Phase 1b/2a study of OPT-302 in combination with aflibercept for persistent central-involved diabetic macular edema

Opthea Protocol Number OPT-302-1003

Clinical Study Sponsor:

Opthea Ltd.
Level 4, 650 Chapel Street
South Yarra, VIC 3141 Australia

[redacted]

Confidentiality Notice

This document contains confidential information belonging to Opthea Ltd. This document must not be disclosed to anyone other than the study staff and members of the independent ethics committee / institutional review board. The information in this document cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Opthea Ltd. For all study-related questions, contact the Key Sponsor Contact(s).

Investigator's Agreement

I have read the attached protocol entitled "Phase 1b/2a study of OPT-302 in combination with aflibercept for persistent central-involved diabetic macular edema", dated 02 August 2018, and agree to abide by all provisions set forth therein.

I agree to comply with the protocol, International Council on Harmonisation Tripartite Guideline on Good Clinical Practice and applicable regulatory requirements, and will make every reasonable effort to complete the study in a timely manner.

I agree to ensure that the confidential information contained in this document will not be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Opthea Ltd. I will provide all study personnel under my supervision who participate in the conduct of this study with copies of the protocol and the investigator's brochure, and with access to all relevant information provided by Opthea. I will discuss this material with them to ensure that they are fully informed about the investigational product and the study.

I agree that Opthea, or their representatives will have direct access to any source documents from which electronic Case Report Form (eCRF) information has been derived. The eCRFs and other data pertinent to this study are the property of Opthea and Opthea may utilize the data in various ways such as, for example, submission to government regulatory authorities, or in publication of the results of the study.

Principal Investigator Signature	Date (DD Month YYYY)
Name of Principal Investigator	Site name

PROTOCOL SYNOPSIS

Protocol Number	OPT-302-1003
Title	Phase 1b/2a study of OPT-302 in combination with aflibercept for persistent central-involved diabetic macular edema
Sponsor	Opthea Ltd
Indication	Diabetic macular edema (DME)
Study Phase	1b/2a
Primary Objective(s)	Phase 1b and Phase 2a: • Evaluate the safety and tolerability of OPT-302 intravitreal (IVT) injection in combination with IVT aflibercept in participants with central-involved DME Phase 2a: • Assess the response rate (≥ 5 letter gain in Best Corrected Visual Acuity [BCVA] from baseline to week 12according to ETDRS criteria) in participants with persistent central-involved DME receiving combination OPT-302 and aflibercept treatment
Secondary Objective(s)	To assess: • the mean change from baseline in BCVA • the mean change from baseline in central subfield thickness (CST) and macular volume (by spectral domain optical coherence tomography [SD-OCT]) • the percent of eyes with ≥ 50% reduction in excess foveal thickness (SD-OCT) • the percent of eyes with CST < 300 µm on SD-OCT • the percent of participants with a ≥ 2 step improvement in ETDRS Diabetic Retinopathy Severity Score • the mean time to, and number of, retreatment injections of aflibercept anti-VEGF-A therapy during long term follow-up (week 12 to 24) • the pharmacokinetics (PK) of OPT-302 • anti-OPT-302 antibody formation
Exploratory Objective(s)	To evaluate: • the CST area under the curve (AUC) • the percent of eyes with resolution of fluid (sub-retinal fluid and intraretinal cysts) on SD-OCT [redacted]
Hypothesis	The combination of OPT-302 with aflibercept administered by repeat IVT injections will achieve acceptable safety / tolerability with clinical activity in participants with persistent center-involving DME.
Study Design	Two part multi-center study consisting of a Phase 1b open-label, sequential dose escalation followed by a Phase 2a randomized, double-masked, dose expansion evaluating intravitreal OPT-302 in combination with aflibercept in participants with persistent central-involved DME.
Planned No. of Subjects	Approximately 117-126
Dose Regimens	Phase 1b dose escalation: The Phase 1b comprises 3 sequential treatment cohorts of 3 to 4 participants per group receiving escalating dose levels of OPT-302 (0.3, 1 or 2 mg) each used in combination with aflibercept (2 mg). OPT-302 and aflibercept will be administered as separate IVT injections (each 0.05 mL) every 4 weeks at Day 1, 29 and 57. When used in combination, the OPT-302 IVT injection will be given after IVT aflibercept, once a post injection safety check has been performed (which will include a check of optic nerve head perfusion, intraocular pressure [IOP] and visual function). The dose regimens for the 3 treatment cohorts in the Phase 1b are as follows: Cohort 1: 2 mg aflibercept and 0.3 mg OPT-302 Cohort 2: 2 mg aflibercept and 1 mg OPT-302 Cohort 3: 2 mg aflibercept and 2 mg OPT-302 There will be no intra-subject dose escalation in this study. Phase 1b cohorts will enroll sequentially, starting with cohort 1. The patient safety data will be reviewed by the data

review team at each cohort dose level review meeting once each subject in the cohort has completed the 14 day safety review period. The decision to dose escalate will be made once the previous dose level has been reviewed and deemed safe. [redacted] Dose escalation will continue through cohorts 2 and 3 until either a maximum tolerated dose (MTD) is reached or the highest dose of OPT-302 has been tested in each subject in cohort 3 and they have completed the 14 day safety review period and the dose level has been reviewed and deemed safe by the data review team.

Phase 2a dose expansion:

The Phase 2a randomized dose expansion will begin dependent upon agreement by the data review team based on their review of safety data from the Phase 1b dose escalation (once all participants have completed the 14 day DLT window). A sample size of at least 108 patients will be randomized in a 2:1 ratio between one of the following two groups:

Cohort 4: 2 mg aflibercept + OPT-302 (*at MTD or highest dose from Phase 1b*)
Cohort 5: 2 mg aflibercept + sham

The dose level for OPT-302 in the Phase 2a will be either at the MTD or highest dose tested in the Phase 1b. OPT-302 (or sham) and aflibercept will be administered as separate IVT injections (each 0.05 mL) every 4 weeks at Day 1, 29 and 57. The OPT-302 IVT injection (or sham) is given sequentially after IVT aflibercept by the unmasked injecting investigator once a post injection safety check has been performed (which will include a check of optic nerve head perfusion, intraocular pressure and visual function).

The planned cohorts and corresponding dose levels of OPT-302 for the Phase 1b and Phase 2a are shown in the table below:

Study Part	Cohort	OPT-302 IVT Dose Level*	OPT-302 IVT Doses (once every 4 weeks)	Aflibercept IVT Doses (2 mg once every 4 weeks)	Subjects (n)#
Phase 1b: *Dose Escalation*	1	0.3 mg	3	3	3-4#
	2	1 mg	3	3	3-4#
	3	2 mg	3	3	3-4#
Phase 2a: *Dose Expansion*	4	*MTD‡*	3	3	72^
	5	*Sham*	-	3	36

IVT = intravitreal; MTD‡ = Maximum Tolerated Dose (or highest dose tested from Phase 1b)

[redacted]

#Phase 1b cohorts of 3 to 4 participants each, should a DLT occur, the cohort will be expanded to 6 participants. ^Note in the Phase 2a that randomization will continue until at least 72 evaluable participants are randomized to the Aflibercept + OPT-302 arm.

Following the dosing period, in the Phase 1b and Phase 2a there will be a 4 week treatment free follow-up to week 12 and then a follow-up to week 24 during which the subject will receive as needed standard of care IVT aflibercept based on retreatment criteria for persistent DME if VA or CST worsens (defined as a ≥ 10% increase in CST or a ≥ 5 letter decline in VA from the last study treatment phase visit [Day 57]).

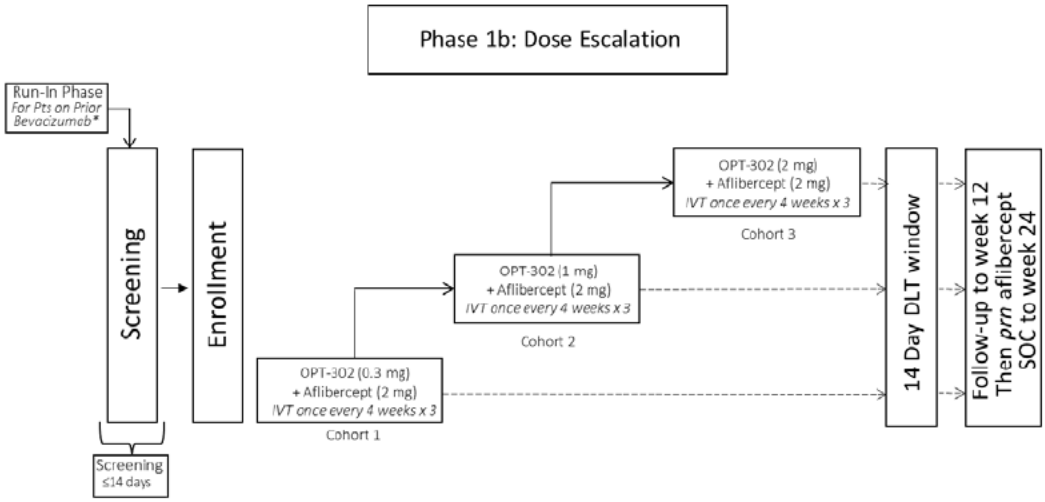
Number of Centers	Approximately 25-50 ophthalmology sites
Key Eligibility Criteria	• Males and females, ≥ 18 years of age • Diabetes mellitus (type 1 or type 2) • Edema that involves the center of the macula as confirmed by the reading center • Eyes with recurrent / persistent DME despite prior intravitreal anti-VEGF-A therapy with a suboptimal response defined as meeting all of the following: ○ Ophthalmoscopic evidence of center-involved DME

	○ Retinal thickness ≥ 320 µm in the central 1 mm subfield on Spectralis (Heidelberg) SD-OCT (or ≥ 305 µm on Cirrus) as confirmed by the reading center ○ DME is the cause of OCT thickening despite ongoing treatment with either aflibercept or ranibizumab with at least ≥ 3 prior IVT injections within 5 months of study Day 1, with the most recent injection being ≤ 42 days prior to study Day 1 (*prior bevacizumab only allowed if subsequently switched to IVT aflibercept or ranibizumab for ≥ 1 most recent injection(s) prior to study Day 1*) • History of center-involved macular edema requiring treatment, including intravitreal anti-VEGF-A therapy, for ≤ 24 months (*maximum number of intravitreal anti-VEGF-A injection cycles is ≤ 24*) • Visual acuity letter score ≤ 73 and ≥ 24 (approximate Snellen equivalent 20/40 to 20/320; Snellen [metric] equivalent 6/12 to 6/96), inclusive, in the study eye
Investigational Product / Study Drug(s)	OPT-302, the investigational product, is a recombinant fusion protein comprising immunoglobulin-like domains 1-3 of the extracellular domain of human vascular endothelial growth factor receptor-3 (VEGFR-3) and the Fc fragment of human immunoglobulin G_1 (IgG_1). It functions by binding and neutralizing the activity of VEGF-C and VEGF-D on endogenous VEGFR-2 and VEGFR-3. OPT-302 study drug will be supplied at a concentration of 40 mg/mL in a 2 mL sterile glass vial to enable a single use IVT injection for a single eye in one subject. Aflibercept (Eylea®), the other study drug, is a fusion protein that includes the key binding domains of human VEGF receptors 1 and 2 with human IgG Fc and acts by binding and inhibiting all VEGF-A isoforms, VEGF-B and placental growth factor. Aflibercept will be [redacted] be stored and prepared for IVT injection in compliance with the manufacturer's instructions.
Control Group	Phase 2a: Intravitreal aflibercept (2 mg) + sham
Study Procedures	The study will be conducted in two parts: Phase 1b - dose escalation and Phase 2a – randomized dose expansion. A signed and dated informed consent form (ICF) must be obtained from each participant prior to performing any study specific procedures. A sub-group of potential study participants who sign the ICF, have been receiving previous intravitreal bevacizumab therapy (≥ 2 most recent prior IVT injections and less than 24 months of treatment) and who meet protocol specified criteria for visual acuity and retinal thickness on SD-OCT in the study eye [redacted] can participate in a run-in phase involving a switch of therapy to at least 1 intravitreal injection of aflibercept (2 mg; 0.05 mL). At the end of the run-in phase, potential participants with persistent DME despite the prior intravitreal anti-VEGF-A therapy and who are eligible [redacted] can be enrolled (Phase 1b) / randomized (Phase 2a) into the study. All participants that enter screening will undergo safety tests and will provide medical / ocular / surgical history to confirm all eligibility requirements of the study have been met (see Section 4). All patients must undergo an ophthalmic examination, fluorescein angiography (FA), color fundus photography and SD-OCT to assess ocular eligibility criteria. [redacted] Participants will also undergo visual function testing to determine if BCVA requirements are met. In the Phase 2a, the investigator, participant and VA examiner will be masked to the treatment assignments. Review of SD-OCT scans, color fundus photographs and FA images will be performed by masked readers, unaware of participant treatment assignment, both at the site and at the reading center. [redacted] All participants will be instructed to return to the clinic for their study visits according to the schedule of assessments (Phase 1b: Appendix A; Phase 2a Appendix B). The investigator or sub-investigator must perform the ophthalmic examinations during the clinic visits. A review of concomitant medications and an assessment of adverse events must be performed at every visit. Medically significant adverse events which are considered to be related to OPT-302 (or sham) and/or aflibercept will be followed until resolved or considered stable. Clinical laboratory safety assessments, vital signs and electrocardiograms (ECG) will be performed at predetermined time-points throughout the

	study according to the schedule of assessments. Participant blood samples will be collected at predetermined time points throughout the study for assessment of OPT-302 PK and immunogenicity (development of anti-OPT-302 antibodies). [redacted] Anatomical and angiographic changes will be evaluated by SD-OCT and FA, respectively. [redacted] Color fundus photography will be used to determine severity of diabetic retinopathy. In addition, changes from baseline in BCVA by ETDRS will also be evaluated (Appendix D).
Primary Endpoints	Phase 1b and Phase 2a: • Safety: Subject incidence of adverse events, DLTs and clinically significant changes in vital signs, ECGs and clinical laboratory tests Phase 2a: • Efficacy: Response rate as defined by proportion of participants receiving combination OPT-302 and aflibercept achieving at least a 5 letter gain in BCVA compared to baseline at week 12 according to ETDRS criteria
Secondary Endpoint(s)	• Mean change in BCVA from baseline to week 12 using ETDRS criteria • Mean change from baseline to week 12 in CST and macular volume on SD-OCT • Percent of eyes with ≥ 50% reduction in excess foveal thickness from baseline to week 12 on SD-OCT • Percent of eyes with CST < 300 µm on SD-OCT through week 12 • Percent of participants with a ≥ 2 step improvement from baseline to week 12 in ETDRS Diabetic Retinopathy Severity Score • The mean time to, and number of, retreatment injections of aflibercept anti-VEGF-A therapy based on protocol specified criteria during week 12 to week 24 follow-up • OPT-302 pharmacokinetics parameters • Incidence of anti-OPT-302 antibody formation
Exploratory Endpoint(s)	• CST area under the curve (AUC) • Percent of eyes with resolution of fluid (sub-retinal fluid and intraretinal cysts) through week 12 on SD-OCT [redacted]
Statistical Considerations	The primary analysis will occur after all participants either complete at least 12 weeks on study or withdraw from the study. The final analysis will occur after all participants complete the long term visit at week 24. All participants that are enrolled (Phase 1b) or randomized (Phase 2a) and receive at least one administration of study drug(s) will be included in the analysis for safety. Assessment of safety will be based on adverse events, DLTs, laboratory parameters, ECG and vital signs. The number and percent of participants reporting adverse events (all, serious, treatment-related and treatment-emergent) will be tabulated. In the Phase 2a dose expansion, patients will be allocated in a 2:1 ratio to one of two treatment groups, aflibercept + OPT-302 or aflibercept + sham, with minimization for two baseline characteristics: BCVA (≤ 55 vs. > 55 letters) and CST (≤ 450 vs. > 450 µm). The primary outcome of the Phase 2a is the proportion of patients with a response of ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group. A one stage design is used based on the response rate primary outcome (Sargent, 2001) of the aflibercept + OPT-302 arm. Assuming the pre-specified "low" (r_0) and "high" (r_A) response rates are $r_0 = 0.28$ and $r_A = 0.45$, and setting the type I and II error rates to 5%, a total of 72 participants need to be randomized to the aflibercept + OPT-302 arm. The combination therapy will be considered to have clinical activity if ≥ 27 of 72 patients have a ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group. Likewise the combination therapy will be considered to have insufficient clinical activity if ≤ 25 of 72 patients have a ≥ 5 letter gain in BCVA from baseline to week 12 in the aflibercept + OPT-302 group. See Section 10 for further details on the statistical considerations.

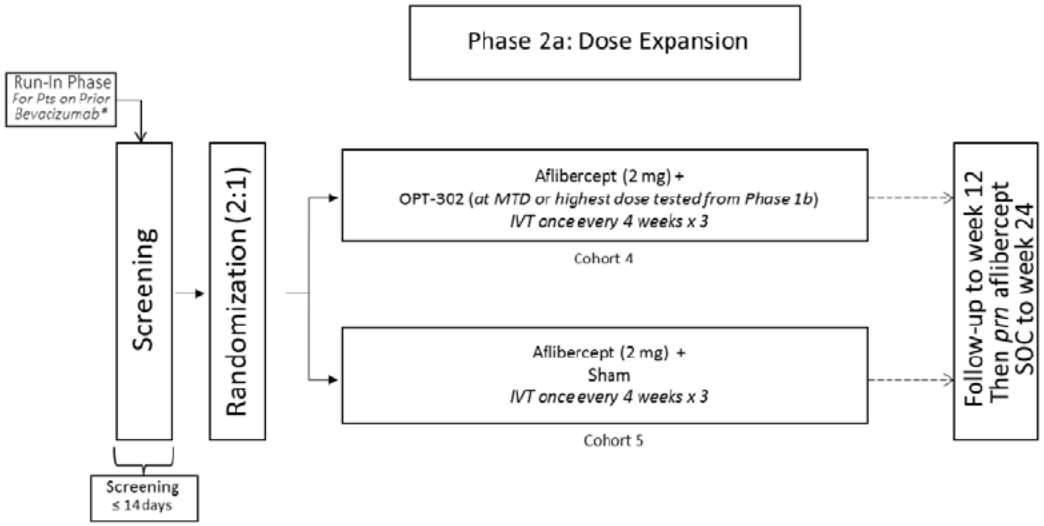
	All participants that are enrolled or randomized and receive at least one administration of OPT-302 (i.e. the safety population) will be included in the analysis for PK. The PK parameters of OPT-302 will be estimated using standard non-compartmental PK methods and summarized using means, standard deviations, medians, minimums and maximums. Descriptive statistics will also be provided for selected demographic, safety, PK and imaging by dose and time as appropriate. Descriptive statistics on continuous data will include means, medians, standard deviations, and ranges, while categorical data will be summarized using frequency counts and percentages. Graphical summaries of the data may be presented. Full details of the statistical analysis will be provided in the Statistical Analysis Plan.

