to the 420 mg QM dose and a 5.3X safety margin to the 420 mg Q2W dose. Effects on fertility were also assessed in the 6 month chronic monkey toxicity study at doses of up to 300 mg/kg/week, which provides 744, 300 and 134X safety margins to the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on AUC. No effects on menstrual cycling in female monkeys or sperm counts and sperm morphology in male monkeys were observed.

The Applicant performed an enhanced pre/postnatal developmental toxicity study in pregnant monkeys with administration of evolocumab at up to 50 mg/kg every other week by the subcutaneous route throughout the periods of embryofetal and pre development (dosing was from day 20 of gestation to parturition). LDL-C was reduced by 15 to 29% compared to offspring of concurrent controls. No evolocumab-related fetal variations or malformations were observed at exposure multiples of 30, 12 and 5.2X the 140 mg Q2W, 420 mg QM and 420 mg Q2W doses, respectively. However, as HDL particles containing ApoE are a major class of lipoproteins in the plasma of human neonates and LDL-C is typically low in newborns (~40% of total cholesterol)<sup>39</sup>; the risk to neonates through in utero evolocumab exposure remains a concern.

#### Immunogenicity:

Maximal pharmacologic effects of evolocumab were maintained in the majority of test animals in the nonclinical program. Overall, rates of production of neutralizing ADA were negligible in hamsters and low in monkeys, and didn't compromise the toxicological assessment of evolocumab in any study.

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<sup>39</sup> Innerarity T L, et al. "Receptor binding activity of high density lipoproteins containing apoprotein E from abetalipoproteinemic and normal neonate plasma" *Metabolism* 1984; **33**: 186-195.

## Section II – Theoretical concerns for evolocumab exposure

The marked plasma LDL-C lowering attainable with clinical administration of evolocumab with or without a statin could be expected to secondarily impact other cholesterol-related processes, including bile acid formation, cholesterol-dependent hormone production, cholesterol-dependent tissue regeneration, and myriad others. These hypothetical concerns and the perceived human risks will be discussed below.

### Increased intestinal bile acids:

Bile acids are synthesized from cholesterol in the liver.<sup>40</sup> The presence of cholesterol up-regulates production of bile acids.<sup>41</sup> High levels of bile acids have been demonstrated in rodent models to promote tumor formation.<sup>42</sup> It is presumed that inhibition of PCSK9 would increase the flow of cholesterol into the liver. Therefore, the potential for increased concentrations of intestinal bile acids was identified as a possible concern for evolocumab therapy. However, no increase in colon tumors were observed in a lifetime hamster carcinogenicity study with evolocumab, which provided exposure multiples of up to 38, 15 and 6.6X the recommended human doses of 140 mg Q2W, 420 mg QW and 420 mg Q2W.

### Hepatitis C virus (HCV) infectivity:

A study by Labonte et al.<sup>43</sup> identified regulation of CD81 by PCSK9 as a potential pathway by which PCSK9 inhibitors might cause increased susceptibility to HCV infection and associated liver tumors. CD81 is a co-receptor for hepatitis C infection in humans. Labonte showed that expression of PCSK9, especially a modified non-secretable form, reduced CD81 and LDLR levels in immortalized human cells and provided resistance to HCV infection in vitro. The Applicant cited an abstract from the 2013 meeting of the American Heart Association, which describes a set of experiments that used a more physiologically relevant soluble form of PCSK9 with Huh-7 cells, in vitro. No regulation of CD81 or increased potential for HCV infectivity was detected in that model. CD81 levels were also evaluated in *PCSK9*<sup>-/-</sup> mice and in hyperlipidemic *PCSK9*<sup>hum/hum, LDLR+/-</sup> mice administered a similar PCSK9 inhibitor monoclonal antibody; no impacts on CD81 levels were reported.<sup>44</sup>

### Adrenal (cortical)-derived hormones:

The adrenal glands are heavily dependent on cholesterol for hormone production, and evolocumab might be expected to affect adrenal function. No effects on the adrenal glands were noted in hamsters administered evolocumab at up to 100 mg/kg/week for 28 days, which represents 112, 48 and 20X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on AUC. Evolocumab did not

<sup>40</sup> Russell DW “The enzymes, regulation, and genetics of bile acid synthesis.” *Annu Rev Biochem* 2003 **72**:137–174.

