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**Clinical Study Phase 2 Protocol
OPI-APXDR-201
ZETA-1**

Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Orally Administered APX3330 in Subjects with Moderately Severe to Severe Non-Proliferative Diabetic Retinopathy or Mild Proliferative Diabetic Retinopathy

Ocuphire Pharma, Inc.
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Farmington Hills, MI 48335

Original:	December 17, 2020
Amendment 1:	January 19, 2021
Amendment 2:	April 26, 2021
Amendment 3:	July 8, 2021
Amendment 4:	September 27, 2021

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SPONSOR SIGNATURE & CONTACTS

Study Title:	Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Orally Administered APX3330 in Subjects with Moderately Severe to Severe Non-Proliferative Diabetic Retinopathy or Mild Proliferative Diabetic Retinopathy
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Person authorized to sign the protocol and protocol amendment(s) for the sponsor, Ocuphire Pharma, Inc.

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Signature

Date

INVESTIGATOR'S AGREEMENT

OPI-APXDR-201 ZETA-1

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Investigator Agreement:

I, the undersigned, have reviewed this protocol and I agree to conduct this protocol in accordance with Good Clinical Practice, the ethical principles set forth in the Declaration of Helsinki and with the US Code of Federal Regulations governing electronic records, electronic signatures (21 CFR 11), the protection of human subjects (21 CFR 50), financial disclosure by Clinical Investigators (21 CFR 54), Institutional Review Boards (21 CFR 56) and the obligations of clinical investigators (21 CFR 312).

Signature: _____ Date: _____

Printed Name: _____

PROCEDURES IN CASE OF EMERGENCY

EMERGENCY CONTACT INFORMATION

Role in Study	Name	Contact Information
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[REDACTED]	[REDACTED]	[REDACTED]

ABBREVIATIONS AND TERMS

<i>Abbreviation</i>	<i>Full term</i>
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
APE1/Ref-1	Apurinic/Apyrimidinic Endonuclease 1/Redox Factor-1
ARP	All Randomized Population
AST	aspartate aminotransferase
BCVA	best-corrected visual acuity
BMI	body mass index
BP	blood pressure
CBC	complete blood count
CI	confidence interval
CRA	Clinical Research Associate
CRF	case report form
CST	central subfield thickness
DME	diabetic macular edema
DR	diabetic retinopathy
DRSS	Diabetic Retinopathy Severity Score
eCRF	electronic case report form
EDC	electronic data capture
eGFR	estimated glomerular filtration rate
ELISA	enzyme-linked immunosorbent assay
ETDRS	Early Treatment Diabetic Retinopathy Study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HbA1c	hemoglobin A1c
HIF-1 α	hypoxia inducible factor 1 alpha

HR	heart rate
ICH	International Council for Harmonisation
IND	Investigational New Drug
IOP	intraocular pressure
IRB	Institutional Review Board
ITT	Intent-to-Treat
IUD	intrauterine device
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LSM	least-squares mean
MDRD	Modification of Diet or Renal Disease
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intention-to-Treat
NFκB	nuclear factor kappa B
NPDR	non-proliferative diabetic retinopathy
OD	oculus dexter (right eye)
OF	odds ratio
OU	oculus uterque (both eyes)
PDR	proliferative diabetic retinopathy
PK	pharmacokinetic
PP	Per Protocol
PRP	panretinal laser photocoagulation
redox	reduction-oxidation
RH	relative humidity
SAE	serious adverse event
SD-OCT	spectral-domain optical coherence tomography
SOC	system organ class
SP	Safety Population
TEAE	treatment-emergent adverse event

US	United States
VA	visual acuity
VEGF	vascular endothelial growth factor
YAG	yttrium aluminum garnet

1. STUDY SUMMARY

Study Number	OPI-APXDR-201
Clinical Phase	Phase 2
Type of Study	Randomized, placebo-controlled, double-masked study of the safety and efficacy of orally administered APX3330 in subjects with moderately severe to severe non-proliferative diabetic retinopathy (NPDR) or mild proliferative diabetic retinopathy (PDR)
Name of Investigational Product	APX3330 – 600 mg dose (5 × 120 mg tablets)
Duration of Study	Up to 28 weeks, including screening, treatment, and follow-up
Rationale	<p>Diabetic retinopathy (DR) is the leading cause of vision loss in adults aged 20 to 74 years. Moderately severe to severe NPDR involves microvascular changes that can be asymptomatic, but eyes with moderately severe to severe NPDR are at high risk of progressing to sight-threatening PDR. Panretinal laser photocoagulation (PRP) had been the standard of care for PDR for more than 40 years and reduces the risk of severe visual loss by 50%, but also destroys tissue by its action and results in scotomas in the visual field. More recently, anti-vascular endothelial growth factor (VEGF) agents have been approved to treat complications of PDR, but this requires frequent intravitreal injections into the eye. Therefore, there is a substantial unmet need for novel treatments that reduce the risk of severe visual loss in PDR that is non-inferior to the current standard of care with fewer side effects.</p> <p>APX3330 is an Apurinic/Apyrimidinic Endonuclease 1/Redox Factor-1 (APE1/Ref-1 or Ref-1) specific inhibitor that can potentially reduce proinflammatory and hypoxic signaling that contributes to the transition to PDR. Ref-1 is an intracellular signaling nexus with important roles in transducing proangiogenic stimuli. Interference of Ref-1 activity with APX3330 blocks angiogenesis and inflammation by simultaneously reducing the activity of several important proangiogenic and proinflammatory transcription factors such as HIF-1α and NF-κB.</p> <p>Previous nonclinical studies showed that AXP3330 significantly decreases angiogenesis, inflammatory agents, and VEGF levels, and reduces the lesion volume in the laser-induced choroidal neovascularization mouse model. Also, Phase 1 and Phase 2 clinical studies in patients with cancer or chronic hepatitis C and B have shown that orally administered APX3330 was generally well tolerated at doses up to 600 mg/day with only mild and infrequent</p>

	systemic effects (mild skin rash and/or diarrhea in less than 10% of patients).
Study Objectives	<p><u>Primary objective</u></p> <ul style="list-style-type: none"> To evaluate the efficacy of APX3330 to improve Early Treatment Diabetic Retinopathy Study (ETDRS) Diabetic Retinopathy Severity Score (DRSS) in subjects with moderately severe to severe NPDR or mild PDR <p><u>Secondary objectives</u></p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p>
Design	<p>Placebo-controlled, double-masked, randomized, Phase 2 study in a maximum of 100 subjects with moderately severe to severe NPDR or mild PDR, evaluating safety and efficacy following oral administration of APX3330 twice daily for 24 weeks.</p> <p>The study will have a 1:1 randomization (placebo: APX3330). Randomization will be stratified by level of disease severity (NPDR or PDR). Subjects with mild PDR will be capped at 20% for each arm.</p> <p>The eligible eye with the highest DRSS will be designated as the study eye for the primary endpoint efficacy analysis. If the PDR cap has been reached, the study eye may be an eye with the lower DRSS if the other eye has mild PDR. In the case where both eyes have the same DRSS, the eye with the worse BCVA will be selected as the study eye. If the DRSS and BCVA are equivalent between eyes, study eye will be the right eye (OD).</p>

Subject Population	A maximum of 100 subjects with moderately severe to severe NPDR (DRSS Level 47 or 53) or mild PDR (DRSS Level 61).
Inclusion Criteria	<ol style="list-style-type: none"> 1. Males or non-pregnant females ≥ 18 years of age 2. At least one eye with DR graded at least moderately severe to severe NPDR or mild PDR (corresponding to DRSS 47, 53, or 61, confirmed by a central reading center) in which PRP and intravitreal injections of an anti-VEGF agent can be safely deferred for ≥ 6 months in the opinion of the Investigator 3. BCVA assessed by ETDRS protocol letters score of ≥ 60 letters (Snellen equivalent $\geq 20/63$) in the study eye 4. Sufficiently clear ocular media, adequate pupillary dilation, and fixation to permit quality fundus imaging in both eyes 5. Able to cooperate sufficiently for ophthalmic visual function testing and anatomic assessment 6. Body mass index (BMI) between 18 and 40 kg/m², inclusive 7. Able and willing to give signed informed consent and follow study instructions 8. Able to self-administer oral study medication or to have study medication administered by a caregiver throughout the study period
Exclusion Criteria	<p>Ophthalmic:</p> <ol style="list-style-type: none"> 1. Retinopathy from causes other than diabetes 2. Presence of center involved diabetic macular edema (DME) defined as a central subfield thickness (CST) ≥ 320 μm on SD-OCT. Center involved DME in the fellow eye is allowed. Intravitreal injections of an anti-VEGF agent in the fellow eye do not exclude the subject 3. Any prior treatment in the study eye with: <ol style="list-style-type: none"> a. Focal or grid laser photocoagulation within the past year or PRP at any time b. Systemic or intravitreal anti-VEGF agents within the last 6 months or likely, in the opinion of the Investigator, to require treatment during the course of the study c. Intraocular steroids including triamcinolone and dexamethasone implant within the last 6 months d. Fluocinolone implant within the last 3 years 4. Clinically significant ocular disease in either eye as deemed by the Investigator to likely interfere with the study procedures and visual acuity measurements (e.g., cataract, pseudophakia)

	<p>without evidence of posterior capsular opacity, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca)</p> <ol style="list-style-type: none"> 5. Presence of other macular or retinal vascular disease including age-related macular degeneration, pattern dystrophy, choroidal neovascularization of any cause, retinal vein occlusion, retinal artery occlusion in the study eye 6. Presence of active vitreous hemorrhage that would prevent adequate clinical imaging in either eye 7. History of retinal detachment or full-thickness macular hole in the study eye 8. Uncontrolled glaucoma in either eye, defined as advanced cup-to-disc ratio > 0.7 and intraocular pressure (IOP) > 25 mmHg, with or without topical antihypertensive eye drops; treatment of ocular hypertension or controlled glaucoma are not criteria for exclusion 9. Ocular incisional surgery including cataract surgery in the study eye within 3 months prior to Day 1 10. Yttrium aluminum garnet (YAG) posterior capsulotomy in the study eye within the last 30 days 11. Aphakia in the study eye 12. Previous pars plana vitrectomy in the study eye 13. Epiretinal membrane, posterior hyaloidal traction, and/or vitreomacular traction in the study eye as determined to be significant by the Investigator 14. Active uveitis and/or vitritis in either eye 15. History of idiopathic or autoimmune-associated uveitis in either eye 16. Active infection in either eye including infectious conjunctivitis, keratitis, scleritis, or endophthalmitis <p>Systemic:</p> <ol style="list-style-type: none"> 17. Poorly controlled diabetes, defined as hemoglobin A1c (HbA1c) $\geq 12.0\%$ or $< 12.0\%$ with uncontrolled diabetes mellitus 18. Known to be immunocompromised or receiving immunosuppressive therapy 19. Any disease or medical condition that in the opinion of the Investigator would prevent the subject from successfully participating in the study or which might confound the study results 20. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine,
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	<p>or cardiovascular disorders) that might interfere with the study as deemed by the Investigator</p> <p>21. Estimated glomerular filtration rate (eGFR) < 30 mL/min by Modification of Diet or Renal Disease (MDRD) or creatinine > 4 mg/dL</p> <p>22. History of allergic reaction to investigational drug or any of its components</p> <p>23. Resting heart rate (HR) outside the specified range of 50-110 beats per minute at the Screening Visit. HR may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position</p> <p>24. Hypertension with resting diastolic blood pressure (BP) > 110 mmHg or systolic BP > 180 mmHg at the Screening Visit. BP may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position</p> <p>25. History of chronic liver disease or presence of elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) consistent with such diagnosis (i.e., AST or ALT > 2 × upper limit of normal)</p> <p>26. Participation in any investigational study within 30 days prior to Screening or planning to participate in any other investigational drug or device clinical trials within 30 days of study completion</p> <p>27. Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. An adult woman is considered to be of childbearing potential unless she is 1 year postmenopausal or 3 months post-surgical sterilization. All women of childbearing potential must have a negative urine pregnancy test result at the Visit 1/Screening examination and must intend to not become pregnant during the study</p>
Screening Visit	<p>Subjects with moderately severe to severe NPDR or mild PDR will be selected for study participation and be screened for study eligibility.</p> <p>Once a subject arrives at the study center, a member of the study center staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed.</p> <p>The start of Screening includes the assignment of a subject identification number, explanation of the study, a medical and</p>

