CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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NON-CLINICAL REVIEW(S)

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:	761235
Supporting document/s:	1
Applicant's letter date:	5-28-2021
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Product:	Vabysmo (faricimab)
Indication:	Treatment of patients with neovascular (wet) age-related macular degeneration (nAMD), diabetic macular edema (DME), and diabetic retinopathy (DR)
Applicant:	Genentech, Inc
Review Division:	Division of Ophthalmology
Reviewer:	María I Rivera, PhD, DPT-RPURM/SM
Supervisor/Team Leader:	Lori E Kotch, PhD, DABT, DPT-RPURM/SM
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Template Version: September 1, 2010

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1 Executive Summary

1.1 Introduction

Genentech is seeking approval of faricimab in the treatment of patients with neovascular (wet) age-related macular degeneration (nAMD), diabetic macular edema (DME), and diabetic retinopathy (DR).

Faricimab is a humanized bi-specific IgG1 monoclonal antibody directed against VEGF-A and Ang-2. The VEGF antigen binding fragment (Fab) is identical to ranibizumab (Lucentis®, BLA 125156, also Genentech). The Ang-2-binding Fab binds Ang-2 with high affinity. The Fc portion of the antibody has been altered so that it does not bind to Fc receptors.

Faricimab is proposed to have anti-angiogenic activity by neutralizing VEGF-A and Ang-2 by binding to the receptor binding domains of each protein and inhibiting binding to its receptors.

The proposed clinical dosing regimen is 6 mg administered by IVT injection every 4 weeks for the first 4 doses, followed by 6 mg at intervals of up to every 16 weeks.

1.2 Brief Discussion of Nonclinical Findings

The main adverse findings identified in the nonclinical studies were ocular inflammation and various findings in the heart in both rabbits and monkeys. The nonclinical data support the ocular findings are primarily related to development of an immunogenic response to faricimab and not a direct pro-inflammatory effect of faricimab. The animal data is insufficient to conclude whether the heart findings are a species-specific ADA response or a direct pro-inflammatory effect. However, together with the clinical data, the weight of data supports minimal clinical concern.

An embryofetal development (EFD) study in cynomolgus monkeys showed an increase incidence of abortions in faricimab treated monkeys at IV doses of 1 or 3 mg/kg administered weekly on gestation days 20 to 48. The increase was not dose dependent. No other maternal or fetal parameter showed a test article-related effect. Based on the increase incidence of the abortions, a test article related effect cannot be ruled out. Serum exposure (C_{max}) in pregnant monkeys at the low dose of 1 mg/kg IV was 158 times the human exposure at the intended dose of 6 mg/eye.

1.3 Recommendations

1.3.1 Approvability

Approval is recommended.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Applicant's proposed text	Reviewer's recommendations
INDICATIONS AND USAGE VABYSMO is a bispecific angiopoietin-2 (Ang-2) and vascular endothelial growth factor (VEGF) inhibitor indicated for the treatment of patients with:	No edits are recommended
8 USE IN SPECIFIC POPULATIONS **8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of VABYSMO administration in pregnant women. (b) (4) Based on the mechanism of action of VEGF and Ang-2 inhibitors, there is a potential risk to female reproductive capacity, and to embryo-fetal development. VABYSMO should not be used during pregnancy unless the potential benefit to the patient outweighs the potential risk to the fetus. All pregnancies have a background risk of birth defect, loss, and other adverse outcomes. The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects is 2%-4% and of miscarriage is 15%-20% of clinically recognized pregnancies.	**8 USE IN SPECIFIC POPULATIONS** **8.1 Pregnancy** Risk Summary There are no adequate and well-controlled studies of VABYSMO administration in pregnant women. Administration of VABYSMO to pregnant monkeys throughout the period of organogenesis resulted in an increased incidence of abortions at IV doses 158 times the human exposure (based on C_{max}) at the maximum recommended human dose [*see Animal Data*]. Based on the mechanism of action of VEGF and Ang-2 inhibitors, there is a potential risk to female reproductive capacity, and to embryo-fetal development. VABYSMO should not be used during pregnancy unless the potential benefit to the patient outweighs the potential risk to the fetus. All pregnancies have a background risk of birth defect, loss, and other adverse outcomes. The background risk of major birth defects and miscarriage for the indicated population is unknown. In the U.S. general population, the estimated background risk of major birth defects is 2%-4% and of miscarriage is 15%-20% of clinically recognized pregnancies.

Data Animal Data (b) (4)	Data Animal Data An embryofetal developmental toxicity study was performed on pregnant cynomolgus monkeys. Pregnant animals received 5 weekly IV injections of VABYSMO starting on day 20 of gestation at 1 or 3 mg/kg. A non-dose dependent increase in pregnancy loss (abortions) was observed at both doses evaluated. Serum exposure (C_{max}) in pregnant monkeys at the low dose of 1 mg/kg was 158 times the human exposure at the maximum recommended intravitreal dose of 6 mg once every 4 weeks. A no observed adverse effect level (NOAEL) was not identified in this study.
8.2 Lactation Risk Summary There is no information regarding the presence of faricimab in human milk, the effects of the drug on the breastfed infant, or the effects of the drug on milk production. (b) (4) many drugs are transferred in human milk with the potential for absorption and adverse reactions in the breastfed child, (b) (4). The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VABYSMO and any potential adverse effects on the breastfed child from VABYSM (b) (4)	**8.2 Lactation** No edits to first and last paragraphs are recommended (b) (4) many drugs are transferred in human milk with the potential for absorption and adverse reactions in the breastfed child, VABYSMO is not recommended during breast feeding.
8.3 Females and Males of Reproductive Potential	**8.3 Females and Males of Reproductive Potential**

Contraception Females of reproductive potential are advised to use effective contraception prior to the initial dose, during treatment and for at least 3 months following the last dose of VABYSMO. Infertility No studies on the effects of faricimab on human fertility have been conducted and it is not known whether faricimab can affect reproduction capacity. (b) (4) Based on the mechanism of action, treatment with VABYSMO may pose a risk to reproductive capacity.	Contraception No edits are recommended. Infertility No studies on the effects of faricimab on human fertility have been conducted and it is not known whether faricimab can affect reproduction capacity. (b) (4) Based on the mechanism of action, treatment with VABYSMO may pose a risk to reproductive capacity.
12 CLINICAL PHARMACOLOGY **12.1 Mechanism of Action** Faricimab is a humanized bispecific IgG1 antibody that acts through inhibition of two distinct pathways by neutralization of both Ang-2 and VEGF-A. (b) (4)	**12 CLINICAL PHARMACOLOGY** **12.1 Mechanism of Action** This reviewer recommends adding language on effects of VEGF-A. VEGF-A has been shown to cause neovascularization and leakage in models of ocular angiogenesis and vascular occlusion, and is thought to contribute to pathophysiology of neovascular AMD, DR, and DME.
13 NONCLINICAL TOXICOLOGY **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**	**13 NONCLINICAL TOXICOLOGY** **13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility**

No carcinogenicity or mutagenicity data are available for VABYSMO injection in animals or humans.	No studies have been conducted to determine the carcinogenic or mutagenic potential of VABYSMO.
(b) (4)	Based on the anti-VEGF mechanism of action of ranibizumab, treatment with VABYSMO may pose a risk to reproductive capacity *[see Females and Males of Reproductive Potential (8.3)].*

2 Drug Information

2.1 Drug

CAS Registry Number: 1607793-29-2

Generic Name: Faricimab

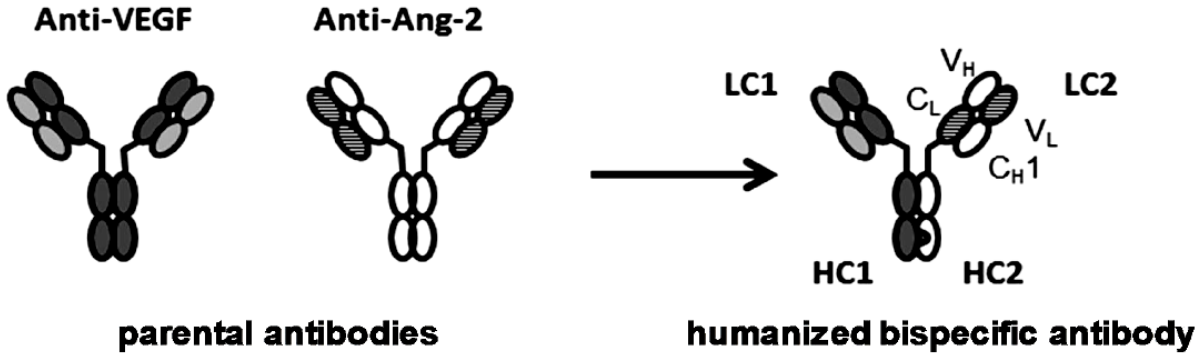
Code Name: RO6867461

Molecular Weight: The calculated molecular mass of intact faricimab is 146,157 Da (peptide chains only, heavy chains without C-terminal lysine residue; HC2 with N-terminal pyroglutamate).

Structure or Biochemical Description

Faricimab is a humanized bispecific antibody of the CrossMAb format (Figure 1) that selectively binds vascular endothelial growth factor A (VEGF-A) and angiopoetin-2 (Ang-2). One arm of faricimab binds to VEGF-A and the other arm independently binds to ANG-2.

Figure 1: Illustration of the CrossMab Design of Faricimab



Note: The figure displays the design of the CrossMab faricimab. Starting from the parental antibodies anti-VEGF and anti–Ang-2, the CrossMab consists of the heavy chain HC1 and the light chain LC1 originating from the anti-VEGF antibody, and the heavy chain HC2 and the light chain LC2 originating from the anti–Ang-2 antibody. The C_H1 and C_L domains from the LC2 and the HC2 are exchanged.

Figure copied from the Applicant's submission (Figure 2.3.S-1).

The recombinant bispecific antibody is produced in CHO cells and consists of two different heavy chains (VEGF-HC: HC1 with 452 amino acid residues, Ang-2-HC: HC2 with 462 amino acid residues; without C-terminal lysine) and two different light chains (VEGF-LC: LC1 with 214 amino acid residues, Ang-2-LC: LC2 with 213 amino acid residues) with inter- and intra-chain disulfide bonds that are typical for IgG1 antibodies plus an additional disulfide bridge in the CH3-CH3 interface.

The VEGF-binding domain is a humanized fragment antigen binding (Fab) and is comparable to other anti-VEGF molecules, e.g., ranibizumab. The Ang-2 binding domain is a human Fab derived from phage display. The constant part is based on a human IgG1 framework. The variable part contains heavy chain V_H3 and light chain $V\kappa1$ subgroup sequences (for the anti-VEGF arm) and heavy chain V_H1 and light chain $V\lambda3$ (from the anti-Ang-2 arm), respectively

Modification of faricimab neonatal Fc receptor (FcRn) and Fc gamma receptor (FcγR) binding sites (b) (4) disables the antibody's Fc-mediated effector functions. (b) (4)

Pharmacologic Class: Angiopoietin-2 (Ang-2) and vascular endothelial growth factor A (VEGF-A) inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 119225

2.3 Drug Formulation

The drug product is composed of 120 mg/mL faricimab in 20 mmol/L L-histidine/ (b) (4) acetate (b) (4) 25 mmol/L sodium chloride, 160 mmol/L sucrose, 7 mmol/L L-methionine, 0.4 mg/mL polysorbate 20, pH 5.5 (Table 2).

The minimum fill volume (b) (4) is sufficient to withdraw and administer the nominal volume (0.05 mL) and dose of 6 mg of faricimab per vial when used as directed.

Table 2: Composition of Faricimab Drug Product

Ingredient	Nominal Amount per Vial	Concentration	Function	Specification
Faricimab	6.00 mg	120 mg/mL	Active ingredient	Module 3.2, Section S.4.1 *Specification*
L-Histidine	155 µg	20 mmol/L	(b) (4)	USP-NF/Ph. Eur./JP
Acetic Acid (b) (4)	QS to pH 5.5			Ph.Helv.[a]
L-Methionine	52.2 µg	7 mmol/L		USP-NF/Ph. Eur./JP
Sodium Chloride	73.1 µg	25 mmol/L		USP-NF/Ph. Eur./JP
D-Sucrose	2.74 mg	160 mmol/L		USP-NF/Ph. Eur./JP
Polysorbate 20	20.00 µg	0.4 mg/mL		USP-NF/Ph. Eur./JPE
Water for Injection	QS to 0.05 mL	—		USP-NF/Ph. Eur./JP

[a] Manufactured by supplier (b) (4) USP-NF/Ph. Eur./JP.

Table copied from the Applicant's submission (Table 2.3.P-1).