STUDY DESIGN AND TREATMENT SCHEMA



Phase 1b Dose Escalation with the following design characteristics

- *Planned cohort size = 3 to 4 participants.*
- [redacted]
- *Three nominal dose levels of IVT OPT-302 (0.3, 1 and 2 mg) will be explored on a fixed schedule of once every 4 weeks for a total of 3 injections.*
- *Dose cohorts (1-3) of OPT-302 in combination with IVT aflibercept (2 mg) will be enrolled sequentially beginning with the lowest dose cohort.*
- *IVT aflibercept given prior to sequential IVT OPT-302 (each 0.05 mL) once every 4 weeks at day 1, 29 & 57.*
- **A sub-group of potential subjects who sign the ICF, have been receiving prior IVT bevacizumab (≥ 2 most recent injections and < 24 months of treatment) and who meet protocol specified criteria for visual acuity and retinal thickness in the study eye can enter a run-in phase involving a switch of therapy to at least 1 IVT injection of aflibercept (2 mg; 0.05 mL). At the end of the run-in phase eligible subjects can be enrolled into the study.*



Phase 2a Dose expansion

- *Patients will be randomized in a 2:1 ratio to one of 2 treatment groups: aflibercept + OPT-302 or aflibercept + sham, with minimization for two baseline characteristics: BCVA (≤ 55 vs. >55 letters) and central subfield thickness (≤ 450 vs. > 450 µm).*
- *Planned cohort size : Cohort 4 = 72 participants.; Cohort 5 = 36 participants*
- *The dose level of IVT OPT-302 will be at the MTD or highest dose tested from the Phase 1b dose escalation).*
- *For cohort 4 IVT aflibercept (2 mg) will be given prior to IVT OPT-302 (each 0.05 mL) once every 4 weeks at day 1, 29 and 57.*
- *For cohort 5 IVT aflibercept (2 mg) will be given (0.05 mL) prior to sham IVT injection once every 4 weeks at day 1, 29 and 57.*
- **A sub-group of potential subjects who sign the ICF, have been receiving prior IVT bevacizumab (≥ 2 most recent injections and < 24 months of treatment) and who meet protocol specified criteria for visual acuity and retinal thickness in the study eye can enter a run-in phase involving a switch of therapy to at least 1 IVT injection of aflibercept (2 mg; 0.05 mL). At the end of the run-in phase eligible subjects can be randomized into the study.*

STUDY GLOSSARY

Abbreviation or Term	Definition/Explanation
°C	Degrees Celsius
µm	Micron(s)
ADA	Anti-Drug Antibody
ADCC	Antibody Dependent Cell Mediated Cytotoxicity
ADR	Adverse Drug Reaction
AE	Adverse Event
ARPE-19	Human Retinal Pigmented Epithelial cell line
AUC	Area Under Curve
$AUC_{(0-\infty)}$	Area Under Curve from time zero to infinity
BCVA	Best Corrected Visual Acuity
cm	Centimeter(s)
C_{max}	Maximum serum concentration
CMC	Complement Mediated Cytotoxicity
CST	Central Subfield Thickness
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DME	Diabetic Macular Edema (Oedema)
DR	Diabetic Retinopathy
DRCR.net	Diabetic Retinopathy Clinical Research Network
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
ETDRS	Early Treatment of Diabetic Retinopathy Study
FA	Fluorescein Angiography
FDA	Food and Drug Administration
FIH	First-in-Human
GLP	Good Laboratory Practice
HbA1c	Glycated Hemoglobin
hr	Hour
HUVEC	Human Umbilical Vein Endothelial Cell
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IgG_1	Immunoglobulin G_1
IND	Investigational New Drug
IOP	Intraocular Pressure
IRB	Institutional Review Board
IRC	Independent Reading Center
ITT	Intent to Treat
IV	Intravenous
IVT	Intravitreal
kg	Kilogram(s)
MedDRA	Medical Dictionary for Regulatory Activities
mg	Milligram(s)
min	Minute(s)
mL	Milliliter(s)
mm	Millimeter(s)
mmHg	Millimeters of mercury
mOsmol	Milliosmole(s)
mRNA	Messenger Ribonucleic Acid
MTD	Maximum Tolerated Dose
nAMD	Neovascular Age-Related Macular Degeneration
ng	Nanogram(s)
NOAEL	No Observed Adverse Effect Level
OCT	Optical Coherence Tomography
[redacted]	
OD	Oculus Dextrus (right eye)
OS	Oculus Sinister (left eye)
OU	Oculus Uterque (both eyes)
PC-3	Prostate Cell line – human

Abbreviation or Term	Definition/Explanation
pg	Picogram(s)
PIGF	Placental Growth Factor
PK	Pharmacokinetics
PP	Per Protocol
SAE	Serious Adverse Event
SD-OCT	Spectral Domain Optical Coherence Tomography
SUSAR	Suspected Unexpected Serious Adverse Reaction
$t_{1/2}$	Half-life
TEAE	Treatment Emergent Adverse Event
t_{max}	Time to maximal concentration
VA	Visual Acuity
VAE	Visual Acuity Examiner
VEGF	Vascular Endothelial Growth Factor
VEGFR	Vascular Endothelial Growth Factor Receptor
YAG	Yttrium Aluminium Garnet

TABLE OF CONTENTS

1. OBJECTIVES

1.1 Primary

Phase 1b and Phase 2a:

- Evaluate the safety and tolerability of OPT-302 intravitreal (IVT) injection in combination with IVT aflibercept in participants with central-involved DME

Phase 2a:

- Assess the response rate (≥ 5 letter gain in BCVA according to ETDRS criteria from baseline to week 12) in participants with persistent central-involved DME receiving combination OPT-302 and aflibercept treatment

1.2 Secondary

To assess:

- the mean change from baseline in BCVA
- the mean change from baseline in central subfield thickness (CST) and macular volume (by spectral domain optical coherence tomography [SD-OCT])
- the percent of eyes with ≥ 50% reduction in excess foveal thickness (SD-OCT)
- the percent of eyes with CST < 300 µm on SD-OCT
- percent of participants with ≥ 2 step improvement in EDTRS Diabetic Retinopathy Severity Score
- the mean time to, and number of, retreatment injections of aflibercept anti-VEGF-A therapy during long term follow-up (week 12 to 24)
- the pharmacokinetics of OPT-302
- anti-OPT-302 antibody formation

1.3 Exploratory

To evaluate:

- the CST Area under the Curve (AUC)
- the percent of eyes with resolution of fluid (sub-retinal fluid and intraretinal cysts) on SD-OCT

[redacted]

2. BACKGROUND AND RATIONALE

2.1 Disease

Diabetic retinopathy (DR) is the most common microvascular complication of diabetes and is the leading cause of vision loss among working age adults in developed countries (Solomon *et al*., 2017). Approximately one-third of patients with DR or up to ~7-10% of diabetics have diabetic macular edema (DME) which is characterized by accumulation of fluid and retinal thickening within the macula and is responsible for most of the central visual loss experienced in the diabetic population (Ding and Wong, 2012; Lee *et al*., 2015). A review of the natural history of DME showed ~50% of eyes lost ≥ 2 lines of visual acuity over two-years, while in the Early Treatment Diabetic Retinopathy Study (ETDRS) a third of untreated eyes with center-involved DME experienced ≥ 15 letter decrease in visual acuity in 3 years (Ferris and Patz, 1984; EDTRS report No.4, 1987). With the increasing prevalence of diabetes worldwide, vision loss from DME represents a significant public health issue with considerable socioeconomic burden (Kiss *et al*., 2016).

Factors associated with increased risk of DR include diabetes duration, hyperglycemia, hypertension and dyslipidemia (Klein et al., 1994; Klein *et al*., 2010; Lee *et al*., 2015; Solomon *et al*., 2017). While management of diabetes with control of blood glucose, blood pressure, and serum lipid levels in conjunction with scheduled eye examinations can delay the onset and progression of DR and decrease the risk of vision loss, a significant proportion of those affected with diabetes will develop DME or proliferative changes that require intervention. (UK prospective diabetes study group, 1998; Chew *et al*., 2010).

Central involved DME is classified as retinal thickening including edema in the macula affecting the central subfield region that is 1 mm in diameter. The pathophysiological mechanisms leading to DME are multifactorial, complex and still to be fully elucidated. However, it is known that hyperglycemia can cause vascular endothelial damage and induce microvascular leakage secondary to breakdown of the inner blood-retinal barrier which leads to thickening or swelling of the macula and potential loss of sight if the edema involves the center of the fovea (Klaassen *et al*., 2013). In addition, ischemia secondary to capillary non-perfusion, caused by diabetic microvascular damage stimulates the release of vascular endothelial growth factor-A (VEGF-A) which is a major contributor to neovascularization, vascular permeability and may also have pro-inflammatory properties (Aiello *et al*., 1994; Senger *et al*., 1990; Ishida *et al*., 2003). Expression of VEGF-A and its receptor, VEGFR-2, have been shown to be greater in diabetic than non-diabetic retinal tissues in humans and VEGFR-2 is concentrated in microvascular epithelial cells, including those in the macula (Sun *et al*., 2014; Witmer *et al*., 2002; Zhao *et al*., 2007). Ocular levels of inflammatory factors which are also regulated and produced by endothelial cells are increased in patients with

DME indicating an important role for inflammation in the disease process (Shin *et al*., 2014; Kocabora *et al*., 2016).

The treatment of central-involved DME has markedly changed over the last decade, from the primary use of focal / grid laser photocoagulation, to the current era of intravitreal pharmacotherapy including anti-VEGF-A agents and corticosteroids. The role of VEGF-A in the pathophysiology of retinal diseases, including wet age-related macular degeneration and DME, has led to the breakthrough development of several inhibitor drugs that target this ligand. The positive clinical data and approval of these drugs have validated the importance of the VEGF family of ligands and receptors in the pathogenesis of DR and DME.

Following the regulatory approval of aflibercept (Eylea®; Regeneron), and ranibizumab (Lucentis®; Genentech), as well as the off-label use of bevacizumab (Avastin®, Genentech), the treatment landscape has significantly altered for patients and currently anti-VEGF-A therapy is the first-line standard of care for central-involved DME (Jampol *et al*., 2014). Aflibercept is a soluble decoy receptor fusion protein created using Trap technology, which targets VEGF-A, placental growth factor (PIGF) and VEGF-B (Regeneron, BLA 125387, 2011). The DA VINCI, VISTA and VIVID studies demonstrated that treatment with aflibercept yielded greater visual gains than macular laser treatment in patients with central involved DME (Korobelnik *et al*., 2014; Payne and Clarke, 2015). The binding affinity of aflibercept to VEGF-A is substantially greater than that of ranibizumab and bevacizumab to VEGF-A, potentially allowing for a less frequent dosing regimen (once every 8 weeks) following an initial monthly loading dose phase (Stewart and Rosenfeld, 2008).

Recent data from the Diabetic Retinopathy Clinical Research Network (DRCR.net) Protocol T comparing all three anti-VEGF-A drugs, indicated that at 1 year for eyes with central-involved DME, each agent improved visual acuity (VA) (Wells *et al*., 2015). There was no difference among the three agents in mean change in VA in eyes with baseline vision of 20/32 to 20/40, whereas aflibercept had more effective vision outcomes in eyes with baseline VA of 20/50 to 20/320 (Wells *et al*., 2015). At 2 years, aflibercept remained superior to bevacizumab, but not ranibizumab, among eyes with baseline VA of 20/50 or worse, while all three drugs had similar safety profiles (Wells *et al*., 2015; Cai and Bressler, 2017). Most patients with central involved DME require near-monthly administration of intravitreal therapy with these anti-VEGF-A agents during the first 12 months of treatment, with fewer injections needed in subsequent years to maintain clinical benefit (Solomon *et al*., 2017).

Intravitreous steroid therapy has also been evaluated in pivotal Phase 3 studies, and the steroid agents dexamethasone and fluocinolone acetonide are approved for use in central involved DME. These agents however, are rarely used as first-line therapy due to inferior visual acuity outcomes

compared to anti-VEGF-A therapy. Patients with persistent DME and who are insufficiently responsive to anti-VEGF-A therapy have shown some treatment benefit with intravitreal corticosteroids (Schwartz *et al*., 2016; Regillo *et al*., 2017). However, as intravitreal corticosteroids are associated with high rates of ocular adverse events including cataract progression and intraocular pressure elevation, switching to corticosteroids from an anti-VEGF-A agent with a sub-optimal response needs to be carefully considered in patients with glaucoma and in young phakic patients (Shah *et al*., 2017). Steroids are therefore preferred in pseudophakic eyes that have chronic / persistent or recurrent disease.

Despite the widespread use of treatments targeting VEGF-A in the management of retinal disorders including DME, there is still room for improvement as many patients demonstrate a sub-optimal response, remain treatment refractory, or require frequent injections for persistent leakage in the macula. A substantial proportion of patients with central involved DME do not show reductions in fluid or clinically significant improvement in visual acuity following anti-VEGF-A treatment (Nguyen *et al*, 2012; Channa *et al*., 2014; Do *et al*., 2013). This resistance may occur as treatment selective anti-VEGF-A monotherapies do not fully address the multifactorial pathogenesis of fovea involving edema. Thus potential combination therapies targeting alternative factors and pathways are currently being investigated for the treatment of central involved DME (Campochiaro *et al*., 2016; Kaiser, 2017).

2.2 OPT-302 Background

2.2.1 Vascular endothelial growth factors C and D

The VEGF-C and VEGF-D members of the VEGF family of secreted glycoproteins are upregulated in response to inhibition of VEGF-A with aflibercept or bevacizumab (Cabral *et al*., 2016; Lieu et al., 2013; Li *et al*., 2014; Rose *et al*., 2010; Fan *et al*., 2011; Grau *et al*., 2011). Such upregulation may be an important mechanism contributing to clinical sub-responsiveness reported with VEGF-A inhibitors. Both VEGF-C and VEGF-D induce angiogenic vessel growth in several *in vivo* models, while VEGF-C can also cause the formation of endothelial cell fenestrations, which increases vascular permeability (Cao *et al*., 1998; Witzenbichler *et al*., 1998; Chung *et al*., 2009; Stacker *et al*., 2001).

VEGF-C contributes to increased vascular permeability and/or retinal angiogenesis through downstream effects of VEGFR-2 and VEGFR-3 activation (Tammela *et al*., 2011; Joukov *et al*., 1998; Joukov *et al*., 1997; Cao *et al*., 2004; Gaal *et al*., 2013; Xu *et al*., 2013). Expression of VEGFR-2 is greater in human diabetic retina than in non-diabetics and the receptor is concentrated in microvascular endothelial cells including those in the macula region (Witmer *et al*., 2002; Sun *et al*., 2014; Zhao *et al*., 2007). Furthermore, VEGF-C can potentiate the angiogenic actions of VEGF-

A as it's binding to VEGFR-2 inhibits apoptosis of microvascular endothelial cells induced by the pro-inflammatory cytokine tumor necrosis factor and hyperglycemia (Zhao *et al*., 2006; Zhao *et al*., 2007). There is also an increase in VEGF-C and VEGF-A mRNA in microvascular endothelial cells of individuals with diabetes (Zhao *et al*., 2007). Evaluation of single nucleotide polymorphisms in diabetic patients found that genetic variation within the VEGF-C gene is associated with DR and DME indicating that VEGF-C and its interaction with VEGFR-2 may play a functional role in the pathogenesis of these diseases (Kaidonis *et al*., 2015).

2.2.2 OPT-302

OPT-302 is a recombinant fusion protein comprising immunoglobulin-like domains 1-3 of the extracellular domain of human vascular endothelial growth factor receptor-3 (VEGFR-3) and the Fc fragment of human immunoglobulin G1 (IgG1). It functions by binding and neutralizing the activity of VEGF-C and VEGF-D on endogenous VEGFR-2 and VEGFR-3.

The activity of OPT-302 both as a targeted inhibitor of VEGF-C and VEGF-D, and also the biological consequences of such inhibition in the eye, have been investigated in a number of *in vitro* and *in vivo* studies. These studies confirm the selective binding of OPT-302 to, and inhibition of, VEGF-C and VEGF-D, but not VEGF-A or placenta growth factor (PIGF). Pre-clinical pharmacology studies of an OPT-302 analogue (VGX-300) in the laser-induced choroidal neovascularization mouse model, demonstrated that targeted inhibition of VEGF-C/D has significant efficacy when used alone or in combination with aflibercept anti-VEGF-A therapy (Lashkari *et al.,* 2014).

The biological effects to induce angiogenesis by VEGF-C and VEGF-D or vascular permeability by VEGF-C, as well as the compensatory upregulation of these two ligands which can occur when VEGF-A is inhibited, support the clinical investigation of OPT-302 in combination with existing anti-VEGF-A therapies for persistent central-involved DME. Combination therapy with a VEGF-A inhibitor and OPT-302 is expected to result in more effective inhibition of central involved macular edema in treatment refractory diabetic patients compared to VEGF-A neutralization alone.