	<p>ophthalmic history, demographics, and a review of prior/concomitant medications. This shall be followed by a urine pregnancy test for women of childbearing potential, physical examination, and measurement of HR/BP.</p> <p>Subsequently, BCVA will be measured and SD-OCT (for CST) and color fundus photographs (for DRSS) will be performed. DRSS eligibility will be determined by a central reading center with 7-field or 4-wide field fundus photography. The central reading center will also determine CST eligibility with SD-OCT.</p> <p>Ophthalmic and non-ophthalmic criteria shall be evaluated, and only eligible subjects will continue.</p> <p>The Screening Visit (Visit 1) occurs 1 to 21 days prior to Qualification/Baseline Visit.</p>
Qualification/Baseline Visit	<p>A Qualification Visit (Visit 2) will occur before dosing on Day 1. BCVA, DRSS, and CST assessments at Screening will be the baseline values.</p> <p>The eligible eye with the highest DRSS, as assessed by the central reading center, will be designated as the study eye for the primary efficacy analysis. If the PDR cap has been reached, the study eye may be an eye with the lower DRSS if the other eye has mild PDR. If both eyes have the same DRSS, the eye with the worse BCVA will be selected as the study eye. If both eyes have equal DRSS and BCVA, OD will be the study eye.</p> <p>If the subject meets all eligibility criteria, then the subject will be randomized into the study and receive study medication. Blood will be drawn for biomarker analysis.</p>
Treatment Visits	<p>There will be 3 scheduled treatment site visits: Visit 4 Week 4 (± 2 days), Visit 6 Week 12 (± 2 days), and Visit 9 Week 24 (± 2 days). In between these visits, subjects will be contacted by telephone on Visit 3 Week 1 (± 2 days), Visit 5 Week 8 (± 2 days), Visit 7 Week 16 (± 2 days), and Visit 8 Week 20 (± 2 days) for a safety assessment to include adverse events (AEs), concomitant medications, and drug compliance.</p> <p>DRSS evaluations by the reading center will be performed with color fundus photographs obtained at the sites during the Visit 6 (Week 12) and Visit 9 (Week 24) Visits. Other assessments performed at visits during the treatment period will include but are not limited to visual acuity, SD-OCT (for CST), and safety measures.</p> <p>In the judgement of the Investigator, if there is any risk to the eye(s) of the subject and if possible, upon approval by the Medical</p>

	<p>Monitor or Sponsor, an appropriate rescue treatment for DR progression will be administered. Eyes may be rescued if there is:</p> <ul style="list-style-type: none"> • Moderate or severe active PDR and/or development of anterior segment neovascularization • Progression to clinically significant center involved DME • ≥ 10-letter loss in BCVA compared to baseline attributable to worsening retinopathy • Any other finding which, in the judgement of the Investigator, requires rescue treatment. In this situation, the Investigator should make every effort to consult with the Medical Monitor or Sponsor prior to initiation of rescue. <p>Prior to initiating rescue, BCVA, color fundus photography for DRSS, CST, biomicroscopy, ophthalmoscopy, and IOP study procedures must be performed before rescue treatment is initiated (only conducted before first instance of rescue treatment). These assessments may be performed as an unscheduled visit.</p> <p>If rescue treatment is given, the subject may continue in the study and will remain on their randomized treatment and complete all remaining study visits per the visit schedule.</p> <p>At Visit 6 Week 12 (± 2 days) prior to the morning dose and 3 hours post-morning dose, blood samples will be collected to establish drug levels of APX3330 from approximately 25 to 30 subjects at a subset of clinical sites. These subjects will be instructed to delay their morning study medication dose on the day of this visit in order to take their study medication at the site.</p> <p>At Visit 2 (Day 1) and Visit 9 Week 24 (± 2 days), biomarker levels in plasma will be measured with an ELISA (Ref-1), and a cytokine panel.</p>
Follow-up Visit	A Follow-up Phone Call will occur on Visit 10 Week 25 (± 2 days), one week after the last dose. Over-the-phone safety assessments will be performed at this follow-up.
Number of Investigational Sites	Approximately 20 to 25 sites
Estimated Total Sample Size	A maximum of 100 randomized subjects, with approximately 96 evaluable subjects
Sample Size Justification	A sample size of 96 subjects who are evaluable for efficacy (i.e., who have at least one post-randomization DRSS evaluation in the study eye, who have missed less than 20% of expected doses, and do not have any major protocol deviations considered to have significant impact on treatment outcome) is needed for the study.

[illegible]

	<p>Measurements:</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p>
Safety Endpoints	<ul style="list-style-type: none"> • Incidence and severity of systemic and ocular AEs • Change from baseline in body system assessments • Change from baseline in vital sign measurements • Change from baseline in clinical laboratory assay results (blood chemistry, hematology) • Change from baseline in IOP • Change from baseline in slit lamp examination parameters • Change from baseline in dilated funduscopy examination parameters • Percent of subjects with a decrease of ≥ 10 letters in BCVA compared to baseline at Week 12 and Week 24 • Percent of subjects progressing to center involved DME (eligible for rescue treatment at Week 12 and Week 24) • Percent of subjects with a worsening in DRSS of ≥ 2 steps in the study eye at Week 12 and Week 24 • Percent of subjects developing anterior segment neovascularization at Week 12 and Week 24 • Percent of subjects rescued (intravitreal anti-VEGF injection, laser PRP, focal/grid laser treatment, or surgery [vitrectomy]) at the discretion of the Investigator at Week 12 and Week 24 • Time to first rescue treatment • Change from baseline in eGFR at Week 12 and Week 24 <p>Urine pregnancy tests for women of childbearing potential will be conducted at each study visit.</p>
Study Medications, Dose and Mode of Administration	<p>APX3330 oral tablets:</p> <p>Five 120 mg tablets will be taken by mouth as follows: 3 tablets every morning and 2 tablets every evening.</p> <p>Placebo oral tablets:</p>

	<p>Placebo tablets are identical to APX3330 tablets except for the absence of the active pharmaceutical ingredient. Five placebo (0 mg) tablets will be taken by mouth as follows: 3 tablets every morning and 2 tablets every evening.</p> <p>Study medication should be taken at approximately the same time each day and may be taken with or without food.</p> <p>If a subject is considering discontinuing from the study due to an AE, the Investigator may offer a dose reduction from 600 mg to 480 mg per day as an alternative (2 tablets every morning and 2 tablets every evening).</p>
Duration of Subject Participation and Study	<p>The total length of subject participation is approximately 28 weeks, with 5 clinic visits, 4 telephone safety calls, and one telephone call follow-up visit summarized below:</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>[REDACTED]</p> <p>The execution of the entire study (first subject screen through last randomized subject completed) is expected to be approximately 12 to 15 months.</p>

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2. INTRODUCTION

2.1. *Investigational products*

Diabetic retinopathy (DR) is the leading cause of vision loss in adults aged 20 to 74 years¹. Moderately severe to severe non-proliferative diabetic retinopathy (NPDR) involves microvascular changes that can be asymptomatic, but eyes with moderately severe to severe NPDR or mild proliferative diabetic retinopathy (PDR) are at high risk of progressing to sight-threatening PDR or diabetic macular edema (DME)². Panretinal laser photocoagulation (PRP) had been the standard of care for PDR for more than 40 years and reduces the risk of severe visual loss by 50%, but also destroys tissue by its action and results in scotomas in the visual field³. More recently, anti-vascular endothelial growth factor (VEGF) agents have been approved to treat complications of PDR⁴, but this requires frequent intravitreal injections. Many patients also have residual loss of vision or persistent DME despite anti-VEGF therapy. Therefore, there is a substantial unmet need for novel treatments that reduce the risk of severe visual loss in patients with diabetic eye disease that are non-inferior to the current standard of care with fewer side effects.

APX3330 is a small-molecule APE1/Ref-1-specific inhibitor that specifically targets Apurinic/Apyrimidinic Endonuclease 1/Redox Factor-1 (APE1/Ref-1 or Ref-1)⁵, a dual-function protein involved in the regulation of transcription factors critical to cell signaling that regulates inflammation, angiogenesis and reduction-oxidation (redox) signaling (Ref-1 function), and DNA repair (APE1 function) that is critical to normal function of neurons⁶⁻⁸.

APX3330 can potentially reduce proinflammatory and hypoxic signaling that contribute to the transition to DR. Ref-1 is an intracellular signaling nexus with an important role in transducing proangiogenic stimuli. Interference of Ref-1 activity with APX3330 blocks angiogenesis and inflammation by simultaneously reducing the activity of several important proangiogenic and proinflammatory transcription factors such as HIF-1 α and NF- κ B⁹. Blocking Ref-1's redox function leads to the targeting of multiple pathways relevant to the pathophysiology of retinal and choroidal vascular diseases, suggesting that it is a particularly good candidate for clinical evaluation of its efficacy and safety in the treatment of this important group of diseases^{6,7,17-21,8,10-16}.

APX3330 has been previously evaluated in preclinical toxicology, and clinical Phase 1 and 2 studies by Eisai for the treatment of hepatic inflammation related to chronic hepatitis and was formerly being developed by Apexian for cancer indications. Previous nonclinical studies showed that AXP3330 significantly decreases angiogenesis, inflammation agents and VEGF levels, and reduces the lesion volume in the laser-induced choroidal neovascularization mouse model^{12,13,16}. Phase 1 and Phase 2 clinical studies in patients with cancer or chronic hepatitis C and B have shown that orally administered APX3330 was generally well tolerated at doses up to 600 mg/day with only mild and infrequent systemic effects (mild skin rash and/or diarrhea in less than 10% of patients). A summary of findings from the collective APX3330 development efforts that directly support Ocuphire's development program are summarized in [Section 2.2](#) and in further detail in the Investigators' Brochure.

In a written response from the United States (US) Food and Drug Administration (FDA) to Investigational New Drug (IND) 142152 dated January 30, 2019, it was noted that the current nonclinical data package was adequate and sufficient for a 6-month clinical trial of APX3330 in

DME patients.

APX3330 is formulated as 120 mg tablets. The dosage for this study will be 600 mg/day dosed as 3 tablets every morning and 2 tablets every evening.

Placebo tablets without the active ingredient will also be dosed 3 tablets every morning and 2 tablets every evening.

2.2. Findings from nonclinical and clinical studies

Nonclinical toxicology studies

Single- and repeat-dose toxicology studies in rats and dogs up to 3 months duration have been conducted together with a series of developmental, genotoxicity, and antigenicity studies. The key toxicology findings that inform the design and conduct of this study include:

Key toxicology findings:

- As a single oral dose, APX3330 was weakly toxic, producing mortality only at the highest dose of 2000 mg/kg.
- Repeated oral doses to rats (100 and 300 mg/kg) produced lysosomal dense eosinophilic inclusions in renal tubules suggestive of accumulation of compound. This finding was reversible within 5 weeks of compound discontinuation. The renal tubule inclusions were not observed in dogs.
- Soft and muddy stool (diarrhea) was the most remarkable finding in dogs treated with doses up to 100 mg/kg for 3 months. In shorter-term repeat-dose studies at 100 or 200 mg/kg, there was an increased leakage of hepatic enzymes and evidence of inflammatory infiltration, but evidence of necrosis was absent.
- APX3330 was not genotoxic and had no toxicologically significant effects in developmental studies.

Preclinical studies

In a laser-induced choroidal neovascularization (L-CNV) mouse model, mice received twice daily gavages of 25 or 50 mg/kg APX3330 or vehicle for 14 days. Optical coherence tomography (OCT), funduscopy, and 3-dimensional quantification of agglutinin stained CNV were performed. At both 25 and 50 mg/kg doses, APX3330 reduced size of L-CNV lesions by >50%. No obvious signs of ocular toxicity were observed during treatment. Oral administration of APX3330 safely and effectively reduced neovascularization in an L-CNV preclinical model.

Clinical studies

APX3330 has been studied in 346 out of 441 patients participating in eleven Phase 1 and 2 non-ocular clinical trials to explore its pharmacokinetic characteristics, safety profile, and effect upon the Ref-1 molecular target. Under the sponsorship of Eisai Co., Ltd., ten clinical trials were conducted involving healthy volunteers and patients with chronic hepatitis C or B, acute severe hepatitis, or alcoholic hepatitis. A Phase 1 oncology trial was conducted by Apexian Pharmaceuticals. Across the clinical studies, daily doses up to 600 mg APX3330 in more than 90 patients and 120 to 240 mg APX3330/day in more than 340 patients were administered. Overall, APX3330 appears to be well tolerated at the identified maximum tolerated dose of 600 mg/day and supports the further clinical development of APX3330 in multiple ophthalmic indications.

Pharmacokinetics

APX3330 exhibits predictable pharmacokinetics with no food-effect that were consistent with the pharmacokinetic data obtained in previous non-clinical studies.

Human pharmacokinetics of APX3330 were predictable and linear, consistent with previous animal studies. In humans, APX3330 demonstrated plasma levels much greater than those seen in animals. In preclinical models, at a dose of 25 mg/kg, (equivalent to a 120 mg daily dose in humans), there was an APX3330 concentration (expressed as blood quinone) of 0.15-2 µg/ml, which was adequate to reach detectable levels in the retina and provide efficacy in reducing neovascularization. In support of these findings, APX3330 was also detected in the eyes of mice using a lesser dose of 10 mg/kg.

In clinical trials, a daily dose of 120 mg resulted in peak blood concentrations of 40 µg/ml, which is 20 times higher than those observed in mouse models. Based on the preclinical and clinical data, the planned clinical dose of 600 mg per day is expected to achieve sufficient exposure to the retina.

Safety

There was a slightly higher incidence (< 10%) of mild to moderate gastrointestinal symptoms and mild to moderate symptoms related to skin rash or irritation in patients given APX3330 compared to placebo. There was also an apparent lack of significant acute toxicity on neurologic, cardiovascular, or pulmonary function at doses up to 600 mg/day treated for up to 360 days.