2.4 Comments on Novel Excipients

A total of four formulations were used during development. (b) (4) The main difference of toxicological concern between the commercial clinical formulation and the nonclinical formulation is the addition of L-methionine in the commercial formulation. Methionine is not listed as an excipient in approved ophthalmic products in the FDA Inactive Ingredient Database.

The safety of IVT use of L-methionine was assessed based on available public data and data from nonclinical studies, primarily, a 2-week IVT toxicology study in cynomolgus monkeys (using (b) (4) methionine as part of vehicle or faricimab formulation, 2 IVT injections 14 days apart). The information was submitted under Module 3.2.P.4.6 of the BLA, but it was a topic of discussion for the type C pre-Phase 3 meeting held on April 3, 2017 (see Section 2.7 Regulatory Background below).

As noted under Section 2.7 Regulatory Background below, Pharm/Tox team concurred that no additional nonclinical studies were warranted to qualify the use of 7 mM methionine (b) (4) (nonclinical review by Andrew McDougal filed in DARRTS on 8-30-2017). Dr. McDougal stated that generally, Pharm/Tox recommends that toxicology studies to qualify excipients be at least 4 weeks duration. However, IVT faricimab caused progressive ocular inflammation after the second dose in the GLP 2-month and 6-month monkey toxicology studies. Therefore, requesting a longer-term study with the Phase 3 clinical formulation of faricimab will not provide additional useful information.

The Applicant referred to additional information from three studies (in cynomolgus monkeys and in New Zealand White rabbits) in which methionine (b) (4) was a component of the vehicle, administered intravitreally up to six times, 14

days apart. The Applicant concluded that no ocular effects of methionine were observed in any of the animals treated with the methionine-containing vehicle. These studies were also referred to by Dr. McDougal on his 8-30-2017 review. Summary information was presented for these additional studies under SD # 59 (Study # 14-0412, pages 54 to 55, and 70; Study # 14-1231, pages 55 to 56, and 71 to 75; Study # 14-1230, pages 57 to 58, 76 to 79).

Dr. McDougal requested further information for a finding of concern, as noted below:

"*For the* (b) (4) *rabbit study (# 14-1230), you reported that the methionine-containing vehicle was associated with "outer retinal degeneration in the left eye of two [2 out of 10] vehicle control group animals, which correlated with minimal unilateral axon degeneration in the optic tract/chiasm of the brain in one animal [1 out of 10]." Report the severity of the retinal degeneration observation. Provide any additional information regarding these eyes, and provide relevant historical control information to support your conclusion that these findings were incidental."*

This reviewer is not aware if this information was presented to the IND. However, Dr McDougal also concluded that based on available information on methionine levels in the eye[1], the amount of methionine added by the proposed IVT injection will be within the range of human variability. Therefore, no safety issue arises from its use as an excipient.

2.5 Comments on Impurities/Degradants of Concern

Pending CMC review.

2.6 Proposed Clinical Population and Dosing Regimen

Treatment of patients with:

- Neovascular (wet) Age-Related Macular Degeneration (nAMD)
- Diabetic Macular Edema (DME)
- Diabetic Retinopathy (DR)

Dosing regimen

- Neovascular (Wet) Age-Related Macular Degeneration (nAMD)

[1] Kanakis MG, Michelakakis H, Petrou P, et al. Case report: aqueous and vitreous amino-acid concentrations in a patient with maple syrup urine disease operated on rhegmatogenous retinal detachment. *BMC Ophthalmol* 2016,16(1): 170. https://www.ncbi.nlm.nih.gov/pubmed/27716111

- 6 mg (0.05 mL) administered by IVT injection every 4 weeks (approximately every 28 ± 7 days, monthly) for the first 4 doses, followed by 6 mg (0.05 mL) at intervals of up to every 16 weeks (4 months)
- Some patients may be dosed as frequently as every 4 weeks (approximately every 28 ± 7 days, monthly)

- Diabetic Macular Edema (DME) and Diabetic Retinopathy (DR)
  - 6 mg (0.05 mL) administered by IVT injection every 4 weeks (approximately every 28 days ± 7 days, monthly) for the first 4 doses, followed by 6 mg (0.05 mL) at intervals of up to every 16 weeks (4 months)
  - Some patients may be dosed as frequently as every 4 weeks (approximately every 28 ± 7 days, monthly)
  - The dosing interval may be adjusted in 4-week increments.

2.7 Regulatory Background

October 29, 2013
Initial IND 119225 submitted – Initial IND nonclinical review filed in DARRTS on 9-23-2013 by Janice Lansita, PhD, DABT

- Nonclinical data supported the starting dose, but not the high dose due to safety concerns of severe ocular inflammation. It was not known if the inflammation was a direct or indirect inflammation through an ADA response in the eye.

September 24, 2013
Sponsor teleconference to discuss the non-clinical data used in support of the proposed doses in the clinical study submitted with the initial IND (meeting minutes filed in DARRTS on 10/2/2013 by Diana Willard, MD)

- The proposed clinical starting dose was supported by the animal data submitted in the IND, however the ocular inflammation observed with higher animal doses raised potential concerns. It was agreed the Division would review the collected data from each of the lower doses in humans before the next higher dose is administered to humans.

January 29, 2014
Pharm/Tox Information Request to Sponsor to submit final reports for 2 nonclinical studies initially submitted as drafts within 120 days of submission of the IND (reviews filed in DARRTS on 1-27-2014 and 2-3-2014 by Andrew McDougal PhD, DABT; Information Request by Diana Willard on 1-29-2014 and 2-4-2014).

- One study was a pivotal study (2-month IVT toxicity in monkeys) for safety assessment of administering multiple ascending doses to humans.

April 3, 2017
FDA Type C face-to-face meeting for chemistry, manufacturing, & control (CMC) and nonclinical (meting minutes filed in DARRTS on 3-24-2017 by Eithu Lwin; nonclinical

review by Andrew McDougal filed in DARRTS on 8-30-2017) – Some nonclinical recommendations included:

- Pharm/Tox team concurred no additional nonclinical studies were warranted to qualify the use of 7 mM methionine (b) (4) for the drug product.
- Pharm/Tox team asked the Sponsor to provide any additional information regarding eyes in the methionine-containing control group with retinal degeneration and to provide relevant historical control data.
- Pharm/Tox team asked the Sponsor to explain the basis to change (b) (4) to histidine acetate, and pH (b) (4) to 5.5. The explanation was found acceptable.
- Pharm/Tox team stated the lesions of heart squamous cyst/plaque in 2 high-dose monkeys (one dose of 1.5 mg/eye and then 3 mg/eye from Day 29 onward) observed in the 6-month ITV toxicity study were considered treatment related and recommended they be summarized in the Investigator's Brochure.

April 24, 2018
FDA Type B (end-of-phase 2 [EOP2]) face-to-face meeting for DME and DR clinical and nonclinical (meeting minutes filed in DARRTS on 5-21-2018 by Diana Willard; nonclinical review by Andrew McDougal filed in DARRTS on 4-16-2018)

- Pharm/Tox team concurred nonclinical program sufficient to support Phase 3 studies.
    - The 26-week IVT monkey study (report # 1057630) was submitted in draft form on 3/24/2015 (SD # 21), and in final form on 7/27/2015 (SD # 24).
- Pharm/Tox team gave recommendations for the conduct of an adequate monkey embryofetal development (EFD) study for BLA submission, including dose(s) that achieves clinically relevant exposures for the period of organogenesis (e.g., gestation days 20-50 for the cynomolgus monkey).
- The Sponsor was told that the determination regarding the need for carcinogenicity studies is formally made at the time of application review. Pharm/Tox team recommended the Sponsor include a written request for waiver of carcinogenicity studies and address the topic of carcinogenicity (e.g., based on mechanisms of action) in the BLA.

August 30, 2018
FDA Type B (EOP2) face-to-face meeting for nAMD clinical and nonclinical (meeting minutes filed in DARRTS on 9-24-2018 by Diana Willard; nonclinical review by Andrew McDougal filed in DARRTS on 8-21-2018)

- Pharm/Tox team agreed that, pending review of the data, the completed nonclinical program to support the DME program also supports the nAMD program, and no further studies were needed prior to initiation of the nAMD Phase 3 trials.
- Additional recommendations were given for the design of the monkey embryofetal toxicity study.

3 Studies Submitted

3.1 Studies Reviewed

- Study # 1048699, Pharmacokinetic Assessment after Intravenous Administration to New Zealand White Rabbits
- Study # 1050473, Pharmacokinetic Assessment following Intravitreal and Intravenous Administration to Cynomolgus Monkeys
- Study # 1104409, Carcinogenicity Assessment
- Study # 1093222, Intravenous Administration Embryofetal Development Study in the Cynomolgus Monkey
- Study # 1120D14, In Vitro Evaluation of RO6867461 in a Human Complement Activation Assay for the Pre-clinical Risk Assessment of Anaphylatoxins and Complement Split Fragment Generation

Previously reviewed under IND 119225:

- Study # 10566781, Binding Affinity to RO6867461 Ligands and Fc receptors
- Study # 1056924, Spontaneous Model of Choroidal Neovascularization in Mice
- Study # 1056925, Laser Induced Model of Choroidal Neovascularization in Cynomolgus Monkey
- Study # 1048818, Pilot Ocular Pharmacokinetic Study Following a Single Intravitreal Administration in Pigmented Rabbit
- Study # 1053033, Pilot Pharmacokinetic Study Following an Intravitreal Administration in Cynomolgus Monkeys
- Study # 1053361, 2-Month Toxicity and Toxicokinetic Study with RO6867461 Following Intravitreous and Intravenous Administration in Cynomolgus Monkeys with a 4-Week Recovery Phase
- Study # 1053362, 2-Week Tolerance Study of RO6867461 following IVT and IV administration in Dutch-belted Rabbits
- Study # 1053363, 2-Week Tolerance Study of RO6867461 following IVT and IV administration in Cynomolgus Monkeys
- Study # 1055832, Pilot Tissue Cross Reactivity Study of RO6867461 in a Limited Panel of Normal Human Tissues
- Study # 1056445, A Tissue Cross-Reactivity Study of RO6867461 in Normal Human Tissues
- Study # 1055400, Evaluation of RO6867461 for the Risk of Cytokine Release and Immune Cell Depletion in an In Vitro 24 h-format in Human Whole Blood
- Study # 8292406, 26-Week Partial Ascending-Dose Toxicity and Toxicokinetic Study Following Once Monthly Intravitreous Injections in Cynomolgus Monkeys with a 13-Week Recovery

3.2 Studies Not Reviewed

- Module 4.2.2.1 Analytical Methods and Validation Reports

3.3 Previous Reviews Referenced

- IND 119225 initial review, by Janice Lansita, PhD, DABT, filed in DARTS on 9-23-2013
- IND 119225, review of 6-month IVT toxicity study submitted under SD # 24, data to support qualification of excipient methionine submitted under SD # 59, by Andrew McDougal, PhD, DABT, filed in DARRTS on 8-30-2017

4 Pharmacology

Faricimab is a humanized bispecific IgG1 antibody that selectively binds to VEGF-A and ANG-2. One arm binds to VEGF-A with high affinity (0.5 to 3 nM; 73 to 438 ng/mL) and the second crossed arm binds to ANG-2 (20 nM; 2923 ng/mL), also with high affinity. Independent and simultaneous binding to both ANG-2 and VEGF-A by faricimab was demonstrated by surface plasmon resonance (SPR). The Fc domain of faricimab has been engineered to abolish binding interactions with Fcγ receptors and with FcRn.

In a laser-induced model of choroidal neovascularization (CNV) in cynomolgus monkeys, faricimab reduced the formation of CNV and subsequent injury to the retina due to lesion formation to a significantly greater degree compared to anti-VEGF-A monoclonal antibody (mAb) alone.

4.1 Primary Pharmacology

Affinity and cross reactivity of the CrossMab VEGF/Ang-2 IgG1 P329G LALA +AAA to its Ligands and Fc effector molecules (Study # 1056781) – This study was previously reviewed under the initial IND (Janice Lansita, PhD, 9-23-2013). Faricimab (RO6867461) bound to full length Ang 2 with high affinity across species including human, cynomolgus monkey, rabbit, and mouse, measured by Surface Plasmon Resonance (SPR). Faricimab also bound with high affinity to the rat receptor binding domain, Ang 2-RBD-Fc. The binding affinity results are summarized in the Applicant's table below.