[redacted]

[redacted]

[redacted]

[redacted]

2.3 Clinical studies

2.3.1 Safety and tolerability

OPT-302 has demonstrated acceptable safety and tolerability up to 2 mg administered by repeat IVT dosing every 4 weeks either alone or in combination with the anti-VEGF-A therapy, ranibizumab (0.5 mg), in a Phase 1 first in human (FIH) study (OPT-302-1001) in 51 patients with nAMD. The Phase 1 study consisted of two parts: Part 1 was an open-label, sequential dose escalation (n=20; 0.3, 1 or 2 mg OPT-302 with 0.5 mg ranibizumab, or 2 mg OPT-302 monotherapy, in 4 cohorts of 5 patients each); Part 2 was a randomized dose expansion (n=31, 2 mg OPT-302 with 0.5 mg ranibizumab [n=23] or 2 mg OPT-302 monotherapy [n=8]). All study drugs were administered by IVT injection for

three consecutive dosing cycles at 4 weekly intervals. Of the 51 patients enrolled, 19 (37%) were male, 32 (63%) were female, 10 (20%) had diabetes mellitus and 25 (49%) were treatment naïve, while 26 (51%) had received ≥ 3 prior IVT injections of anti-VEGF-A therapy.

No dose-limiting toxicities (DLTs) were observed with OPT-302 at any IVT dose level up to 2 mg either in combination with anti-VEGF-A therapy or as a monotherapy, and the MTD was not reached. In addition, there was no evidence of OPT-302 related immunogenicity after IVT administration of OPT-302 doses up to 2.0 mg/eye.

[redacted]

Overall, 100% of evaluable participants at Week 12 (n=49) maintained BCVA, defined as ≤15 letter loss from baseline. In addition, changes from baseline in anatomic measures (CST) on SD-OCT through to week 12, demonstrated no adverse safety signals in treatment naïve patients and those who showed a sub-optimal response to prior anti-VEGF-A therapy.

In summary, OPT-302 was well tolerated at doses up to 2 mg when administered by repeat intravitreal injections once every 4 weeks, either in combination with anti-VEGF-A therapy (0.5 mg ranibizumab) or as a monotherapy.

[redacted]

2.4 Risk Assessment

Dose selection for this study is based on the current safety and PK data of OPT-302, assessed in a Phase 1 FIH study in 51 patients with nAMD and in nonclinical studies [redacted]. Based on the available data, administration of 0.3, 1 or 2 mg OPT-302 by IVT injection once every 4 weeks on Days 1, 29 and 57 is anticipated to have an acceptable risk benefit profile with acceptable safety and tolerability in participants with central involved DME.

The other study drug to be used in the study is aflibercept which has been approved and is widely used globally for retinal vascular diseases including central involved DME. Overall, IVT aflibercept is a highly effective and safe therapy for improving vision and reducing vision loss in patients with DME. Cumulative safety data to date do not show an increased risk of any ocular or systemic adverse events with this anti-VEGF-A agent compared to other similar drugs used to treat these indications (Wells *et al*., 2015; Wells *et al*., 2016). There is therefore no additional risk to the use of this drug over and above standard care and the participants would likely be treated in the clinic with IVT injections of aflibercept or a similar anti-VEGF-A agent, even if not enrolled in this study

[redacted]

[redacted]

Participants enrolled in this study will be monitored closely in the clinic following dosing with aflibercept and OPT-302.

[redacted] Further details of the safety data for aflibercept may be found in the package insert and the approved label for the drug.

2.5 Rationale

Intravitreal anti-VEGF-A therapy has become the standard of care for most cases of center involving DME. However, even with monthly or near monthly IVT injections for the first 12 months utilized in controlled clinical trials, > 35 % of patients with DME treated with anti-VEGF-A therapy fail to achieve ≥ 2 lines improvement in VA from baseline at 2 years, while approximately a third or more of eyes still had CST ≥ 300 µm at 1 year, or did not have complete resolution of retinal thickening (< 250 µm on time domain OCT) by 2 years (Elman *et al.,* 2011; Rajendram *et al*., 2012; Nguyen *et al*., 2012). In addition, data suggest that sub-optimally responding eyes may be identified after as few as 3 anti-VEGF injections and should be considered for alternate therapies (Gonzalez *et al*., 2016). A post hoc analysis of the DRCR network Protocol I study showed that ~40% of eyes had a poor mean BCVA response of < 5 letter gain from baseline, while ~23% of eyes showed a moderate mean VA gain of 5 - 9 letters from baseline after 3 monthly injections of ranibizumab, and both groups then only achieved a further mean VA improvement of < 3 letters at 1 and 3 years (Gonzalez *et al*., 2016). Within the subset of patients with a poor BCVA of < 5 letter at 12 weeks, a minority of eyes (23%) continuing to receive regular monthly anti-VEGF-A therapy slowly improved their response to at least a 10 letter gain from baseline at 52 weeks (Gonzalez *et al*., 2016).

Tolerance or tachyphylaxis, differences in drug levels between patients and resistance have all been proposed as potential reasons for suboptimal results in retinal diseases treated with anti-VEGF-A

therapy (Schaal *et al*., 2008; Eghoj and Sorensen 2012). Various reasons for this, including differences in individual VEGF-A gene expression, up-regulation of alternative factors and pathways, and a shift to chronic inflammation mechanisms have been implicated (Forooghian *et al.,* 2011). To overcome this suboptimal response, strategies involving more intensive dosing regimens or switching drugs within the same anti-VEGF-A pharmacologic group have been used with alternative anti-VEGF-A agents reportedly showing further anatomic improvements but limited changes in VA (Lim *et al.,* 2015; Rahimy *et al*., 2016; Spooner *et al.,* 2017; Mira *et al*., 2017). However, many of these studies have either been retrospective and / or employ varied methodologies including differing retreatment regimens, variable entry criteria and non-standardized VA measurements. Thus, refractory eyes responding sub-optimally to first-line therapy characterized by persistent or recurrent retinal thickening and/or poor gains in VA represent a therapeutic challenge with a high unmet medical need.

Since treatment selective anti-VEGF-A monotherapies do not fully address the multifactorial pathogenesis of center involving DME, potential adjunctive therapies targeting alternative factors and pathways are currently being investigated (Campochiaro *et al*., 2016; Kaiser, 2017). Increased levels of the ligands VEGF-C/D upregulated in response to inhibition of VEGF-A with aflibercept or bevacizumab may represent an alternate mechanism contributing to sub-responsiveness reported with VEGF-A inhibitors (Cabral *et al*., 2016; Lieu *et al*., 2013; Li *et al*., 2014; Rose *et al*., 2010; Fan *et al*., 2011; Grau *et al*., 2011). In the Phase 1 FIH study (OPT-302-1001), nAMD patients (n=19) showing a sub-optimal response to prior IVT anti-VEGF-A therapy (mean number of prior injections = 17 [range 3 to 76]), and subsequently receiving combination OPT-302 with ranibizumab for 3 monthly IVT injections, had a change from baseline to week 12 in mean BCVA of +4.9 letters and a reduction in mean CST of -54 µm. A total of 10/19 (53%) of these patients receiving combination OPT-302 with ranibizumab had a ≥ 5 letter gain from baseline at week 12 in BCVA despite being treatment refractory to prior anti-VEGF-A therapy.

OPT-302 which blocks the alternative VEGF-C/D angiogenesis and vascular leakage ligand pathways, therefore represents a novel therapeutic candidate to be used in combination with the anti-VEGF-A inhibitor, aflibercept as a potential treatment of center involved DME.

2.5.1 Rationale for OPT-302 dose selection

OPT-302 in combination with aflibercept will be investigated in the Phase 1b / 2a study. The pre-specified nominal doses of OPT-302 for use in the dose escalation are a starting dose at 0.3 mg, escalating to 1 mg and a potential maximum dose of 2 mg, administered by IVT injection once every 4 weeks for a total of 3 injections. [redacted] The doses for OPT-302 were selected based on

the clinical and non-clinical safety data [redacted] and PK modelling derived from [redacted] clinical PK samples.

[redacted]

The clinical and non-clinical data support the proposed dosing regimen for OPT-302 at dose levels up to 2 mg when used in combination with intravitreal anti-VEGF-A therapy (aflibercept) administered by sequential intravitreal injection once every 4 weeks.

2.6 Clinical Hypothesis

The combination of OPT-302 with aflibercept administered by repeat IVT injections will achieve acceptable safety / tolerability with clinical activity in participants with persistent center-involving DME.

3. EXPERIMENTAL PLAN

3.1 Study Design

This is a two part multi-center study consisting of Phase 1b open-label, sequential dose escalation followed by a Phase 2a randomized, double-masked, dose expansion evaluating intravitreal OPT-302 in combination with aflibercept in participants with persistent central-involved DME.

3.1.1 Run-in Phase for Potential Participants receiving Prior Intravitreal Bevacizumab.

A sub-group of potential study participants who have been receiving previous intravitreal bevacizumab therapy (≥ 2 most recent prior IVT injections and less than 24 months of treatment) for center involved DME can participate in a run-in phase.

[redacted]

During the run-in phase, study eyes will receive switch therapy of at least 1 intravitreal injection of aflibercept (2 mg; 0.05 mL). At the end of the run-in phase, potential participants with persistent DME despite the prior intravitreal anti-VEGF-A therapy and who are eligible [redacted] can be enrolled (Phase 1b) / randomized (Phase 2a) into the study. [redacted]

3.1.2 Phase 1b – Dose Escalation

The Phase 1b dose escalation is aimed at determining the MTD or highest dose tested, safety, pharmacokinetics and pharmacodynamics of OPT-302 in combination with aflibercept. It will consist of up to three cohorts (3 combination cohorts) of 3 to 4 participants each. [redacted] The 3 treatment cohorts consist of 3 sequential, escalating cohort dose levels of OPT-302 (0.3, 1 and 2 mg) each used in combination with aflibercept (2 mg). OPT-302 and aflibercept will be administered as separate IVT injections (each 0.05 mL) once every 4 weeks at Day 1, 29 and 57. The OPT-302 IVT injection is given sequentially after IVT aflibercept once a post injection safety check has been performed (which may include a check of optic nerve head perfusion, intraocular pressure and / or visual function).

The dose regimens for the 3 treatment cohorts in the Phase 1b are as follows:

- Cohort 1: 2 mg aflibercept and 0.3 mg OPT-302
- Cohort 2: 2 mg aflibercept and 1 mg OPT-302

- Cohort 3: 2 mg aflibercept and 2 mg OPT-302

Following the third IVT injection participants will have a 4 week treatment free follow-up through week 12 and an additional longer-term follow-up through week 24 when standard of care IVT aflibercept retreatment will be based on protocol-specified retreatment criteria for persistent DME if visual acuity (VA) or central subfield thickness (CST) on SD-OCT worsens (defined as a ≥ 10% decrease increase in CST or a ≥ 5 letter decline in VA from the last study treatment phase visit [Day 57]).

Dose escalation cohorts in the Phase 1b will enroll sequentially, starting with cohort 1. [redacted] At least 3 patients must complete the 14 day DLT period at each dose level before the data review team can consider dose escalation. [redacted]

3.1.3 Phase 2a – Randomized Dose Expansion

The Phase 2a randomized dose expansion will begin dependent upon agreement by the data review team based on their review of emerging safety data from the Phase 1b dose escalation phase (once all participants have completed the 14 day DLT window).

In the Phase 2a, at least 108 participants will be assigned randomly in a 2:1 ratio to one of the following two treatment groups:

- Cohort 4: 2 mg aflibercept + OPT-302 (*at MTD or highest dose tested from the Phase 1b*)
- Cohort 5: aflibercept + sham

Cohort 4 will dose a minimum of 72 participants using IVT aflibercept (2 mg in 0.05 mL) in combination with OPT-302 (0.05 mL) at the MTD or highest tested dose from the Phase 1b, given once every 4 weeks (Day 1, 29 and 57) for a total of 3 sequential injections.

Cohort 5 will dose a minimum of 36 participants with IVT aflibercept (2 mg in 0.05 mL) + sham once every 4 weeks (Day 1, 29 and 57) for a total of 3 injections. Randomization to cohorts 4 and 5 will continue until 72 evaluable participants are available in the aflibercept + OPT-302 experimental arm.

The OPT-302 IVT injection (or sham) is given sequentially after IVT aflibercept by the unmasked injecting investigator once a post injection safety check has been performed (which will include a

check of optic nerve head perfusion, intraocular pressure and visual function). [redacted]

Participants will have a further 4 week treatment free follow-up through week 12 and an additional longer-term follow-up through week 24 when standard of care IVT aflibercept retreatment will be based on protocol-specified retreatment criteria for persistent DME if VA or CST on SD-OCT worsens (defined as a ≥ 10% increase in CST or a ≥ 5 letter decline in VA from the last study treatment phase visit [Day 57]).

In the Phase 2a randomized dose expansion the investigator ophthalmologist and VA examiners will be "masked" to the individual treatment assignments. Evaluation of SD-OCT scans, color fundus photographs and FA images will be performed by a masked observer, unaware of participant treatment assignment, both at the site and at the Independent Reading Center.

The overall study design is described by a study design and treatment schema at the end of the protocol synopsis section.

3.2 Number of Centers

The study will be conducted at up to ~25-50 Ophthalmology sites with expertise in retinal disorders.

3.3 Number of Participants

In the Phase 1b dose escalation, at least 9 adult participants ≥ 18 years with persistent central-involved DME who have received prior IVT anti-VEGF-A therapy with the need for additional therapy are expected to be enrolled. The sample size for the Phase 1b dose escalation was determined empirically and is consistent with historical precedence for initial human clinical study of therapies including in retinal eye disease. In the Phase 2a dose expansion, at least 108 participants will be randomized in a 2:1 ratio to two treatment groups of (i) OPT-302 at the MTD or highest dose tested from the Phase 1b, in combination with aflibercept (2 mg) or (ii) aflibercept (2 mg + sham.

The rationale for the number of participants in the Phase 2a study is detailed in Section 10.3.

3.4 Estimated Study Duration

It is estimated that the study duration for participants in the active treatment phase will be 6 months consisting of 14 days for screening, 2 months of treatment (at Day 1, 29 and 57) depending on tolerability to aflibercept ± OPT-302 (or sham) and week 12 and 24 follow-up visits. For the study participants who enter the run-in phase, there will be an additional ~4 to 6 weeks prior to entry into the active treatment phase.

3.4.1 End of Study

Primary Completion: The primary completion of study will occur when target enrollment or randomization is complete and each participant does at least one of the following:

- Has had the opportunity to complete the week 12 visit on study, or
- Withdraws from study

End of Trial: The end of trial will occur when target enrollment or randomization is complete and each participant either withdraws from study or completes the week 12 follow up visit or the week 24 long-term follow-up.

[redacted]

4. SUBJECT ELIGIBILITY

Adult participants ≥ 18 years with diabetes mellitus with central-involved macular edema with a suboptimal response despite prior intravitreal anti-VEGF-A therapy which in the opinion of the investigator might benefit from additional treatment. [redacted]

4.1 Inclusion Criteria

4.1.1 Able and willing to provide written informed consent

4.1.2 Age ≥ 18 years of either gender

4.1.3 Diabetes mellitus (type 1 or type 2)

4.1.4 Edema that involves the center of the macula as confirmed by the reading center

4.1.5 Eyes with recurrent / persistent DME despite prior intravitreal anti-VEGF therapy with a suboptimal response and defined as meeting all of the following:

- Ophthalmoscopic evidence of center-involved DME
- Retinal thickness ≥ 320 µm in the central 1 mm subfield on Spectralis (Heidelberg) SD-OCT (or ≥ 305 µm on Cirrus) as confirmed by the reading center
- DME is the cause of OCT thickening despite ongoing treatment with either aflibercept or ranibizumab with ≥ 3 prior IVT injections within 5 months of study Day 1, the most recent injection being ≤ 42 days prior to study Day 1 (*prior bevacizumab allowed only if therapy subsequently switched to intravitreal aflibercept or ranibizumab for ≥ 1 most recent injection(s) prior to study Day 1*)

4.1.6 History of center-involved macular edema requiring treatment, including intravitreal ant-VEGF-A therapy, for ≤ 24 months (*maximum number of intravitreal anti-VEGF-A injection cycles is ≤ 24*)

4.1.7 BCVA letter score ≤ 73 and ≥ 24 (approximate Snellen equivalent 20/40 to 20/320; Snellen [metric] equivalent 6/12 to 6/96) in the study eye, inclusive

4.1.8 If female and of child-bearing potential: Pregnancy test at screening and Day 1 is negative, and agrees to use a highly effective method of contraceptive for the duration of the study and for at least 3 months following the last dose of study medication. The following are considered "highly effective methods": *i.e.* hormonal contraceptive (oral, intravaginal, or implant, but excluding progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action); intrauterine device; or documented vasectomy of partner. A participant will not be considered to be of child-bearing potential if she is post-menopausal and has not had menses for at least 12 months prior to screening (by history), or if surgically sterile

4.1.9 Only one eye will be enrolled in the study. (*If both eyes meet the entry criteria the study eye chosen is the worse eye (based on investigator assessment of SD-OCT and / or VA). If both eyes are equal, the participant and investigator will select the eye for entry*).

4.2 Exclusion Criteria

4.2.1 Eyes in which scatter (panretinal) photocoagulation is needed now or is likely to be needed within the next 3 months (*e.g. eyes with high risk proliferative diabetic retinopathy [defined as neovascularization of the disc > 1/3 disc area or neovascularization elsewhere > 1/2 disc area and presence of vitreous hemorrhage regardless of size of neovascularization] not already adequately treated with photocoagulation*)

4.2.2 Macula edema is considered to be due to a cause other than DME in the study eye (*e.g. clinical exam by investigator and OCT as confirmed by the reading center suggest that vitreoretinal interface abnormalities such as taut posterior hyaloid or epiretinal membrane are the primary cause of macular edema*)

4.2.3 Presence of any abnormality that in the opinion of the investigator would be likely to confound assessment of visual acuity improvement in the study eye in which macular edema resolves, or improves, such as dense subfoveal hard exudates, neovascular glaucoma, or presence of chorioretinal / foveal atrophy involving the center of the macula

4.2.4 Vitreoretinal traction confirmed by OCT, or seen clinically within 1 disc diameter of the center of the macula of the study eye as confirmed by the reading center

4.2.5 Any retinal vein occlusion involving the macula in the study eye as confirmed by the reading center

4.2.6 Any intraocular surgery in the study eye within 4 months of study entry or anticipated within the next 3 months following dosing on Day 1.