In the phase 1 studies, AEs reported in which subjects administered APX3330 orally from 10 mg/day to 600 mg/day for up to 300 days comprised 5 out of 19 subjects with mild diarrhea (at doses of 120 mg, 180 mg, or 240 mg a day).

In the five Phase 2 studies to assess efficacy and safety in the treatment of hepatitis, 279 patients were administered oral APX3330 (120 mg/day or 240 mg/day), and 68 were administered placebo for approximately 12 weeks. In these studies, the percentages of patients with any reported AE were similar for those receiving APX3330 and for those receiving placebo. Serious adverse events (SAEs) and AEs leading to discontinuation were higher for those administered placebo compared to those given APX3330. Similar percentages of AEs classified as mild, moderate, or severe were observed between patients administered APX3330 or placebo. APX3330 had a higher reported rate compared to placebo for gastrointestinal disorders and skin and subcutaneous tissue disorders. All but one of these AEs were classified as mild or moderate.

Ref-1 target engagement.

In APX_CLN_0011, biopsy analyses of patients participating in the trial confirmed that APX3330 directly targets the Ref-1 protein and that the targeting produces subsequent regulation of transcription factors such as NF-κB and HIF-1α, regulators of VEGF and other inflammatory molecules. This mechanism of action provides significant rationale for testing APX3330 in diseases in which inflammation and neo-vascular development play a critical pathogenic role.

2.3. Benefit/Risk assessment

Safety data derived from prior human studies in healthy volunteers, as well as patients with advanced hepatitis, and patients with advanced cancers indicate that APX3330 is safe for additional clinical development and does not result in significant toxicity when administered in doses at or below 600 mg per day. Additionally, preclinical data derived over the past decade has

confirmed the pro-inflammatory role played by the transcription factors under the control of the Ref-1 protein. These findings provide a rationale for studying the effect of APX3330 in patients with diseases such as DR and DME. In summary, based on the clinical and nonclinical studies, there is sufficient data to support the use of APX3330 for the potential treatment of DR/DME, and safety risks are acceptable for the proposed current study.

Some notable risks to the patient of which sites should be aware include gastrointestinal (diarrhea) and skin (rash) side effects, noted in patients taking APX3330. However, these were present in less than 10% of patients receiving the drug and were described as mild to moderate for all but one patient. Nonclinical toxicology studies showed dose-limiting toxic effects (200 mg/kg) primarily to the liver. More detail on these effects may be found in the Investigator's Brochure.

There is abundant nonclinical data to suggest that APX3330 will provide significant benefit for patients with diabetic eye disease. However, to date there have been no studies in this patient population. Thus, the direct benefit of this treatment to patients participating in this study is unknown.

2.4. Route of administration, dosage regimen, and treatment period

APX3330 oral tablets:

Five 120 mg tablets will be taken by mouth as follows: 3 tablets every morning and 2 tablets every evening.

Placebo oral tablets:

Placebo tablets are identical to APX3330 tablets except for the absence of the active pharmaceutical ingredient. Five placebo (0 mg) tablets will be taken by mouth as follows: 3 tablets every morning and 2 tablets every evening.

Study medication should be taken at approximately the same time each day and may be taken with or without food.

If a subject is considering discontinuing from the study due to an AE, the Investigator may offer a dose reduction from 600 mg to 480 mg per day as an alternative (2 tablets every morning and 2 tablets every evening).

Specific instructions on dosing regimen will be given to subjects on a form separate from the blister packs.

2.5. Compliance

This study will be conducted in compliance with the protocol and in accordance with Good Clinical Practice (GCP), the ethical principles set forth in the Declaration of Helsinki and with the US Code of Federal Regulations.

2.6. Study population

A maximum of 100 male or female subjects aged ≥ 18 years with DR rated as moderately severe to severe NPDR (Diabetic Retinopathy Severity Score [DRSS] Level 47 or 53) or mild PDR (DRSS Level 61) will be randomized in a 1:1 ratio to one of two treatment arms, with the expectation that approximately 96 subjects will be evaluable for efficacy. Randomization will be stratified by level of disease severity (NPDR or PDR), and subjects with mild PDR will be

capped at 20% for each arm. Patients with DME in the fellow eye will be eligible for enrollment into the study, however center involved DME in the study eye is exclusionary.

3. OBJECTIVES AND PURPOSE

The ZETA-1 study is a placebo-controlled, double-masked, randomized, Phase 2 study in a maximum of 100 randomized subjects with moderately severe to severe NPDR or mild PDR, evaluating safety and efficacy following oral administration of APX3330 twice daily for 24 weeks.

Primary objective

- To evaluate the efficacy of APX3330 to improve Early Treatment Diabetic Retinopathy Study (ETDRS) DRSS in subjects with moderately severe to severe NPDR or mild PDR.

[illegible]

4. STUDY DESIGN

This is a placebo-controlled, double-masked, randomized, Phase 2 study in a maximum of 100 subjects with moderately severe to severe NPDR or mild PDR, evaluating safety and efficacy following oral administration of APX3330 twice daily for 24 weeks.

The study will have a 1:1 randomization (placebo: APX3330). Randomization will be stratified by level of disease severity (NPDR or PDR). Subjects with mild PDR will be capped at 20% for each arm.

The eligible eye with the highest DRSS will be designated as the study eye for the primary endpoint efficacy analysis. If the PDR cap has been reached, the study eye may be an eye with the lower DRSS if the other eye has mild PDR. If eyes have the same DRSS, the eye with the worse BCVA will be selected as the study eye. If the DRSS and BCVA are equivalent between eyes, study eye will be the right eye (OD).

4.1. Primary and secondary endpoints

Efficacy:

The primary efficacy endpoint is the percent of subjects with a ≥ 2 -step improvement in DRSS in the study eye at Week 24.

Secondary efficacy endpoints

APPENDIX 1: DRSS PRIMARY ENDPOINT

APPENDIX 2: ORIGINAL SERIES

SLOAN LETTER ETDRS CARD

-

Primary and secondary endpoints will be evaluated in the study eyes, fellow eyes, all qualified eyes (study eyes and fellow eyes that meet all study eye eligibility criteria), and either eye (i.e., best response). All of the efficacy endpoints will also be analyzed by mITT and PP populations. Other subpopulations may be identified and analyzed.

Exploratory efficacy endpoints:

Measurements:

- DRSS will be measured with 7-field or 4-wide field color fundus photographs
- CST will be measured using SD-OCT
- BCVA will be measured by Standard ETDRS chart at 4 m (letters)

Every effort will be made to have the same person perform the measurements at all timepoints and at all visits.

Safety:

Safety endpoints will include:

- Incidence and severity of systemic and ocular AEs
- Change from baseline in body system assessments
- Change from baseline in vital sign measurements
- Change from baseline in clinical laboratory assay results (blood chemistry, hematology)
- Change from baseline in intraocular pressure (IOP)
- Change from baseline in slit lamp examination parameters
- Change from baseline in dilated funduscopy examination parameters
- Percent of subjects with a decrease of ≥ 10 letters in BCVA compared to baseline at Week 12 and Week 24
- Percent of subjects progressing to center involved DME (eligible for rescue treatment at Week 12 and Week 24)
- Percent of subjects with a worsening in DRSS of ≥ 2 steps in the study eye at Week 12 and Week 24
- Percent of subjects developing anterior segment neovascularization at Week 12 and Week 24
- Percent of subjects rescued (intravitreal anti-VEGF injection, laser PRP, focal/grid laser treatment, or surgery [vitrectomy]) at the discretion of the Investigator at Week 12 and Week 24
- Time to first rescue treatment
- Change from baseline in estimated glomerular filtration rate (eGFR) at Week 12 and Week 24

Urine pregnancy tests for women of childbearing potential will be conducted at each study visit.

Please see [Table 1](#) for details on assessments to be conducted at each visit.

4.2. Description and schedule of visits and procedures

A maximum of 100 subjects with DR rated as moderately severe to severe NPDR (DRSS Level 47 or 53) or mild PDR (DRSS Level 61) \geq 18 years of age will be randomized in a 1:1 ratio to one of two treatment arms, with the expectation that approximately 96 subjects will be evaluable for efficacy. Randomization will be stratified by level of disease severity (NPDR or PDR) and subjects with mild PDR will be capped at 20% for each arm.

The visit schedule, details of assessments and blood draws to be completed at each visit is presented in [Table 1](#) and [Table 2](#).

Table 1: Screening, Qualification/Baseline, Treatments, and Follow-up Visits and Procedures

	██████	██████	██	██████	██	██████	██	██	██████	██████
Day^	██████	█	██████	██████	██████	██████	██████	██████	██████	██████
Visit	█	█	█	█	█	█	█	█	█	█
Informed Consent	█	█		█		█				█
Subject Identification Number Assigned	█	█		█		█				█
Randomization Number Assigned		█								
Medical/Ophthalmic History	█	█		█		█				█
Demographics	█	█		█		█				█
Drug Accountability				█		█			█	
Drug Compliance			█	█	█		█	█		
Concomitant Medications	█	█	█	█	█	█	█	█	█	█
Urine Pregnancy	█	█	█	█	█	█	█	█	█	
Physical Examination	█								█	
HR/BP/Vital Signs	█			█		█			█	
BCVA (ETDRS)	█			█		█			█	
DRSS (Color Fundus Photographs*)	█					█			█	
CST (SD-OCT)	█					█			█	
Biomicroscopy	█			█		█			█	
Ophthalmoscopy	█			█		█			█	
IOP	█			█		█			█	
PK**						█				
Blood Chemistry (Compr. Metabolic Panel)	█					█			█	
Blood Hematology (Complete Blood Count [CBC])	█					█			█	
Confirmation of HbA1c***	█									
Kidney Function (eGFR)	█					█			█	
Exploratory Biomarkers****		█							█	
Adverse Events	█	█	█	█	█	█	█	█	█	█
Meds Dispensed/ Re-Dispensed		█		█		█				

BCVA=best-corrected visual acuity; BP=blood pressure; CST=central subfield thickness; DRSS=Diabetic Retinopathy Severity Score; eGFR=estimated glomerular filtration rate; ELISA=enzyme-linked immunosorbent assay; ETDRS=Early Treatment Diabetic Retinopathy Study; HR=heart rate; IOP=intraocular pressure; PK=pharmacokinetic; SD-OCT=spectral-domain optical coherence tomography.

^ Day of study refers to number of weeks and days after date of randomization.

^^If the subject meets all the inclusion criteria and none of the exclusion criteria, this Qualification Visit becomes the Baseline Visit. A subject identification number is assigned after the subject is qualified.

*DRSS. The ETDRS classification can be found in Report Number 10 and is based on fundus photography, which includes 7 overlapping stereoscopic 30° photographic fields. 4-wide field photographs have subsequently been shown to provide equivalent results.

**PK. At Visit 6 (Week 12), two blood samples will be collected to establish drug levels from approximately 25 to 30 subjects, one sample prior to the morning dose and one sample 3 hours post-morning dose, at a subset of sites. These subjects will be instructed to delay their AM study medication dose on the day of this visit in order to take their study medication at the site.

***Either by subject-provided report, primary care physician, or local or central lab test.

****Exploratory biomarkers in plasma will be examined with an ELISA (Ref-1 levels) and a cytokine panel.

Table 2: Number and Volume of Blood Samples and Total Blood Volume Collected in the Study (Qualification/Baseline through Week 24)

Assessment	Type of Lab Procedure (Maximum Number of Samples Per Subject)	Estimated Volume of Blood per Sample per Subject (mL)
Clinical laboratory (at Screening, Week 12 and Week 24)	Blood Chemistry (Comprehensive Metabolic Panel) (3) Hematology (CBC) (3) HbA1c (1) ^	Blood Chemistry (3 x 4 mL) Hematology (3 x 3 mL) HbA1c (1 x 3 mL)^
Pharmacokinetics (at designated sites at Week 12 only)	LC-MS/MS (2)	PK (2 x 3 mL)
Exploratory Biomarkers (ELISA and cytokine panel at Day 1 and Week 24)	Ref-1 ELISA (2) Cytokine panel (2)	ELISA (2 x 4 mL) Cytokine panel (2 x 4 mL)
Estimated Total Volume with (without) PK per Subject in the Study		46 (40) mL^

ELISA=enzyme-linked immunosorbent assay; HbA1c=hemoglobin A1c; LC-MS/MS=liquid chromatography-tandem mass spectrometry; PK=pharmacokinetic.

^If necessary to conduct HbA1c lab test, an additional 3 mL sample will be drawn.

4.3. Measures taken to minimize/avoid bias

This is a placebo-controlled, double-masked, 1:1 randomized, 2-arm, Phase 2 study. The appearance of the placebo tablets is identical to the APX3330 tablets and will be provided in identical packaging to ensure masking to Investigators, site staff, subjects, and Ocuphire.

4.4. Study medications

APX3330 is formulated as immediate-release, pale orange to light yellow film-coated tablets containing 120 mg of APX3330 per tablet. The tablets consist of a mixture of intragranular components to which an extragranular layer is applied, all being compressed as circular disks and coated with Opadry Yellow. The intragranular components are the active pharmaceutical ingredient (APX3330) and the following excipients: lactose monohydrate, microcrystalline cellulose NF (Avicel PH101), starch 1500, sodium carboxymethylcellulose (Aqualon 7MF PH), and methylcellulose A15LV USP. The extragranular components are microcrystalline cellulose NF (Avicel PH102), sodium carboxymethylcellulose (Aqualon 7MF PH), and magnesium stearate.