Table 3: Binding of Faricimab to Various Ang-2 Antigens

Ang-2 species	Full length Ang-2: Affinity (Method)	Ang-2-RBD-Fc: Affinity (Method)
Human	20 nM (SPR, solution affinity)	21 nM (SPR, solution affinity) 22 nM (ITC)
Cynomolgus	13 nM (SPR, solution affinity)	Material not available
Mouse	13 nM (SPR, solution affinity)	5 nM (SPR, solution affinity)
Rabbit	11 nM (SPR, solution affinity)	Material not available
Rat	Material not available	8 nM (SPR, solution affinity)

Faricimab did not bind to full length human Ang-1, whereas it bound Ang-2.

Faricimab showed high affinity binding to VEGF121 in human and rat and VEGF165 in human (Table 4; Applicant's table). The cynomolgus monkey, rhesus monkey, and human VEGF121 share 100% sequence homology. Therefore, faricimab was not formally tested for binding to cynomolgus monkey VEGF121.

Table 4: Binding of Faricimab to VEGF Antigens

VEGF species	VEGF-A121: Affinity (Method)	VEGF-A165: Affinity (Method)
Human	1 nM (SPR, kinetic affinity) 0.5 nM (SPR, solution affinity) 3 nM (ITC)	3 nM (ITC)
Cynomolgus	Sequence Identical to human (see Section "VEGF-A121 amino acid sequence alignment")	Material not available
Mouse	No binding	Material not available
Rabbit	Material not available	Material not available
Rat	14 nM (SPR, kinetic affinity)	Material not available

The Fc region of RO6867461 was mutated to prevent binding to Fc receptors. No binding to human FcγRI, FcγRII, and FcγRIIIa (V158), or FcRn (from human, cynomolgus monkey, and mouse) were observed by surface plasmon resonance.

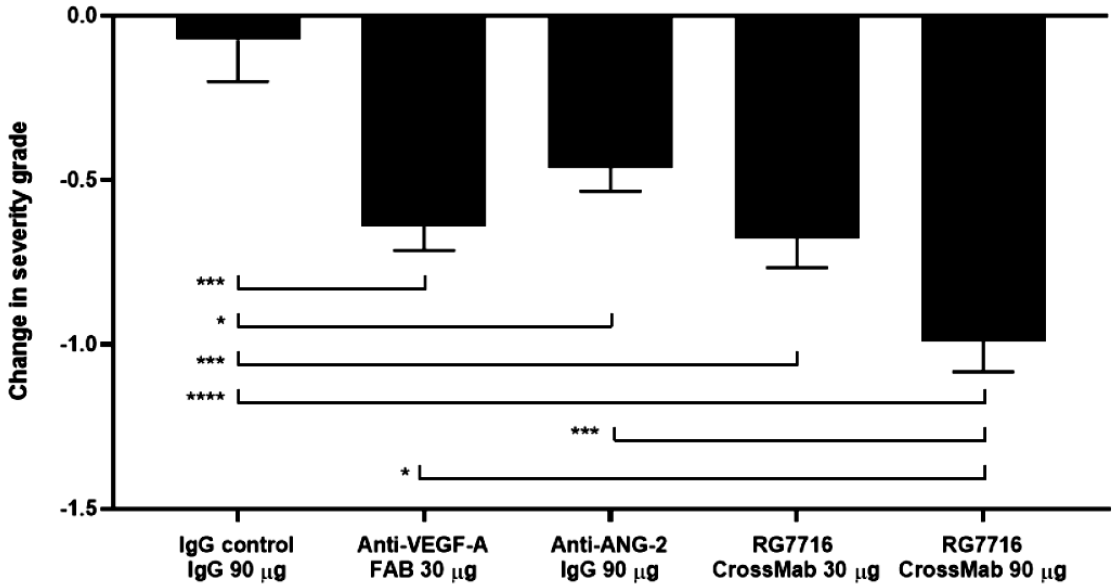
Efficacy and Pharmacokinetics following Intravitreal Administration to Cynomolgus Monkeys with Experimental Laser-Induced Choroidal Neovascularization (Study # 1056925; corrected data under Study # 1103767) – This study was previously reviewed under the initial IND (Janice Lansita, PhD, 9-23-2013). The study aim was to characterize the pharmacokinetic (PK) and

pharmacodynamic (PD) profiles of faricimab (CrossMab; RO6867461), Lucentis® (a standard of care in wet AMD), a monoclonal antibody targeting Ang-2 (RO5485202), and an IgG negative control following IVT administration to cynomolgus monkeys with experimental laser-induced choroidal neovascularization (CNV).

Cynomolgus monkeys (n=3/group) were dosed IVT with 30 µg or 90 µg of faricimab, 90 µg of anti-Ang-2 antibody, 30 µg of Lucentis®, or 90 µg of IgG control (dose volume 50 µL). Both eyes were injected a single IVT injection of the drugs at Day 14 after application of laser. The severity of the CNV lesions was evaluated on Day 30 by fluorescence angiography.

No change in CNV lesion severity was observed for the isotype control. The severity of leakiness was reduced by anti-Ang-2 and anti-VEGF-A (Lucentis®) treatment by -0.46 and -0.63, respectively. Treatment with 30 and 90 µg faricimab reduced the severity score by -0.68 and -0.99, respectively. At an equimolar number of binding sites, 90 µg of faricimab (MW~150kDa) reduced the lesion severity significantly more than 30 µg of Lucentis® (MW~50kDa); (analysis of variance [ANOVA] followed by Tukey's multiple comparison, $p < 0.05$ compared with Lucentis®). The figure below (Applicant's figure) shows the comparative reduction in lesion severity grade.

Figure 2: Reduction in CNV Lesion Severity Grade



IgG = immunoglobulin; FAB = fragment antigen binding; IVT = intravitreal; RG7716 CrossMab = faricimab (anti-VEGF-A/ANG-2 antibody); VEGF = vascular endothelial growth factor.

Inhibition of neovascularization measured in severity grades, change of severity from baseline is shown for each treatment; all treatments significantly reduced the severity grade compared to IgG control. In addition, efficacy of RG7716 (150-kDa molecule at 90 µg/50 µl injected IVT) was significantly better at equal molar concentration of binding sites than anti-VEGF-A (ranibizumab, 50-kDa molecule at 30 µg/50 µl injected IVT) and anti-ANG-2.

Error bars show SEM of n = 12 eyes from 6 cynomolgus monkeys and 9 spots per eye in the groups treated with anti-VEGF-A, anti-ANG-2, and RG7716 CrossMab 90 µg, and SEM of n = 10 and n = 9 eyes from 6 and 5 cynomolgus monkeys and 9 spots per eye in the groups treated with RG7716 CrossMab 30 µg and IgG control, respectively.

The symbol "*" denotes significance after one-sided ANOVA and Tukey's multiple *t*-test. IgG control is significantly different from anti-VEGF-A (***, $P = 0.0002$), anti-ANG-2 (*, $P = 0.0262$), RG7716, 30 µg (***, $P = 0.0001$), and RG7716, 90 µg (****, $P < 0.0001$). Furthermore, RG7716, 90 µg is significantly different from anti-ANG-2 (***, $P = 0.0002$) and anti-VEGF-A (*, $P = 0.0319$).

Overall, data demonstrated that better efficacy is observed with faricimab treatment than treatment with anti-VEGF alone at equimolar anti-VEGF-A binding sites.

Faricimab systemic exposure (C_{max} and AUC) was about 4-fold higher than that observed for Lucentis® (MW below glomerular filtration cut point) and about 5-fold lower than that for RO5485202 and RO5489789 (both with FcRn binding). ADA formation affected only two timepoints at 30 µg (monkey # 1208, Days 28 and 35) and one timepoint at 90 µg (monkey # 2907, Day 32).

4.2 Secondary Pharmacology

Evaluation of RO6867461 for the Risk of Cytokine Release and Immune Cell Depletion in an in Vitro 24h-Format Human Whole Blood Cell Assay (Study # 1055400; Module 4.2.3.7.7) - This study was previously reviewed under the initial IND (Janice Lansita, PhD, 9-23-2013). The potential for cytokine release was evaluated in an in vitro assay using human whole blood from 30 healthy human donors. Faricimab was incubated with whole blood samples for 24 hours at 0.1, 1, 10, 100 µg/ml.

Faricimab generally led to secretion of IL-6, IL-8, TNF-a and IFN-g with maximum median upregulation by a factor of 1.57, 0.90, 1.0 and 1.18, respectively, compared to the negative comparator Erbitux®. However, when compared to Campath®/ MabCampath® (positive control; a humanized anti-CD52 IgG1 known to induce cytokine release syndrome in greater than 90% of recipients), 0.1-100 µg/ml faricimab induced at least 70-, 16-, 5- and 50-fold lower levels of IL-6, IL-8, TNF-α and IFN-γ, respectively.

Faricimab showed increases in IL-6 (1.57X) and IFN-γ (1.18X) compared to the negative control indicating some cytokine induction and no effect on peripheral immune cell subsets (T-cells, B-cells, NK-cells, monocytes, or granulocytes).

4.3 Safety Pharmacology

Stand-alone safety pharmacology studies were not performed. Safety pharmacology endpoints were included as part of the 2-month cynomolgus monkey study (neurological exam, respiration rate, body temperature) following IVT (1.5 to 6 mg/eye, once monthly, one eye dosed) and IV dosing (5 mg/kg once monthly), and 6-month cynomolgus monkey ocular toxicity study (neurological exam, heart rate, EKG) following IVT dosing (0.5 to 3 mg, once monthly, one eye dosed). No toxicologically relevant findings were observed.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

The PK parameters of faricimab via IVT and IV routes were investigated in rabbits and monkeys following a single dose administration and after multiple-dose administration, as part of the toxicokinetic studies.

As the metabolism/catabolism of antibodies generally involves degradation to smaller peptides and amino acids, classical biotransformation studies were not conducted.

Studies # 1048818, *“Pilot Ocular Pharmacokinetic Study Following a Single*

Intravitreal Administration in Pigmented Rabbit", and # 1053033, *"Pilot Pharmacokinetic Study Following an Intravitreal Administration in Cynomolgus Monkeys"* were previously reviewed under the initial IND (Janice Lansita, PhD, 9-23-2013). Per Dr. Lansita's review:

- Single dose IVT studies of faricimab (RO6867461) in the rabbit and cynomolgus monkey showed greater vitreous, aqueous and serum exposure in the rabbit at a lower IVT dose of 0.5 mg/eye or 0.5 mg/mL compared with the monkey dosed at 1.5 mg/eye or 0.75 mg/mL.
- Bioavailability was 2.2% in the rabbit and 13% in the cynomolgus monkey. However, in the 2-week rabbit study at higher doses, bioavailability was higher at 17%.
- The Applicant notes that due to ADA formation in the rabbit, the rabbit was ruled out as a toxicology species.
- In Study # 1053033 in cynomolgus monkeys, fine, bright white particulates were distributed throughout the vitreous following a single IVT dose at 1.5 mg/eye. ADA in serum was detected at the last time points *(≥336 hours)*.
- A comparison of the single dose PK parameters of faricimab following IVT administration are summarized in the Applicant's table below.

Table 5: Mean Pharmacokinetic Parameters of Faricimab after a Single Intravitreal Administration in New Zealand White Rabbits and Cynomolgus Monkeys

		Faricimab	
Parameter	**Unit**	**Rabbit**	**Monkey**
Dose		0.5 mg/eye	1.5 mg/eye
Number of Animals per Group	—	16	3
Vitreous Humor			
C_{max}	µg/mL	547	107
$t_{1/2}$	hr	103	72.5
$AUC_{0\text{-}inf}$	(µg•hr)/mL	76,500	23,700
Aqueous Humor			
C_{max}	µg/mL	—	40.8
$t_{1/2}$	hr	—	93.5
$AUC_{0\text{-}inf}$	(µg•hr)/mL	—	7210
Serum			
C_{max}	µg/mL	0.427	2.16
$t_{1/2}$	hr	—	68.9
$AUC_{0\text{-}inf}$	(µg•hr)/mL	57.1	318

AUC=area under the concentration–time curve; C_{max}=maximum concentration; hr=hour; $t_{1/2}$=half-life.

Source: [1048818, 1053033].

Pharmacokinetic Assessment after Intravenous Administration to New Zealand White Rabbits (Study # 1048699) – After a single IV injection of 0.1 mg/kg or 1.25 mg/kg faricimab (RO6867461), blood levels were below the lower limit of quantitation (<0.001 µg/mL) at ≥168 hours postdose. Summary PK data is shown in Table 6 (Applicant's table). ADA formation was positive for all serum samples.