4.2.7 Previous vitrectomy or scleral buckling surgery in the study eye

4.2.8 HbA1C level ≥12% and/or recent signs of uncontrolled diabetes (*3 or more episodes of severe hypoglycemia within 3 months of baseline, or hospitalization for hyperglycemia, or 2 or more episodes of ketoacidosis within 1 year of baseline, or an episode of ketoacidosis within 3 months of baseline*).

4.2.9 Renal failure, dialysis, or history of renal transplant

4.2.10 Myocardial infarction, other cardiac event requiring hospitalization, stroke, transient ischemic attack, or treatment for congestive heart failure within 6 months prior study Day 1.

4.2.11 Uncontrolled hypertension ≥180 mmHg systolic or ≥110 mmHg diastolic. (*If blood pressure is brought below 180/110 mmHg by anti-hypertensive treatment, the individual can become eligible. Participants with a history of controlled hypertension on medication may have their blood pressure taken at a second visit to qualify based on the repeat testing*).

4.2.12 Pregnant or lactating

4.2.13 Major surgery (*defined as intra-abdominal or surgery requiring general anesthesia*) within 28 days prior to dosing on study Day 1 or major surgery planned during the next 6 months.

4.2.14 Previous treatment with photodynamic therapy or external beam radiation in the study eye

4.2.15 Subjects who have received panretinal or focal / grid photocoagulation, YAG laser, or peripheral retinal cryoablation (for retinal tears only) in the study eye within the previous 4 months

4.2.16 Concurrent or prior use of systemic anti-VEGF agents

4.2.17 Concurrent or prior use of intravitreal bevacizumab in the study eye, unless therapy switched to intravitreal aflibercept or ranibizumab for ≥ 1 most recent injection(s) prior to Day 1

4.2.18 Most recent intravitreal injection of aflibercept or ranibizumab less than 28 days or greater than or equal to 42 days prior to Day 1 dosing in the study eye

4.2.19 Administration of systemic steroids within 4 months prior to Day 1

4.2.20 Concurrent or prior use of any intravitreal injections of steroids within 4 months prior to Day 1 in the study eye

4.2.21 Concurrent or prior use of dexamethasone implant in the study eye

4.2.22 Concurrent or prior use of fluocinolone implant in the study eye

4.2.23 Concurrent or prior administration of experimental therapy within 30 days of screening

4.2.24 Concurrent treatment for active systemic (non-ocular) infection at screening, if in the opinion of the investigator, doing so will place the participant at undue risk

4.2.25 Concurrent treatment in either eye for any ocular condition with an investigational drug or device that has not received regulatory approval

4.2.26 Concurrent or prior use of thiazolidinediones within 6 months prior to Day 1 dosing

4.2.27 Active or recent (within 4 weeks) intraocular inflammation (grade trace or above) in the study eye

4.2.28 Any active periocular or intraocular infection or inflammation (*e.g. conjunctivitis, keratitis, scleritis, uveitis or endophthalmitis*)

4.2.29 Subjects with other ocular diseases that can in the opinion of the investigator compromise the visual acuity of the study eye such as amblyopia and anterior ischemic optic neuropathy

4.2.30 History of idiopathic or autoimmune-associated uveitis in either eye.

4.2.31 Current vitreous hemorrhage at the screening assessments in the study eye as confirmed by the reading center

4.2.32 Uncontrolled glaucoma (>30 mmHg) either untreated or on anti-glaucoma medication at screening

4.2.33 Known allergy to any component of the study drug(s)

4.2.34 Prior participation in this clinical trial

4.2.35 History or evidence of clinically significant disorder, condition or disease (with the exception of those outlined above) that, in the opinion of the investigator or medical monitor would pose a risk to participant safety or interfere with study evaluation, procedures or completion

5. SUBJECT ENROLLMENT / RANDOMIZATION

Before participants may be entered into the study, the site must obtain IRB / IEC written approval of the protocol, informed consent form, and other participant information and/or recruitment material, if applicable [redacted]. The informed consent form must be signed and dated by the participant or by their legal representative and by the person who conducted the informed consent discussion before commencement of study-specific procedures. [redacted]

[redacted]

Participants will be enrolled in the Phase 1b after all screening visit and safety assessments have been completed and eligibility criteria have been met. In the Phase 2a dose expansion a participant is considered randomized when they have met all eligibility criteria and have received the randomized treatment allocation [redacted].

[redacted] Participants who are deemed ineligible will be documented as screen failures. Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently enrolled (Phase 1b) or randomized (Phase 2a).

[redacted]

6. INVESTIGATIONAL PRODUCT / STUDY INTERVENTION(S)

OPT-302 is the only investigational product administered in this study. The other study drug to be used in the study is aflibercept. A pharmacy manual containing detailed information regarding the storage, preparation, and administration of OPT-302 will be provided as a separate document.

6.1 OPT-302

OPT-302 will be manufactured [redacted] in accordance with current Good Manufacturing Practice. The investigational product will be supplied [redacted] at a concentration of 40 mg/mL. [redacted]

[redacted] Each glass vial will contain sufficient study medication to enable a single use only treatment for a single eye in one participant. OPT-302 will be given by IVT injection.

[redacted] Instructions for the preparation of OPT-302 for IVT administration are specified in the pharmacy manual.

[redacted]

6.1.2 Dosage, Administration, and Schedule

The OPT-302 investigational product and aflibercept study drug will be administered by intravitreal injection carried out under aseptic conditions according to institutional medical standards and applicable guidelines by a qualified ophthalmologist experienced with this route of administration.

[redacted]

6.1.2.2 Post Intravitreal Injection Procedures

All participants receiving study drug(s) will be carefully monitored throughout the injection and immediately after the administration of study drug(s).

[redacted]

[redacted]

6.1.2.4 Phase 1b Dose Escalation

The Phase 1b dose escalation is open label and eligible participants who enroll will receive OPT-302 by IVT injection (0.05 mL) at doses of 0.3 mg (Cohort 1), 1 mg (cohort 2) or 2 mg (Cohort 3) once every 4 weeks (Day 1, 29 and 57) each used in combination with aflibercept (2 mg) for a total of 3 injections. The OPT-302 IVT injection is given sequentially after IVT aflibercept once a post injection safety check has been performed (which may include a check of optic nerve head perfusion, intraocular pressure and/or visual function. Dose escalation will occur at the planned dose levels until the MTD is determined or until the highest dose level is tested. [redacted]

6.1.2.5 Phase 2a Randomization and Masking

For the Phase 2a dose expansion, at least 108 eligible participants will be centrally allocated in a 2:1 ratio to one of the two treatment groups (combination OPT-302 + aflibercept [n=72]: aflibercept + sham [n=36]) by a dynamic minimization procedure stratifying by two baseline characteristics: ETDRS Best Corrected Visual Acuity (BCVA ≤ 55 or > 55 letters) and Central Subfield Thickness (CST ≤ 450 vs. > 450 µm). The dynamic minimization will use a stochastic treatment allocation

algorithm based on the variance method (Pockock and Simon, 1975). Randomization will continue until 72 evaluable participants are randomized to the aflibercept + OPT-302 experimental arm.

The Phase 2a is double masked: the participant, investigator, VA assessors, image technician readers / photographers and other site staff involved in participant care will be masked to study medication. [redacted] OPT-302 and aflibercept will be administered as separate IVT injections (each 0.05 mL) every 4 weeks at Day 1, 29 and 57. The OPT-302 IVT injection (or sham) is given sequentially after IVT aflibercept by the unmasked injecting investigator once a post injection safety check has been performed (which may include a check of optic nerve head perfusion, intraocular pressure or visual function). [redacted]

[redacted]

6.2 Phase 2a Sham Control

In the Phase 2a the control arm will consist of aflibercept IVT injection followed by a sham injection. For participants receiving sham injections in the control group, the procedure will be done by the injecting ophthalmologist and involves pressing the hub of an identical syringe (but without the needle) against the eye wall to mimic the active doses injected into the vitreous cavity, whilst also following other standard preparation steps used for OPT-302 such as aseptic techniques etc. [redacted]

6.3 Other Study Drug - Aflibercept

Aflibercept (Eylea®) is a fusion protein that includes the key binding domains of human VEGF receptors 1 and 2 with human IgG Fc and acts by binding and inhibiting all VEGF-A isoforms, VEGF-B and placental growth factor, preventing increased permeability and macular edema in central involved DME. Aflibercept will be [redacted] stored and prepared for IVT injection in compliance with the manufacturer's instructions. Aflibercept will be administered to the study eye at a dose of 2 mg in 0.05 mL. Refer to the aflibercept package insert for more details about the study drug and its physical, chemical and pharmacological properties and formulation.

6.3.1 Aflibercept Retreatment Criteria – Week 12 to Week 24

Following the dosing period, in the Phase 1b and Phase 2a there will be a 4 week treatment free follow-up to week 12 and then a follow-up to week 24 during which the subject will receive as needed standard of care IVT aflibercept based on retreatment criteria for persistent DME if visual acuity (VA) or central subfield thickness (CST) worsens (defined as a ≥ 10% increase in CST or a ≥ 5 letter decline in VA from the last study treatment phase visit [Day 57]).

6.5 Dose Escalation / Expansion Safety Review

The Phase 1b dose escalation is aimed at determining the MTD or highest dose tested, and evaluating the safety and tolerability of OPT-302 (0.3, 1 or 2 mg) in combination with aflibercept (2 mg). Dose limiting toxicity (DLT) and stopping criteria will be incorporated into the Phase 1b of the study. There will be a 14 day DLT window following the first dose for determination of whether patient dosing will continue and a data review team will meet to review participant safety to make recommendations on dose escalation to the higher dose level cohort.

[redacted]

Dose escalation will occur at the planned dose levels until the MTD is determined or until the highest dose level is tested. A DLT is defined as any related ocular or systemic adverse event [redacted] occurring during the first 14 days in each dose escalation cohort:

- Any adverse event ≥ grade 3 deemed related to the study drug(s) by the investigator

[redacted]

[redacted]

6.6 Data Review Team

During the trial, Opthea will assess subject safety and tolerability of investigational product through periodic review of individual safety data (unmasked review for the Phase 1b and masked review for the Phase 2a). A data review team will review accumulating safety data and make recommendations to the study team regarding the conduct of the study in order to safeguard the interest of the trial participants while preserving the integrity of the study. [redacted]

6.6.1 Phase 1b Dose Level and Safety Data Review

For the Phase 1b dose escalation, a dose level review meeting will be held for each cohort. Escalation to a higher dose cohort will only proceed when the previous dose regimen(s) has (have) been found to be reasonably tolerated based on available study data through the DLT period for all participants enrolled in the cohort [redacted]

6.6.2 Phase 2a Safety Data Review

For the Phase 2a, the data review team will review masked safety data once 30, 60 and 90 patients have been randomized and completed at least 14 days follow-up post first dose. [redacted]

6.6.3 Determination of Adverse Event Severity and Relatedness

Determination of the severity of adverse events will be consistent with Common Terminology Criteria for Adverse Events (CTCAE) v 4. If the adverse event is not specified in CTCAE, then the severity will be assessed on a standard adverse event scoring system [redacted]. The relationship of an adverse event to investigational product will be determined by the investigator. An event should be considered related to treatment if, in the Investigator's medical judgment, there is a reasonable possibility that the event may have been caused by OPT-302 and or aflibercept [redacted].

[redacted]

[redacted]

[redacted]

6.7 Concomitant Therapy

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care except for those listed in Section 6.9. Any medication including over the counter or prescription medicines, vitamins and/or herbal supplements that the participant is receiving at the time of enrollment / randomization or receives during the study through to week 24 must be recorded in the eCRF along with reason for use, dates of administration (including start and end dates) and dosage information (*e.g.* dose). [redacted]

[redacted]

[redacted]

6.8 Concomitant Therapy for the Non-Study Eye

Treatment of DME that develops in the non-study eye during the study should follow the investigators best medical judgment for the patient. Other investigational agents must not be used for treatment of the non-study eye.

6.9 Excluded Treatments During or Prior to Study Period

The following medications and/or therapies should not be administered within the timeframes specified prior to enrollment or randomization or during the study (Day 1 through week 24):

- Eyes in which scatter (panretinal) photocoagulation is needed now or is likely to be needed within the next 3 months (*e.g. eyes with high risk proliferative diabetic retinopathy [defined as neovascularization of the disc > 1/3 disc area or neovascularization elsewhere > 1/2 disc area and presence of vitreous hemorrhage regardless of size of neovascularization] not already adequately treated with photocoagulation*)
- Previous treatment with photodynamic therapy, thermal laser or external beam radiation in the study eye
- Subjects who have received panretinal or focal/grid photocoagulation, YAG laser, or peripheral retinal cryoablation (for retinal tears only) in the study eye within the previous 4 months
- Concurrent or prior use of systemic anti-VEGF agents
- Concurrent or prior use of intravitreal bevacizumab in the study eye, unless therapy switched to intravitreal aflibercept or ranibizumab for ≥ 1 most recent injection(s) prior to study Day 1
- Most recent intravitreal injection of aflibercept or ranibizumab less than 28 days or greater than or equal to 42 days prior to Day 1 dosing in the study eye
- Administration of systemic steroids within 4 months prior to Day 1
- Concurrent or prior use of any intravitreal injections of steroids within 4 months prior to Day 1 in the study eye
- Concurrent or prior use of dexamethasone implant in the study eye
- Concurrent or prior use of fluocinolone implant in the study eye
- Concurrent or prior administration of experimental therapy within 30 days of screening
- Concurrent treatment for active systemic (non-ocular) infection at screening if in the opinion of the investigator, doing so will place the participant at undue risk
- Concurrent treatment in either eye for any ocular condition with an investigational drug or device that has not received regulatory approval
- Concurrent or prior use of thiazolidinediones within 6 months prior to Day 1 dosing

6.10 Other Procedures

The following procedures should not be undertaken within the timeframes specified prior to enrollment or randomization or during the study (unless otherwise specified below):

- Any intraocular surgery within 6 months of study entry or anticipated within the next 3 months following dosing on Day 1
- Previous posterior vitrectomy or scleral buckling surgery
- Major surgery (defined as intra-abdominal or surgery requiring general anesthesia) within 28 days prior to dosing on study Day 1 or major surgery planned during the next 6 months.

6.11 Contraception

All women of child bearing potential must have a negative pregnancy test performed at screening and at regular intervals throughout the study.

Women of child bearing potential must agree not to attempt to become pregnant or undergo *in vitro* fertilization and, must use highly effective contraceptive methods during the study and for 3 months following the last dose administration of study drug(s). The following are considered "highly effective methods": *e.g.* hormonal contraceptive (oral, intravaginal, or implant, but excluding progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action); intrauterine device; or documented vasectomy of partner. Male participants with female partners of child bearing potential must agree to use effective contraception (*e.g.* condom) during the study and for 3 months following the last dose administration of study drug(s).

Note: Women who are postmenopausal for at least 1 year (by history and defined as 12 consecutive months with no menses without an alternative medical cause), women with documented total hysterectomy, documented bilateral oophorectomy, and women with documented bilateral tubal ligation are considered of non-childbearing potential and are not required to use contraception.

7. STUDY PROCEDURES

7.1 General Study Procedures

All assessments for each participant will be performed by the study investigator(s) and / or appropriately delegated center staff according to the Schedule of Assessments [redacted] Every effort must be made to adhere to the Schedule of Assessments. Participants who are unable to make a required study visit should be encouraged to return to the clinic within the specified visit window to complete study assessments. In general, scheduled visits will include assessment of adverse events, concomitant medications, treatments and blood samples for various laboratory tests. All adverse events, concomitant medications, treatments and visit assessments will be documented on eCRFs.

All participants must sign the current IRB / IEC approved, protocol-specific informed consent form prior to undergoing any protocol specific evaluations and or procedures. [redacted]

7.2 Run-In Phase for Participants receiving Prior Intravitreal Bevacizumab

[redacted]

Only potential participants who meet the study inclusion criteria and no exclusion criteria [redacted] may enter the Run-In Phase.

Participants in the Run-in Phase will receive:

- At least one Intravitreal injection of aflibercept (2mg; 0.05 mL) in the proposed study eye
- Post injection safety checks will be performed in accordance with local site standard of care

[redacted]

At the end of the run-in phase, participants with persistent DME despite the prior intravitreal anti-VEGF-A therapy and who continue to be eligible [redacted] can be enrolled (Phase 1b) / randomized (Phase 2a) into the study. [redacted]

7.3 Screening

All screening assessments to determine eligibility must be completed within 14 days prior to study Day 1.

The following assessments will be performed at the screening visit after the informed consent has been obtained [redacted]

- Demographics

- Medical / Ocular / Surgical history including current conditions will be obtained
- Concurrent medications and any medication taken within 14 days prior to study Day 1
- Previous treatments for center involved DME. [redacted]
- Physical Exam [redacted]
- Vital signs measured
- ECG recorded
- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- Collection of Hematology, Chemistry and HbA1c samples
- Urine sample for Dipstick Urinalysis and Pregnancy test (*women of child bearing potential only*)
- SD-OCT [redacted]
- Fluorescein angiography [redacted]
- Color Fundus Photography [redacted]
- [redacted]

Medical, surgical and ocular history will be collected during screening and must include documented diagnosis of diabetic macular edema. [redacted] Angiographic and / or OCT confirmation of disease status must be performed using the same techniques and equipment as are planned throughout the study. When all screening procedures have been performed and the investigator has confirmed the subject's eligibility for the study, the participant will return for the Day 1 visit.

7.4 Week 1 Day 1

7.4.1 Pre-dose / Baseline

Prior to the participant being enrolled (Phase 1b) or randomized (Phase 2a) into the study the following assessment will occur.

- Vital signs measured
- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- Collection of Hematology and Chemistry samples
- [redacted]
- Urine sample for Pregnancy test (*women of child bearing potential only*)
- Any changes in Medical History or concomitant medications

[redacted]

Upon completion of screening and pre-dose tests and assessments, and review of all results including imaging eligibility performed by the independent reading center, the investigator will confirm final subject eligibility.