<sup>41</sup> Russell DW “Bile acid biosynthesis.” *Biochemistry* 1992; **31**(20):4737-4749.

<sup>42</sup> Weisburger JH, et al. “Bile acids, but not neutral sterols, are tumor promoters in the colon in man and in rodents.” *Env Health Perspect* 1983; **50**:101-107.

<sup>43</sup> Labonte, P et al. “PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression” *Hepatology* 2009; **50**(1):17-24.

<sup>44</sup> Ramanathan A, et al. “Role of alirocumab (proprotein convertase subtilisin/kexin type 9 antibody) on CD81 levels and hepatitis C virus entry into hepatocytes” *American Heart Association* 2013; Abstract 12052.

cause any adrenal effects in monkeys at up to 300 mg/kg/week for up to 6 months, which represents 744, 300 and 134X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively. Adrenal-derived hormones were not directly measured in any study.

#### Impaired liver regeneration:

A concern for liver injury and evolocumab treatment comes from a published study conducted with the PCSK9 knockout mouse<sup>45</sup>. When compared to littermates, *PCSK9-null* mice (but not *PCSK9<sup>+/-</sup>* mice) were markedly delayed in their ability to regenerate liver tissue following partial hepatectomy. Furthermore, the regenerating liver tissue exhibited necrotic foci. In these foci, the liver architecture was disrupted with swollen hepatocytes undergoing ballooning degeneration. Infiltration of red blood cells and leukocytes was also observed at the border of the necrotic areas. Whether this deficit is likely to be associated only with catastrophic liver injury (e.g., partial hepatectomy) or would also manifest following other liver injury (e.g., acetaminophen toxicity) is unknown. Of particular theoretical concern is the often transient, but sometimes severe, liver injury induced by statins, which could theoretically be exacerbated by pharmacologically-induced loss of PCSK9 analogous to the *PCSK9<sup>-/-</sup>* mouse phenotype. However, liver toxicity was not exacerbated in a 3 month combination toxicity study with evolocumab at doses that provided 50, 10 and 8.8X the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, when coadministered with rosuvastatin in monkeys. A limitation of the study was that rosuvastatin was not dosed at a level sufficient to cause any toxicity when administered alone, although the dose was high enough to provide a 2-fold safety margin to the 40 mg clinical dose of rosuvastatin. It is unknown whether recovery from more serious liver damage would be impacted by PCSK9 inhibitor therapies, including evolocumab. One possible explanation for the failure of liver to properly regenerate, owes to the discovery that HDL-C concentrations regulate bone marrow-derived endothelial progenitor cells<sup>46</sup>, including the precursors of liver sinusoidal cells. Upon significant liver damage, liver regeneration is dependent upon endothelial progenitor cells to migrate to the liver from the bone marrow, where they are responsible for directing repair of damaged hepatic blood vessels and tissues. This process is required for proper liver repair after partial hepatectomy.<sup>47</sup> It is tempting to speculate low HDL-C impaired the production and migration of progenitor cells to direct liver regeneration in *PCSK9<sup>-/-</sup>* mice. Hamsters administered evolocumab had low HDL-C in plasma, but no sinusoidal cell defects were observed in the liver. HDL-C has not been observed to be reduced in humans administered evolocumab.