Table 6: Serum Raw Data and Descriptive Statistics of RO6867461 after Single Intravenous Administration of 1.25 mg/kg RO6867461-000-002 to New Zealand White Rabbits

time [h]	Rabbit12/04 [µg/mL]	Rabbit12/05 [µg/mL]	Rabbit12/06 [µg/mL]	N	mean [µg/mL]	CV [%]	median [µg/mL]	range [µg/mL]
1	36.0	41.1	46.8	3	41.3	13.1	41.1	36.0-46.8
7	17.7	28.8	30.9	3	25.8	27.5	28.8	17.7-30.9
25.7	5.60	10.0	9.40	3	8.33	28.6	9.40	5.60-10.0
72	2.20	3.60	2.60	3	2.80	25.8	2.60	2.20-3.60
168	<0.001	<0.001	<0.001	3	0	NC	0	0-0
337	<0.001	<0.001	<0.001	3	0	NC	0	0-0
504	<0.001	<0.001	<0.001	3	0	NC	0	0-0
672	<0.001	<0.001	<0.001	3	0	NC	0	0-0

Compared with wild-type IgG (RO6867488), faricimab exhibited similar exposure ($AUC_{0\text{-last}}$) at the low dose (0.1 mg/kg for both molecules) and lower exposure at the high dose (1.25 mg/kg for faricimab and 1 mg/kg for wild-type IgG), in line with the expected faster clearance due to mutations in the FcRn binding domain of the Fc region to not bind Fc receptors.

Pharmacokinetic Assessment following Intravitreal and Intravenous Administration to Cynomolgus Monkeys (Study # 1050473) - Following a single 0.3 mg/kg IV administration to cynomolgus monkeys (n=2/group), faricimab (RO6867461) exhibited faster systemic clearance (0.0107 mL/min/kg vs. 0.006 mL/min/kg for wild-type IgG [RO6867488]) associated with shorter systemic $t_{1/2}$ compared with wild-type IgG (32 hours vs. 108 hours for wild-type IgG). The results are in line with the expected faster clearance due to mutations in the FcRn binding domain of the Fc region to not bind Fc receptors.

Note: The first statement in the "Conclusions" in the Study Report appears to be incorrect, as it is stated: *"RO6867461 showed low clearance and volume of distribution, associated with long terminal half-life*."

For all test articles studied (3 in total), fine, bright white particulates distributed throughout the vitreous, which were observed on Day 4 (approximately 72 hours postdose) in 5 of the 6 treated eyes. The presence of these opacities decreased over time such that they were perceptible by Day 31 (approximately 720 hours postdose) in only 2 of the 6 eyes (treated with RO6892065 Fab2 fragment). The composition of the particulates was not determined.

The summary PK data in serum and aqueous humor following a single IVT injection is shown below (Applicant's table):

Table 7: Summary PK Data in Serum and Aqueous Humor following a Single IVT Injection of Faricimab (RO6867461), Wild-Type IgG (RO6867488), and Fab2 Fragment (RO6892065)

Parameter	Unit	Group 1 RO6867461 1500 µg/eye		Group 2 RO6867488 1500 µg/eye		Group 3 RO6892065 1000 µg/eye	
		Serum	Aqueous humor	Serum	Aqueous humor	Serum	Aqueous humor
Cmax	[µg/mL]	3.79	98.7	4.60	68.5	0.436	33.3
tmax	[h]	24	72	24	72	24	72
t1/2	[h]	89.3	68.4	143	66.2	56.9	53.6
tlast	[h]	672	672	588	672	504	672
AUC(0-tlast)	[(µg·h)/mL]	295	18100	622	13100	46.8	5950
AUC(0-inf)	[(µg·h)/mL]	296	18200	655	13100	46.9	5950
AUC(rest, tlast-inf)	[%]	0.4	0.1	6.7	0.1	0.2	0.0
MRT(tot)	[h]	95.3	97.0	187	107	81.8	87.9
F	[%]	12.7	-	15.4	-	15.8	-

RO6867461 is an IgG with no FcRn and FcγRIIIa binding affinities.
RO6867488 is an IgG with no FcγRIIIa binding (but intact FcRn binding).
RO6892065 is a Fab2 fragment.
Aqueous humor samples were collected at 72, 336, and 672 hours postdose
Serum samples were collected predose and from 0.083 to 672 hours postdose.

Anti-drug antibody (ADA) development was observed in aqueous humor samples following IVT dosing. ADA in the serum was present at ≥336 hours postdose following IVT administration. ADA measurements were not performed in serum samples collected following IV administration.

5.2 Toxicokinetics

In the toxicology studies in cynomolgus monkeys, there was a dose-proportional increase in the mean values for maximum observed serum concentration (C_{max}) and area under the concentration-time curve (AUC) following both IVT and IV administration of faricimab. A correlation between ADA development and reduced systemic exposure was observed in the 2-month and 6-month GLP toxicology studies. This did not compromise the overall readout of the studies, because there was still sufficient exposure in ADA-positive animals.

6 General Toxicology

6.1 Single-Dose Toxicity

As stated in Dr. Lansita's initial IND review, tolerability was evaluated in the context of the single-dose PK studies. Faricimab (RO6867461) appeared to be well-

tolerated in the single dose IVT PK studies. In cynomolgus monkeys, fine, bright white particulates were distributed throughout the vitreous following a single IVT dose at 1.5 mg/eye.

6.2 Repeat-Dose Toxicity

The following studies were reviewed under the initial IND by Dr Lansita (9-23-2013).

- Study # 1053361, 2-Month Toxicity and Toxicokinetic Study with RO6867461 following Intravitreous and Intravenous Administration in Cynomolgus Monkeys with a 4-Week Recovery Phase
- Study # 1053362, 2-Week Tolerance Study of RO6867461 following IVT and IV Administration in Dutch-Belted Rabbits
- Study # 1053363, 2-Week Tolerance Study of RO6867461 following IVT and IV Administration in Cynomolgus Monkeys

Per Section **1.2 Brief Discussion of Nonclinical Findings** of Dr. Lansita's review, key findings/clinically relevant issues were:

"*The RO6867461 nonclinical studies in the rabbit and monkey identified toxicities that included severe ocular inflammation (rabbit and monkey), coagulative liver necrosis (rabbit), degeneration/necrosis of the myocardium (rabbit), and minimal mixed-cell inflammation in the aorta (monkey). The ocular inflammation in the monkey appeared to partially reverse 42 days after the 2nd dose of RO6867461 with anti-inflammatory treatment. In the rabbit, evidence of less severe ocular inflammation was observed in rabbits that did not receive a 2nd dose which indicates animals were responsive to anti-inflammatory therapy. The Sponsor claims that the nonclinical toxicities are secondary to an ADA response and are not relevant to human. The Sponsor provided preliminary data that support immune complex deposition in the aorta, however, similar data were not provided in the eye, therefore the safety concern of severe ocular inflammation remains. The proposed clinical starting dose of 0.5 mg/eye is supported by the nonclinical data but the high dose of 6.0 mg/eye is not. Since it is not known whether RO6867461 causes direct inflammation or indirect inflammation through an ADA response in the eye, it is recommended that the clinical protocol be adjusted to accommodate safety concern."*

Dr. Lansita agreed that the rabbit does not appear to be a suitable species, due to the severe toxicity observed at low IVT doses (1.5 mg/eye) following a single dose; chronic studies to support future clinical trials do not appear to be feasible in the rabbit unless lower doses were explored. She agreed, as the Applicant proposed, that the monkey alone should be used in the planned 6-month chronic toxicity study.

Per Section **1.3.3 Additional Recommendation(s) (Non-hold comments/ advice to sponsor)** of Dr. Lansita's review, nonclinical recommendations included:

"The preliminary immunohistochemistry (IHC) data provided by the Sponsor indicate the presence of immune complexes and complement, in the absence of drug, in the aorta of monkeys. However, since drug was not detected in association with monkey IgG, monkey IgM, and complement, it remains unclear whether RO6867461 mediates inflammation directly or indirectly via an ADA response, although the preliminary data are compelling.

- *Please conduct in vitro or in vivo studies or provide data to demonstrate that RO6867461 does not cause direct inflammatory effects (beyond the cytokine release study).*
- *In addition, proinflammatory cytokines, complement factors, and Ang1, can be monitored in the planned 6-month chronic monkey study.*
- *Please also evaluate ocular tissues by IHC for the presence of immune complex, complement factors, and RO6867461 in the 2-month monkey study (if samples are available) and in the planned 6-month monkey study."*

The 26-week IVT monkey study (report # 1057630) was submitted in draft form on 3/24/2015 (SD # 21), and in final form on 7/27/2015 (SD # 24). Dr. Lansita's recommendations for the 26-week monkey study were incorporated in the study design. The study was previously reviewed by Andrew McDougal, PhD. As this is the most pivotal ocular toxicity study, Dr. McDougal's review is copied below (additional comments by this reviewer are shown in italics).

Study title: RO6867461: 26-Week Partial Ascending-Dose Toxicity and Toxicokinetic Study Following Once Monthly Intravitreous Injections in Cynomolgus Monkeys with a 13-Week Recovery	
Study no.:	• Sponsor's report # 1057630 • Study laboratory # 8292406
Study report location:	IND module 4.2.3.2 (Toxicology → Repeat-Dose Toxicity → Nonhuman Primate – Other – Medium): • Draft report submitted 3/24/2015 (SD #21), accessible via: \\cdsesub1\evsprod\ind119225\0021\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\1057630-draft.pdf and \\cdsesub1\evsprod\ind119225\0021\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\1057630-final-phase-report.pdf • Final report submitted 7/27/2015 (SD # 24),

	accessible via: \\cdsesub1\evsprod\ind119225\0024\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\1057630-final.pdf • A summary of changes from the draft to the final report was included: (\\cdsesub1\evsprod\ind119225\0024\m4\42-stud-rep\423-tox\4232-repeat-dose-tox\1057630-content-changes.pdf). This reviewer reviewed these changes; they are acceptable and not concerning for the adequacy of the study.
Conducting laboratory and location:	(b) (4)
Final report date	July 17, 2015
Date of study initiation:	October 24, 2013
GLP compliance:	Yes, with the exceptions of : • Electroretinography • Optical coherence tomography (OCT) • Bioanalysis of vitreal fluid • Analysis of plasma for target (Ang-2, Ang-1, VEGF-A) • Immunohistochemistry
QA statement:	Yes
Drug, lot #, and % purity:	RO6867461, batch # GJD0005, purity 97.0%

Key Study Findings

- For ocular toxicity, the NOAEL was 0.5 mg/eye (the low dose). The LOAEL was 1.5 mg/eye (the mid-dose), due to ocular inflammation (observed by ophthalmology and confirmed by histopathology).
- For systemic toxicity, the NOAEL = 1.5 mg/eye (the mid-dose). The LOAEL = 1.5/3 mg/eye (the high dose), based on heart squamous cyst/plaque detected in two high-dose monkeys (2/12).
- Review note: this primary P/T review was performed only on the final report (dated 7/17/2015), rather than the draft report (dated 2/26/2015)

Group	No. of Animals[a] Male	Female	Dosing Regimen Right eye	Left eye	Right Eye Dose Level (mg/eye/dose)	Dose Concentration (mg/mL)	Left Eye Dose Level (mg/eye/dose)
1[b]	5	5	Vehicle	Vehicle	0	0	0
2	3	3	Test Article	Vehicle	0.5	10	0
3	5	5	Test Article	Vehicle	1.5	30	0
4	8	8	Test Article	Vehicle	1.5/3[c]	30/60[c]	0

a Animals designated for the recovery sacrifice (two animals/sex in Groups 1 and 3 and three animals/sex in Group 4, dependent on survival) underwent 13 weeks of recovery following the dosing phase.

b Group 1 received vehicle control article ([(b) (4)] histidine/[(b) (4)] sodium chloride, [(b) (4)] polysorbate 20, and [(b) (4)] sucrose, pH [(b) (4)] only in both eyes.

c Right eyes of animals in Group 4 were given 1.5 mg/eye/dose on Day 1 and 3 mg/eye/dose from Day 29 onward.

Doses: 0, 0.5, 1.5, or 3.0 mg/eye

per dose:
- Group 1 (vehicle control): 5/sex
- Group 2 (0.5 mg/eye OD): 3/sex
- Group 3 (1.5 mg/eye OD): 5/sex
- Group 4 (1.5 mg on D1, 3.0 mg thereafter): 8/sex

Frequency of dosing: Monthly x 7 (D1, 29, 58, 85, 113, 141, and 169)
- Note: dosing holidays for groups 3 and 4 (1.5 and 3.0 mg/eye)
- Main-group sacrifice was D184.
- Recovery-group sacrifice was after 92 days of recovery (i.e. 13 weeks)

Route of administration:
- Intravitreal (ivt) injection
- "by an OSOD representative" (page 29)
- Under anesthesia, and with topical anesthetic, and topical povidone iodine
- "Injections in the right eye were alternated, such that the first injection was administered at approximately the 11 o'clock position, and the second injection was administered at approximately the 10 o'clock position. Injections in the left eye were alternated from approximately the 1 o'clock and 2 o'clock positions."