Placebo tablets are immediate release, identical in shape and color to APX3330 tablets except for the absence of the active pharmaceutical ingredient.

4.4.1. Packaging and labeling

APX3330 immediate-release tablets and their placebo are packaged in PVC/Aclar[®] thermoform blister cards lidded with aluminum foil. The blister cards are comprised of five 7-count blister strips sealed into a card yielding a total of 35 tablets per card (7 × 5 configuration). A row of 5 comprises a daily dose of 600 mg. A blister card provides for a week of treatment.

Each blister card will be labeled with an investigational label showing the study protocol number, subject identification number, and other relevant information, including a statement “Caution – New Drug – Limited by Federal (US) Law to Investigational Use”.

4.4.2. Storage of study medication

APX3330 tablets are stable, when packaged in bottles, for at least 36 months when stored at 25°C/60% RH. Some discoloration with only minimal instability was observed at higher temperatures and humidity levels. In blisters, there is limited stability data for APX3330 tablets immediate release. The clinical supply will be placed in stability studies, and retest dates will be minimally set through the duration of the clinical trial. Expiry dates for dispensing purposes will be provided as soon as stability study results are provided.

Prior to dispensing, all investigational material must be stored in a secure location with strictly limited access documented by signature of authorized persons who may dispense investigational materials. The products can be shipped and stored at the site at room temperature (15°C to 25°C, 59°F to 77°F).

4.4.3. Study medication accountability

4.4.3.1. Receipt and disposition of study medication

All investigational study medication must be stored in a secure facility, with access limited to the Investigator and authorized staff.

The Investigator or designee (e.g., study coordinator or pharmacist) will maintain a full accountability record for the study medication and will be responsible for recording the receipt, dispensing, and return of all supplies of the study medication using the inventories supplied by Ocuphire. The Investigator or designee will account for all study medication.

Study medication will be dispensed initially at Visit 2 (Week 1, Day 1 of administration) by the site and then at Visit 4 (Week 4) and Visit 6 (Week 12) at the site. Subjects will bring all unused study medication to each site visit for drug accountability.

The study monitor at each site will review dispensing and study medication accountability records during site visits and at the completion of the study and will note any discrepancies.

4.4.3.2. Study medication compliance

On visits involving a phone call, the Investigator will ask subjects about compliance with their study medication dosing schedule. If subjects report missed doses, the Investigator will record, to the best of their ability, the Week number and Day that doses were missed.

4.4.3.3. Return of study medication

When the study is completed, subject is withdrawn or the study is terminated by Ocuphire, all study material including used and unused study medication will be returned to Ocuphire (or its designee) or destroyed under the direction of same. All study medication accounting procedures must be completed before the study is considered complete. A final study medication disposition will be completed by the study coordinator.

4.5. Expected duration of subject participation

The total length of subject participation is approximately 28 weeks, with 5 clinic visits, 4 telephone safety calls, and one telephone call follow-up visit summarized below:

- Screening Visit 1 (up to 21 days prior to Baseline Visit)
- Qualification/Baseline Visit 2 (1 day)

■ Treatment-study period ■



The execution of the entire study (first subject screen through last randomized subject completed) is expected to be approximately 12 to 15 months.

4.6. Randomization and procedure for breaking the code

At the initiation of study-related procedures (Visit 1), every subject who is screened is assigned a subject identification number in numerical order within site. Once a subject is qualified for the study (Visit 2), the subject is assigned a randomization number provided by the biostatistician in the electronic data capture (EDC) system. Randomization will be stratified by level of disease severity (NPDR or PDR) and subjects with mild PDR will be capped at 20% for each arm.

Study medication will be masked to both Investigator and study subjects, as well as Ocuphire. Assignment to treatment sequence will be masked to the Investigator, Ocuphire, and the subjects. Only in case of medical emergency or occurrence of SAEs will the randomization code be unmasked by the Principal Investigator using the EDC system and made available to other personnel involved in monitoring the safety of this study.

4.7. Collection of data

Study-specific data that have been outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator. Data are verified electronically using a series of online programmed edit checks that have been created by the Clinical Data Manager and programmed by the Clinical Data Programmer or designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the Clinical Research Associate (CRA) and site staff. CRAs will review and verify a targeted subset of data collected in the electronic case report form (eCRF) against any applicable source documentation during remote review or scheduled monitoring visits. The specific data selected for source data verification will be detailed in the Clinical Monitoring Plan. The CRA will work closely with the site staff to address any discrepancies which have been found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

4.8. Completed subject

A completed subject is defined as one who completes all planned dosing and procedures through the end of Visit 9 (Week 24).

4.9. Non-completing subject

A non-completing subject is defined as one who exits the study by their own volition or at the discretion of the Investigator and/or the Medical Monitor prior to completing all of the study procedures required in this protocol. Any subject may decide to voluntarily withdraw from the study at any time without prejudice. Every effort should be made to perform all Visit 9 (Week 24) procedures at a visit prior to discontinuation.

4.9.1. Study medication reduction or discontinuation

The study medication may be discontinued for the following reasons:

- **Adverse Events:** AEs include clinically significant laboratory abnormalities and intercurrent diseases reported by the subject or observed by the Investigator with documentation on the case report form (CRF).
- **Death:** If a subject dies, the AE that caused the death should be documented on the CRF and be noted as serious and fatal.
- **Disallowed concurrent medication:** Any medication not allowed by the protocol would be a protocol violation.
- **Lack of efficacy:** A subject may elect to discontinue participation in the study for a perceived lack of efficacy.

- **Investigator decision:** A subject may be discontinued for reasons other than those bulleted previously if the Investigator thinks it is not in the best interest of the subject to continue.
- **Pregnancy:** A subject may be discontinued from study medication if pregnancy occurs while on study.
- **Other:** If there is any other reason for subject discontinuation, this should be noted on the CRF.

The reason for premature study medication discontinuation should be entered onto the appropriate CRF.

If a subject is considering discontinuing from the study due to an AE, the Investigator may offer a dose reduction from 600 mg to 480 mg per day as an alternative (2 tablets every morning and 2 tablets every evening).

4.9.2. Reasons for withdrawal from study

- Subject withdraws consent.
- Subject is lost to follow-up.
- Subject withdraws for other reason.

4.9.3. Entire study terminated

The entire study may be terminated by Investigators or Ocuphire. Prompt, written notice of reasonable cause to the other party (Ocuphire or Investigators, respectively) is required. Prompt notice to the Institutional Review Board (IRB) and to regulatory authorities is also required.

4.9.4. Actions after discontinuation

All subjects who discontinue study medication due to a report of an AE **must** be followed up and provided appropriate medical care until their signs and symptoms have remitted or stabilized or until abnormal laboratory findings have returned to acceptable or pre-study limits.

For any subject who chooses to withdraw consent or who is non-compliant, every possible effort should be made by the Investigator to ensure there is a final follow-up telephone call that includes assessments for AEs and concomitant medications.

4.10. Completed study

The study is completed when all randomized subjects have completed the study, all CRFs have been completed, and all CRF data entered into the database. Final database lock will occur after the last randomized subject completes, all data have been entered, and all queries resolved.

4.11. Procedure after the completion of the study

When the study is completed, the CRO will provide Ocuphire and the Investigator with a brief (i.e., 1-3 pages) report, containing a description of the study, the number of subjects enrolled, the number of subjects completed, the number of subjects who dropped out and why, efficacy findings, and AEs.

5. SUBJECT INCLUSION AND EXCLUSION CRITERIA

5.1. Subject inclusion criteria

1. Males or non-pregnant females ≥ 18 years of age
2. At least one eye with DR graded at least moderately severe to severe NPDR or mild PDR (corresponding to DRSS 47, 53, or 61, confirmed by a central reading center) in which PRP and intravitreal injections of an anti-VEGF agent can be safely deferred for ≥ 6 months in the opinion of the Investigator
3. BCVA assessed by ETDRS protocol letters score of ≥ 60 letters (Snellen equivalent $\geq 20/63$) in the study eye
4. Sufficiently clear ocular media, adequate pupillary dilation, and fixation to permit quality fundus imaging in both eyes
5. Able to cooperate sufficiently for ophthalmic visual function testing and anatomic assessment
6. Body mass index (BMI) between 18 and 40 kg/m², inclusive
7. Able and willing to give signed informed consent and follow study instructions
8. Able to self-administer oral study medication or to have study medication administered by a caregiver throughout the study period

5.2. Subject exclusion criteria

Ophthalmic:

1. Retinopathy from causes other than diabetes
2. Presence of center involved diabetic macular edema (DME) defined as a central subfield thickness (CST) ≥ 320 μ m on SD-OCT. Center involved DME in the fellow eye is allowed. Intravitreal injections of an anti-VEGF agent in the fellow eye does not exclude the subject.
3. Any prior treatment in the study eye with:
 - a. Focal or grid laser photocoagulation within the past year or PRP at any time
 - b. Systemic or intravitreal anti-VEGF agents within the last 6 months or likely, in the opinion of the Investigator, to require treatment during the course of the study
 - c. Intraocular steroids including triamcinolone and dexamethasone implant within the last 6 months
 - d. Fluocinolone implant within the last 3 years
4. Clinically significant ocular disease in either eye as deemed by the Investigator to likely interfere with the study procedures and visual acuity measurements (e.g., cataract, pseudophakia without evidence of posterior capsular opacity, glaucoma, corneal edema, uveitis, severe keratoconjunctivitis sicca)
5. Presence of other macular or retinal vascular disease including age-related macular degeneration, pattern dystrophy, choroidal neovascularization of any cause, retinal vein occlusion, retinal artery occlusion in the study eye

6. Presence of active vitreous hemorrhage that would prevent adequate clinical imaging in either eye
7. History of retinal detachment or full-thickness macular hole in the study eye
8. Uncontrolled glaucoma in either eye, defined as advanced cup-to-disc ratio > 0.7 and intraocular pressure (IOP) > 25 mmHg, with or without topical antihypertensive eye drops; treatment of ocular hypertension or controlled glaucoma are not criteria for exclusion
9. Ocular incisional surgery including cataract surgery in the study eye within 3 months prior to Day 1
10. Yttrium aluminum garnet (YAG) posterior capsulotomy in the study eye within the last 30 days
11. Aphakia in the study eye
12. Previous pars plana vitrectomy in the study eye
13. Epiretinal membrane, posterior hyaloidal traction, and/or vitreomacular traction in the study eye as determined to be significant by the Investigator
14. Active uveitis and/or vitritis in either eye
15. History of idiopathic or autoimmune-associated uveitis in either eye
16. Active infection in either eye including infectious conjunctivitis, keratitis, scleritis, or endophthalmitis

Systemic:

17. Poorly controlled diabetes, defined as hemoglobin A1c (HbA1c) $\geq 12.0\%$ or $< 12.0\%$ with uncontrolled diabetes mellitus
18. Known to be immunocompromised or receiving immunosuppressive therapy
19. Any disease or medical condition that in the opinion of the Investigator would prevent the subject from successfully participating in the study or which might confound the study results
20. Clinically significant systemic disease (e.g., uncontrolled diabetes, myasthenia gravis, cancer, hepatic, renal, endocrine, or cardiovascular disorders) that might interfere with the study as deemed by the Investigator
21. Estimated glomerular filtration rate (eGFR) < 30 mL/min by Modification of Diet or Renal Disease (MDRD) or creatinine > 4 mg/dL
22. History of allergic reaction to investigational drug or any of its components
23. Resting heart rate (HR) outside the specified range of 50-110 beats per minute at the Screening Visit. HR may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position
24. Hypertension with resting diastolic blood pressure (BP) > 110 mmHg or systolic BP > 180 mmHg at the Screening Visit. BP may be repeated only once if outside the specified range following at least a 5-minute rest period in the sitting position

25. History of chronic liver disease or presence of elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) consistent with such diagnosis (i.e., AST or ALT $> 2 \times$ upper limit of normal)
26. Participation in any investigational study within 30 days prior to Screening or planning to participate in any other investigational drug or device clinical trials within 30 days of study completion
27. Women of childbearing potential who are pregnant, nursing, planning a pregnancy, or not using a medically acceptable form of birth control. Acceptable methods include the use of at least one of the following: intrauterine device (IUD), hormonal (oral, injection, patch, implant, ring), barrier with spermicide (condom, diaphragm), or abstinence. An adult woman is considered to be of childbearing potential unless she is 1 year postmenopausal or 3 months post-surgical sterilization. All women of childbearing potential must have a negative urine pregnancy test result at the Visit 1/Screening examination and must intend to not become pregnant during the study

6. TREATMENT OF SUBJECTS

A maximum of 100 male or female subjects aged ≥ 18 years with DR rated as moderately severe to severe NPDR (DRSS Level 47 or 53) or mild PDR (DRSS Level 61) will be randomized in a 1:1 ratio to one of two treatment arms, with the expectation that approximately 96 subjects will be evaluable for efficacy. Randomization will be stratified by level of disease severity (NPDR or PDR) and subjects with mild PDR will be capped at 20% for each arm. Patients with DME in the fellow eye will be eligible for enrollment into the study, however center involved DME in the study eye is exclusionary.