#### Immune function perturbation in adult animals:

Inhibition of PCSK9 produces profound lowering of circulating cholesterol. The immune system is dependent on cholesterol for proper function. Clonal expansion of rapidly

<sup>45</sup> Zaid A, et al. "Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9): Hepatocyte-specific Low-Density Lipoprotein Receptor Degradation and Critical Role in Mouse Liver Regeneration" *Hepatology* 2008; **48**:646.

<sup>46</sup> Noor R, et al. "High-density lipoprotein cholesterol regulates endothelial progenitor cells by increasing eNOS and preventing apoptosis" *Atherosclerosis* 2007; **192**:92-99.

<sup>47</sup> DeLeve L "Liver sinusoidal endothelial cells and liver regeneration" *J Clin Invest* 2013 **123**:1861-1866.

dividing immune cells (e.g., B cells, T cells, etc.) and cell-cell signaling are heavily dependent on cholesterol and cholesterol derivatives<sup>48</sup>. No significant effects of evolocumab on immune function, including immunophenotyping and T cell dependent antibody response (TDAR), were observed in adult monkeys subcutaneously administered evolocumab at up to 300 mg/kg/week, which provides a 744, 300 and 134X safety margin for the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on AUC exposures. No effects of evolocumab on immune function, including immunophenotyping, TDAR and NK cell function, were observed in monkeys administered a combination of 100 mg/kg evolocumab subcutaneously administered every other week with 5 mg/day oral rosuvastatin, which provides an 50, 10 and 8.8X safety margin to the recommended human doses of 140 mg Q2W, 420 mg QM and 420 mg Q2W, respectively, based on AUC. The 5 mg/kg dose of rosuvastatin provides a 2X exposure multiple to the 40 mg QD maximum recommended human dose, based on AUC. It should be noted that rosuvastatin showed no evidence of pharmacodynamic activity in monkeys at this dose; the expected plasma AUC for rosuvastatin was observed, however.

#### Insulin sensitivity:

A potential signal for increased transition from pre-diabetes to type 2 diabetes mellitus has been identified in clinical studies with statins<sup>49</sup>. Decreased insulin sensitivity was observed in PCSK9 knockout mice compared to wild-type mice<sup>50</sup>. Compared to wild-type mice, PCSK9 knockout mice were hypoinsulinemic, hyperglycemic and glucose-intolerant. Islets of PCSK9 knockout mice exhibited signs of dysfunction. The authors hypothesized that the observed pancreatic islet cell inflammation and apoptosis could be the result of sterol accumulation in  $\beta$ -cells or a failure of  $\beta$ -cell replacement and renewal. However, no effects on plasma glucose or pancreas structure/function were observed in studies with healthy monkeys and hamsters administered high doses of evolocumab.

#### Neurocognitive assessments:

Adverse clinical neurocognitive events (e.g., transient confusion and memory loss) have been described, primarily through patient reporting, in adults on chronic statin therapy. Cholesterol and other sterols are important for nerve function in both the central and peripheral nervous system. The brain is a cholesterol-rich organ, which depends almost completely on de novo cholesterol biosynthesis for its sterols; peripheral blood lipids are unavailable to the CNS, due to blockade by the blood-brain-barrier. PCSK9 is highly expressed in brain tissues, although its function there is uncertain.<sup>51</sup> Evolocumab is a 150 kDa immunoglobulin, with very low access to the brain. Therefore, it is considered unlikely that evolocumab could directly affect the structure or function of the CNS, but

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<sup>48</sup> Norata GD, et al. "Emerging role of high density lipoproteins as a player in the immune system" *Atherosclerosis* 2012; **220**:11-21.

<sup>49</sup> Van de Woestigne AP, et al. "Effect of statin therapy on incident type 2 diabetes mellitus in patients with clinically manifest vascular disease" *Am J Cardiol* 2015; **115**(4): 441-446.

<sup>50</sup> Mbikay M, et al. "PCSK9-deficient mice exhibit impaired glucose tolerance and pancreatic islet abnormalities" *FEBS Letters* 2010; **584**:701-706.