Dose volume: 50 µl/eye

Formulation/Vehicle: Aqueous solution:
- [(b) (4)] histidine/[(b) (4)]
- [(b) (4)] sodium chloride
- [(b) (4)] (w/w) polysorbate 20
- [(b) (4)] sucrose
- pH [(b) (4)]

Species/Strain:	Cynomolgus monkeys • obtained from (b) (4) (b) (4)
Age:	3 years 11 months, to 6 years 3 months old
Weight at start of dosing:	Males: 4.4 to 7.3 kg Females:3.0 to 4.1 kg
Medication regiment:	• After each dose: topical antibiotic (Tobrex®), and an agent to reverse the anesthetic (atipamezole) • D1 and D29: an NSAID (flunixin meglumine) im prior to sedation, and an opioid (buprenorphine) at 6 hours after dosing • D58 onward: an oral NSAID (meloxicam) and topical antibiotic/anti-inflammatory ointment (neomyxin and polymyxin with dexamethasone) from 2 days prior to 2 days after dosing
Unique study design:	• Two interim sacrifices were scheduled: ○ one group 3 male (# I00117) on D135 (2 doses + 13 week recovery), ○ one group 3 male (# I00115) on D218 (5 doses + 13 week recovery) • Vitreous fluid (100 µl/eye) was collected OU at necropsy. After collection, each eye was injected with Davidson's fixative until turgid, stored for 24-48 hours in Davidson's, and then transferred to 70% alcohol.

Observations and Results

Mortality

- No treatment-related mortalities or early sacrifices.
- Two unscheduled sacrifices: control male # I00110 on D64 (accidental humerus fracture), and control female # I00130 on D169 (moribundity attributed to renal dysfunction secondary to urinary bladder hemorrhage/inflammation).

Endpoints

"mortality, clinical observations, qualitative food consumption, body weights, neurological examinations, jacketed external telemetry electrocardiography (JET-ECG), ophthalmic observations, intraocular pressure measurements (IOP), spectral domain optical coherence tomography (sdOCT), fundus photography, fluorescein angiography (FA), electroretinography (ERG), and clinical and anatomic pathology, as well as immunohistochemistry. Blood samples were collected for toxicokinetic and antidrug antibody evaluations; Ang-2, Ang-1, and VEGF-A analyses; and compliment factors and proinflammatory cytokine assessments. Vitreous samples were collected at necropsy and analyzed for test article concentrations and antidrug antibody content.

Standard endpoints: Authors report **"**No test article-related morbidity or mortality occurred. No changes in clinical observations, food consumption, body weights, neurological examination, ECG, ERG, clinical pathology, organ weight, or macroscopic observations were attributable to RO6867461." (page 21)

Clinical signs

- No NOAEL for ocular irritation
- The low dose (0.5 mg/eye) was "well tolerated" with mild aqueous and vitreous cell.
- At 1.5 and 1.5/3.0 mg/kg, "severe anterior and posterior segment inflammatory response was sporadically observed"
    - aqueous flare, aqueous and vitreous cell, fibrin in the anterior chamber, keratic precipitates, inflammatory debris on the anterior lens capsules, incomplete pupil dilation following the topical application of a mydriatic, vitreous haze, white vitreous floaters, and white perivascular sheathing around retinal blood vessels
    - Dose suspended for two males at 1.5 mg (Group 3) and one male in Group 4 (1.5 → 3.0 mg) *due to severe ocular inflammation. One male at 1.5 mg only received 2 doses; the other animals received 5 or 6 doses.*
        - *One high-dose male was maintained during the recovery phase. The animals fully recovered by the end of the recovery phase.*

Ophthalmoscopy

- Digital fundus photography and OCT were consistent:
    - Fundus photography detected perivascular sheathing and hazy media at 1.5 and 1.5/3 mg/eye, beginning as early as *Day 88 (i.e., after the fourth dose)*
    - OCT detected increased retinal nerve fiber layer (RNFL) thickness at 1.5 and 1.5/3 mg/eye *and increased blood vessel size at 1.5/3 mg/eye* beginning at Week 13.

Histopathology

Adequate battery?	Yes, standard systemic battery, including: • Eye with bulbar conjunctiva • Eyelids (upper and lower, to include palpebral conjunctiva) • Lacrimal gland • Nasal turbinates • Nasopharynx • Optic nerve

- No treatment-related effects at 0.5 mg/eye
- At 1.5 and 1.5/3 mg/eye, minimal-to-slight mixed cell infiltrate was observed: inner retina, optic disk, and vitreous (Table 8: Applicant's table). The authors noted that these observations correlate with the perivascular sheathing and

vitreous cells noted by indirect ophthalmoscopy. *The finding was partially reversible*.

- Plasma cells were present in mixed cell infiltrate in the mid-dose male.

Table 8: Incidence and Severity of RO6867461-Related Microscopic Findings – Terminal Sacrifice

	RO6867461							
Sex	Males				Females			
Dose Level (mg/eye/dose)	0	0.5	1.5	1.5/3	0	0.5	1.5	1.5/3
Right Eye								
Number Examined	3	3	3	5	3	3	3	5
Infiltrate, mixed cell, inner retina/optic disc/vitreous								
Not Present	3	3	2	4	3	3	3	2
Minimal	0	0	1	0	0	0	0	1
Slight	0	0	0	1	0	0	0	2

- *Immunohistochemistry was conducted in 3 high-dose monkeys (# I00122, # I00142, and # I00143).* Immunohistochemistry detected:
  - "granular deposits containing test article *(human IgG),* monkey immunoglobulins"*, IgM and/or C3* in these areas of cellular infiltration *(considered to be evidence of immune complex-mediated reactions)*. The authors consider this evidence of an ADA-response.
  - *test article (human IgG), monkey IgG, IgM, and/or C3-containing granular deposits in the endothelium/subendothelium and tunica media of blood vessels in the retina in right eye tissue. These findings are characteristic of immune complex-mediated reactions.*
  - *increased staining of human IgG, monkey IgG, IgM, albumin, and/or C3 in resident/inflammatory cells (monocytes/macrophages).*

Systemic Histopathology

- The authors did not identify treatment-related systemic effects.
- This reviewer considers the heart squamous cyst/plaque finding to be treatment related, and consistent with the previously observed effects. As was noted previously, it is not clear whether these cardiac lesions are the result of the primary pharmacology of RO6867461 (and therefore relevant to patient safety), or if they are species-specific responses caused by ADA formation.
  - *The finding was not present in recovery animals.*
- Data from pdf pages 460, 473, 494, 540. These effects were graded as minimal, except for the squamous cyst/plaque (graded as present).

Lesion	Males				Females			
	0	0.5	1.5	1.5/3	0	0.5	1.5	1.5/3
Main-group (N = 3/sex/dose)								
Heart – mononuclear cell infiltrate	2/3	2/3	1/3	3/3	1/3	0/3	1/3	1/3
Inflammation, vessel	0/3	0/3	0/3	0/3	0/3	**1/3**	0/3	0/3
Squamous cyst/plaque [severity = present]	0/3	0/3	0/3	**2/3**	0/3	0/3	0/3	0/3
Recovery-group								
Heart – mononuclear cell infiltrate	**1/1**	0	0	0/3	0/1	0	0/2	0/3
Heart – degeneration / necrosis, myocardium [severity = minimal]	**1/1**	0	0	**1/3**	0/1	0	0/2	0/3

Right eyes were dosed with 0, 0.5, 1.5, or 1.5/3 mg/eye of RO687461. Left eyes were dosed with vehicle only.

- The literature[2] indicates that heart squamous cysts and squamous epithelial plaques are “exceedingly rare” in cynomolgus monkeys. Chamanza et al. 2006[3] reports an incidence of 0.3% (6 out of 2050 cynomolgus monkeys).
- The individual monkeys were:
  - # I00120 (high-dose male). Eye histopathology found minimal mononuclear cell infiltration in multiple ocular tissues. Serum ADA response was negative on D1 (pre-dose), positive on D29, D141, D184.
  - # I00125 (high-dose male). Eye histopathology found minimal mononuclear cell infiltration in multiple ocular tissues. Serum ADA was

[2] Kaspareit J, Friderichs-Gromoll S, Habermann G, et al. Spontaneous squamous cysts and squamous epithelial plaques in the heart of cynomolgus monkeys (Macaca fascicularis). Exp. *Toxicol Pathol* 2003: 54(4): 301-303. https://www.ncbi.nlm.nih.gov/pubmed/12710713

[3] Chamanza E, Parry N M A, Rogerson P, et al. Spontaneous lesions of the cardiovascular system in purpose-bred laboratory nonhuman primates. *Toxicol Pathol* 2006, 34: 357-363. http://journals.sagepub.com/doi/pdf/10.1080/01926230600809737

negative on D1 (pre-dose), negative on D29, positive on D141, positive on D184.
- Vitreous ADA was not detected in either animal.

Toxicokinetics

- The authors report that the "majority" of monkeys tested positive for ADA, which was associated with loss of systemic exposure (page 23).
  - *With repeated dosing, test article mean serum concentrations were lower than on Day 1 (comparative data at 24 hours postdose) (Table 10; Applicant's table)*
  - *Some animals had levels below LLOQ (2 ng/mL). These included, mid-dose female I00137 on Days 113 onward, mid-dose males I00115, I00116, and I00117 from Day 29 or Day 58 onward, high-dose female I00142 on Days 113 onward and high-dose males I00122, I00123, and I00126 from Day 85 or Day 113 onward (pages 2770 to 2773 in the Study Report).*
  - *Overall, these animals had high ADA titers (pages 2905 to 2918).*
- The TK summary in the Investigator Brochure is clearer than the summary in the Study Report:

Table 10 Mean Pharmacokinetic Parameters of RO6867461 after Multiple Intravenous and Intravitreal Administrations in Cynomolgus Monkeys in the Six Month GLP-Toxicology Study

		RO6867461, Mean (% CV)		
		Intravitreal Administration		
Parameter	Unit	0.5 mg/ eye/dose	1.5 mg/ eye/dose	1.5/3.0 mg/ eye/dose
Systemic PK Data after the First Dose				
No.	Male/Female	3/3	13/13	—
C_{max}	µg/mL	0.5 (36.0)	1.9 (36.6)	—
C_{max}/D	(µg/mL)/ (mg/kg)	1.0 (36.0)	1.2 (36.6)	—
t_{max}	h	48.0 (54.8)	48.5 (55.1)	—
$AUC_{(0-72)}$	(µg/h) • mL	29.7 (40.3)	107 (36.8)	—
$AUC_{(0-72)}/D$	(µg/h) • mL/ (mg/kg)	59.4 (40.3)	71.3 (36.8)	—
Systemic PK Data after the Last Dose				
No.	Male/Female	3/3	2/4	5/7
$C_{max\ (D141)}$	µg/mL	0.2 (64.2)	1.6 (27.1)	2.2 (66.0)
$C_{max\ (D141)}/D$	(µg/mL)/ (mg/kg)	0.4 (64.2)	1.1 (27.1)	0.7 (66.0)
t_{max}	h	48.0 (54.8)	56.0 (44.3)	64.0 (29.2)
$AUC_{(3360-3432)}$	(µg/h) • mL	11.6 (87)	91.4 (27.9)	130 (66.7)
$AUC_{(3360-3432)}/D$	(µg/h) • mL/ (mg/kg)	23.2 (87)	60.9 (27.9)	43.3 (66.7)
Vitreal Concentration at Termination				
No.	Male/Female	3/3	3/3	5/5
Vitreal concentration	µg/mL	4.9 (43.5)	15.8 (74.5)	24.5 (103)
Vitreal concentration/D	(µg/mL)/ (mg/kg)	9.7 (43.5)	10.5 (74.5)	8.2 (103)

AUC = area under the concentration–time curve; AUC/D = dose-normalized area under the concentration–time curve; C_{max} = maximum observed concentration; C_{max}/D = dose-normalized maximum observed concentration; t_{max} = time to maximum concentration.

Notes: Mean is calculated for both genders combined.

Vitreous humor was collected at terminal time point.