Subjects will be screened at Visit 1 and those successfully completing eligibility requirements will return to site for their [REDACTED]

Subjects will take five 120 mg tablets (APX3330 or placebo) by mouth each day, with 3 tablets every morning and 2 tablets every evening for 24 weeks. Study medication should be taken at approximately the same time each day and may be taken with or without food.

6.1. Treatment adherence

All subjects will be instructed on the importance of following the dosing regimen (5 tablets per day dosed as 3 tablets in the morning and 2 tablets in the evening). Study medication should be taken at approximately the same time each day and may be taken with or without food. Dosing will commence in the morning of Day 2 and continue daily for 24 weeks.

If a subject is considering discontinuing from the study due to an AE, the Investigator may offer a dose reduction from 600 mg to 480 mg per day as an alternative (2 tablets every morning and 2 tablets every evening).

Subjects are to bring unused study medication into the study site at [REDACTED]. Treatment adherence will be measured by counting the number of unused tablets at each study visit.

6.2. Concomitant medications

As noted in the exclusion criteria ([Section 5.2](#)), the following are prohibited:

- Systemic or intravitreal anti-VEGF agents within the last 3 months
- Intraocular steroids including triamcinolone and dexamethasone implant within the last 6 months
- Fluocinolone implant within the last 3 years

Use of all medications should be documented on the appropriate CRF. Investigators are encouraged to contact Ocuphire or the Medical Monitor for any questions regarding allowed medications. Judgment of continued study participation by the subject, and inclusion of this subject's subsequent visits in the safety and efficacy analysis will be made by Ocuphire.

All medications which the subject has taken within 30 days prior to the Screening Visit and during the study will be recorded in the CRF. The name of the drug, dose, route of administration, duration of treatment and indication will be recorded for each medication. For combination products (e.g., Contac[®], Cosopt[®]), the brand name is desired. For non-combination products, the generic name is desired. The use of routine ophthalmic diagnostic pharmaceutical agents (e.g., fluorescein and local anesthetic) will be allowed, and individual documentation is not required. Any change in dosing parameters should also be recorded in the CRF.

Refer to [Section 6.3](#) for details regarding concomitant treatment for DME or PDR during the study.

6.3. Treatment for DME or PDR during study

6.3.1. Continued standard of care treatment for DME or PDR in fellow eye

A fellow eye receiving continued standard of care treatment for DME or PDR at study entry may continue to receive treatment during the study per the discretion of the Investigator. The subject will receive randomized study medication and follow the study schedule of assessments without alteration.

6.3.2. Initiation of rescue treatment for DME or PDR in either eye

Whenever possible the Investigator should consult with the Medical Monitor or Sponsor prior to initiation of rescue treatment. Eyes may be rescued if there is:

- Moderate or severe active PDR and/or development of anterior segment neovascularization
- Progression to clinically significant center involved DME
- ≥ 10 -letter loss in BCVA compared to baseline attributable to worsening retinopathy
- Any other finding which, in the judgement of the Investigator, requires rescue treatment. In this situation, the Investigator should make every effort to consult with the Medical Monitor or Sponsor prior to initiation of rescue.

Prior to initiating rescue, BCVA, color fundus photography for DRSS, CST, biomicroscopy, ophthalmoscopy and IOP study procedures must be performed before rescue treatment is initiated (only conducted before first instance of rescue treatment). These assessments may be performed as an unscheduled visit.

If rescue treatment is given, the subject may continue in the study and will remain on their randomized treatment and complete all remaining study visits per the visit schedule.

7. ASSESSMENT OF EFFICACY

7.1. Specification of the efficacy parameters

The primary efficacy endpoint is the percent of subjects with a ≥ 2 -step improvement in DRSS in the study eye at Week 24.

Secondary efficacy endpoints can be found in [Section 4.1](#).

Primary and secondary endpoints will be evaluated in the study eyes, fellow eyes, all qualified eyes (study eyes and fellow eyes that meet all study eye eligibility criteria), and either eye (i.e., best response).

All of the efficacy endpoints will also be analyzed by mITT and PP populations. Other subpopulations may be identified and analyzed.

Exploratory endpoints will be compared between treatment groups.

7.2. Assessing, recording, and analyzing of efficacy parameters

The timing for assessment of efficacy parameters may be found in [Section 4.2](#), [Table 1](#).

For pharmacokinetics analysis, at Visit 6 (██████████ 2 days) prior to the morning dose and 3 hours post-morning dose, blood samples will be collected to establish drug levels of APX3330 from approximately 25 to 30 subjects at a subset of clinical sites. These subjects will be instructed to delay their morning study medication dose on the day of this visit in order to take their study medication at the site. Five mL of blood will be drawn immediately pre-dosing to establish a steady-state drug level. A second 5-mL sample will be drawn 3 hours later to establish the C_{\max} drug level. Analysis of plasma samples for APX3330 concentration determinations will be performed by a central PK laboratory using a validated liquid chromatography-mass spectrometry and liquid chromatography-tandem mass spectrometry (LC-MS/MS) method.

Every effort will be made to have the same person perform the measurements at all timepoints and at all visits.

7.2.1. Screening/Visit 1 (Day -21 to -1)

Individuals who are potential subjects (subjects with DR rated as moderately severe to severe NPDR [DRSS Level 47 or 53] or mild PDR [DRSS Level 61] ≥ 18 years of age) will be contacted by the study center to schedule the Screening Visit.

Once a subject arrives at the study center, a member of the study center staff will interview the individual as to their qualifications for participation in the study, and if the subject wishes to continue, the informed consent form is signed. The start of Screening includes the assignment of a subject identification number, an explanation of the study, a medical and ophthalmic history, demographics, and a review of prior/concomitant medications. This shall be followed by a urine pregnancy test for women of childbearing potential, physical examination, and measurement of HR/BP.

Subsequently, BCVA will be measured and SD-OCT (for CST) and color fundus photographs (for DRSS) will be performed. DRSS eligibility will be determined by a central reading center with 7-field or 4-wide field fundus photography. The central reading center will also determine CST eligibility with SD-OCT.

This will be followed by blood sample collection for assessments of blood chemistry and hematology, ophthalmic examination that includes biomicroscopy and direct or indirect ophthalmoscopy, IOP assessment, eGFR, and AEs. If the subject does not have an available HbA1c, they should be sent to a local laboratory to get an HbA1c level to determine eligibility; alternatively, a blood sample can be collected for assessment by the central laboratory.

The Screening Visit occurs 1 to 21 days prior to Qualification/Baseline Visit. Eligibility criteria can be found in [Section 5.1](#).

7.2.2. Qualification/Baseline – Visit 2/Day 1

Subjects who meet all eligibility criteria (including DRSS and SD-OCT) will return for their Qualification Visit.

Subjects will be assessed for concomitant medications ([Section 6.2](#)), urine pregnancy test for women of childbearing potential and AEs.

BCVA, DRSS, CST and other safety assessments performed at Screening will be the baseline values.

The eligible eye with the highest DRSS will be designated as the study eye for the primary endpoint efficacy analysis. If the PDR cap has been reached, the study eye may be an eye with the lower DRSS if the other eye has mild PDR. In the case where both eyes have the same DRSS, the eye with the worse BCVA will be selected as the study eye. If the DRSS and BCVA are equivalent between eyes, OD will be the study eye.

The subject will then be randomized into the study. Randomization will be stratified by level of disease severity (NPDR or PDR) and subjects with mild PDR will be capped at 20% for each arm. Study medication will be dispensed in accordance with the subjects randomized treatment arm.

Blood samples will be drawn pre-dose for baseline levels for exploratory biomarker processing (i.e., cytokine and Ref-1 levels) to evaluate pharmacodynamic properties. Please refer to the lab manual for instructions on sample preparation, shipping and analysis.

Subjects will be instructed to administer their study medication (APX3330 or placebo) each day, with 3 tablets every morning and 2 tablets every evening. Study medication should be taken at approximately the same time each day and may be taken with or without food.

7.2.3. Telephone Safety Call Visit 3 (██████ ± 2 Days)

Subjects will be contacted by the site by telephone for a safety assessment to include:

- Drug compliance
- Concomitant medications
- AEs
- Urine pregnancy test at home (women of childbearing potential only)

7.2.4. Treatment Visit 4 ([REDACTED] ± 2 days)

Subjects will return to the site at Week 4 (Visit 4) for a series of safety and efficacy assessments as follows:

- Drug accountability
- Concomitant medications
- Urine pregnancy test (women of childbearing potential only)
- HR/BP/vital signs
- BCVA (ETDRS)
- Biomicroscopy
- Ophthalmoscopy
- IOP
- AEs

Following the completion of the assessments, used kits will be collected for accountability and new study medication kits will be dispensed.

7.2.5. Telephone Safety Call Visit 5 ([REDACTED] ± 2 days)

Subjects will be contacted by the site by telephone for a safety assessment to include:

- Drug compliance
- Concomitant medications
- AEs
- Urine pregnancy test at home (women of childbearing potential only)

7.2.6. Treatment Visit 6 ([REDACTED] ± 2 days)

Subjects will return to the site at Week 12 (Visit 6) for a series of safety and efficacy assessments as follows:

- Drug accountability
- Concomitant medications
- Urine pregnancy test (women of childbearing potential only)
- HR/BP/vital signs
- BCVA (ETDRS)
- DRSS
- CST (SD-OCT)
- Blood draw for PK (prior to the morning dose and at 3 hours post dose; at select sites)
- Blood chemistry

- Blood hematology
- Biomicroscopy
- Ophthalmoscopy
- IOP
- eGFR
- AEs

Following the completion of the assessments, used kits will be collected for accountability and new study medication kits will be dispensed.

7.2.7. Telephone Safety Call Visit 7 (██████ ± 2 days)

Subjects will be contacted by the site by telephone for a safety assessment to include:

- Drug compliance
- Concomitant medications
- AEs
- Urine pregnancy test at home (women of childbearing potential only)

7.2.8. Telephone Safety Call Visit 8 (██████ ± 2 days)

Subjects will be contacted by the site by telephone for a safety assessment to include:

- Drug compliance
- Concomitant medications
- AEs
- Urine pregnancy test at home (women of childbearing potential only)

7.2.9. Treatment Visit 9 (██████ ± 2 days)

Subjects will return to the site at Week 24 (Visit 9) for a series of safety and efficacy assessments as follows:

- Drug accountability
- Concomitant medications
- Urine pregnancy test (women of childbearing potential only)
- Physical examination
- HR/BP/vital signs
- BCVA (ETDRS)
- DRSS
- CST (SD-OCT)
- Blood chemistry

- Blood hematology
- Biomicroscopy
- Ophthalmoscopy
- IOP
- eGFR
- AEs
- Blood draw for exploratory biomarkers (ELISA, cytokine panel, comprehensive metabolic panel)

Visit 9 () is the end of the treatment period. Study medication will be returned for accountability and no further study medication will be dispensed.

7.2.10. Follow-Up Visit 10 (Week 25 ± 2 days)

Subjects will receive a Follow-up Phone Call to evaluate:

- AEs
- Concomitant medications

7.2.11. Unscheduled visits

An Unscheduled Visit may be any visit to the Investigator other than the specific visits requested in the protocol as possibly required for the subject's ophthalmic condition. The Investigator will perform all procedures necessary to evaluate the study participant at these visits and record any AEs in the CRF.

As noted in [Section 4.9.4](#), every possible effort should be made by Investigators to assure that subjects who discontinue from the study have a final Follow-up Phone Call that includes assessments of AEs and concomitant medications. Every effort should be made to perform all Week 24 procedures at a visit prior to discontinuation.

7.2.12. Visit variation

The timings of visits may vary as specified above and in the Schedule of Assessments ([Table 1](#)). Completion of assessments outside of the specified windows may be considered a protocol deviation.

8. ASSESSMENT OF SAFETY

8.1.Safety review committee

This study will have an independent safety review committee consisting of the Medical Monitor as well as an independent ophthalmologist. They will meet at a minimum quarterly and review all safety data and provide a written summary of safety including a specific statement regarding the safe continuation of the trial. As specified in the committee charter, committee members may request further information on cases of concern and may request additional testing be performed to elucidate the nature of any adverse event of concern.

8.2.Specification of safety parameters

The assessment of safety is the secondary objective of this study. The assessment of safety will be evaluated by:

- HR/BP/vital signs (as per the site's normal equipment and procedures)
- DRSS (worsening of ≥ 2 steps)
- BCVA (decreases, as measured by ETDRS)
- Blood chemistry
- Blood hematology
- IOP
- Biomicroscopy of the anterior segment including evaluation of cornea, conjunctiva, and anterior chamber; fluorescein staining will be used
- Ophthalmoscopy (dilated fundus exam including optic nerve, macula, vessels, and periphery)
- eGFR
- AEs

8.3.Assessing, recording, and analyzing safety parameters

The timing for recording safety parameters may be found in [Section 4.2 \(Table 1\)](#). The assessment of safety parameters is described in [Section 8.1](#).