<sup>51</sup> Liu M, et al. "PCSK9 is not involved in the degradation of LDL receptors and BACE1 in the adult mouse brain" *J Lipid Res* 2010; **51**:2611-2618.

this leaves the possibility of effects on peripheral neurons. No significant evolocumab-related effects on peripheral neurons were observed with evolocumab in toxicity studies in hamsters and in monkeys.

### Summary

#### Toxicokinetics and Determination of Safety Margins:

Evolocumab was administered to hamsters for three months and to monkeys for 6 months at up to 300 mg/kg/week. No dose-limiting test item-related toxicity was observed at any duration in hamsters or monkeys. The highest dose tested in repeat-dose toxicity studies for both species was 300 mg/kg/week, which are considered to be the NOAEL doses. Based on plasma exposures measured in toxicity studies in hamsters, the safety margins for the 140 mg Q2W, 420 mg QM, and 420 mg Q2W doses are 112, 48 and 20X, respectively. In monkeys, the safety margins are 744, 300, and 134X the recommended human doses of 140 mg Q2W, 420 mg QM, and 420 mg Q2W, respectively. Evolocumab was not carcinogenic in hamsters with lifetime dosing at up to 38, 15 and 6.6X the human exposures at the 140 mg Q2W, 420 mg QM and 420 mg Q2W doses. No effects on fertility and mating were observed in hamsters at up to 30, 12 and 5.3X the 140 mg Q2W, 420 mg QM and 420 mg Q2W doses, based on AUC. No effects on pre- or postnatal development were observed in monkeys at exposures up to 30, 12 and 5.2X greater than those in humans administered the 140 mg Q2W, 420 mg QM and 420 mg Q2W doses. Administration of evolocumab generally led to predictable and dose-proportional increases in exposure in animals. These data, combined with robust pharmacodynamic lowering of cholesterol, confirm adequate exposures throughout the toxicity studies in hamsters and monkeys. Safety margins are shown in Table 41, below.

**Table 41: Summary table of safety margins for nonclinical assessment of evolocumab**

Type of Study	Toxicity	Species	NOAEL & dosing frequency (duration)	Clinical Doses	Safety Margin Based on AUC <sup>†,‡</sup>
General Toxicity	None	Golden Syrian hamsters	300 mg/kg/week (3 month)	140 mg Q2W	112X
				420 mg QM	48X
				420 mg Q2W	20X
General Toxicity	None	Cynomolgus monkeys	300 mg/kg/week (6 months)	140 mg Q2W	744X
				420 mg QM	300X
				420 mg Q2W	134X
General toxicity in combination with rosuvastatin	No additive/synergistic toxicity	Cynomolgus monkeys	100 mg/kg/2week evolocumab 5 mg/kg/day rosuvastatin (3 months)	140 mg Q2W	50X
				420 mg QM	10X
				420 mg Q2W	8.8X
Carcinogenicity	No drug-related tumors	Golden Syrian hamsters	100 mg/kg/2week (lifetime)	140 mg Q2W	38X
				420 mg QM	15X
				420 mg Q2W	6.6X
Fertility and mating	None	Golden Syrian hamsters	100 mg/kg/2week (2-3 doses, females; 5 doses, males)	140 mg Q2W	30X
				420 mg QM	12X
				420 mg Q2W	5.3X
Enhanced pre/postnatal development	None	Cynomolgus monkeys	50 mg/kg/2week maternal (gestation day 20 to parturition); offspring (in utero exposure)	140 mg Q2W	30X
				420 mg QM	12X
				420 mg Q2W	5.2X

<sup>†</sup>AUC in human: Human AUC<sub>0-tau</sub> for evolocumab were determined following evolocumab SC 140 mg Q2W (387 day·µg/mL for 2 doses, or 194 day·µg/mL for one dose) or 420 mg QM (962 day·µg/mL) as monotherapy in primary hyperlipidemia or mixed dyslipidemia patients (Study 20101154). Human AUC<sub>Week8-10</sub> following evolocumab SC 420 mg Q2W (1,080 day·µg/mL) was determined in severe familial hypercholesterolemia patients (Study 20110271).