Source: Roche Report 1057630

Table 11 Detection of Anti-RO6867461 Antibodies in Cynomolgus Monkeys after Intravitreal Administration of RO6867461 during the Six Month GLP-Toxicology Study

Day of Treatment	RO6867461 ADA Positive/Total Number of Animals			
	Intravitreal Administration			
	Vehicle	0.5 mg/eye	1.5 mg/eye	1.5/3.0 mg/eye
Day 1	0/10	0/6	0/10	0/16
Day 29	—	—	—	7/16
Day 141	0/9	4/6	5/9	10/16
Day 184	0/8	4/6	4/9	9/16
Day RP 92	0/2	—	0/2	2/6

ADA = anti-drug antibody; RP = recovery period.
Source: Roche Report 1057630

- *In general, on Days 1 or 29 and 141, $AUC_{0\text{-}72\ hr}$ increased in a dose-proportional manner over the dose range tested (0.5, 1.5, and 3 mg/eye).*
- *The systemic exposure to faricimab on Day 141 decreased when compared to Day 1 or 29 for all dose levels.*
- *Overall, during the dosing and recovery phases, 20 out of 32 faricimab- treated animals were confirmed positive for ADA on one or more occasions postdose across dose levels.*
- *There was a correlation of positive ADA with reduced systemic exposure of faricimab in multiple animals, as noted above.*
- *Faricimab was below the detection limit in all the vitreous samples after 13 weeks of recovery phase.*
- *ADA analysis in vitreous samples showed that 1 out of 6 animals given 0.5 mg/eye was confirmed ADA positive in the right eye; 3 out of 10 animals given 1.5 mg/eye were confirmed positive for ADA in left or both eyes, and 5 out of 16 animals given 1.5/3 mg/eye were ADA positive in the right or both eyes.*

Special Assessments

Platelet Depleted Plasma Analysis

- *IVT administration of RO6867461 to cynomolgus monkey had no impact on Ang-1 or Ang-2 levels in the systemic circulation; VEGF levels in plasma-depleted samples were not quantifiable, likely due to use of platelet-depleted plasma.*
    - *VEGF was not quantifiable in all samples from vehicle and all dosed groups (the lower limit of quantitation [LLOQ] was 31.3pg/mL). It was initially expected that measurable levels of VEGF would be present in*

plasma, based on legacy data generated using standard plasma. In this study, platelet-depleted plasma was prepared, and as large amounts of VEGF are stored in platelets, this may explain why all measurements here were below the LLOQ.

- *No correlation was observed between Ang-1 concentrations and drug levels. Overall, Ang-1 levels ranged from BLQ (< 234pg/mL) to 44,100 pg/mL in the vehicle group and from BLQ to 77,500 pg/mL in the RO6867461-treated groups.*
- *No correlation was observed between Ang-2 concentrations and drug levels. Overall, Ang-2 levels ranged from 4530 pg/mL to 21,000 pg/mL in the vehicle group and from 2990 pg/mL to 23,000 pg/mL in the RO6867461-treated groups.*
- *Note: Ang-1 and Ang-2 concentration data was not fully reviewed because tabulated mean data was not included. There was high intergroup variability, which could confound the determination of a test article related change.*

Complement Factors and Proinflammatory Cytokine Plasma Assessments

- *For both complement analytes tested (C3a and C5a), no clear signs of test article-related increases were noted. Both C3a and C5a remained essentially at Day 1 predose levels in all groups at all time points.*
- *For the cytokine s and chemokines measured, no clear test article-related effects were noted. These included TNFα, IFNγ, MCP-1, IL-1β, IL-6, IL-8, IL-12/23.*

Dose Formulation Analysis

The results of Day 1, Day 29, and Day 141 formulation analysis were within +/- 10% of the targeted protein concentrations. The result from UV concentration measurement of the end-of-use sample showed the test article was stable over the whole course of the study.

7 Genetic Toxicology

In accordance with ICH S6(R1) guidance, standard genotoxicity studies were not conducted with faricimab. Direct DNA interaction is not expected for a recombinant humanized IgG1 bi-specific mAb.

8 Carcinogenicity

No standard rodent carcinogenicity studies have been conducted as faricimab showed no relevant binding to mouse or rat VEGF-A. The Applicant provided a

Carcinogenicity Assessment Document (Study # 1104409; Module 4.2.3.7.7). Some excerpts that support low risk for carcinogenicity include:

- Monoclonal antibodies (mAbs) are not deemed to have an intrinsic carcinogenic potential. Therefore, faricimab and its metabolites (oligo peptides and amino acids) are unlikely to be tumor initiators.
- In oncology, high levels of ANG-2 correlated with increased angiogenic, metastatic, and invasive potential in multiple cancer types.
- Targeting both ANG-2 and VEGF-A, has demonstrated potent anti-tumoral and anti-angiogenic activity in various cancer models[4, 5].
- VEGF-A$^{-/-}$ embryonic stem (ES) cells exhibit a dramatically reduced ability to form tumors in nude mice[6]. Wild-type ES cells formed rapidly growing tumors, whereas VEGF$^{-A-/-}$ ES cells had dramatically impaired ability to grow in vivo.
- The molecular structure of proteins such as mAbs does not suggest provoking carcinogenicity or malignant transformation of cells and tissues via mechanisms involving direct DNA damage: IgG macromolecules do not penetrate cell membranes like small molecules and are, therefore, not considered to have direct interactions with cellular DNA.
- There was no evidence of hyperplasia or pre-neoplastic changes in the histopathological examinations of organs and tissues in toxicology studies conducted with faricimab in rabbits or cynomolgus monkeys.
    - In monkeys, animals were sacrificed following a 6-month dosing period, or a 13-week recovery phase. In the 6-month monkey toxicology study, systemic exposures at the highest dose were 8-10-fold greater than faricimab steady-state exposure estimates in nAMD and DME patients, respectively. Overall, the systemic exposure observed in clinical studies following IVT dosing of 6 mg/eye of faricimab was very low.
- The anti-angiogenic mechanism of action of faricimab is per se not considered to impair immune surveillance. This is supported by data from the clinical development program indicating that the achieved low systemic exposures to faricimab were not associated with overt negative immune-modulatory activities on the innate or adaptive immune system. In line with these results, toxicology studies in cynomolgus monkeys up to 6 months provided no evidence of any impact of faricimab on the immune system, i.e., for hematology examinations and histopathological examinations of lymphoid organs.
- There is clinical experience with various marketed antibodies or antibody-based therapies (bevacizumab and ranibizumab) targeting VEGF-A, which is blocked by faricimab. There are currently no approved therapies targeting ANG-2, or

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[4] Bessho H, Wong B, Huang D, et al. Effect of Ang-2-VEGF-A bispecific antibody in renal cell carcinoma. *Cancer Invest* 2015, 33(8): 378-386.

[5] Mueller T, Freystein J, Lucas H, et al. Efficacy of a bispecific antibody co-targeting VEGFA and Ang-2 in combination with chemotherapy in a chemoresistant colorectal carcinoma xenograft model. *Molecules* 2019, 24(16): 2865.

[6] Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996, 380: 439-442.

inhibiting both VEGF-A and ANG-2. For these marketed antibodies, no studies have been reported to evaluate carcinogenicity. For bevacizumab and ranibizumab, no increased risk for tumors in patients has been reported.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

In the 6-month repeat-dose IVT toxicity study in sexually mature cynomolgus monkeys (Study # 1057630), no effects on the male or female reproductive organs were observed up to the highest dose level tested, i.e., an initial injection of 1.5 mg followed by 6 monthly injections of 3 mg in the right eye. The assessment included organ weights (epididymis, prostate, testis, ovary, uterus) and histopathology (epididymis, prostate, testis, ovary, uterus/cervix, vagina). The systemic exposure after the last dose at 1.5/3.5 mg (right eye) in the 6-month IVT toxicity study was 2.2 µg/mL, providing an exposure margin of ~10X the clinical C_{max} values (0.22 or 0.23 µg/mL, see proposed label).

As stated by the Applicant (Module 2.6.6 Toxicology Written Summary, page 17), the pharmacological inhibition of angiogenesis by faricimab is generally expected to have adverse consequences on the female reproductive cycle, since angiogenesis plays a critical role in ovarian and endometrial function. The Applicant explained that menstrual cycling was not explicitly monitored in the 6-month monkey study as it would not have contributed to human risk assessment in view of the already established risk of perturbation of female reproductive function.

As the mechanism of action support a theoretical risk for effects on fertility, this reviewer agrees with including language in the proposed label that faricimab may pose a risk to reproductive capacity (Sections 8.3 and 13.1). It is worth noting that although no effects were observed in organ weights and histopathology of reproductive organs in the 6-month ocular toxicity study, the study did not include evaluations of more thorough measures (e.g., sperm motility and viability, menstrual cyclicity, etc.) to help minimize clinical concern.

9.2 Embryonic Fetal Development

Study title: RO6867461: Intravenous Administration Embryofetal Development Study in the Cynomolgus Monkey

Study no.:	1093222
Study report location:	Docubridge Module 4.2.3.5 \\CDSESUB1\evsprod\bla761235\0001\m4\42-stud-rep\423-tox\4235-repro-dev-tox\42352-embryo-fetal-dev\1093222.pdf
Conducting laboratory and location:	(b) (4)
Date of study initiation:	May 17, 2019
GLP compliance:	Yes Exceptions: The following investigation was performed under non-GLP conditions: • Monkey Chorionic Gonadotropin (mCG) Analysis • Interpretation of Toxicokinetic and Immunogenicity Results
QA statement:	Yes
Drug, lot #, and % purity:	Faricimab (RO6867461), lot # GLI0271-01, 99.2%

Key Study Findings

- There was an increase incidence in pregnancy loss in test article-treated monkeys. The increase was not dose dependent. The Applicant provided a reference to support the incidence was within normal variability for the species. However, this reviewer believes that given the increased incidence compared to the concurrent control, a test article-related effect cannot be totally ruled out. The lack of a dose-response is not unusual for antibody-like molecules.
- No other maternal or fetal adverse effects were observed.
- The low dose, 1 mg/kg, is 10X the recommended clinical dose of 6 mg (0.1 mg/kg).
- Per information in the proposed label, mean (±SD) free faricimab (unbound to VEGF-A and Ang-2) plasma C_{max} are 0.23 (0.07) µg/mL and 0.22 (0.07) µg/mL in nAMD and in DME/DR patients, respectively. Using a C_{max} of 36.4 µg/mL observed on GD 20 (1-hour postdose) in the monkey at the low dose, the exposure margin is 158X.
    - As the abortions occurred from GD 26 to GD 30, the GD 48 exposure was not used.

Methods

Doses:	0, 1, and 3 mg/kg
Frequency of dosing:	Once weekly on gestation days (GD) 20, 27, 34, 41, and 48
Dose volume:	1 mL/kg
Route of administration:	IV
Formulation/Vehicle:	Vehicle for Faricimab
Species/Strain:	Cynomolgus monkeys
Number/Sex/Group:	18 pregnant monkeys/group
Satellite groups:	None
Study design:	An EFD study was conducted (instead of an ePPND), with dosing only during organogenesis, to reduce the impact of immunogenicity in the pregnant cynomolgus monkeys. C-section was conducted on GD100. Maternal and fetal TK and ADA development were measured.
Deviation from study protocol:	None with an impact on data interpretation or study validity

Observations and Results

Mortality (Twice daily)

None test article related; one control animal was prematurely sacrificed on GD 80. The animal had poor health condition, severe body weight loss, and an intrauterine death.

Clinical Signs (Twice daily; detailed inspection once a week)

Abortion was confirmed in the following animals (see more details below):

Animal #	Sex	Group	Category, Observation	Days Seen
P0018	F	1	Abortion, abortion confirmed (Ultrasound)	Gestation: 80
P0104	F	2	Abortion, abortion confirmed (Ultrasound)	Gestation: 38
P0108	F	2	Abortion, abortion confirmed (Ultrasound)	Gestation: 26
P0111	F	2	Abortion, abortion confirmed (Ultrasound)	Gestation: 44
P0112	F	2	Abortion, abortion confirmed (Ultrasound)	Gestation: 27
P0117	F	2	Abortion, abortion confirmed (Ultrasound)	Gestation: 30
P0206	F	3	Abortion, abortion confirmed (Ultrasound)	Gestation: 30
P0214	F	3	Abortion, abortion confirmed (Ultrasound)	Gestation: 30
P0217	F	3	Abortion, abortion confirmed (Ultrasound)	Gestation: 27

Group 1: control
Group 2: low dose, 1 mg/kg
Group 3: high dose, 3 mg/kg

Body Weight (Weekly starting on GD 20)

No test article-related effects

Toxicokinetics (Blood samples collected from maternal animals on GD 20 and GD 48 predose and 1-hour postdose. On GD 100 (day of c-section), blood samples collected from maternal and fetal blood [from umbilical cord])

All animals showed exposure to faricimab at the 1-hour postdose evaluations on GD 20 and GD 48. After the first IV administration, plasma concentration increased proportionally with the dose (1 and 3 mg/kg). The between-animal variability, as determined from the coefficient of variation (CV in %), of faricimab levels was low, with values of 16.7% at the low dose and 16.8% at the high dose.