8.4.Adverse events and serious adverse events

All AEs and SAEs that occur following consent and until the final study visit should be collected and recorded on the AE or SAE CRF page. Only treatment-emergent adverse events/adverse reactions (TEAEs) will be summarized ([Section 9.3.5](#)).

All AEs/adverse reactions occurring during the study (i.e., once the subject has signed the informed consent) must be documented, regardless of the assumption of causal relationship, on the respective CRF. All TEAEs/adverse reactions must be documented from the time the subject receives the first dose of study medication until the subject's participation in the study has been completed. If a subject has ongoing AEs/adverse reactions at the time of study completion or discontinuation from the study, the ongoing AEs/adverse reactions must be followed-up and provided appropriate medical care until the signs and symptoms have remitted or stabilized or until abnormal laboratory findings have returned to acceptable or pre-study limits.

Documentation of AEs/adverse reactions includes start date and end date, severity, relationship to study medications, action(s) taken, seriousness and outcome.

8.4.1. Adverse event definitions

The following definitions of terms apply to this section:

Adverse event. An AE is any untoward medical occurrence in a patient or clinical study subject administered a study medication (pharmacological/biological product) that does not necessarily have a causal relationship to this medication. An AE can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporarily associated with the use of the study medication, whether or not related to the study medication. Study medication includes the investigational drug under evaluation and the comparator product or vehicle placebo that is given or administered during any phase of the study.

Medical conditions/diseases present before starting the investigational treatment are only considered AEs if they worsen after starting the investigational treatment. Abnormal test results constitute AEs only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

The occurrence of AEs should be sought by open-ended questioning of the subject at each visit during the study. At each clinic assessment/visit, study personnel should ask the following question: “Have you had any problems since your last visit?”. AEs also may be detected when they are volunteered by the subject during or between visits or through study assessments.

Life-threatening adverse event or life-threatening suspected adverse reaction. An AE or suspected adverse reaction is considered “life-threatening” if, in the view of either the Investigator or Ocuphire, its occurrence places the subject or subject at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction. An AE or suspected adverse reaction is considered “serious” if, in the view of either the Investigator or Ocuphire, it results in any of the following outcomes at any dose:

- Death
- Life threatening
- Initial or prolonged hospitalization
- Disability or permanent damage
- Congenital anomaly or birth defect
- Needs intervention to prevent impairment
- Other medically important serious event

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject or subject may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Treatment on an outpatient emergency basis that does not result in hospital admission, or a hospitalization that is elective or is a preplanned treatment for a pre-existing condition that is unrelated to the medication under study and has not worsened since the start of the study, is not considered an SAE.

Suspected/Related adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, “reasonable possibility” means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than an expected adverse reaction, which means any AE caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction. An AE or suspected adverse reaction is considered “unexpected” if it is not listed in the Investigators’ Brochure or is not listed at the specificity or severity that has been observed; or, if an Investigators’ Brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. “Unexpected,” as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigators’ Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

The study medication relationship for each AE/adverse reaction should be determined by the Investigator using these explanations:

- Not related
- Unlikely related
- Possibly related
- Probably related
- Definitely related
- Unknown

Unless the relationship is considered to be “Not related” or “Unlikely related” and there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the study medication and the occurrence of the AE, then the AE should be considered “related.”

If the relationship between the AE/SAE and the investigational product is determined by Ocuphire to be anything other than “Not related” or “Unlikely related” the event will be considered to be related to the investigational product for the purposes of expedited regulatory reporting.

Severity of an AE is defined as a qualitative assessment of the level of discomfort of an AE as is determined by the Investigator or reported to him/her by the subject. The assessment of severity is made irrespective of study medication relationship or seriousness of the event and should be evaluated according to the following scale:

- 1 = Mild: Present, but not distressing, and no disruption of normal daily activity
- 2 = Moderate: Discomfort sufficient to reduce or affect normal daily activity

- 3 = Severe: Incapacitating, with inability to work or perform normal daily activity

A change in severity for a reported AE will require an end date for the previous severity and a new start and end date for the new severity. For example, a change in severity may go from mild to severe or from severe to moderate. In either case, the start or end dates should be recorded.

The term “severe” is used to describe the intensity of an event/reaction; the event/reaction itself may be of relatively minor medical significance (such as a severe headache). This is not the same as a “serious” AE, which is based on a subject/event outcome or action criteria usually associated with events that pose a threat to the subject’s life or vital functions. “Seriousness” (NOT severity) serves as a guide for defining regulatory reporting obligations.

Action taken in response to an AE is coded as:

- Dose not changed: An indication that a medication schedule was maintained.
- Dose reduced: An indication that a medication schedule was modified by subtraction, either by changing the frequency, strength, or amount.
- Dose interrupted: An indication that a medication schedule was modified by temporarily terminating a prescribed regimen of medication.
- Drug withdrawn: An indication that a medication schedule was modified through termination of a prescribed regimen of medication.
- Not applicable: Determination of a value is not relevant in the current context.
- Unknown: Not known, not observed, not recorded, or refused.

Additional Other Action Taken:

- Concomitant medication
- Hospitalization

Outcome of an AE is coded as:

- Fatal: The termination of life as a result of an AE
- Not recovered/not resolved: One of the possible results of an AE outcome that indicates that the event has not improved or recuperated
- Recovered/resolved: One of the possible results of an AE outcome that indicates that the event has improved or recuperated
- Recovered/Resolved with sequelae: One of the possible results of an AE outcome where the subject recuperated but retained pathological conditions resulting from the prior disease or injury
- Recovering/resolving: One of the possible results of an AE outcome that indicates that the event is improving
- Unknown: Not known, not observed, not recorded, or refused

Expedited reporting of Serious and Unexpected Adverse Events: All SAEs (related and unrelated) will be recorded following subject signature of the informed consent and until the

final study visit (Visit 10/Week 25). Any SAEs “suspected” to be related to the study medication and discovered by the Investigator at any time after the study should be reported.

Any SAE that occurs must be reported to the CRO/Ocuphire within 24 hours of its occurrence or within 24 hours of learning of its occurrence. Recurrent episodes, complications, or progression of the initial SAE must be reported to the CRO/Ocuphire as follow-up to the original episode within 24 hours of the Investigator receiving the information. Information about all SAEs will be collected and recorded on the SAE form. All pertinent medical records and information collected during the treatment and follow-up of the subject should be maintained at the site with a copy emailed to Zeta1_Safety@MedTrials.com. The Investigator must assess the SAE relationship and complete the SAE form. The CRO/Ocuphire may request additional information. Follow-up information (e.g., discharge summary) will be retained in the subject’s chart and a copy will be emailed to Zeta1_Safety@MedTrials.com. In addition, all SAEs should be recorded on the AE CRF page with the serious question marked “Yes”.

It is the Investigator’s responsibility to notify the approving IRB of any SAEs on a timely basis as instructed by Ocuphire following Ocuphire’s determination of causality. All subjects who experience an SAE should be followed clinically and undergo the appropriate diagnostic evaluations until stabilization or resolution of the event. Ocuphire will report all SAEs to the US FDA on the appropriate schedule depending if the event is drug related or not drug related, expected, unexpected (based on the available information in the Investigators’ Brochure).

Any death occurring during the study and follow-up period must be reported as an SAE. For any death occurring through the end of the study, regardless of the degree of relationship to study medication, the SAE resulting in the death must be reported to the CRO/Ocuphire. A death occurring after completion of the study including the Safety Follow-up Visits, that is not reasonably associated with study medication administration, does not require completion of the SAE form.

8.4.2. Follow-up of subjects after adverse events

If an AE/adverse reaction occurs, the Investigator will institute support and/or treatment as deemed appropriate. All SAEs ongoing at the time of the last visit, or discontinuation from the study, will be followed up until the AE/adverse reaction is resolved or stabilized, the subject is lost to follow-up, or there is other resolution to the event.

8.4.3. Pregnancy and contraception

If any study subject becomes or is found to be pregnant while receiving study medication during the active treatment period or within 30 days of discontinuing the study medication, the Investigator is to submit this information on a Pregnancy Notification Cover Sheet along with a completed Serious Adverse Event Form. This must be done irrespective of whether an AE has occurred. Pregnancy during this time frame of the female partner of a male subject should also be reported. To ensure subject safety, each pregnancy must be reported to the Sponsor’s Medical Monitor or CRO designee within 24 hours of awareness of the pregnancy.

If the pregnancy of a female study participant is discovered during the Active Treatment period, the study medication should be discontinued.

The Investigator will follow the subject (or female partner of a male subject) until completion of the pregnancy.

Within 24 hours of awareness, Investigator sites will notify the Sponsor's Medical Monitor or CRO designee of abnormal pregnancy outcomes. Normal outcomes may be reported within 5 days of awareness. Reporting of outcome will be provided on the Pregnancy Reporting Form.

In addition to reporting the outcome on the Pregnancy Reporting Form, if the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion, stillbirth, neonatal death within one month of birth, or congenital anomaly [including that in an aborted fetus]), the Investigator should also follow the procedures for reporting a SAE within 24 hours of awareness.

Additional information about pregnancy outcomes follows:

- Note that "spontaneous abortion" includes miscarriage and missed abortion.
- All neonatal deaths that occur within one month of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after one month that the Investigator assesses as possibly or probably related to the in-utero exposure to the study medication should also be reported.
- In the case of a live birth, the "normality" of the newborn can be assessed at time of birth.

The "normality" of an aborted fetus can be assessed by gross visual inspection unless there are pre-abortion laboratory findings suggestive of a congenital anomaly.

9. STATISTICS

9.1. *Sample size*

A sample size of 96 subjects who are evaluable for efficacy (i.e., who have at least one post-randomization DRSS evaluation in the study eye, who have missed less than 20% of expected doses, and do not have any major protocol deviations considered to have significant impact on treatment outcome) is needed for the study. The primary treatment comparison will be APX3330 vs placebo ($\alpha = 0.05$ significance, two-tailed).

Ninety-six subjects will provide greater than 80% power to detect a difference of 24% between the APX3330 and placebo groups in percent of subjects with ≥ 2 steps in DRSS improvement in the study eye compared to Baseline at Week 24. This calculation is based on a 6% placebo effect and a 30% APX3330 treatment effect.

All subjects will be randomized into the study in a 1:1 ratio to one of the treatment arms (APX3330 or placebo).

It is assumed that approximately 20% of subjects will drop out between Baseline/Day 1 and Week 24 and that approximately 5% will not be evaluable. To account for drop-out, a maximum of 100 subjects will be randomized into the study.

9.2. *Analysis populations*

Modified Intention-to-Treat (mITT): The mITT will include all randomized subjects who received at least one dose of study treatment and at least one post-dose efficacy measurement. The mITT will be to analyze efficacy endpoints.

Per Protocol Population (PP): The PP population will include all subjects in the mITT who have missed less than 20% of expected doses and do not have any major protocol deviations considered to have significant impact on treatment outcome. The PP population will be used for primary endpoint analysis and to analyze efficacy endpoints.

All Randomized Population (ARP): The ARP will include all randomized subjects. This population is also known as the Intent-to-Treat (ITT) population. The ARP will be used in confirmatory efficacy analyses.

Safety Population (SP): The SP will include all randomized subjects who have received at least one dose of study treatment. The SP will be used to summarize safety variables.

9.3. *Statistical methods*

9.3.1. **General considerations**

All continuous variables will be summarized by treatment and timepoint (as applicable) using descriptive statistics (n, mean, median, standard deviation, minimum, and maximum). All categorical variables will be summarized by treatment and timepoint (as applicable) using frequency counts and percentages.

All study data will be listed by treatment, subject, and timepoint (as applicable).

All statistical tests will be performed using a significance level of 5% (two-tailed). The p-values for the analysis of secondary efficacy endpoints will be considered descriptive.

9.3.2. Demographic and baseline characteristics

Demographic and baseline characteristics such as age, race and sex, will be summarized by treatment group using the mITT, PP population, ARP, and the SP. These data will also be provided in by-subject listings.

9.3.3. Subject disposition

Subject disposition, including randomization, and completion and withdrawal from the study will be summarized using the ARP. These data will also be provided in by-subject listings.

9.3.4. Medical history and prior/concomitant medications

Medical history will be coded using the latest version of Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by treatment group using the SP.

Prior medications (medications with an end date prior to the date of randomization) and concomitant medications (medications with a start or end date after the date of randomization) will be coded using World Health Organization Drug Dictionary and will both be summarized by treatment group using the SP.

Medical history and prior and concomitant medications will also be provided in by-subject listings.

9.3.5. Analysis of efficacy

Efficacy will be assessed using the mITT and PP population with subjects included in the treatment arm in which they were randomized. For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations or for subjects requiring rescue if applicable; details will be provided in the study Statistical Analysis Plan. If the analysis using the PP population shows a positive effect for APX-3330 at the 0.05 level of significance, the primary endpoint will be considered met. Confirmatory analyses may be performed using the ARP, with imputation performed for missing data. If warranted, confirmatory analyses with imputation for missing data or subjects requiring rescue will also be performed for the secondary efficacy endpoints.

For all efficacy endpoints, Baseline values are defined as the last observation prior to randomization.