<sup>‡</sup>AUC in animals: Hamster AUC for evolocumab was determined following once-weekly subcutaneous administration for 3 months at 300 mg/kg/week (AUC<sub>last</sub> of 11,083 day·µg/mL). Monkey AUC for evolocumab was determined following once-weekly subcutaneous administration for 6 months at 300 mg/kg/week (AUC<sub>0-168</sub> of 72,083 day·µg/mL). Monkey AUC for evolocumab was determined following once every other week subcutaneous administration of 100 mg/kg for 3 months with coadministration of rosuvastatin (AUC<sub>0-tau</sub> of 9,541 day·µg/mL). Hamster AUC was determined during lifetime exposure to evolocumab dosing of 100 mg/kg once every other week via the subcutaneous route (AUC<sub>last</sub> of 7,167 day·µg/mL). Hamster AUC for fertility and early embryonic development was estimated following once-weekly subcutaneous administration for 3 months at 100 mg/kg/week (AUC<sub>last</sub> of 5,750 day·µg/mL). Monkey AUC was determined for pregnant females for enhanced pre-/postnatal development following once every other weekly subcutaneous administration of 50 mg/kg evolocumab during the period of embryo-fetal development to birth (AUC<sub>0-336</sub> of 5,600 day·µg/mL).

**OVERALL conclusions and recommendations**

Based on review of the totality of the available nonclinical data, Pharmacology/ Toxicology recommends that evolocumab be approved for the treatment of adults with primary hyperlipidemia (mixed dyslipidemia or heterozygous familial hypercholesterolemia) and adults and patients  $\geq 12$  years with homozygous familial hypercholesterolemia.

**12 Appendix/Attachments****Appendix 1: Executive Carcinogenicity Assessment Committee Minutes****Executive CAC****Date of Meeting: December 9, 2014**

**Committee:** Abby Jacobs, Ph.D., OND IO, Acting Chair  
Paul Brown, Ph.D., OND IO, Member  
Linda Fossom, Ph.D., DPP, Alternate Member  
Karen Davis-Bruno, Ph.D., DMEP, Pharm Tox Supervisor  
C. Lee Elmore, Ph.D., DMEP, Presenting Reviewer

**Author of Minutes:** C. Lee Elmore, Ph.D., DMEP

The following information reflects a brief summary of the Committee discussion and its recommendations.

**BLA #125522****Drug Name:** Repatha (Evolocumab, AMG-145)**Sponsor:** Amgen, Inc.**Background**

Evolocumab is a PCSK9 inhibitor monoclonal antibody being developed by Amgen, Inc. for the chronic treatment of hypercholesterolemia. No genetic toxicology studies have been conducted. The Executive CAC provided concurrence on dose selection for the hamster carcinogenicity study protocol (minutes dated 1 September 2011).

**Hamster Carcinogenicity Study**

Golden Syrian hamsters (60/sex/group) were administered evolocumab subcutaneously at 0, 10, 30, and 100 mg/kg once every two weeks in (b) (4)

Doses were selected based on achievement of a maximal pharmacologic effect in the hamster that is at least comparable to the anticipated systemic clinical exposure.

The applicant terminated the entire female study prematurely at Week 86 with concurrence from CDER's Executive CAC, based on excess mortality in the control group. The male study was terminated as scheduled at Week 105.

There was no significant effect of evolocumab on male or female Golden Syrian hamster

mortality or body weight. The pharmacodynamic effect of evolocumab was maintained throughout the study, which indicates exposure was durable for the duration of the study. No drug-related tumors were observed in a lifetime carcinogenicity study with evolocumab at up to 100 mg/kg administered once every two weeks.