7.4.2 Enrollment (Phase 1b) / Randomization (Phase 2a)

Eligible participants only will be enrolled (Phase 1b) or randomized (Phase 2a) into the study.

In Phase 1b, a subject is considered enrolled when study drug(s) is administered. The dose regimens for the 3 treatment cohorts in the Phase 1b are as follows:

Cohort 1: 2 mg aflibercept and 0.3 mg OPT-302
Cohort 2: 2 mg aflibercept and 1 mg OPT-302
Cohort 3: 2 mg aflibercept and 2 mg OPT-302

For the Phase 2a, the subject is considered randomized when the unmasked site representative obtains the randomization number and treatment allocation [redacted]. Participants will be randomized in a 2:1 ratio to one of the two following arms:

Cohort 4: Aflibercept (2 mg) + OPT-302 (*at the MTD or highest dose tested from the Phase 1b*)
Cohort 5: Aflibercept (2 mg) + sham

7.4.3 Study Drug(s) Administration

Eligible participants will receive study drug(s) as follows:

- Intravitreal injection of aflibercept in study eye
- Post injection safety check must be performed and will include a check of optic nerve head perfusion, intraocular pressure and visual function
- Intravitreal injection of OPT-302 (*or sham in Phase 2a*) to the study eye

[redacted]

7.6 Week 3 Day 15 [redacted] *(Phase 1b only)*

At Day 15 [redacted] the following assessments / procedures will be performed:

- Vital signs measured

- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- SD-OCT [redacted]
- OCT-A [redacted]
- Record any adverse event and concomitant medications

7.7 Week 5 Day 29 [redacted]

On Day 29 [redacted] the following assessments / procedures will be performed prior to study drug administration:

- Vital signs measured
- ECG performed
- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- Collection of Hematology and Chemistry samples
- [redacted]
- Urine sample for Dipstick Urinalyses and Pregnancy test (*women of child bearing potential only*)
- SD-OCT [redacted]
- [redacted]
- Record any adverse events and concomitant medications administered

Study drug(s) Administration:

- Intravitreal injection of aflibercept in study eye
- Post injection safety check must be performed and will include a check of optic nerve head perfusion, intraocular pressure and visual function
- Intravitreal injection of OPT-302 (*or sham in Phase 2a*) to the study eye

[redacted]

- [redacted]

7.8 Week 9 Day 57 [redacted]

On Day 57 [redacted] the following assessments / procedures will be performed prior to study drug Administration:

- Vital signs measured
- ECG performed
- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- Collection of Hematology and Chemistry samples

- [redacted]
- Urine sample for Dipstick Urinalyses and Pregnancy test (*women of child bearing potential only*)
- SD-OCT [redacted]
- [redacted]
- Record any adverse events and concomitant medications administered

Study drug Administration:

- Intravitreal injection of aflibercept in study eye
- Post injection safety check must be performed and will include a check of optic nerve head perfusion, intraocular pressure and visual function
- Intravitreal injection of OPT-302 (*or sham in Phase 2a*) to the study eye

[redacted]

- [redacted]

[redacted]

- [redacted]

7.10 Week 12 Day 85 [redacted] – Follow Up

On Day 85 [redacted] the following assessments / procedures will be performed:

- Vital signs measured
- ECG recorded
- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- Collection of Hematology, Chemistry and HbA1c samples
- Urine sample for Dipstick Urinalysis and Pregnancy test (women of child bearing potential only)
- [redacted]
- SD-OCT (both eyes)
- Fluorescein angiography (both eyes)
- Color Fundus Photography (both eyes)
- [redacted]
- Record any adverse events and concomitant medications administered

[redacted]

7.11 Week 24 Day 168 [redacted] – Long Term Follow Up

On Day 168 [redacted] the following assessments / procedures will be performed:

- Ocular Examination [redacted]
- BCVA by EDTRS [redacted]
- SD-OCT [redacted]
- Fluorescein angiography [redacted]
- Color Fundus Photography [redacted]
- Record any adverse events and concomitant medications administered

7.12 Study Assessments

7.12.1 Demographics

Date of birth, gender, and race will be collected for all participants.

7.12.2 Medical / Ocular / Surgical History

Clinically significant medical, ocular and surgical history will be obtained from medical records, the physical examination and by interviewing the participant. The original diagnosis of central-involved DME must be recorded in the source documents. If a subject is referred to the study center, a copy of all applicable reports and imaging evidence, confirming the diagnosis must be provided to the study center before enrollment or randomization.

7.12.3 Concomitant Medications

All prescription and nonprescription medications taken within 14 days prior to treatment until the long-term follow-up visit will be recorded with start and stop dates captured or indicated as ongoing.

All prior IVT anti-VEGF-A therapy administered for central involved DME should also be recorded [redacted]

At each visit following Day 1 participants will be questioned by study staff to assess if they have commenced or had any changes in concomitant medications since the previous visit. Study staff should record all concomitant medications administered to the participant whilst in the clinic.

7.12.4 Physical Examination

A physical examination, including a review of body systems, will be performed at screening. [redacted]

7.12.5 Vital Signs

The following vital signs will be measured:

- Body temperature (degrees Celsius [°C])
- Respiratory rate (breaths / min)

- Pulse rate (beats / min),
- Blood pressure (mm mercury [mmHg])

Blood pressure and pulse are to be recorded after the participant has been resting semi-supine for at least 5 minutes. [redacted]

7.12.6 Electrocardiograms

ECG assessments will be collected in participants who have been supine for no less than 5 minutes. The following parameters will be reported: QRS, QT, QTcF, RR and PR intervals. [redacted] Repeat measurements will be performed if there are any clinical abnormalities observed or artifacts are present. [redacted]

7.12.7 Ocular Examination

A standard ophthalmic examination using slit lamp biomicroscopy and dilated fundus examination, will be performed on the study eye at all visits and fellow eye at selected visits by a study certified ophthalmologist. [redacted]

7.12.8 Best Corrected Visual Acuity

Visual acuity tests will be performed in the study eye and fellow eye by certified assessors using validated vision charts according to the ETDRS refraction [redacted]. In the Phase 2a dose expansion, visual acuity examiners will be masked to the participants' treatment assignments.

[redacted]

[redacted]

7.12.9 Clinical Laboratory Assessments

All laboratory samples are to be obtained by venipuncture before study drug(s) administration. All analyses will be performed by the study central laboratory unless otherwise indicated [redacted] Required clinical safety laboratory tests are as follows:

Biochemistry: Sodium; potassium; chloride; bicarbonate; albumin; calcium; magnesium; phosphorous; glucose; blood urea nitrogen; creatinine; creatinine kinase; total bilirubin; aspartate aminotransferase; alanine transferase; alkaline phosphatase.

Hematology: Red blood cells; hemoglobin; hematocrit; mean corpuscular volume; mean corpuscular hemoglobin; platelets; white blood cells with differential (absolute count or percentage of: neutrophils, eosinophils, basophils, lymphocytes and monocytes).

HbA1c: Glycated hemoglobin

Urine: Urine pregnancy testing (using a standard pregnancy kit) for women of child bearing potential only and urinalysis (dipstick testing) will be performed in the clinic.

[redacted] All testing materials will be provided as kits by the central laboratory. Each study site will follow the laboratory manual for proper collection, processing, labelling, and transport to the central laboratory.

[redacted]

[redacted]

7.12.10.1 Pharmacokinetics Samples

The PK parameters of OPT-302 will be estimated using standard non-compartmental methods. Pharmacokinetics data will include, but may not be limited to, C_{max}, t_{max} and area under the curve (AUC), and half-life ($t_{1/2}$), where feasible. [redacted]

The handling and processing of laboratory samples is described in detail in the study Laboratory Manual. All testing materials will be provided as kits to the site.

7.12.10.2 Anti-OPT-302 Antibody Analysis

Blood samples will be collected from all participants for the measurement of anti-OPT-302 binding antibodies. [redacted]

The handling and processing of laboratory samples is described in detail in the study laboratory manual. All testing materials will be provided as kits to the site.

7.12.11 Spectral Domain Optical Coherence Tomography (SD-OCT)

SD-OCT scans of both the study eye and fellow eye will be taken at each pre-determined time-point throughout the study by a masked and certified OCT technician, according to the IRC imaging standardized procedures. [redacted]

[redacted]

7.12.12 Fundus Fluorescein Angiography (FA)

A study IRC certified photographer (masked) will perform FA in both the study eye and fellow eye at each pre-determined time-point throughout the study according to the IRC imaging standardized procedures. [redacted]

7.12.13 Color Fundus Photography

A study IRC certified photographer (masked) will perform color fundus photography in both the study eye and fellow eye at each pre-determined time-point throughout the study according to the IRC imaging procedures. [redacted]

[redacted]

7.12.15 Independent Reading Center (IRC)

An Independent Reading Center (IRC) will conduct independent review of all imaging for the study to reduce variability of interpretation, and for the Phase 2a dose expansion will be masked to study treatment allocation. These masking procedures will avoid both performance and detection bias.

The IRC will assess and register / certify all imaging equipment and each technician involved in imaging at each site. [redacted] The images will be anonymized prior to submission to the IRC. [redacted]

Confirmation of eligibility is to be provided by the IRC prior to enrollment of any participant into the study. Determination of on study clinical management by imaging of participants will be assessed at the local site.

[redacted]

[redacted]

8. REMOVAL AND REPLACEMENT OF PARTICIPANTS

8.1 Removal of Participants

Participants have the right to withdraw fully or partially from the study at any time and for any reason without prejudice to their future medical care by the physician or at the institution.

Participants may decline to continue receiving investigational product(s) or other protocol-required therapies at any time during the study. If this occurs, the investigator will discuss with the participant

appropriate procedures for withdrawal from investigational product(s) or other protocol-required therapies. [redacted]

Should a participant (or a legally acceptable representative) request or decide to withdraw from the study, all efforts will be made to complete and report the observations as thoroughly as possible up to the date of withdrawal. [redacted]

The investigator's clinical judgment will be used to determine whether a participant should be removed from treatment or from the study due to an adverse event. A participant may also voluntarily withdraw from treatment or from the study due to an adverse event.

[redacted]

[redacted]

9. SAFETY DATA COLLECTION, RECORDING, AND REPORTING

It is the responsibility of the Investigator to ensure that all adverse events and other clinically significant findings that occur during the clinical study are documented and reported accurately.

9.1 Adverse Events

9.1.1 Definition of Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a clinical trial subject. The event does not necessarily have a causal relationship with study treatment. The investigator is responsible for ensuring that any AEs observed or reported by the participant are recorded in the medical record [redacted]

The definition of AEs includes worsening of a pre-existing medical condition. Worsening indicates the pre-existing medical condition (*e.g.*, diabetes, migraine headaches, gout) has increased in severity, frequency, and/or duration, and/or has an association with a significantly worse outcome. An AE does not include: medical / surgical procedures (but the condition that leads to the procedure may be and AE; situation where an untoward medical occurrence has not occurred (*e.g.* hospitalization for cosmetic surgery); overdose of study drug(s) or concomitant medication that does not result in any signs or symptoms (if signs or symptoms are present, then will be recorded as an AE); or underlying disease progression (DME in the study eye).

A treatment emergent adverse event (TEAE) is an AE that was not present prior to treatment with the study drug(s), or an event that was present prior to treatment, but worsens either in intensity or frequency following treatment.

9.1.2 Reporting Procedures for Adverse Events

The investigator is responsible for ensuring that all AEs observed or reported by the subject that occur after signing of the informed consent throughout the study until the final study visit are reported. [redacted]

At each visit, the investigator / delegate will determine whether any AEs have occurred. [redacted]

[redacted]

If known, the medical diagnosis of an adverse event should be recorded in preference to the listing of individual signs and symptoms. The investigator will evaluate any changes in laboratory values if laboratory testing is performed, and make a determination as to whether or not the change is clinically important to be reported as an adverse event, and whether or not the changes were related to the study drug(s). In general, abnormal laboratory findings without clinical significance (based on the investigator's judgment) should not be recorded as AEs. However, laboratory value changes that the investigator considers clinically significant or requiring treatment / adjustment in current therapy are considered AEs. Where applicable, the clinical sequelae (not the laboratory abnormality) should be recorded as the adverse event.

Every adverse event must be assessed and the eCRF entry reviewed and confirmed by the investigator.

All ocular AEs should indicate which eye the adverse event occurred (Oculus sinister (OS) [left], oculus dextrus (OD) [right], oculus uterque (OU) [both]).

The investigator has overall responsibility to ensure that the following adverse event attributes are assigned:

- Adverse event diagnosis or syndrome(s), if known (if not known, signs or symptoms)
- Dates of onset and resolution
- Severity [and/or toxicity per protocol]
- Assessment of relatedness to study drug(s)
- Action taken

If any AEs are present when a participant completes the study or when a participant is discontinued from the study, the Investigator / delegate should make every effort to follow-up the participant until the adverse event has resolved or stabilized. All follow-up information (and attempted follow-up contacts) should be documented in the participant's medical records.

If an adverse event changes in severity, it should be a single entry in the eCRF, and assigned the highest severity experienced. The adverse event toxicity grading scale used will be the National Institutes of Health Common Terminology Criteria for Adverse Events (CTCAE, Version 4).

If the adverse event is not specified in the CTCAE, the severity will be assessed on the following scale with appropriate clinical definitions:

Grade	Standard Adverse Event Severity Scoring System
1	MILD: An adverse event where there is an awareness of the sign or symptom, but no or minimal interference with normal daily activities.
2	MODERATE: An adverse event that causes interference with normal daily activities
3	SEVERE: An adverse event that causes inability to perform normal daily activities.
4	LIFE-THREATENING: An adverse event that causes inability to perform basic self-care functions and/or urgent medical or urgent operative intervention is indicated to prevent permanent impairment, persistent disability or death.
5	FATAL: An adverse event that directly leads to death.

It should be noted that an adverse event that is considered to be "severe" may not necessarily be considered to be "serious" or of major medical significance.

The relationship to study drug(s) should be assessed, and characterized as either study drug related, injection-related, or not related. Those AEs assessed as study drug related should further be assessed as possibly, probably or definitely related. [redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

The degree of certainty with which an AE is attributed in an individual participant to study drug(s) or alternative cause, (*e.g.* natural history of the underlying diseases, concomitant therapy, *etc.*) will be determined by how well the event can be understood in terms of:

- Known pharmacology of the study drug(s);
- Reaction of a similar nature previously observed with similar drugs;
- The event having often been reported in the literature for similar drugs as product related;
- The event being related by time to drug use/termination, product discontinuation, or reproduced on rechallenge;
- The plausibility of other explanation for the AE, based on the natural history of a given disease, or the safety profile of other drugs or interventions.

Medically significant AEs considered related to the study drug(s) by the investigator or the sponsor will be followed until resolved or considered stable.

9.2 Adverse Drug Reaction (ADR)

An adverse drug reaction (ADR) is defined by the International Council for Harmonisation (ICH) as any noxious and unintended response to a medicinal product related to any dose. Therefore, it is any AE where there is reasonable possibility of a causal relationship between the study drug(s) and the AE. An unexpected ADR is defined as an ADR, the nature, severity or frequency of which, is not consistent with the applicable drug information (i.e. not listed in the investigator's brochure for OPT-302 or in the summary of product characteristics for aflibercept).

9.3 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A suspected unexpected serious drug reaction (SUSAR) is defined as a SAE that is suspected to be an ADR, but is not consistent with the information as provided in the Investigators' Brochure - i.e. either is not listed as an expected ADR in the Investigator's Brochure, occurred at a greater severity than was listed, or there is an increase in the rate of occurrence that is judged to be clinically important. All SUSARs must be reported to the applicable regulatory agencies within the timelines as stipulated by local law and guidelines by the Sponsor, and reported to each IRB / IEC by each Investigator.

9.4 Serious Adverse Events

9.4.1 Definition of Serious Adverse Events

A serious adverse event (SAE) is defined as an AE that meets at least one of the following serious criteria:

- Results in death;
- Is life-threatening;
- Requires in-participant hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability / incapacity;
- Is a congenital anomaly / birth defect;
- Is an important medical event.

Death is an outcome of a SAE, and not a SAE in itself. All deaths must be reported for participants on study and for deaths occurring within 30 days of last study drug(s) administration or within 30 days of last study evaluation, whichever is longer, to [redacted] ensure immediate [redacted] reporting to all appropriate regulatory bodies within the required timelines as applicable. The Investigator should provide any additional requested information as available (*e.g.* autopsy reports and terminal medical reports). The term "life-threatening" in the

definition of "serious" refers to an event in which the participant was at immediate risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more serious.

Hospitalization is defined as the participant being hospitalized overnight, or the participant's hospital stay being prolonged for at least an additional overnight stay. Hospital admissions for a pre-existing condition or for normal disease management procedures (*e.g.* chemotherapy) will not be considered a SAE. [redacted] Complications that occur during hospitalizations, prolonging hospitalization by ≥ 24 hrs are an SAE.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered to be SAEs when, based upon appropriate medical judgment, they may jeopardize the study participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or convulsions, or the development of drug dependency or drug abuse.

9.4.2 Reporting Procedures for Serious Adverse Events

The investigator is responsible for ensuring that all SAEs observed by the investigator or reported by the subject that occur after signing of the informed consent through the week 24 follow-up visit are recorded in the participant's medical record. Any SAE (including death) that occurs during the course of the study, whether or not related to the study drug(s) due to any cause, must be reported immediately on the SAE form (within 24 hours of the investigator becoming aware of the event) [redacted]

The investigator (or designee) will be requested to complete the SAE form associated with the study including as much information regarding the event that is available at the time of the initial report. [redacted] The investigator must review and sign-off each SAE report to confirm that they have reviewed the SAE and the details are correct; however, this sign-off must not delay the initial reporting and may be undertaken after the initial report has been made. Prompt notification is

essential so that legal requirements and ethical obligations to the participants participating in the study can be met. [redacted]

The Investigator must also:

- Report all SAEs to the reviewing IEC / IRB within the time-line specified by the reviewing body per the respective reporting requirements;
- [redacted]

Opthea will report SAEs and / or SUSARs as required to regulatory authorities, investigators / institutions, and IRBs / IECs as applicable in compliance with all reporting requirements according to local regulations and good clinical practice.