The primary efficacy endpoint is the difference between treatment groups in percent of subjects with a ≥ 2 -step improvement from baseline in DRSS in the study eye at Week 24. The primary efficacy endpoint will be analyzed using a logistic regression model with treatment as factor and the baseline DRSS as a covariate. The percent of subjects in each treatment group meeting the criteria, the odds ratio (OR) with 95% confidence interval (CI), and p-value will be provided. The analysis will be performed using the mITT and PP population, with subjects included in their randomized treatment regardless of the treatment they actually received.

Secondary efficacy endpoints are indicated in [Section 4.1](#).

Each of the continuous secondary efficacy endpoints will be analyzed using analysis of covariance (ANCOVA) with change from baseline as the dependent variable, treatment as factor, and the respective baseline value included as the covariate. Each ANCOVA will be performed using the mITT and PP population, with subjects included in their randomized treatment regardless of the treatment they actually received. The output from each ANCOVA will include

the least-squares mean (LSM) and standard error for both treatment groups, along with the placebo-corrected LSM, its 95% CI, and associated p-value.

For each of the secondary endpoints related to percent of subjects achieving certain criteria, the analysis will be performed using a logistic regression model with treatment and the respective baseline as a covariate. For each analysis, the percentage of subjects in each treatment group meeting the criteria, the OR with 95% CI and p-value will be provided. For these endpoints, the mITT and PP population will be used with subjects included in their randomized treatment regardless of the treatment they actually received.

Efficacy endpoints for other subpopulations may be identified and analyzed.

9.3.6. Analysis of safety

Safety will be assessed using the SP with subjects included in the treatment group they actually received, regardless of their randomized treatment. Observed case data will be used; no imputation will be performed for missing safety data.

Continuous safety endpoints collected at each visit will be summarized with number of subjects, mean, standard deviation, median, minimum, and maximum for each treatment group and for all subjects. Qualitative variables collected at each visit will be summarized using counts and percentages for each treatment group and for all subjects. Summaries will also include change from baseline and shift tables, where appropriate. Individual subject safety data will be listed.

Verbatim descriptions of AEs will be coded using MedDRA. Only TEAEs (those that occur after the first dose of study medication *or increasing in severity after initiation of study medication*) will be summarized. TEAEs and SAEs will be summarized by treatment group, by system organ class (SOC), severity, and relationship to study medication. Deaths, withdrawal from study medication due to AEs, and withdrawal from the study due to AEs will each be summarized by treatment group. Note that in MedDRA, ocular events are coded to the SOC of “special senses”. Thus, using SOC in the summaries will provide a separation of ocular and non-ocular AEs.

9.4. Procedure for accounting for missing, unused, or spurious data

For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations or subjects requiring rescue, if applicable. Confirmatory efficacy analyses for secondary endpoints may also be performed using imputation for missing data or subjects requiring rescue. Details for all imputation methods will be provided in the study Statistical Analysis Plan. For the summarization of safety data, observed case data only will be used.

If there are significant disruptions to data collection due to the COVID-19 pandemic, the analysis of efficacy may be impacted. If so, any adjustments to the analysis will be consistent with the FDA Guidance for Industry: Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency. This guidance outlines considerations for the statistical analysis of efficacy endpoints in a study affected by COVID-19 to help ensure that the study will provide interpretable findings with correct statistical quantification of uncertainty. Details of any changes to the efficacy analysis, if required due to the COVID-19 pandemic, will be provided in the study Statistical Analysis Plan.

9.5.Procedure for reporting deviations from the statistical plan

Any deviations from the statistical plan will be described and a justification given in the final Clinical Study Report.

10. DIRECT ACCESS TO SOURCE DATA AND DOCUMENTS

The Investigator will permit study-related monitoring visits, audits, IRB review, and regulatory inspection(s) by providing direct access to source data and documents.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The progress of the study will be monitored by on-site, remote, written, and telephone communications between personnel at the Investigator's site and the Medical Monitor. Should the COVID-19 pandemic restrict monitors from traveling to a site, remote review will be conducted to the extent possible, while still ensuring the study is monitored appropriately per applicable regulations and guidelines. The Investigator will allow the CRO/Ocuphire, the Study Monitor, and the Medical Monitor to inspect all CRFs, subject records (source documents), signed consent forms, records of study medication receipt, storage, preparation, and disposition, and regulatory files related to this study.

12. ETHICAL CONSIDERATIONS AND GCP COMPLIANCE

12.1.GCP compliance

The proposed study is subject to all applicable governmental rules and regulations concerning the conduct of clinical trials on human subjects. This includes, but is not necessarily limited to, the approval of IRBs, the Helsinki Declaration, US FDA Law, International Council for Harmonisation (ICH) GCP guidelines, obtaining prospective informed consent, monitoring of the conduct of the study and the completeness of the CRFs by Ocuphire or its designee(s), and appropriate record retention by the Investigator.

12.2.Institutional Review Board (IRB)

This protocol, materials used to recruit subjects, and materials used to document consent must be approved by the IRB prior to initiation of the study. Written IRB approval must adequately identify the protocol and informed consent. In addition to approving the protocol, the IRB must also approve the Subject Information and Consent Form, as well as any advertising tools that will be used for the study. Written approval also must indicate whether approval was granted based on full committee review or expedited review. Copies of all approved materials, all correspondence with the IRB and written approval from the IRB must be made available to Ocuphire, *prior* to the start of subject enrollment into the study.

12.3.Protocol deviations/violations

The Investigator should not deviate from the requirements of this protocol without prior written approval of the Medical Monitor or Ocuphire except in the event of a medical emergency.

A reportable protocol deviation is defined as nonadherence to the protocol that involves inclusion/exclusion criteria, affects subject safety, rights or welfare, or has the potential to affect the integrity of the data. Examples of major protocol deviations include study enrollment by ineligible subject, loss of key data such as equipment malfunction (e.g., pupillometer), and/or use of a prohibited medication during the study.

All protocol deviations will be reported by entering the event in the appropriate eCRF page. Protocol deviations should be reported to the IRB in accordance with IRB-specific guidelines. If there is any question as to whether the deviation is reportable, Ocuphire or designee and the IRB should be contacted.

All changes to the protocol will be made by the Sponsor or designee as an approved amendment to the protocol, submitted to the FDA, and approved by the IRB prior to implementation.

Changes implemented without prior approval will be considered protocol violations.

12.4. Informed consent requirements

Written informed consent will be obtained from each subject prior to the performance of any study assessments. A copy of the signed and dated consent document will be given to each subject. The original signed and dated informed consent document must be maintained in the study files at the Investigator's site.

The Investigator is responsible for ensuring that no subject is subject to any study-related examination or activity before that subject has given informed consent. The subject must give written consent after the receipt of detailed information. The verbal explanation will cover all the elements specified in the written information provided for the subject.

It should be emphasized that the subject is at liberty to withdraw consent to participate at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects who refuse to give, or withdraw, written informed consent may not be included or continued in this study, but this will not impact on their subsequent care.

The Investigator will inform the subject of the aims, methods, anticipated benefits, and potential hazards of the study, including any discomfort it may entail. The subject must be given every opportunity to clarify any points he/she does not understand and if necessary, ask for more information. At the end of the interview, the subject may be given time to reflect if this is required, or if the subject requests more time. Subjects and/or legal guardian will be required to sign and date the informed consent form.

A copy of the signed and dated consent document will be given to each subject. The original signed and dated informed consent document must be maintained in the study files at the Investigator's site. Signed informed consent must be obtained prior to the conductance of any study procedures.

13. DATA HANDLING AND RECORD KEEPING

All procedures for the handling and analysis of data will be conducted using good computing practices meeting ICH and US FDA guidelines for the handling and analysis of data for clinical trials.

13.1. Data entry

Study-specific data that have been outlined in the protocol will be entered into the clinical database by individual(s) designated by the Investigator.

13.2. Data quality control and reporting

Data are verified electronically using a series of programmed edit checks that have been created by the Clinical Data Manager and programmed by the Clinical Data Programmer or designee. Data discrepancies will be brought to the attention of the clinical team and investigated by the CRA and site staff. CRAs will review and verify all data collected in the CRF against source documentation during scheduled monitoring visits. The CRA will work closely with the site staff to address any discrepancies which have been found so that proper resolutions can be made and documented in the clinical database. An audit trail within the system will track all changes made to the data.

13.3. Archiving of data

Archived versions of the database will be saved by Ocuphire consistent with ICH GCP Guidelines, complying with whichever of the requirements is longer. Ocuphire will notify the Investigator when documents should be returned.

13.4. Records retention

The Investigator's site and clinical laboratory will retain all records related to the study in compliance with ICH GCP Guidelines.

13.5. Amendments to the protocol

Modifications of the signed protocol are only possible by approved protocol amendments and with the agreement of all responsible persons. The procedure for approval of a protocol amendment is identical to that for approval of the protocol. The IRB must be informed of all protocol amendments and should be asked for its opinion as to whether a full re-evaluation of the ethical aspects of the study is necessary by the committee. This should be fully documented.

The Investigator must not implement any deviation from or change to the protocol, without discussion with and agreement by Ocuphire and prior review and documented approval/favorable opinion of the amendment from the relevant ethics committee, except where it is necessary to eliminate an immediate hazard to study subjects, or where the change(s) involves only logistical or administrative aspects of the study [e.g., change in monitor(s), change of telephone number(s)].

Protocol amendments will be submitted to the appropriate authority or authorities as required by the applicable regulatory requirement(s).

SUMMARY OF CHANGES

Bold underlined text shows additions; strikethrough text shows deletions. For changes that affect multiple sections of the protocol, the change is listed once at the first instance in the table below, and each subsequent protocol section incorporating that change is also listed at that point. Administrative and minor editing changes that do not affect the content or conduct of the protocol have been made; these are not listed.

Protocol OPI-APXDR-201 Amendment 1

Protocol OPI-APXDR-201 Amendment 1, issued 19 January 2021, makes the following changes to the original protocol dated 17 December 2020:

Table 3 Protocol OPI-APXDR-201 Amendment 1 Summary of Changes

Section/Location	Description of Change	Rationale for Change
Synopsis: Design (p.9) Synopsis: Qualification/ Baseline Visit (p.13) 4. Study Design (p.26) 7.2.2. Qualification/ Baseline – Visit 2/ Day 1 (p.41)	Added text: <u>If the PDR cap has been reached, the study eye may be an eye with the lower DRSS if the other eye has mild PDR.</u>	To allow patients with 2 eligible eyes (one with mild PDR) to enroll in the study even if 20% cap for mild PDR has been met
Synopsis: Exclusion Criteria (p.12) 5.2 Subject Exclusion Criteria (p.36)	Current text: Hypertension with resting diastolic blood pressure (BP) > 105 mmHg or systolic BP > 200 mmHg at the Screening Visit. New text: Hypertension with resting diastolic blood pressure (BP) > 105 110 mmHg or systolic BP > 200 180 mmHg at the Screening Visit.	To change blood pressure eligibility criterion to be consistent with precedence in this patient population (180/110 instead of 200/105). See below table.

Diabetic Retinopathy Study #	MOA	BP criterion
NCT04418427	anti-VEGF	160/100
NCT04265261	Unknown	180/100
NCT02435862	anti-integrin	180/110
NCT01805297	anti-VEGF	200/120
NCT01769183	anti-microbial	180/110
NCT01489189	anti-VEGF	180/110
NCT00711490	mTOR	180/110
NCT00336323	anti-VEGF	180/110
NCT00231023	corticosteroid	180/110
NCT00105404	corticosteroid	180/110

Protocol OPI-APXDR-201 Amendment 2

Protocol OPI-APXDR-201 Amendment 2, issued 26 April 2021, makes the following changes to protocol Amendment 1 dated 19 January 2021:

Table 4 Protocol OPI-APXDR-201 Amendment 2 Summary of Changes

Section/Location	Description of Change	Rationale for Change
1. Study Summary: Exclusion Criteria (p.10); 5.2. Subject Exclusion Criteria (p.35)	2. Presence of center involved diabetic macular edema (DME) defined as a central subfield thickness (CST) ≥ 300 μm on SD-OCT or the presence of intra- or subretinal fluid within the central subfield . Center involved DME in the fellow eye is allowed. Intravitreal injections of an anti-VEGF agent in the fellow eye do not exclude the subject	To revise the definition of center involved DME (removing the requirement of no fluid within the central subfield)
1. Study Summary: Screening Visit (p.13) 1 Study Summary: Duration of Subject Participation and Study (p.17); 4.5. Expected duration of subject participation (p.32) Table 1 (p.29) 7.2.1. Screening/Visit 1 (p.40,41)	The Screening Visit (Visit 1) occurs 31 to 710 days prior to Qualification/Baseline Visit. • Screening Visit 1 (up to 710 days prior to Baseline Visit) Screening window: -710 to -31 Screening/Visit 1 (Day -710 to -1) The Screening Visit occurs 31 to 710 days prior to Qualification/Baseline Visit. Eligibility criteria can be found in Section 5.1 .	To extend the Screening Visit window from -3 to -7 days to -1 to -10 days

Protocol OPI-APXDR-201 Amendment 3

Protocol OPI-APXDR-201 Amendment 3, issued 8 July 2021, makes the following changes to the protocol Amendment 2, dated 26 April 2021:

Table 5 Protocol OPI-APXDR-201 Amendment 3 Summary of Changes

Section/Location	Description of Change	Rationale for Change
1. Study Summary: Exclusion Criteria (p.10) 5.2. Subject Exclusion Criteria (p.35)	2. Presence of center involved diabetic macular edema (DME) defined as a central subfield thickness (CST) \geq 300 320 μ m on SD-OCT. Center involved DME in the fellow eye is allowed. Intravitreal injections of an anti-VEGF agent in the fellow eye does not exclude the subject.	To change the threshold for DME from a CST of 300 μ m to 320 μ m
1. Study Summary: Exclusion Criteria (p.11) 5.2. Subject Exclusion Criteria (p.36)	17. Poorly controlled diabetes, defined as hemoglobin A1c (HbA1c) \geq 12.0% or $<$ 12.0% with uncontrolled diabetes mellitus or no HbA1c available	To clarify that subjects must have an HbA1c value to determine eligibility, and that for those without an HbA1c value available at Screening, they should obtain an HbA1c from a local laboratory
Table 1: Screening, Qualification/Baseline, Treatments, and Follow-up Visits and Procedures (p.29)	Confirmation of HbA1c*** ***Either by subject-provided report, primary care physician, or local lab test.	
7.2.1. Screening/Visit 1 (Day -10 to -1) (p.41)	If the subject does not have an available HbA1c, they should be sent to a local laboratory to get a HbA1c level to determine eligibility.	