At the predose blood collection on GD 48, individual animal data showed the exposure was low or below limit of quantification (BLQ). After the fifth and the final IV administration to the mothers on GD 48, mean plasma concentration values of 23400.7 ng/mL and 47734.0 ng/mL were observed at the low and high dose, respectively. The exposure in terms of plasma concentration increased less than proportionally with the dose. The between animal variability, as determined from the CV of faricimab levels was high, 67.4% for Group 2 animals and 107.9% for Group 3 animals. At least 3 animals (low dose # P0114 and # P0118, and high dose # P0205) showed low faricimab levels (5.19 to 29.5 ng/mL) at 1 hour postdose on GD 48. These animals had high ADA titer, but other animals with similar ADA titers did not show a marked decrease in faricimab concentration. The high variability was considered related to ADA development.

None of the maternal animals or their fetuses showed exposure to faricimab at cesarean section on GD 100 (~52 days after last IV administration to the mother).

Table 8 (Applicant's table) shows the maternal and fetal mean exposure.

Table 9: Maternal and Fetal PK Parameters

Treatment Duration	Species/ Test System	No of Animal	Dose (mg/kg/dose) (Group)	Mean observed C_{max} (µg/mL)	CV (%)	Mean observed C_{max}/Dose (µg/mL)/ (mg/kg/dose)
4 weeks (once weekly)	Monkey Cynomolgus Females			Day GD 20 1 hour postdose, all animals		
		18	0 (G1)	BLQ	NA	BLQ
		18	1 (G2)	36.4	16.7	36.4
		18	3 (G3)	108.7	16.8	36.2
				Day GD 48 1 hour postdose, all animals		
		18	0 (G1)	BLQ	NA	BLQ
		13	1 (G2)	23.4	67.4	23.4
		15	3 (G3)	47.7	107.9	15.9
				Day GD 48 1 hour postdose, animals with ADA titer > 320 excluded		
		18	0 (G1)	BLQ	NA	BLQ
		5[b]	1 (G2)	37.5	13.7	37.5
		5[c]	3 (G3)	114.2	16.7	38.1
				Day GD 100		
		17	0 (G1)	BLQ	NA	BLQ
		13	1 (G2)	BLQ	NA	BLQ
		15	3 (G3)	0.0002[a]	NA	BLQ
	Fetus	17	0 (G1)	BLQ	NA	BLQ
		13	1 (G2)	BLQ	NA	BLQ
		15	3 (G3)	BLQ	NA	BLQ

a Animal P0207 had a concentration of 0.00275 µg/mL

b Animals P0106, P0110, and P0116 were ADA negative. Animal P0113 revealed a titer of 80 and Animal P0103 had a titer of 320 at GD 48, 0 hours. The exposure results from these 5 animals were used for the calculation of mean plasma concentration. For 4 animals (P0107, P0109, P0114, and P0115), there was not sufficient sample volume left to perform titration of the GD 48 positive ADA samples. Because the titer for these 4 animals is unknown, the TK results could not be taken into consideration for the calculation of Mean plasma concentration.

c All animals in G3 developed ADAs. The following 5 animals developed a weak ADA response with titers ≤ 320 at GD 48 (P0202, titer 80; Animal P0204 titer 320; Animal P0210 titer 160; Animal P0212 titer 10, Animal P0213 titer 10). These 5 animals were included in the calculation of the mean plasma concentration. For 3 animals (Animals P0203, P0205, and P0216), there was not sufficient sample volume left to perform titration of the GD 48 positive ADA samples. Because the titer for these three animals is unknown, the TK results could not be taken into consideration for the calculation of mean plasma concentration.

ADA Formation (Same collection timepoints as for TK):

Right before the fifth and final dose on GD 48, ADAs were detected in 1/18 control mothers (titer 10), 10/13 low dose mothers (titer range 80 to 1280) and 15/15 high dose mothers (titer range 10 to 40960).

At the end of the study on GD 100, ADAs were detected in 0/17 control mothers, 10/13 low-dose mothers (titer range 160 to 20480) and 14/15 high-dose mothers

(titer range 640 to 40960).

The ADA incidence in fetuses were 0/17, 7/13 and 14/15 in control, low-dose, and high-dose groups, respectively.

Dosing Solution Analysis

Formulations prepared for the first and last doses were tested. The protein concentration results for all tested dose formulation samples were within ± 10% of the theoretical concentration.

Necropsy (GD 100 ±1 day)

No test article-related findings

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

There was an increase incidence in pregnancy loss in test article-treated monkeys (see Table 9 below; Applicant's table):

Table 10: Pregnancy Loss

Test Item	(dosage)	1	2	3
RO6867461	mg/kg on GD 20, 27, 34, 41, 48	0	1	3

Group/Sex:	1/F	2/F	3/F
Number of Pregnant Females:	18	18	18
Total number of females with abortion	1	5	3
Percentage of pre-natal loss	5.6	27.8	16.7
Total number of dead fetus at cesarian section	0	1	0
Total number of live fetus at cesarian section	17	12	15

Control group: The control animal showed a body weight loss of 700 g from GD 55 to GD 76. Due to this high body weight loss an unscheduled ultrasound was performed on GD 80. No fetal heartbeat was detected, and the uterine content did not correspond to a live pregnancy on the day of gestation.

Low-dose group: Five animals aborted. Since 4 of 5 abortions in this group were detected prior to or on GD 30, the serum sample was analyzed for chorionic gonadotropin (mCG) level on GD 20. The values exceeded 5 ng/mL and the animals were considered pregnant. Some of these animals showed vaginal bleeding starting at GD 23, GD 26, or GD 30. The ultrasound showed no detectable embryonic heartbeat or was negative for pregnancy (abortion was presumed).

High-dose group: Three animals aborted, all of them detected on GD 30. The animals had negative ultrasound or heavy vaginal bleeding starting on GD 20.

The results of mCG determination indicated that all animals with negative ultrasound results prior to or on GD 30 were initially pregnant on GD 20.

It was stated in the Study Report that pregnancy loss, although higher in test article-treated monkeys, it remained within normal variability for the species[7]. Considering the unusually low incidence of pregnancy loss in the control group, the lack of dose-relationship and comparison with historical control data, the greater incidence in the treated groups was considered incidental.

The publication referenced evaluated studies conducted by (b) (4) (performing lab). The data showed that pregnancy losses were most likely to occur up until GD 50 and around birth and were lowest between GD 100 and GD150. Based on the percentage presented on Table 1 of the publication (copied below), the data showed a 3% pregnancy loss for GD 21-30 (data from 1990 and later). The overall incidence in test article-treated groups in the current study is 8/36 or 22%. As such, it appears that a test article-related effect cannot be totally ruled out. The lack of a dose-response is not unusual for antibody-like molecules.

Table 1
Distribution of the Number of Losses Between Gestation Days 21 and 100 by Year Study Commenced and Route of Administration

		Route of administration					
		ig	im	iv	iv inf	sc	Total
Studies with pre-1990 start date	Number of pregnant animals at start of study	228	18	53	12	36	347
	No. losses day 21–30 (%)	20 (9)	0 (0)	4 (8)	2 (17)	5 (14)	31 (9)
	No. losses day 31–50 (%)	28 (12)	2 (11)	6 (11)	2 (17)	5 (14)	43 (12)
	No. losses day 51–75 (%)	15 (7)	1 (6)	4 (8)	3 (25)	3 (8)	26 (7)
	No. losses day 76–100 (%)	4 (2)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1)
Studies with start date of 1990 or later	Number of pregnant animals at start of study	262	245	93	108	14	722
	No. losses day 21–30 (%)	10 (4)	2 (1)	3 (3)	4 (4)	1 (7)	20 (3)
	No. losses day 31–50 (%)	14 (5)	17 (7)	1 (1)	5 (5)	1 (7)	38 (5)
	No. losses day 51–75 (%)	11 (4)	5 (2)	2 (2)	6 (6)	1 (7)	25 (3)
	No. losses day 76–100 (%)	2 (1)	7 (3)	0 (0)	2 (2)	0 (0)	11 (2)

Ig, intragastral; im, intramuscular; iv, intravenous injection; if inf, intravenous infusion; sc, subcutaneous. Difference in losses between studies that started prior to 1990 and thereafter are statistically significant ($P < 0.0001$). Therefore, the analysis of the effects of route of vehicle administration was confined to studies with start date of 1990 or later. Differences in losses did not attain statistical significance ($P > 0.05$).

Mean placental weight at the faricimab-treated groups was slightly higher than that in the concurrent control group (Table 10; Applicant's table). Statistical significance was not attained. It was stated in the Study Report that since the placental weight range

[7] Jarvis P, Srivastav S, Vogelwedde E, et al. The Cynomolgus monkey as a model for developmental toxicity studies: variability of pregnancy losses, statistical power estimates, and group size considerations. *Birth Defects Res B Dev Reprod Toxicol* 2010, 89(3): 175-187.

in the dose groups was close to that in the control group and within the normal variability, this difference was considered incidental. Therefore, this reviewer agrees with the Study Report authors assessment.

Table 11: Mean Placental Weight and Weight Range

	Mean	Range
Group 1	53.5 g	39.6 - 64.7 g
Group 2	57.4 g	42.5 - 73.0 g
Group 3	59.8 g	44.0 - 77.1 g

Mean fetal weight was slightly greater at 3 mg/kg faricimab (Table 10; Applicant's table). Statistical significance was not attained and the fetal weight range in the high dose group was close to that in the concurrent control group. The Applicant noted that the values were within the range of normal variability. Therefore, this finding was not considered to be related to faricimab in the Study Report. This reviewer agrees with the Study Report authors assessment.

Table 12: Mean Fetal Weight and Weight Range

	Mean	Range
Group 1	111.6 g	77.8 - 128.6 g
Group 2	112.2 g	95.6 - 126.8 g
Group 3	116.6 g	87.4 - 135.6 g

Offspring (Malformations, Variations, etc.)

No test article-related findings

9.3 Prenatal and Postnatal Development

No studies were conducted.

Additional reproductive toxicity comments:

Per information in the Carcinogenicity Assessment Document (Study # 1104409; Module 4.2.3.7.7), there is a theoretical risk for embryofetal developmental toxicity and postnatal development supported by the published literature, as noted below:

Genetically engineered rodent models for knockout of the targets VEGF-A and ANG-2 are available.

In heterozygous VEGF-A-deficient (VEGF-$A^{+/-}$) embryos, formation of blood vessels was abnormal, but not abolished. In contrast, the level of impairment in the blood vessel formation resulted in death at mid-gestation in homozygous VEGF-A-deficient (VEGF-$A^{-/-}$) embryos. As a consequence, no viable F1 VEGF-$A^{+/-}$ offspring

were obtained at birth and embryos displayed macroscopic and microscopic abnormalities in embryonic development and vascular growth. VEGF-A deficiency impaired most steps of early vascular development[8, 9].

ANG-2 is dispensable for embryonic vascular development, but is a requisite for subsequent angiogenic remodeling. Mice lacking ANG-2 also exhibit major lymphatic vessel defects. The primary role of ANG-2 is considered in postnatal angiogenic remodeling involving vessel sprouting and regression. Mice lacking ANG-2 generally die within two weeks of birth. Although overtly normal at birth, homozygous ANG-$2^{-/-}$ mice suffer from severe chylous ascites and lymphatic dysfunction shortly after feeding with hypoplasia of the lymphatic vasculature[10]

Additional references to published studies showing that ANG-2 is expressed at sites of vascular remodeling in the embryo and placenta and is required for postnatal angiogenesis were provided by the Applicant in Module 2.6.6 Toxicology Written Summary, pages 17 to 18).

As noted by the Applicant (Toxicology Written Summary, page 18): "*IgGs are transferred across the placenta in humans and in non-human primates by the FcRn transport system starting during the second half of gestation [DeSesso et al. 2012]. Faricimab is a humanized bispecific IgG1 antibody with abolished FcRn binding and does not therefore cross the placenta, as confirmed in the EFD study. The lack of embryonic exposure, however, does not preclude the risk of harm to pregnancy due to the important role of angiogenesis in formation of the placenta. Human safety information on faricimab related to pregnancy and lactation is not available, precautions for pregnant women should be proposed (refer to 2.7.4 Summary of Clinical Safety, Section 12.4). Based on the anti-angiogenic mechanism of action, treatment with faricimab may pose a hazard to embryofetal development (including teratogenicity) and reproductive capacity.*"

This reviewer agrees with the Applicant conclusions. It is also worth noting that faricimab binding affinities in humans, 0.5 to 3 nM (73 to 438 ng/mL) for VEGF and 20 nM (2923 ng/mL) for Ang-2, support anti-VEGF activity at the clinical C_{max} of 220 to 230 ng/mL.