9.5 Regulatory Reporting Requirements

Opthea has a legal responsibility to notify local regulatory authorities, about the safety of the study drug(s) under clinical investigation. Prompt notification of SAEs by the investigator is essential so that legal obligations and ethical responsibilities towards the safety of other participants are met. All SUSARs qualify for expedited reporting as soon as possible after Sponsor confirmation that the case meets the criteria for expedited reporting, and no later than the timelines stipulated by each relevant regulatory authority. Fatal or life-threatening SUSARs must be reported very rapidly by Sponsor (or designee) to the Regulatory authorities [redacted]

[redacted] Therefore, it is essential that all SAEs are reported as soon as the site becomes aware that the event has taken place, in order for rapid triaging and notification to occur.

9.6 Institutional Review Board / Independent Ethics Committee Reporting

It is the Investigators' responsibility to comply with the requirements for IRB / IEC notification. [redacted]

9.7 Pregnancy Reporting

Participants who become pregnant during the study period up to and including 30 days after the last use of study drug(s) are to be instructed that they immediately notify the Investigator, and must not receive any further study drug(s). [redacted]

10. STATISTICAL CONSIDERATIONS

All statistical considerations and analyses will be fully described in the statistical analysis plan, which will be finalized prior to database lock (and un-masking for the Phase 2a). [redacted]

10.1 Study Design

This is a two part (Phase 1b open-label, sequential dose escalation followed by a Phase 2a double masked, randomized, dose expansion) study evaluating OPT-302 in combination with aflibercept in participants with central-involved DME.

10.2 Study Endpoints, Subsets, and Covariates

10.2.1 Primary Endpoints

Phase 1b and Phase 2a:

- Safety: Subject incidence of adverse events, DLTs and clinically significant changes in vital signs, ECGs and clinical laboratory tests

Phase 2a:

- Efficacy: Response rate as defined by proportion of participants receiving combination OPT-302 and aflibercept achieving at least a 5 letter gain in BCVA compared to baseline at week 12 according to ETDRS criteria

10.2.2 Secondary Endpoints

- Mean change in BCVA from baseline to week 12 according to standardized ETDRS criteria
- Mean change from baseline to week 12 in CST and macular volume on SD-OCT
- Percent of eyes with ≥ 50% reduction in excess foveal thickness from baseline to week 12 on SD-OCT
- Percent of eyes with CST < 300 µm on SD-OCT through week 12
- Percent of participants with a ≥ 2 step improvement from baseline to week 12 in ETDRS Diabetic Retinopathy Severity Score
- The mean time to, and number of, retreatment injections of aflibercept anti-VEGF-A therapy based on protocol specified criteria during week 12 to 24 follow-up
- OPT-302 PK parameters
- Incidence of anti-OPT-302 antibody formation

10.2.3 Exploratory Endpoints

- CST area under the curve (AUC)
- Percent of eyes with resolution of fluid (sub-retinal fluid and intraretinal cysts) through week 12 on SD-OCT
- [redacted]

Additional details on endpoints will be included in the statistical analysis plan.

10.2.4 Analysis Subsets

10.2.4.1 Intent-to-treat (ITT) Analysis Set

The Intent-to-Treat (ITT) analysis sets will include all participants enrolled (Phase 1b) or randomized (Phase 2a) into the study, irrespective of whether study drug(s) was administered or not. These analysis sets will be used to report participant disposition and to provide a sensitivity analysis of the safety and efficacy endpoints only.

10.2.4.2 Safety Analysis Set

The safety analysis sets will comprise all participants in the ITT analysis sets, but excluding those not receiving at least one dose of study drug(s) (aflibercept or OPT-302). These analysis sets will be employed to determine the safety endpoints. Note that this population includes the 'unevaluable' participants (see per protocol population below) in the Phase 2a that received at least one administration of study drugs.

10.2.4.3 Dose Escalation Analysis Set

The analysis of dose limiting toxicity (DLT) will be conducted on the Phase 1b dose escalation analysis set defined as all DLT evaluable participants. A participant is classified as DLT-evaluable if they have had the opportunity to complete the DLT interval (Days 1 to 14) and received 1 dose of planned OPT-302 or experienced a DLT at any time during the first 14 days on study. [redacted]

10.2.4.4 Per-Protocol Analysis Set

The Per-Protocol (PP) analysis sets will comprise participants in the safety dataset who were compliant with study medication, and who are considered sufficiently compliant with the protocol. The participants pertaining to the PP datasets are considered 'evaluable' participants. Efficacy analyses performed using the PP datasets will be considered as primary analysis. The PP analysis sets are intended to represent the subset of participants who were in the intended study population, received the intended study medication (a total of 3 scheduled IVT injections once every 4 weeks), and could be evaluated for study outcomes.

10.2.5 Covariates

Due to the small sample size, the impact of baseline characteristics on study outcomes will not be explored, and no subgroup analyses will be performed in the primary analyses of safety and efficacy endpoints. Covariates could be included in secondary analyses for these purposes and will be defined in the statistical analysis plan.

10.3 Sample Size Considerations

In the Phase 1b dose escalation, at least 9 participants are expected to be enrolled. The sample size was determined empirically and is consistent with those used in this type of initial human clinical study. A total of at least 108 additional participants are expected to be treated in the Phase 2a (dose expansion) with OPT-302 at the MTD or highest dose tested from the Phase 1b, in combination with aflibercept (72 participants) or with aflibercept alone (36 participants).

Increasing numbers of participants will be exposed in the various phases of the trial:

- In the Phase 1b dose escalation, if 3 participants will be treated with OPT-302 per cohort (without dose limiting toxicity), a total of at least 9 participants will be treated in this phase of the trial.
- In the Phase 2a dose expansion, 72 additional participants will be enrolled at the MTD or the highest dose level of OPT-302 (determined from the Phase 1b). In case one or more of these 72 participants are not evaluable, randomization will continue and more patients will receive the same dose.

- A total of up to at least 81 participants receiving OPT-302 is anticipated to be enrolled in the trial (9 in Phase 1b and 72 in Phase 2a).

The table below shows the true proportion of patients with an AE that can be ruled out at various confidence levels, assuming no such event is observed in the trial.

Number of participants without adverse event	Confidence level		
	80%	90%	95%
3	0.48	0.57	0.63
9	0.24	0.30	0.35
81*	0.04	0.05	0.06

** If more than 72 participants are randomized to the experimental arm (aflibercept + OPT-302) in Phase 2a, the confidence levels of the total group of participants receiving OPT-302 will be higher than N=81.*

In the Phase 2a dose expansion, subjects will be allocated in a 2:1 ratio to one of two treatment groups, aflibercept with OPT-302 or aflibercept with sham, with minimization for two baseline characteristics: BCVA (≤ 55 vs. > 55 letters) and CST (≤ 450 vs. > 450 µm). The primary outcome of the Phase 2a is the proportion of evaluable patients with a response of ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the combination aflibercept + OPT-302 group.

The design of the trial is non-comparative in so far as the sample size is calculated for the aflibercept + OPT-302 arm only. A one-stage design is used for the primary outcome (Sargent 2001) of the aflibercept + OPT-302 arm. A formal rule allows for the assessment of the observed response rate as compared with pre-specified "low" and high response rates. Specifically, the hypotheses of interest are H_0: $r \leq r_0$ ("low" response rate) against H_A: $r \geq r_A$ ("high" response rate).

The following assumptions were made for sample size calculations:

- the pre-specified "low" and "high" response rates are $r_0 = 0.28$ and $r_A = 0.45$
- the type I error rate (α, probability of accepting a treatment whose true response rate is lower than r_0, a false positive outcome) is set to 5%
- the type II error rate (ß, probability of rejecting a treatment whose true response rate is higher than r_A, a false negative outcome) is set to 5%
- the probability of correctly rejecting a treatment whose true response rate is lower than r_0 is set to at least 90%
- the probability of correctly accepting a treatment whose true response rate is higher than r_A is set to at least 90%

Under these assumptions, a total of 72 evaluable subjects need to be randomized to the aflibercept + OPT-302 arm. With the 2:1 randomization, a sample size of at least 108 subjects is randomized between aflibercept + OPT-302 (n=72) or aflibercept + sham (n=36). Non-evaluable participants will

be replaced. The conclusions based on the results of 72 evaluable participants in the combination aflibercept + OPT-302 arm will be:

- clinical activity if ≥ 27 of 72 patients have a ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group
- insufficient clinical activity if ≤ 25 of 72 patients have a ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group

[redacted]

10.4 Access to Individual Subject Treatment Assignments

The study will be conducted in 2 parts: Phase 1b open label dose escalation and Phase 2a randomized dose expansion. Participants in this study will either receive aflibercept + OPT-302, or aflibercept + sham. The Phase 2a is a double-masked trial. The sponsor, investigator, VA assessor, imaging readers and subject will be masked to the treatment to which the subject was randomized.

10.5 Interim Analysis, Safety Review [redacted]

No formal interim analysis is planned. A data review team will advise on periodic safety review of the study [redacted] If concerns arise from either planned or unplanned safety reviews, the data review team may request additional review or recommend that the study at any time be modified or discontinued due to documented safety findings.

10.5.1 Phase 1b - Dose Escalation

In the open label Phase 1b, the unmasked patient safety data for each cohort will be reviewed by the data review team [redacted] After reviewing at least 14 days of safety data (DLT window) for a cohort, the data review team will decide on escalation to the next dose level for the subsequent cohort (or whether to stop dose escalation). Dose escalation will occur at the planned dose levels of OPT-302 until the MTD is determined or until the highest dose level is tested. On completion of Phase 1b the MTD or highest dose tested will be used to inform the OPT-302 dose selected for Phase 2a dose expansion.

10.5.2 Phase 2a - Dose Expansion

No interim analyses or stopping rules are planned based on the evaluation of efficacy. During the Phase 2a randomized dose expansion, there will be masked reviews of safety data by the data review team once 30, 60 and 90 participants have been randomized, received OPT-302 treatment and completed at least 14 days on study [redacted] If concerns arise from either

planned or unplanned safety reviews, the data review team may request additional review or recommend that the study at any time be modified or discontinued due to documented safety findings.

10.6 Statistical Analysis Plan

A final statistical analysis plan will be developed and approved by both Opthea and the study biostatistician prior to database lock. The primary efficacy analysis will occur when target enrollment or randomization is complete and each participant either completes the week 12 follow-up visit or withdraws from the study. The final analysis for all other endpoints will occur after all participants complete the week 24 follow-up visit.

[redacted] The following is a summary of the planned analyses; full details are captured in the statistical analysis plan.

Continuous variables will be summarized using descriptive statistics (N, mean, standard deviation, median, minimum and maximum), while categorical variables will be summarized as counts and percentages of participants in each category. Results will be presented by study arm as appropriate.

The primary efficacy analysis will be presented using the PP analysis set. Secondary efficacy analyses will be presented using the PP and ITT populations. For efficacy analyses, no missing values will be imputed as the primary efficacy analysis is on the PP population. Safety analyses will be presented using the safety and ITT populations.

All baseline and demographic summaries will be based on the ITT dataset.

10.7 Planned Methods of Analysis of Key Study Endpoints

10.7.1 Efficacy Endpoints

The primary efficacy endpoint of the Phase 2a is the proportion of evaluable participants (i.e. in the PP set) with a response of ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the combination aflibercept + OPT-302 group. Based on the results of 72 evaluable participants in the aflibercept + OPT-302 arm the combination therapy will be considered to have clinical activity if ≥ 27 of 72 participants have a ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group. Likewise the combination therapy will be considered to have insufficient clinical activity if ≤ 25 of 72 participants have a ≥ 5 letter gain in BCVA from baseline to week 12 according to ETDRS criteria in the aflibercept + OPT-302 group.

[redacted]

The difference in the mean change from baseline to week 12 between treatments will be tested for three secondary efficacy endpoints: mean BCVA, mean CST and mean macular volume. A model for repeated measures, fitted by restricted maximum likelihood method, will be used for the analysis. This model takes into account the presence of missing data and yields valid estimates under the assumption of data missing at random. [redacted]

In addition, the following secondary outcomes will be estimated in the Phase 2a by treatment arm, and their 95% confidence intervals will be obtained:

- Percent of eyes with ≥ 50% reduction in excess foveal thickness from baseline to week 12 on SD-OCT
- Percent of eyes with CST < 300 µm on SD-OCT through week 12
- Percent of participants with a ≥ 2 step improvement from baseline to week 12 in ETDRS Diabetic Retinopathy Severity Score
- The mean time to, and number of, retreatment injections of aflibercept anti-VEGF-A therapy based on protocol specified criteria during week 12 to week 24 follow-up

Further details of the analysis methods will be provided in the statistical analysis plan.

10.7.2 Safety Endpoints

10.7.2.1 Adverse Events

All safety summaries will be presented by study arm using the safety dataset. The extent of exposure to study medication will be quantified using total dose (mg), number of doses, and duration of exposure (days). Total dose and duration of exposure will be summarized using descriptive statistics (mean, median, standard deviation, minimum and maximum); number of doses and duration of exposure will be presented by counts and percentages. Percent compliance and the number of missed doses will be summarized using descriptive statistics. Compliance will also be presented according to range categories and summarized by counts and percentages.

The number of participants reporting AEs and TEAEs will be summarized by system organ class and preferred term; a participant will only be counted once per system organ class and once per preferred term within a study arm. Participant counts and percentages and event counts will be presented for each study arm by decreasing frequency and presented as all AEs, ocular AEs and non-ocular AEs for the following summaries: all TEAE, all SAEs, all TEAE by maximum severity, all TEAE by relationship to study medication, all TEAE related to study medication.

Participant listings will also be presented for all AEs as well as for SAEs leading to discontinuation from the study and AEs leading to discontinuation from study medication. These listings will include study arm and study period along with variables describing the nature, duration, and resolution of the event.

Adverse events will be coded using Medical Dictionary for Regulatory Activities [redacted] The number and percentage of participants reporting AEs will be evaluated for each dose and across doses and will also be tabulated by relationship to study drug(s). AEs resulting in treatment discontinuation will be identified.

Adverse events will be listed for participants enrolled in the study.

10.7.2.2 Dose Limiting Toxicities

A listing and summary of the subject incidence of DLTs will be provided should they occur.

10.7.2.3 Clinical Laboratory Tests

Clinical laboratory data will be listed for each subject. Values outside the normal laboratory reference ranges will be flagged as high or low on the listings.

10.7.2.4 Vital Signs

Vital signs data will be listed for each subject.

10.7.2.5 Electrocardiograms

ECG data will be listed for each subject and summarized.

10.7.2.6 Anti-OPT-302 Antibodies

Anti-OPT-302 antibody data will be listed for each subject and summarized.

10.7.3 Pharmacokinetic (PK) Analyses

The PK parameters of OPT-302 will be estimated using standard non-compartmental methods and summarized by cohort and dose level using means, standard deviations, medians, minimum and maximums. Individual serum / time profiles will be summarized by dose level. OPT-302 concentrations at each time point along with PK parameter values may be listed for each subject. Summary statistics will be computed for each sampling time and parameter as appropriate. Additional analyses of OPT-302 concentration-time data using compartmental methods may be performed.

[redacted]

11. REGULATORY OBLIGATIONS

11.1 Informed Consent

Before a subject's participation in the clinical study, the investigator or delegate (*e.g.* sub-investigator) is responsible for obtaining written informed consent from the subject or legally acceptable representative after adequate explanation of the aims, methods, anticipated benefits, and

potential hazards of the study and before any protocol-specific screening procedures or any study drug(s) are administered. A legally acceptable representative is an individual or other body authorized under applicable law to consent, on behalf of a prospective subject, to the subject's participation in the clinical study.

The investigator is also responsible for asking the subject if they have a primary care physician and if the subject agrees to have his/her primary care physician informed of their participation in the clinical study. If the subject agrees to such notification, the investigator shall inform the subject's primary care physician of the subject's participation in the clinical study. If the subject does not have a primary care physician and the investigator will be acting in that capacity, the investigator should document such in the subject's medical record. The original signed informed consent form should be retained in accordance with institutional policy, and a copy of the signed consent form should be provided to the subject or legally acceptable representative.

If a potential subject is illiterate and does not have a legally acceptable representative, the investigator must provide an impartial witness to read the informed consent form to the subject and must allow for questions. Thereafter, both the subject and the witness must sign the informed consent form to attest that informed consent was freely given and understood.

11.2 Regulatory Considerations

Opthea or their agents will submit appropriate documents for review and approval to the local regulatory agencies and IRB / IECs affiliated to each site prior to study commencement. This study will be conducted in accordance with the following guidelines and regulations:

- International Council for Harmonisation (ICH) Guidelines for Good Clinical Practice;
- The Declaration of Helsinki;
- US Food and Drug Administration (FDA) Human Participant Protection Regulations (Title 21 Code of Federal Regulations, Parts 50, 54, 56 & 312).

11.3 Institutional Review Board / Independent Ethics Committee

A copy of the protocol, proposed informed consent form, other written subject information, and any proposed advertising material must be submitted to the IRB / IEC for written approval. A copy of the written approval of the protocol and informed consent form must be received before recruitment of participants into the study and shipment of Opthea investigational product (OPT-302).

The investigator must submit and, where necessary, obtain approval from the IRB / IEC for all subsequent protocol amendments and changes to the informed consent document. The investigator should notify the IRB / IEC of important deviations from the protocol or SAEs occurring at the site and other AE reports received from Opthea, in accordance with local procedures.

The investigator will be responsible for obtaining annual IRB / IEC approval /renewal throughout the duration of the study. The investigator must also notify the IRB / IEC of the end of the study.