Protocol OPI-APXDR-201 Amendment 4

Protocol OPI-APXDR-201 Amendment 4, issued 27 September 2021, makes the following changes to the protocol Amendment 3, dated 08 July 2021:

Table 6 Protocol OPI-APXDR-201 Amendment 5 Summary of Changes

Section/Location	Description of Change	Rationale for Change
Title (p.1) Sponsor Signature & Contacts (p.3) Investigator's Agreement (p.3) 1. Study Summary: Type of Study (p.8)	Randomized, Placebo-Controlled, Double-Masked Study of the Safety and Efficacy of Orally Administered APX3330 in Subjects with Moderately Severe to Severe Non-Proliferative Diabetic Retinopathy and or Mild Proliferative Diabetic Retinopathy	To clarify that subjects were to have either moderately severe to severe NPDR or mild PDR
1. Study Summary: Design (p.9)	Placebo-controlled, double-masked, randomized, Phase 2 study in approximately a maximum of 100 subjects with moderately severe to severe NPDR or mild PDR	To clarify that a maximum of 100 subjects will be enrolled
1. Study Summary: Subject Population (p.10)	A maximum of One hundred (100) subjects with moderately severe to severe NPDR (DRSS Level 47 or 53) or mild PDR (DRSS Level 61).	
1. Study Summary: Estimated Total Sample Size (p.14)	A maximum of One hundred (100) randomized subjects, with approximately 96 evaluable subjects	
2.6. Study Population (p.23)	A maximum of One hundred (100) male or female subjects aged ≥ 18 years with DR rated as moderately severe to severe NPDR (Diabetic Retinopathy Severity Score [DRSS] Level 47 or 53) or mild PDR (DRSS Level 61) will be randomized in a 1:1 ratio to one of two treatment arms, with the expectation that approximately 96 subjects will be evaluable for efficacy.	
3. Objectives and Purpose (p.25)	The ZETA-1 study is a placebo-controlled, double-masked, randomized, Phase 2 study in approximately a maximum of 100 randomized subjects with moderately severe to severe NPDR or mild PDR	
4. Study Design (p.26)	This is a placebo-controlled, double-masked, randomized, Phase 2 study in approximately a maximum of 100 subjects with moderately severe to severe NPDR or mild PDR	
4.2. Description and schedule of visits and procedures (p.28) 6. Treatment of Subjects (p.38)	A maximum of One hundred (100) subjects with DR rated as moderately severe to severe NPDR (DRSS Level 47 or 53) or mild PDR (DRSS Level 61) ≥ 18 years of age will be randomized in a 1:1 ratio to one of two treatment arms, with the expectation that approximately 96 subjects will be evaluable for efficacy.	

1. Study Summary: Duration of Study (p.8)	Up to 26 28 weeks, including screening, treatment, and follow-up	To extend the Screening Visit window from -1 to -10 days to -1 to -21 days.
1. Study Summary: Screening Visit (p.13) 7.2.1 Screening/Visit 1 (p.41)	The Screening Visit (Visit 1) occurs 1 to 10 21 days prior to Qualification/Baseline Visit.	
Table 1. Screening, Qualification/Baseline, Treatment and Follow-up Visits and Procedures (p.29) 7.2.1 Screening/Visit 1 (p.40)	Day (-10 -21 to 01)	
1. Duration of Subject Participation and Study (p.17) 4.5. Expected duration of subject participation (p.32)	The total length of subject participation is approximately 26 28 weeks, with 5 clinic visits, 4 telephone safety calls, and one telephone call follow-up visit summarized below: Screening Visit 1 (up to 10 21 days prior to Baseline Visit)	
Table 1: Screening, Qualification/Baseline, Treatments, and Follow-up Visits and Procedures (p.30)	Confirmation of HbA1c*** ***Either by subject-provided report, primary care physician, or local or central lab test.	To clarify that subjects without an HbA1c value available at Screening may obtain an HbA1c from a local or central laboratory.
7.2.1. Screening/Visit 1 (Day -10 to -1) (p.41)	If the subject does not have an available HbA1c, they should be sent to a local laboratory to get an HbA1c level to determine eligibility; alternatively, a blood sample can be collected for assessment by the central laboratory.	
Table 2: Number and Volume of Blood Samples and Total Blood Volume Collected in the Study (Qualification/Baseline through Week 24) (p.30)	HbA1c (1) ^ HbA1c (1 x 3 mL) ^ ^If necessary to conduct HbA1c lab test, an additional 3mL sample will be drawn.	To add the volume of blood required for HbA1c.
1. Number of Investigational Sites (p.14)	Approximately 15 20 to 25 sites	To increase the number of investigational sites from 15 sites to 20 to 25 sites.
1. Sample Size Justification (p.14) 9.1 Sample size (p.51)	A sample size of 96 subjects who are evaluable for efficacy (i.e., who have at least one post-randomization DRSS evaluation in the study eye, who have missed less than 20% of expected doses, and do not have any major protocol deviations <u>considered to have significant impact on treatment outcome</u>) is needed for the study.	
1. Safety Endpoints (p.16) 4.1 Primary and secondary endpoints: Safety (p.27)	<ul style="list-style-type: none"> Time to first rescue treatment 	To add time to first rescue treatment as a safety endpoint.
1. Treatment Visits (p.14)	In the judgement of the Investigator, if there is any risk to the eye(s) of the subject and if possible, upon	To provide additional clarity on treatment for







	<p>approval by the Medical Monitor or Sponsor, an appropriate rescue treatment for DR progression will be administered. Eyes may be rescued if there is:</p> <ul style="list-style-type: none"> • Moderate or severe active Level 71 or higher PDR and/or development of anterior segment neovascularization • Progression to clinically significant center involved DME • ≥ 10-letter loss in BCVA compared to baseline attributable to worsening retinopathy • Any other finding which, in the judgement of the Investigator, requires rescue treatment. In this situation, the investigator should make every effort to consult with the Medical Monitor or Sponsor prior to initiation of rescue. <p>Rescue can be administered in either eye during the study. If the rescue is administered to the study eye, the subject will be considered a study rescue subject and the subject will be discontinued from the study. Every effort should be made to complete all Visit 9 (Week 24) study procedures prior to rescue.</p> <p>Prior to initiating rescue, BCVA, color fundus photography for DRSS, CST, biomicroscopy, ophthalmoscopy, and IOP study procedures must be performed before rescue treatment is initiated (only conducted before first instance of rescue treatment). These assessments may be performed as an unscheduled visit.</p> <p>If rescue treatment is given, the subject may continue in the study and will remain on their randomized treatment and complete all remaining study visits per the visit schedule.</p>	<p>DME or PDR during the study; and to modify the protocol to allow subjects who initiate rescue for DME or PDR in either eye to continue in the study.</p>
9.3.5 Analysis of efficacy (p.52)	<p>For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations <u>or for subjects requiring rescue</u> if applicable; details will be provided in the study Statistical Analysis Plan.</p> <p>For the analysis of the secondary efficacy endpoints, only observed case data will be used. If warranted, confirmatory analyses using the ARP with imputation for missing data <u>or subjects requiring rescue</u> will also be performed for the secondary efficacy endpoints.</p>	<p>To modify text for subjects requiring rescue.</p>
9.4 Procedure for accounting for missing, unused, or spurious data (p.53)	<p>For the analysis of the primary efficacy endpoint, appropriate imputation techniques will be performed for missing observations <u>or subjects requiring rescue</u>, if applicable. Confirmatory efficacy analyses for secondary endpoints may also be performed using imputation for missing data <u>or subjects requiring rescue</u>.</p>	

<p>6.3. Treatment for DME or PDR during study (p.39)</p>	<p>6.3 Treatment for DME or PDR during study</p> <p>6.3.1 Continued standard of care treatment for DME or PDR in fellow eye A fellow eye receiving continued standard of care treatment for DME or PDR at study entry may continue to receive treatment during the study per the discretion of the Investigator. The subject will receive randomized study medication and follow the study schedule of assessments without alteration.</p> <p>6.3.2 Initiation of rescue treatment for DME or PDR in either eye Whenever possible the Investigator should consult with the Medical Monitor or Sponsor prior to initiation of rescue treatment. Eyes may be rescued if there is:</p> <ul style="list-style-type: none"> • Moderate or severe active PDR and/or development of anterior segment neovascularization • Progression to clinically significant center involved DME • ≥ 10-letter loss in BCVA compared to baseline attributable to worsening retinopathy • Any other finding which, in the judgement of the Investigator, requires rescue treatment. In this situation, the Investigator should make every effort to consult with the Medical Monitor or Sponsor prior to initiation of rescue. <p>Prior to initiating rescue, BCVA, color fundus photography for DRSS, CST, biomicroscopy, ophthalmoscopy and IOP study procedures must be performed before rescue treatment is initiated (only conducted before first instance of rescue treatment). These assessments may be performed as an unscheduled visit.</p> <p>If rescue treatment is given, the subject may continue in the study and will remain on their randomized treatment and complete all remaining study visits per the visit schedule.</p>	<p>To provide additional clarity on treatment for DME or PDR during the study; and to modify the protocol to allow subjects who initiate rescue for DME or PDR in either eye to continue in the study.</p>
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APPENDIX 1: DRSS PRIMARY ENDPOINT

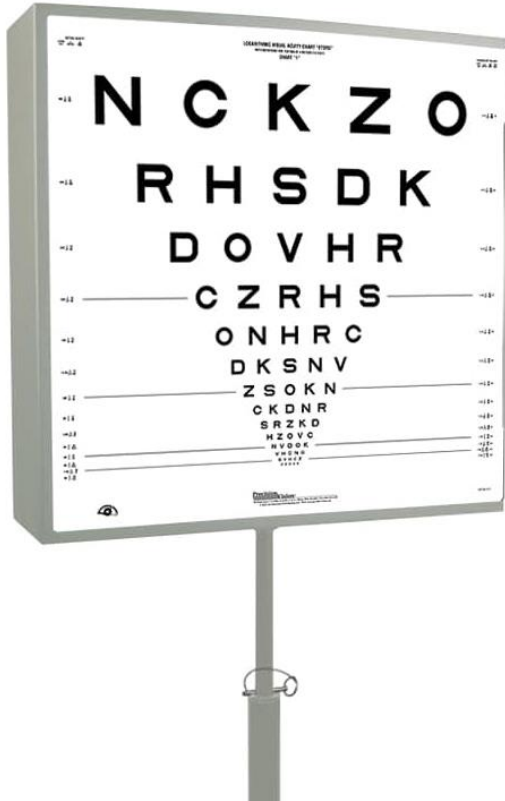
Primary Endpoint of APX3330 ZETA-1 Trial

Percent of patients
with a ≥ 2 step
improvement on the
DRSS score at
week 24

Patients included in the ZETA-1 Trial						
DRSS Score	1 (10)	2 (20)	3 (35)	4 (43)	5, 6 (47, 53)	7 – 13 (60, 61, 65, 71, 75, 85, 90)
Description	DR Absent	Micro-aneurysm only	Mild NPDR	Moderate NPDR	Moderately Severe NPDR	PDR – Mild, Moderate, and Severe
Retinal Image	 <p>Healthy blood vessels with no bulges</p>	 <p>Small bulges in blood vessel walls as well as other signs in the retina</p>	 <p>More changes in the blood vessels in the retina and small spots of blood can become more visible</p>	 <p>More blood vessels in larger areas of the retina show changes</p>	 <p>Many of the blood vessels in the retina show visible changes</p>	 <p>Increased growth of new, damaged blood vessels</p>

APPENDIX 2: ORIGINAL SERIES SLOAN LETTER ETDRS CARD

BCVA (distance) will be measured with a Standard ETDRS illuminated chart (on wall or stand) at 4 m.



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