### **Executive CAC Recommendations and Conclusions**

Hamster:

- The Committee concurred that the study was acceptable, noting prior Exec CAC concurrence with the protocol.
- The Committee concurred that there were no drug-related neoplasms in the study.

Abby Jacobs, Ph.D.  
Acting Chair, Executive CAC

**Appendix 2: Tumor Rates in a Two-year Hamster Carcinogenicity Bioassay**

**Table 3A: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Male Hamster**

Organ Name	Tumor Name	0 mg	10 mg	30 mg	100 mg	P_Value	P_Value	P_Value	P_Value
		Veh C N=60	Low N=60	Med N=60	High N=60	Dose Resp	L vs Veh C	M vs Veh C	H vs Veh C
#####									
Adrenal, Cortex	B-Adenoma, cortical cell	12	12	7	10	0.6251	0.5930	0.8220	0.5511
	B-Adenoma, subcapsular ce	22	18	20	23	0.2525	0.7571	0.5000	0.5000
	M-Carcinoma, cortical cel	0	2	2	1	0.4342	0.2581	0.2418	0.5000
	M-Carcinoma, subcapsular	0	0	6	7	0.5002	0.4102	0.5797	0.5000
Adrenal, Medull	B-Pheochromocytoma	0	2	0	1	0.4344	0.2581	.	0.5000
	M-Malignant pheochromocyt	1	0	1	0	0.6216	0.5106	0.7473	0.5000
Body, Whole/Cav	B-Hemangioma	1	5	4	0	0.9329	0.1118	0.1737	0.5000
	M-Hemangiosarcoma	3	3	0	4	0.2472	0.3487	0.8709	0.4878
	M-Histiocytic Sarcoma	0	0	0	1	0.2486	.	.	0.5000
	M-Lymphosarcoma	6	3	5	1	0.9506	0.7570	0.4695	0.9377
	M-Mast cell tumor	1	0	1	0	0.6216	0.5106	0.7473	0.5000
	M-Plasma cell tumor	3	0	1	0	0.9251	0.8868	0.6834	0.8791
Cecum	M-Carcinoma	0	0	1	0	0.2486	.	0.4045	.
Colon	M-Leiomyosarcoma	0	0	0	1	0.2486	.	.	0.5000
Foot	M-Fibrosarcoma	0	1	0	0	0.4919	0.5106	.	.
Harderian Gland	B-Adenoma	0	1	0	2	0.1106	0.5106	.	0.2527
Liver	B-Adenoma, hepatocellular	4	5	3	6	0.2353	0.5135	0.4754	0.3560
	M-Carcinoma, hepatocellul	1	0	1	1	0.3045	0.5106	0.7473	0.7527
Mesentery	B-Lipoma	0	0	0	1	0.2486	.	.	0.5000
Pancreas	B-Adenoma, islet cell	3	0	2	0	0.8040	0.8868	0.4895	0.8791
	M-Carcinoma, islet cell	0	0	1	0	0.2486	.	0.4045	.
Parathyroid	B-Adenoma	6	12	2	6	0.7243	0.1035	0.8520	0.6053
Penis	M-Carcinoma, squamous cel	0	1	0	0	0.4919	0.5106	.	.
Pituitary	B-Adenoma	1	1	0	1	0.5323	0.2581	0.4045	0.7527
Skin/Subcutis	B-Adenoma, sebaceous	1	0	0	0	0.7514	0.5106	0.4045	0.5000
	B-Basal cell tumor	0	1	0	0	0.4919	0.5106	.	.
	B-Melanoma	0	0	0	1	0.2486	.	.	0.5000
	B-Papilloma, squamous cel	0	0	0	1	0.2486	.	.	0.5000
	M-Fibrosarcoma	1	0	0	0	0.7514	0.5106	0.4045	0.5000
	M-Sarcoma, NOS	0	0	1	0	0.2473	.	0.5000	.
Stomach, Nongla	B-Papilloma, squamous cel	1	0	0	0	0.7514	0.5106	0.4045	0.5000
Subcutaneous In	B-Schwannoma	0	0	0	1	0.2486	.	.	0.5000
Thyroid	B-Adenoma, C-cell	3	3	4	4	0.3279	0.3592	0.5000	0.5000
	B-Adenoma, follicular cel	0	1	0	2	0.1073	0.5106	.	0.2473
	M-Carcinoma, C-cell	2	1	1	2	0	0.8471	0.5000	0.6750 0.7473

**Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Hamster**

Organ Name	Tumor Name	0 mg	10 mg	30 mg	100 mg	P_Value	P_Value	P_Value	P_Value
		Cont N=60	Low N=60	Med N=60	High N=60	Dose Resp	L vs Cont	M vs Cont	H vs Cont
#####									
Adrenal, Cortex	B-Adenoma, cortical cell	19	10	11	9	0.8852	0.9644	0.9220	0.9470
	B-Adenoma, subcapsular ce	12	18	15	11	0.7573	0.3021	0.4857	0.5000
	M-Carcinoma, cortical cel	0	0	1	0	0.2333	.	0.5333	.
	M-Carcinoma, subcapsular	3	0	2	2	0.4214	0.8908	0.5209	0.4782
Adrenal, Medull	B-Pheochromocytoma	3	3	0	0	0.9879	0.3951	0.8833	0.8663
	M-Malignant pheochromocyt	2	0	0	1	0.5198	0.7660	0.7556	0.4829
Body, Whole/Cav	B-Hemangioma	0	0	1	0	0.2360	.	0.5227	.
	M-Hemangiosarcoma	1	2	4	1	0.5875	0.5489	0.2305	0.7558
	M-Lymphosarcoma	2	2	4	0	0.8820	0.3369	0.3951	0.7326
	M-Plasma cell tumor	0	1	0	0	0.4944	0.5333	.	.
Cervix	B-Stromal tumor	0	1	0	0	0.4944	0.5333	.	.
	M-Adenocarcinoma	0	0	0	1	0.2444	.	.	0.5116
	M-Carcinoma	0	0	1	0	0.2333	.	0.5333	.
Harderian Gland	B-Adenoma	0	0	1	0	0.2360	.	0.5227	.
Jejunum	B-Polyp	0	0	1	0	0.2333	.	0.5333	.
Kidney	M-Nephroblastoma	0	1	0	0	0.4944	0.5333	.	.
Liver	B-Adenoma, hepatocellular	2	1	0	0	0.9397	0.5333	0.7667	0.7442
Lymph Node, Oth	M-Sarcoma, NOS	0	1	0	0	0.4889	0.5435	.	.
Mammary Gland,	B-Fibroadenoma	1	0	1	0	0.6183	0.5217	0.2667	0.4884
	M-Carcinoma	2	1	0	2	0.3341	0.5319	0.7556	0.6791
Mandibular Sali	M-Carcinoma	1	0	0	0	0.7556	0.5217	0.5111	0.4884
Ovary	B-Granulosa/theca cell tu	2	3	3	2	0.5299	0.5624	0.5426	0.6065
	B-Thecoma	0	0	1	0	0.2360	.	0.5227	.
	M-Granulosa/theca cell tu	0	0	0	1	0.2444	.	.	0.5116
	M-Leiomyosarcoma	0	0	1	0	0.2333	.	0.5333	.
Pancreas	B-Adenoma, islet cell	0	1	0	1	0.3159	0.5333	.	0.5116
	M-Carcinoma, acinar Cell	0	0	1	0	0.2333	.	0.5333	.
Parathyroid	B-Adenoma	6	8	8	7	0.4625	0.5111	0.4791	0.5345
Pituitary	B-Adenoma	6	10	8	4	0.8530	0.3286	0.4791	0.6093
	M-Carcinoma	0	0	1	1	0.1853	.	0.5333	0.5116
Skin/Subcutis	B-Adenoma, sebaceous	0	0	0	1	0.2444	.	.	0.5116
	B-Fibroma	0	1	0	0	0.4944	0.5333	.	.
	B-Lipoma	1	0	0	2	0.1439	0.5217	0.5111	0.5000
	B-Papilloma, squamous cel	0	0	0	1	0.2444	.	.	0.5116
	M-Liposarcoma	0	0	1	0	0.2333	.	0.5333	.