[redacted]

[redacted]

[redacted]

11.5 Subject Confidentiality

The investigator and members of the site staff must ensure that the subject's confidentiality is maintained:

[redacted]

[redacted] All electronic information regarding study participants will be kept on a password protected computer. [redacted] The study sites' data management and clinical staff will be the only personnel with access to the protected health information of the study participant. All research records will be kept according to ethics committee, ICH and regulatory requirements (whichever is the longer duration) following closure of the study. Identifiable information will not be stored in the eCRF and will not leave the site. [redacted]

Original records pertaining to this study may be inspected / audited at any time by Opthea employees or their duly authorized representatives, a regulatory authority or the IEC / IRB. All records accessed will be strictly confidential. Consent to participate in this study includes consent to direct access to records and these inspections / audits.

11.6 Investigator Signatory Obligations

For multicenter studies the clinical study report should be signed by the coordinating investigator identified by Opthea, who will either be:

- a recognized expert in the therapeutic area
- an investigator who provided significant contributions to the design or interpretation of the study
- an investigator contributing a high number of eligible participants

12. ADMINISTRATIVE AND LEGAL OBLIGATIONS

12.1 Clinical Trial Agreement

Prior to commencement of the study, the principal investigator must sign a clinical trial agreement that will clearly delineate the responsibilities and obligations of the investigator and Sponsor and will form the contractual basis under which the clinical trial will be conducted. [redacted]

12.2 Protocol Amendments and Study Termination

If Opthea amends the protocol, agreement from the investigator must be obtained. The IRB / IEC must be informed of all amendments and give approval.

Opthea reserves the right to terminate the study at any time either at a particular site or at all sites at any time and for any reason. Both Opthea and the investigator reserve the right to terminate the investigator's participation in the study according to the study contract. If such action is taken, Opthea will discuss this with the investigator(s) at that time and notify the investigator(s) in writing. If the study is suspended or terminated for safety reasons all investigators conducting the study will be immediately notified of the action as well as the reason for it, as will the relevant regulatory agencies. The Investigator will advise the IRB / IEC overseeing the study at their site.

Upon closure of the study (whether at the expected conclusion or prematurely), the following activities will be performed by the Sponsor in conjunction with the Investigator:

- Return of all study data
- Data clarification and resolution of queries
- Study drug accountability, reconciliation and final disposition
- Review of site study records for completeness
- Shipment of all relevant samples to the central laboratory

The investigator should notify the IRB / IEC in writing of the study's completion or early termination and send a copy of the notification to Opthea.

[redacted]

12.3 Study Documentation and Archive

The investigator should maintain a list of appropriately qualified persons to whom he / she has delegated study duties. All persons authorized to make entries and / or corrections on eCRFs will be included on the Delegation of Authority Form.

Source documents are original documents, data, and records from which the subject's eCRF data are obtained. These include but are not limited to hospital records, clinical and office charts, laboratory and pharmacy records, images, microfiches, radiographs, and correspondence. The principal investigator will be responsible for ensuring that source documents are filed in a suitably secure location to ensure source data verification can be undertaken throughout the study.

The investigator and study staff are responsible for maintaining a comprehensive and centralized filing system of all study-related (essential) documentation, suitable for inspection at any time by representatives from Opthea and / or applicable regulatory authorities.

In addition, all original source documents supporting entries in the eCRFs must be maintained and be readily available.

Following completion of the study the investigator will retain copies of the approved protocol, completed eCRFs, informed consent documents, relevant source documents, and all other supporting documentation related to the project in accordance with the applicable institutional review board, ethics committee, ICH and regulatory requirements (whichever is the longer). Documents must be retained for a minimum of 15 years from the date of termination of the study or for at least 2 years after the last approval of a marketing application in an ICH region, or at least 2 years after the formal discontinuation of the clinical development of an investigational product in accordance with local regulatory requirements.

In the event that the investigator retires or relocates, custody of the records may be transferred to another suitable person who will accept responsibility for the records. Notice of such transfer should be given to Opthea in writing. The investigator must notify Opthea prior to destroying any study related documents.

12.4 Study Monitoring, Data Collection and Quality Management

Monitoring for this study will be conducted both during monitoring visits (both masked and unmasked) and via centralized review of eCRF data. The investigator will permit Opthea and their

agents to monitor the study as frequently as Opthea deems necessary to determine that data recording and protocol adherence are satisfactory. A designated representative of Opthea in the form of a study monitor will verify participant data on the eCRFs for the purpose of analysis. The investigator will allow Opthea and their agents direct access to the related source documents for monitoring purposes as frequently as the sponsor deems necessary. This includes tests performed as a requirement for participation in this study and may also include other medical records required to confirm information contained in the eCRF such as past history and secondary diagnoses.

At each participant visit, the Investigator or delegate should record all data generated since the last visit on the eCRF. The Investigator and his / her staff will be expected to cooperate with the monitor to assist in providing any missing information. The study monitor will require access to the investigator's study file to ensure completeness of all study-related documentation.

The date the study monitor visits the study site will be recorded in the site visit log. During monitoring visits, the study site co-coordinator and Investigator should be available, the source documentation will be accessible and a suitable environment will be provided for the study monitor to review study related documentation.

The Opthea monitor is responsible for verifying the eCRFs at regular intervals throughout the study to verify adherence to the protocol; completeness, accuracy, and consistency of the data; and adherence to local regulations on the conduct of clinical research. The monitor should have access to subject medical records and other study-related records needed to verify the entries on the eCRFs. The key purposes for monitoring visits by the study monitor include the following:

- Review of all study documentation for completion, adherence to the protocol
- Notation of inconsistencies or missing data.
- Ensuring all study materials are correctly stored and dispensed.
- Verification of study data with source documents as per monitoring guidelines.
- Checking fulfilment of the obligations of the Investigator.
- Review of consent forms and date of consent.
- Inspection of investigational product (storage, labelling and documentation).

[redacted]

The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits, including delays in completing eCRFs, are resolved.

The study may be subject to an audit by an authorized representative of Opthea and / or an authorized Regulatory Authority (*e.g.* Food and Drug Administration [FDA]). Regulatory authorities may request access to all study documentation, including source documents for inspection and

copying, in keeping with local regulations. Opthea will immediately notify the Investigator of an upcoming audit / inspection. The principal investigator must also notify Opthea if they are made aware of an audit that may involve this study, or the facilities.

In the event of an audit, all pertinent study-related documentation must be made available. If an audit or inspection occurs, the Investigator will permit the auditor/inspector direct access to all relevant documents and allocate his / her time as well as the time of relevant staff to discuss the findings and any relevant issues.

[redacted]

[redacted] A detailed monitoring plan and data management plan will be developed detailing the quality control and quality assurance checks to be undertaken.

12.5 Language

All written information and other material to be used by participants and investigative staff must use vocabulary and language that are clearly understood.

12.6 Transfer of Sponsor Obligations

Transfer of Sponsor obligations may occur for certain activities such as project management, monitoring and data management. Such transfer of obligations will be outlined in a specific agreement, and will not discharge Sponsor of the obligation to ensure proper oversight of all aspects of the study.

12.7 Publication Policy

Authorship of any publications resulting from this study will be determined on the basis of the Uniform Requirement for Manuscripts Submitted to Biomedical Journals (International Committee of Medical Journal Editors)

[redacted]

13. REFERENCES

Aiello LP, Avery RL, Arigg PG, *et al*. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994; 331:1480-1487.

Avery RL, *et al*. Intravitreal injection technique and monitoring. Updated guidelines of an expert panel. Retina 2014; 34:S1-S18.

Cabral T, Lima L, Polido J, *et al*. Angiogenesis agents levels after bevacizumab intravitreal injection in patients with neovascular age-related macular degeneration. Invest Ophthalmol Vis Sci 2016; 57:3341.

Cai S, Bressler NM. Aflibercept, bevacizumab or ranibizumab for diabetic macular oedema: recent clinically relevant findings from DRCR.net Protocol T. Curr Opin Ophthalmol 2017 Aug 23 (Epub).

Campochiaro PA, Khanani A, Singer M, *et al*. Enhanced benefit in diabetic macula edema from AKB-9778 Tie2 activation combined with vascular endothelial growth factor suppression. Ophthalmology 2016; 123:1722-30.

Cao Y, *et al*. Vascular endothelial growth factor C induces angiogenesis in vivo. Proc Natl Acad Sci USA 1998; 95:14389-94.

Cao R, *et al*. Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogenesis, lymphangiogenesis, vascular fenestrations, and permeability. Circ Res 2004; 94:664-70.

Channa R, Sophie R, Khwaja AA, *et al*. Factors affecting visual outcomes in patients with diabetic macular edema treated with ranibizumab. Eye (Lond) 2014; 28:269-78.

Chew EY, *et al*. (ACCORD Study Group and Eye Study Group). Effects of medical therapy on retinopathy progression in type 2 diabetes. N Engl J Med 2010; 363:233-244.

Chung ES, *et al*. Contribution of macrophages to angiogenesis induced by vascular endothelial growth factor receptor-3-specific ligands. Am J Pathol 2009; 175:1984-92.

Ding J, Wong TY. Current epidemiology of diabetic retinopathy and diabetic macular edema. Curr Diab Rep 2012; 12:346-354.

Do DV, Nguyen QD, Khwaja AA, *et al*. Ranibizumab for edema of the macula in diabetes study: 3-year outcomes and the need for prolonged frequent treatment. JAMA Ophthalmol 2013; 131:139-45.

Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema. ETDRS report number 4. Int Ophthalmol Clin 1987; 27:265-272.

Eghøj MS, Sørensen TL. Tachyphylaxis during treatment of exudative age-related macular degeneration with ranibizumab. Br J Ophthalmol 2012; 96:21–23.

Elman MJ, Bressler NM, Qin H, *et al*. Expanded 2-year follow-up of ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. Ophthalmology 2011; 118:609–614.

Fan F, Samuel S, Gaur P, *et al*. Chronic exposure of colorectal cancer cells to bevacizumab promotes compensatory pathways that mediate tumor cell migration. Brit J Cancer 2011; 104:1270-7.

Ferris FL, Patz A. Macula edema. A complication of diabetic retinopathy. Surv Ophthalmol 1984; 28 Suppl: 452-61.

Forooghian F, Chew EY, Meyerle CB, *et al*. Investigation of the role of neutralizing antibodies against ranibizumab as mediators of tachyphylaxis. Acta Ophthalmol 2011; 89:e206–e207.

Gaal EI, *et al*. Comparison of vascular growth factors in the murine brain reveals placenta growth factor as prime candidate for CNS revascularization. Blood 2013; 122:658-65.

Genentech. Lucentis™, ranibizumab, Biologics License Application 125156. 2005.

Goldberg RA. How to prevent endophthalmitis after intravitreal injections. Int J Retin Vitr 2015; 1:12-14.

Gonzalez VH, Campbell J, Holekamp NM, *et al*. Early and long-term responses to anti-vascular endothelial growth factor therapy in diabetic macular edema: Analysis of Protocol I data. Am J Ophthalmol 2016; 172:72-79.

Grau S, Thorsteinsdottir J, von Baumgarten L, *et al*. Bevacizumab can induce reactivity to VEGF-C and -D in human brain and tumor derived endothelial cells. J Neurooncol 2011; 104:103-12.

Gregori NZ, Flynn HW, Schwartz SG, *et al*. Current infectious endophthalmitis rates after intravitreal injections of anti-vascular endothelial growth factor agents and outcomes of treatment. Ophthalmic Surg Lasers Imaging Retina 2015; 46:643-648.

Hasler PW, Bloch SB, Villumsen J, *et al*. Safety study of 38,503 intravitreal injections performed mainly by physicians in training and nurses in a hospital setting. Acta Ophthalmol 2015; 93:122-125.

Ishida S, Usui T, Yamashiro K, *et al*., VEGF164-mediated Inflammation Is Required for Pathological, but Not Physiological, Ischemia-induced Retinal Neovascularization. J Exp Med 2003; 198:483–489.

Jager RD, Aiello LP, Patel SC, Cunningham ET. Risks of intravitreous injection: a comprehensive review. Retina 2004; 24:676-698.

Jampol LM, Bressler NM, Glassman AR. Revolution to a new standard treatment of diabetic macular edema. JAMA 2014; 311:2269-2270.

Joukov V, *et al*. Proteolytic processing regulates receptor specificity and activity of VEGF-C. EMBO J 1997; 16:3898-911.

Joukov V, *et al*. A recombinant mutant vascular endothelial growth factor-C that has lost vascular endothelial growth factor receptor-2 binding, activation, and vascular permeability activities. J Biol Chem 1998; 273:6599-602.

Kaidonis G, Burdon KP, Gillies MC, *et al*. Common sequence variation in the VEGFC gene is associated with diabetic retinopathy and diabetic macular edema. Ophthalmology 2015; 122:1828-36.

Kaiser PK. Anti-integrin therapy in treatment of DME. Retina Today 2017; July/Aug: 57-59.

Kiss S, Chandwani HS, Cole AL, *et al*. Comorbidity and health care visit burden in working-age commercially insured patients with diabetic macular edema. Clin Ophthalmol 2016; 10:2443-2453.

Klaassen I, Van Noorden CJF, Schlingemann RO. Molecular basis of the inner blood-retinal barrier and its breakdown in diabetic macular edema and other pathological conditions. Prog Retin Eye Res 2013; 34:19-48.

Klein R, Klein BEK, Moss MA. The Wisconsin epidemiologic study of diabetic retinopathy XIV. Ten year incidence and progression of diabetic retinopathy. Arch Ophthalmol 1994; 112:1217-1228.

Klein R, Lee KL, Gangnon REG, *et al*. The 25-year incidence of visual impairment in type 1 diabetes mellitus: The Wisconsin epidemiologic study of diabetic retinopathy. Ophthalmology 2010; 117:63-70.

Kocabora MS, Telli ME, Fazil K, *et al*. Serum and aqueous concentrations of inflammatory markers in diabetic macular edema. Ocul Immunol Inflamm 2016; 24:549-554.

Korobelnik JF, Do DV, Schmidt-Erurth U, *et al*. Intravitreal aflibercept for diabetic macular edema. Ophthalmology 2014; 121:2247-2254.

Lashkari KM, et. al. Expression of VEGF-C, VEGF-D and their cognate receptors in experimental and clinical choroidal neovascularisation. ARVO Annual Meeting 2013; Abstract 4999-A0128.

Lashkari KM, *et al*. VEGF-C and VEGF-D Blockade by VGX-300 Inhibits Choroidal Neovascularization and Leakage in a Mouse Model of Wet AMD. ARVO Annual Meeting 2014; Abstract 4802.

Lee R, Wong TY, Sabanayagam C. Epidemiology of diabetic retinopathy, diabetic macular edema and related vision loss. Eye Vis (Lond) 2015; 2:17.

Li D, Xie K, Ding G, Li J, *et al*. Tumor resistance to anti-VEGF therapy through up-regulation of VEGF-C expression. Cancer Lett 2014; 346;45-52.

Lieu CH, Tran H, Jiang ZQ, *et al*. The association of alternate VEGF ligands with resistance to anti-VEGF therapy in metastatic colorectal cancer. PLoS One 2013; 8:e77117.

Lim LS, Ng WY, Mathur R. Conversion to aflibercept for diabetic macular edema unresponsive to ranibizumab or bevacizumab. Clin Ophthalmol 2015; 9:1715-1718.

Mira F, Paulo M, Henriques F, *et al*. Switch to aflibercept in diabetic macular edema patients unresponsive to previous anti-VEGF therapy. J Ophthalmol 2017; doi: 10.1155/2017/5632634.

Nguyen QD, Brown DM, Marcus DM, *et al*. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. Ophthalmology 2012; 119:789-801.

OPT-302 Investigator's Brochure. Opthea Limited, South Yarra, VIC, Australia.

Payne JF, Clark WL. Intravitreal aflibercept for diabetic macular edema. Retinal Physician 2015; 12(Jan/Feb):43-47

Pockock SJ, Simon R: Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. Biometrics 1975; 31: 103-115.

Rahimy E, Shahlaee A, Khan MA, *et al*. Conversion to aflibercept after prior anti-VEGF therapy for persistent diabetic macular edema. Am J Ophthalmol 2016; 164:118-127.

Rajendram R, Fraser-Bell S, Kaines A, *et al*. A 2-year prospective randomized controlled trial of intravitreal bevacizumab or laser therapy (BOLT) in the management of diabetic macular edema: 24-month data: report 3. Arch Ophthalmol 2012; 130:972–979

Regeneron. Aflibercept ophthalmic solution, Biologics License Application 125387. 2011.

Regillo CD, Callanan DG, Do DV *et al*. Use of corticosteroids in the treatment of patients with diabetic macular edema who have a suboptimal response to anti-VEGF: Recommendations of an expert panel. Ophthalmic Surg Lasers Imaging Retina 2017; 48:291-301.

Rose SD, Aghi MK. Mechanisms of evasion to antiangiogenic therapy in glioblastoma. Clin Neurosurg 2010; 57:123-8.

Sargent DJ, Chan V, Goldberg RM. A three outcome design for Phase II clinical trials. Contemp Clin Trials 2001; 22:117-125.

Schaal S, Kaplan HJ, Tezel TH. Is there tachyphylaxis to intravitreal anti-vascular endothelial growth factor pharmacotherapy in age-related macular degeneration? Ophthalmology 2008; 115:2199–2205.

Schwartz SG, Scott IU, Stewart MW, *et al*. Update on corticosteroids for diabetic macular edema. Clin Ophthalmol 2016; 10:1723-1730.

Shah P, Schwartz SG, Scott IU. Updating your diabetic macular edema treatment protocol. Incorporating the latest data from randomized clinical trials. Retinal Physician 2017; 14(Jul/Aug):48-50.

Shin ES, Sorenson CM, Sheibani N. Diabetes and retinal vascular dysfunction. J Ophthalmic Vis Res 2014; 9:362-373.

Senger DR, Connolly DT, Van De Water L, *et al*., Purification and NH2-Terminal Amino Acid Sequence of Guinea Pig Tumor Secreted Vascular Permeability Factor 1. Cancer Res 1990; 50:1774-78.

Solomon SD, Chew E, Duh EJ, *et al*. Diabetic Retinopathy: A position statement by the American Diabetes Association. Diabetes Care 2017; 40:412-418.

Spooner K, Hon T, Wijeyakumar W, Chang AA. Switching to aflibercept among patients with treatment resistant neovascular age-related macular degeneration: a systematic review with meta-analysis. Clin Ophthalmol 2017; 11:161-177.