10 Special Toxicology Studies

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[8] Carmeliet P, Ferreira V, Breier G, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature* 1996, 380(6573): 435-359.

[9] Ferrara N, Carver-Moore K, Chen H, et al. Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature* 1996, 380(6573): 439-442.

[10] Gale N W, Thurston G, Hackett S F, et al. Angiopoietin-2 is required for postnatal angiogenesis and lymphatic patterning, and only the latter role is rescued by Angiopoietin-1. *Dev Cell* 2002, 3(3): 411-423.

A Tissue Cross-Reactivity Study of RO6867461 in Normal Human Tissues (Study # 1056445) – This study was previously reviewed under the initial IND (Janice Lansita, PhD, 9-23-2013). Per Dr Lanista's conclusions, faricimab (RO6867461) showed positive staining of cytoplasm and/or cytoplasmic processes in the vascular endothelium, reticular cells, hematopoietic cells, mononuclear leukocytes, vascular smooth muscle, and placental trophoblasts; as well as extracellular material in bone marrow, kidney, lung, lymph node, salivary gland, skin, and uterus-endometrium

Pilot tissue cross-reactivity study (# 1055832) in a limited panel of human tissues was also reviewed under the initial IND. The results were similar to those of the pivotal study. The cytoplasm and extracellular material distribution was consistent with the secreted nature of both VEGF and Ang2.

In Vitro Evaluation of RO6867461 in a Human Complement Activation Assay for the Pre-clinical Risk Assessment of Anaphylatoxins and Complement Split Fragment Generation (Study # 1120D14) - This assay was based on ELISA measurements of the anaphylatoxins C3a and C5a released in plasma after a 45 min-incubation of the test item with fresh human blood from 10 healthy individuals. Following faricimab (0.1 to 100 µg/ml) stimulation, no dose-dependent increase of C3a and/or C5a were observed. The median ratios (faricimab/PBS) ranged from 0.93 to 1.06 for C3a and 1.42–1.47 for C5a. In contrast, the positive control (Lemtrada) median ratios (Lemtrada/PBS) were up to 3.19 for C3a and 3.47 for C5a.

The results do not support a significantly increased risk of anaphylatoxin release (<1.5-fold increase) or a dose-dependent activation of the complement cascade for faricimab.

11 Integrated Summary and Safety Evaluation

The monkey was selected as the most relevant species based on 100% sequence homology (VEGF) and binding affinity (Ang-2) comparable to humans. In initial toxicology studies, the rabbit developed severe toxicity observed at low IVT doses (1.5 mg/eye) following a single dose. The rabbit was not considered to be a suitable species for repeat-dose chronic toxicology studies.

In monkeys administered monthly faricimab IVT doses, the main ocular finding was severe anterior and posterior ocular inflammation. In the pivotal 6-month IVT toxicity study, ophthalmoscopy findings at doses of 1.5 mg and/or 1.5 mg/3.0 mg (initial dose of 1.5 mg/eye, followed by 3.0 mg/eye) included aqueous flare, aqueous and vitreous cell, fibrin in the anterior chamber, keratic precipitates, inflammatory debris on the anterior lens capsules, incomplete pupil dilation following the topical application of a mydriatic, vitreous haze, white vitreous floaters, and white perivascular sheathing around retinal blood vessels. Dosing was suspended for two males (males # I00115 and # I00117) at the mid-dose (1.5 mg/eye) and one male (# I00126) at the high dose (1.5

mg/3.0 mg) due to severe ocular inflammation. One male at 1.5 mg only received 2 doses, the other animals received 5 or 6 doses.

Fundus photography detected perivascular sheathing and hazy media at 1.5 and 1.5/3 mg/eye, beginning as early at Day 88 (i.e., after the fourth dose). OCT detected increased retinal nerve fiber layer (RNFL) thickness at 1.5 and 1.5/3 mg/eye and increased blood vessel size at 1.5/3 mg/eye beginning at Week 13. Microscopically, minimal-to-slight mixed cell infiltrate was observed in several ocular tissues (inner retina, optic disk, and vitreous).

Some key findings and correlation with the presence of ADA in the vitreous or serum are shown in the table below (reviewer's table).

Table 13: Key Findings and Correlation with ADA in Vitreous and Serum

Dose	Animal #[a]	Keratic precipitate – Slit lamp	RNFL-OCT	↑Blood vessel size - OCT	Perivascular Sheathing – indirect ophthalmoscopy/fundus photography	Vitreous haze 3+,4+/Degraded view fundus – indirect ophthalmoscopy	Mixed-cell infiltrate-histopath	ADA Titer	
								Vitreous[b]	Serum (Day 141)
1.5 mg	M114							N	0.0205
	M115	X	X		X	X	X	2.84	3.05
	M116		X				X	0.488	3.10
	M117	X	X		X	X		QNS	3.05
	M118							N	0.0210
1.5/3.0 mg	M119							N	0.0235
	M120							N	3.00
	M121							N	0.0210
	M122		X	X	X		X	0.364	3.10
	M123							0.0795	3.18
	M124							N	0.0215
	M125							N	0.134
	M126	X	X	X	X	X		2.71	3.11
1.5 mg	F135							N	0.0215
	F136							N	0.0470
	F137							N	3.13
	F138							N	0.0210
	F139							N	0.0996
1.5/3.0 mg	F140							N	1.82
	F141		X	X			X	QNS	3.09
	F142	X	X	X	X		X	2.82	3.09
	F143		X	X	X		X	2.78	3.08
	F144		X					N	0.0575
	F145							N	0.0190
	F146							N	0.0215
	F147							N	0.0215

[a]Animal number only include the last 3 digits; actual numbers start with I00 (e.g., I00114); M = male; F = female
[b]QNS = Quantity Not Sufficient; insufficient sample volume for analysis; N = Negative results

As shown in Table 13, most monkeys with ocular findings were positive for vitreous ADAs and also had high serum ADA titers. The ocular findings were not present in animals negative for ADAs in the vitreous; serum ADAs were low in most of these animals showing no ocular findings. These data support that the ocular findings were primarily related to ADA formation and not a direct pro-inflammatory effect of faricimab.

In addition, to support that the ocular inflammation was primarily related to the formation of ADAs, the Applicant conducted immunohistochemistry evaluation in 3 high-dose animals. The key findings included:

- granular deposits containing test article (human IgG), monkey immunoglobulins, IgM and/or C3 in the ocular tissues with mixed cell infiltrates
- test article (human IgG), monkey IgG, IgM, and/or C3-containing granular deposits in the endothelium/subendothelium and tunica media of blood vessels in the retina
- increased staining of human IgG, monkey IgG, IgM, albumin, and/or C3 in resident/inflammatory cells (monocytes/macrophages).

As noted by the Applicant, the immunohistochemistry findings were consistent with an immune-mediated (immune-complex) basis with complement activation for the associated pathology in the right (treated) eye from all 3 high-dose monkeys examined.

The NOAEL for ocular inflammation was the low dose (0.5 mg/eye; 0.25 mg/mL vitreous), 0.17X the intended clinical dose of 6 mg (1.5 mg/mL vitreous). Immunogenicity in animals is not always predictive of immunogenicity in humans. The Applicant stated on page 16 of the Toxicology Written Summary:

"The overall incidence of ADAs and intra-ocular inflammations was low, and no meaningful impact of ADA was observed on overall safety. Although a higher incidence of intra-ocular inflammation was observed in ADA-positive patients compared to ADA-negative patients, this observation is not currently considered to be clinically relevant."

If immunogenicity in the monkey is considered not predictive of the potential immunogenicity of faricimab in humans, the NOAEL is the high dose of 3.0 mg/eye (1.5 mg/mL vitreous), 1X the intended human dose.

Heart as a target organ

This issue was previously addressed by Dr McDougal (8/30/2017, IND 119225). Pharm/Tox considered these lesions to be treatment related. Some excerpts in the nonclinical review of Dr. McDougal are copied below:

From the previous P/T review (Lansita, 9/23/2013, IND 119225):

- GLP 2-month IVT and IV toxicology study in monkeys (report # 1053361)

  - 0, 1.5, 3, or 6 mg OD IVT once monthly x 3 (D1, D29, D57). Main-group 3/sex/dose. Recovery (2/sex) for the control and high-dose group.
  - 0 or 5 mg/kg IV (also once monthly x 3) [5/sex/dose]
  - Two high-dose recovery males, one at 6 mg/eye IVT and one at 5 mg/kg IV, exhibited "minimal mixed cell inflammation of the aortic root/valve of the heart". The authors attributed this finding to species-specific "immune-mediated (immune-complex) basis with complement activation." P/T concluded that insufficient information was available to reach a conclusion as to whether the effect was direct pharmacology relevant to patients, or a species-specific ADA response.
- Non-GLP 2-week tolerance IVT and IV study in rabbits (report # 1053362)
  - Dutch Belted rabbits, 2/sex/group [no untreated or vehicle control group]
  - IVT: 1.5, 3 or 6 mg/eye
  - IV: 3 or 10 mg/kg
  - Dosing: every 14 days x 2 (D1 and D15)
  - Histopathology of the heart detected "degeneration/necrosis, myocardium" at 3 mg IV (minimal for 1/2 males and 1/2 females), 10 mg IV (minimal for 1/2 males, slight for 2/2 females).
  - No heart lesions detected for the IVT groups.
  - The effect was considered adverse, and restricted to the right ventricular free wall.
- The non-GLP 2-week monkey IVT and IV tolerability study (report # 1053363) identified the IVT and IV high-doses as NOAELs. The P/T review notes one presumably incidental finding, "vasculopathy of an extraocular vessel adjacent to the optic nerve in one male given 3 mg/kg IV"'
- The 6-month IVT toxicology study (report # 1057630) detected heart squamous cyst/plaque in 2 high-dose monkeys (2/12).
  - Systemic PK for the high dose: Cmax = 2.2 µg/ml and AUC = 130 µg•hr/ml after the last dose.
  - For clinical trial PB28936, the 6.0 mg/eye dose MAD resulted in a mean C_{max} of 0.116 µg/ml and a mean AUC of 35.2 µg•hr/ml.

	6-month monkey data: high-dose (1.5/3.0 mg/eye) PK [LOAEL – heart toxicity]	Clinical trial BP28936: data for the high-dose (6.0 mg/eye MAD)	**Exposure margin**
Cmax (µg/ml)	2.2	0.116	**18.9655 x**
AUC (µg*hr/ml	130	35.2	**3.693 x**

Under the initial IND, the Applicant provided preliminary data that support immune complex deposition in the aorta (see Dr. Lansita's review, 9-23-2013). However, Dr Lansita stated that since the test article was not detected in association with monkey IgG, monkey IgM, and complement, it remained unclear whether faricimab mediates inflammation directly or indirectly via an ADA response. In the 6-month study, immunohistochemistry data supported immune complex deposition in the eye, but the

heart tissue was not evaluated. Overall, the heart findings were of minimal severity. The Applicant stated that faricimab was well tolerated in the clinical trials and based on all available data to date, no meaningful impact of ADA was observed on efficacy, pharmacodynamics, or on overall safety in the clinical studies (Module 2.5 Clinical Overview, page 36). Therefore, the weight of evidence supports the heart findings are of minimal clinical concern.

No unexpected tissue binding of faricimab was observed in cross-reactivity studies of normal human tissues, and the results of in vitro cytokine release assays indicated no substantial risk of cytokine release syndrome, direct complement activation, or peripheral immune-cell depletion.

An embryofetal development (EFD) study in cynomolgus monkeys showed an increase incidence of abortions in faricimab treated monkeys at IV doses of 1 or 3 mg/kg administered weekly on gestation days 20 to 48. No other maternal or fetal parameter showed a test-article related effect (see more details under Section 9 Reproductive and Developmental Toxicology of this review). Based on the increase incidence of the abortions, a test article related effect cannot be ruled out. At the low dose, the exposure margin (based on C_{max}) is 158X the intended 6 mg/eye clinical dose.

In conclusion, the nonclinical data provide adequate information to support labeling and safety characterization. Although, the nonclinical data is insufficient to address whether the heart findings are a species-specific ADA response or a direct pro-inflammatory effect of faricimab, together with the clinical data, the weight of evidence supports minimal clinical concern. From the nonclinical perspective, approval is recommended.

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/s/

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