**Table 3B: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons  
Female Hamster**

Organ Name	Tumor Name	0 mg	10 mg	30 mg	100 mg	P_Value	P_Value	P_Value	P_Value
		Cont N=60	Low N=60	Med N=60	High N=60	Dose Resp	L vs Cont	M vs Cont	H vs Cont
#####									
Stomach, Nongla	B-Papilloma, squamous cel	0	1	0	0	0.4944	0.5333	.	.
Subcutaneous In	B-Lipoma	0	0	0	1	0.2444	.	.	0.5116
	M-Melanoma	0	0	0	1	0.2444	.	.	0.5116
Thyroid	B-Adenoma, C-cell	3	4	2	1	0.8699	0.5713	0.5209	0.6791
	B-Adenoma, follicular cel	2	2	3	3	0.3011	0.3546	0.5426	0.5218
	M-Carcinoma, C-cell	3	0	1	0	0.9248	0.8908	0.7121	0.8663
	M-Carcinoma, follicular c	3	0	1	0	0.9248	0.8908	0.7121	0.8663
Uterus	B-Adenoma	0	0	0	1	0.2444	.	.	0.5116
	B-Papilloma	0	0	0	1	0.2444	.	.	0.5116
	B-Polyp, endometrial stro	0	2	3	1	0.4728	0.2899	0.1515	0.5116
	M-Adenocarcinoma	0	0	0	1	0.2444	.	.	0.5116
	M-Carcinoma, endometrial	0	1	0	0	0.4944	0.5333	.	.
	M-Leiomyosarcoma	0	2	3	0	0.7335	0.2899	0.1426	.
	M-Sarcoma, endometrial st	1	0	0	0	0.7556	0.5217	0.5111	0.4884
Vagina	B-Papilloma, squamous cel	1	3	2	1	0.6358	0.3546	0.5333	0.7558
	M-Adenocarcinoma	0	0	1	1	0.1840	.	0.5227	0.5116

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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CALVIN L ELMORE  
05/15/2015

KAREN L DAVIS BRUNO  
05/15/2015

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

**NDA/BLA Number:** 125522     **Applicant:** Amgen

**Stamp Date:** 08/27/2014

**Drug Name:** Evolocumab     **NDA/BLA Type:** NME BLA

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

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		Yes, per ICH S6
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		Tox formulation – Phase 1/2/3: <span style="background-color: #cccccc; padding: 2px;">(b) (4)</span>  Clinical formulation – Phase 3: 220 mM proline, 20 mM acetate, 0.01% (w/v) polysorbate 80, pH 5.0  Note: Privagen (BLA 125201) for monthly intravenous injection contains 250 mM proline. Therefore, this matter is not a concern for filing.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement  
010908

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

	<b>Content Parameter</b>	<b>Yes</b>	<b>No</b>	<b>Comment</b>
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/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		serum plasma levels
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)			no issues identified
11	Has the applicant addressed any abuse potential issues in the submission?			n/a
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			n/a

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

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

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

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Reviewing Pharmacologist Date

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Team Leader/Supervisor Date

File name: 5\_Pharmacology\_Toxicology Filing Checklist for NDA\_BLA or Supplement 010908

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

Appears this way on original

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**This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.**  
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/s/  
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CALVIN L ELMORE  
10/09/2014

KAREN L DAVIS BRUNO  
10/09/2014