Stacker SA, *et al*., VEGF-D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med 2001; 7:186-91.

Stewart MW, Rosenfeld PJ. Predicted biological activity of intravitreal VEGF Trap. Br J Ophthamol 2008; 92:667-8.

Stewart MW. Pharmacokinetics, pharmacodynamics and pre-clinical characteristics of ophthalmic drugs that bind VEGF. Expert Rev. Clin Pharmacol 2014; 7:167–180

Sun D, Nakao S, Xie F, *et al*. Molecular imaging reveals elevated VEGFR-2 expression in retinal capillaries in diabetes: a novel biomarker for early diagnosis. FASEB J 2014; 28:3942-51.

Tammela T, *et al*. VEGFR-3 controls tip to stalk conversion at vessel fusion sites by reinforcing Notch signalling. Nat Cell Biol 2011; 13:1202-13.

UK Prospective Diabetes Study Group. Tight blood pressure control and risk of macrovascular and microvasculature complications in type 2 diabetes: UKPDS 38. BMJ 1998; 317:703-713.

Wells J, Glassman A, Ayala A. The Diabetic Retinopathy Clinical Research Network. Aflibercept, bevacizumab or ranibizumab for diabetic macular edema. N Engl J Med 2015; 372:1193-1203.

Wells JA, Glassman AR, Ayala AR, *et al*. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema; two-year results from a comparative effectiveness randomized clinical trial. Ophthalmology 2016; 123:1351-1359.

Witmer AN, Blaauwgeers HG, Weich HA, *et al*. Altered expression patterns of VEGF receptors in human diabetic retina and in experimental VEGF-induced retinopathy in monkey. Invest Ophthalmol Vis Sci 2002; 43:849-57.

Witzenbichler B, *et al*. Vascular endothelial growth factor-C (VEGF-C/VEGF-2) promotes angiogenesis in the setting of tissue ischemia. Am J Pathol, 1998; 153:381-94.

Xu H, *et al*. Vascular endothelial growth factor C is increased in endometrium and promotes endothelial functions, vascular permeability and angiogenesis and growth of endometriosis. Angiogenesis 2013; 16:541-51.

Zhao B, Ma A, Cai J, *et al*. VEGF-A regulates the expression of VEGF-C in human retinal pigment epithelial cells. Br J Ophthalmol 2006; 90:1052-9.

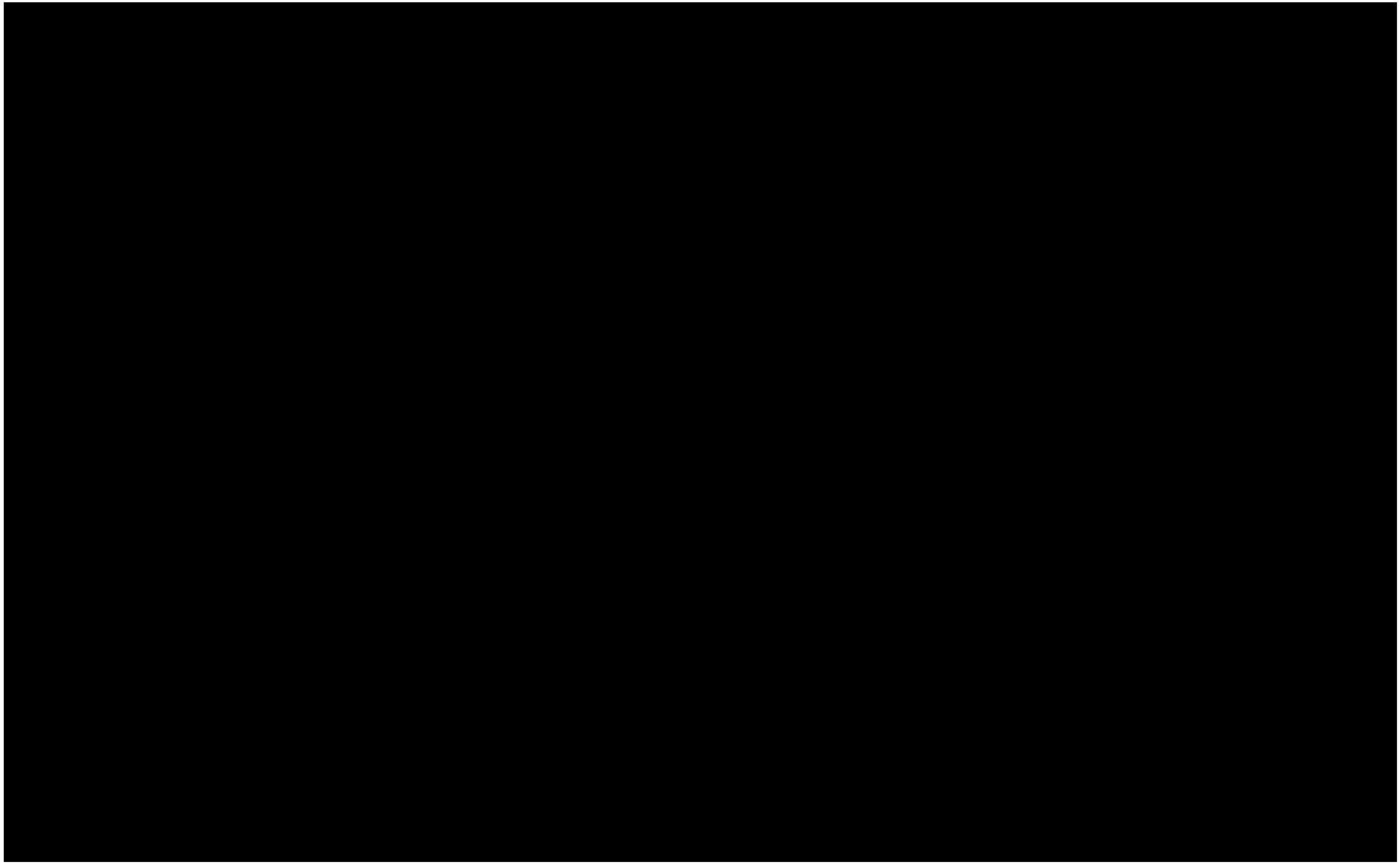
Zhao B, Smith G, Cai J, *et al*. Vascular endothelial growth factor C promotes survival of retinal vascular endothelial cells via vascular endothelial growth factor receptor-2. Br J Ophthalmol 2007; 91:538-45.

14. APPENDICES

Appendix A. Phase 1b Schedule of Assessments

Study Procedures	Run-In Phase*	Screen Visit(s)	IVT Treatment 1							IVT Treatment 2			IVT Treatment 3				Follow-up[14]	Long term follow-up[14]
Week (*beginning*)			1					2	3	5			9				12	24
Day	≤ -42 to -21	≤ -14	1		2	4		8	15	29			57			58	85	168
[redacted]																		
Informed Consent	X*	X																
Demographics	X*	X																
Run-In Eligibility	X[a]																	
Inclusion / exclusion criteria	X[b]	X	X															
Medical / Ocular / Surgical History	X*	X																
Physical Exam[1]		X																
Safety Assessments																		
Vital signs[2]		X	X		X				X	X		X	X		X		X	
ECG		X								X			X				X	
Eye Examination[3]		X	X		X				X	X		X	X		X		X	X
BCVA by ETDRS[4]		X	X						X	X			X				X	X
Concomitant Medications[5]			*Continuous assessment (through week 24)*															
Adverse Events			*Continuous assessment (through week 24)*															
Laboratory Assessments																		
Safety labs[6]		X	X							X			X				X	
HbA1c		X															X	
Urinalysis		X								X			X				X	
Pregnancy Test (urine)[7]		X	X							X			X				X	
Anti-OPT-302 antibody samples[8]	[redacted]																	
PK Samples																		
Imaging																		
SD-OCT[9]		X							X	X			X				X	X
Fluorescein angiography[10]		X															X	X
Color Fundus Photography[10]		X															X	X
[redacted]																		
Study Drug IVT Admin																		
Aflibercept 2 mg	X[c]			X							X			X			[13]X*prn*	
OPT-302[12]				X							X			X				

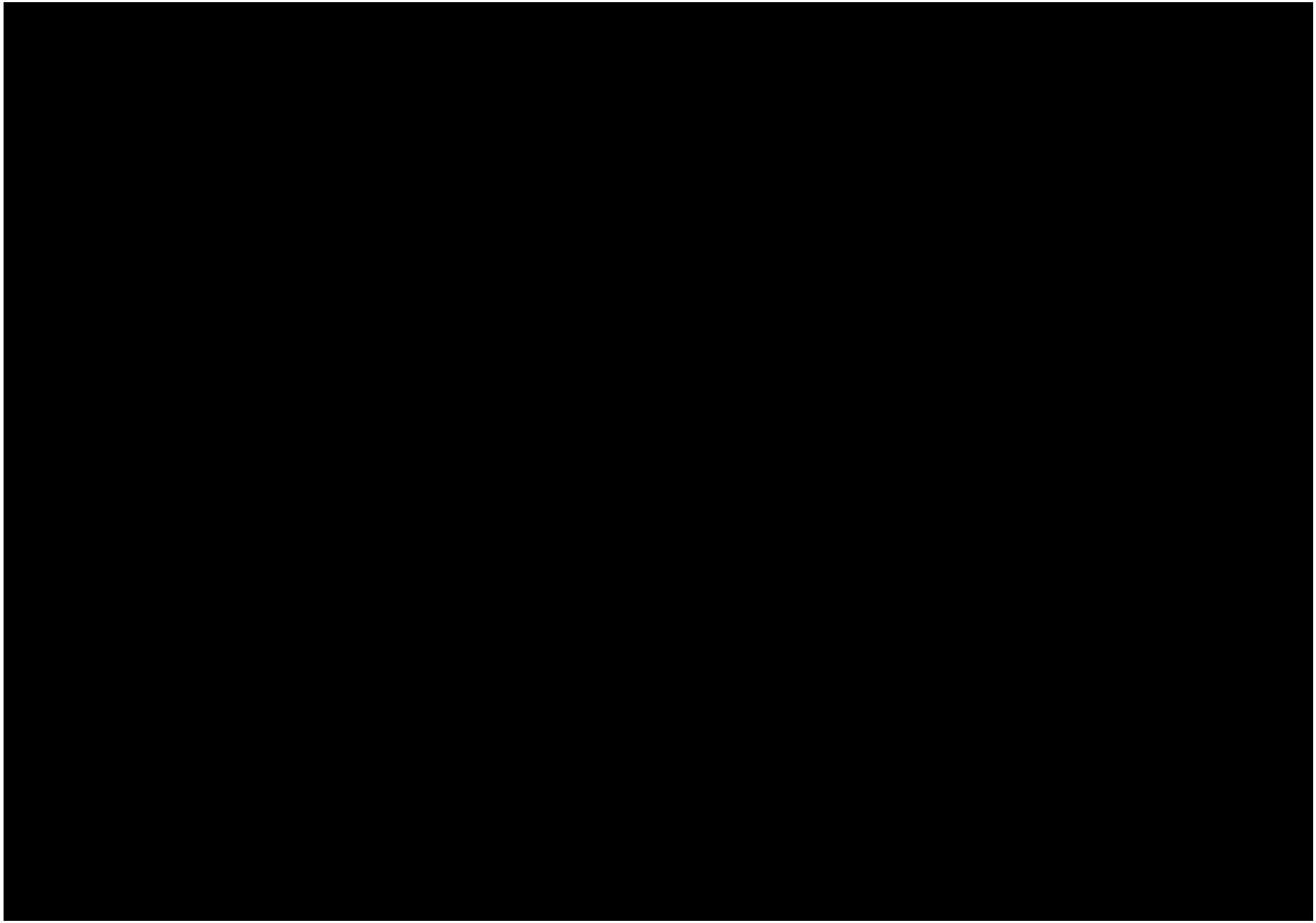
ADA = Anti-Drug Antibody for OPT-302; BCVA = Best Corrected Visual Acuity; DLT = Dose Limiting Toxicity; ETDRS = Early Treatment of Diabetic Retinopathy Study; FA = Fluorescein Angiography; PK = Pharmacokinetic; SD-OCT = Spectral Domain Optical Coherence Tomography



Appendix B. Phase 2a Schedule of Assessments

Study Procedures	Run-In Phase*	Screen Visit(s)	IVT Treatment 1			IVT Treatment 2			IVT Treatment 3			Follow-up[16]	Long term follow-up[16]
Week (*beginning*)			1			5			9			12	24
Day	≤ -42 to -21	≤ -14	1			29			57			85	168
[redacted]													
Informed Consent	X*	X											
Demographics	X*	X											
Run-In Eligibility*	X[a]												
Inclusion / exclusion criteria	X[b]	X	X										
Medical / Ocular / Surgical History	X*	X											
Physical Exam[1]		X											
Safety Assessments													
Vital signs[2]		X	X		X	X		X	X		X	X	
ECG		X				X			X			X	
Eye Examination[3]		X	X		X	X		X	X		X	X	X
BCVA by ETDRS[4]		X	X			X			X			X	X
Concomitant Medications[5]			*Continuous assessment (through week 24)*										
Adverse Events			*Continuous assessment (through week 24)*										
Laboratory Assessments													
Safety labs[6]		X	X			X			X			X	
HbA1c		X										X	
Urinalysis		X				X			X			X	
Pregnancy Test (Urine)[7]		X	X			X			X			X	
Anti-OPT-302 antibody samples[8]	[redacted]												
[redacted]													
Imaging													
SD-OCT[10]		X				X			X			X	X
Fluorescein angiography		X										X	X
Color Fundus Photography[11]		X										X	X
[redacted]													
Study Drug IVT Admin & Randomization													
Interactive Randomization system, IXRS[13]		X	X										
Aflibercept 2 mg	X[c]			X			X			X		[15]X*prn*	
OPT-302 or Sham[14]				X			X			X			

ADA = Anti-Drug Antibody for OPT-302; BCVA = Best Corrected Visual Acuity; DLT = Dose Limiting Toxicity; ETDRS = Early Treatment of Diabetic Retinopathy Study; FA = Fluorescein Angiography; PK = Pharmacokinetic; SD-OCT = Spectral Domain Optical Coherence Tomography



[redacted]

Appendix D. Refraction and Vision Testing Protocol (ETDRS)

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

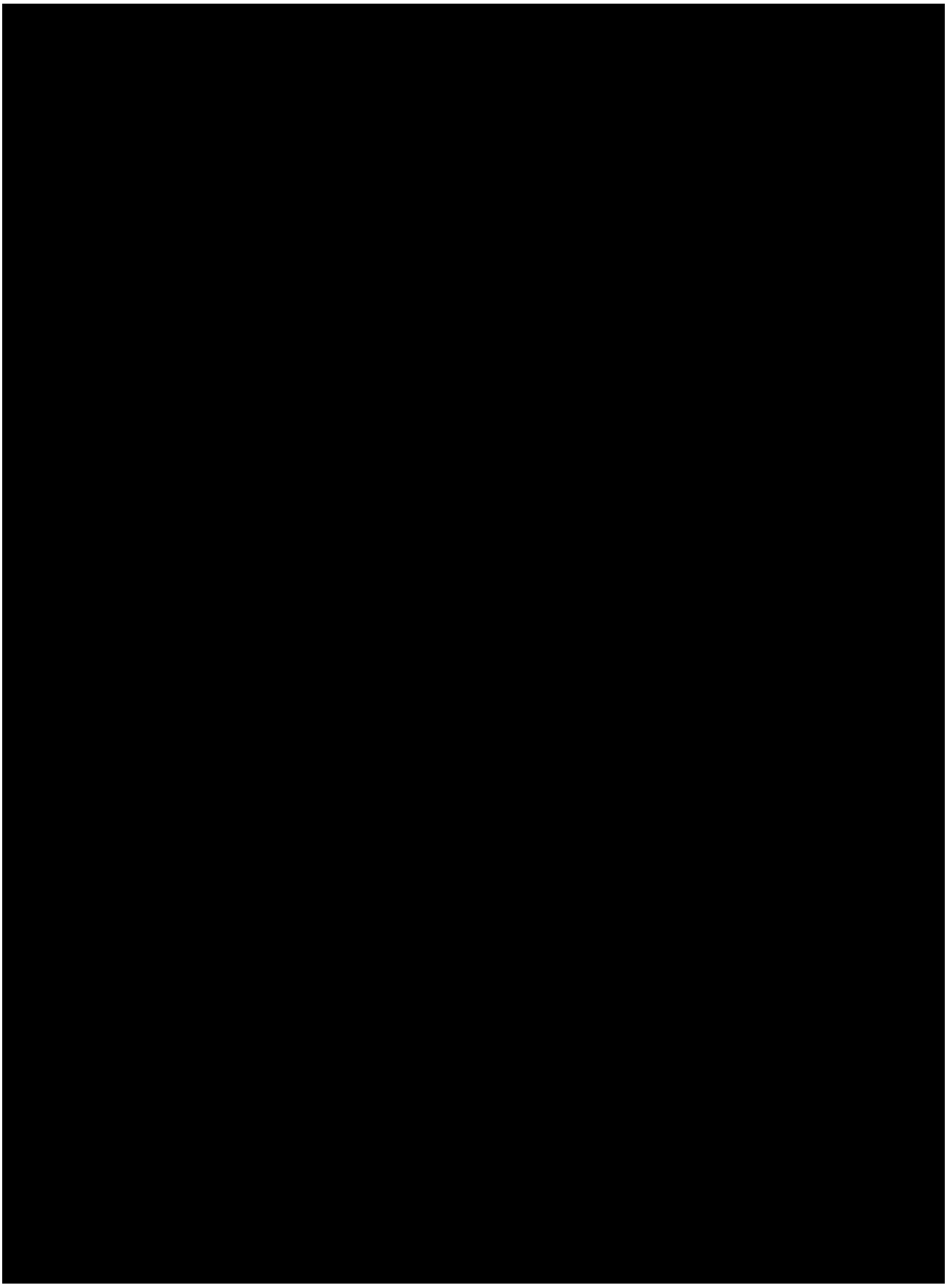
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]



[redacted]