OTT166-201 A Phase 2 Randomized, Double-Masked, Vehicle-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of OTT166 Ophthalmic Solution in the Treatment of Diabetic Retinopathy (DR)

Protocol Number: OTT166-201

Brief Title: OTT166 in DR

Version: 5.0

Sponsor Name and Address:

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PROTOCOL AMENDMENT SUMMARY OF CHANGES

Document History	Date
Amendment 04 (V5.0)	10 October 2023
Amendment 03 (V4.0)	21 December 2022
Amendment 02 (V3.0)	14 October 2022
Amendment 01 (V2.0)	11 May 2022
Original Protocol (V1.0)	02 March 2022

Amendment 04 (V5.0), 10 OCT 2023

A description of important changes in this amendment is provided in the table below:

Section # and Name	Description of Change	Brief Rationale
1.0 Study Synopsis 2.20.2 Outcome Measures	Secondary endpoints updated to include: "Proportion of participants developing VTC at Week 24", "Time to development of PDR worse than mild (DRSS 65 and above) or CI-DME", and "Time to rescue therapy for participants who meet objective criteria for rescue therapy". "Mean and median step change in DRSS step from baseline to Week 24" was removed.	To clarify and further define the endpoints.
1.0 Study Synopsis 2.9 Exclusion Criteria	Exclusion #1 updated to include "and there is a normal foveal contour as determined by the Central Reading Center".	To specify the exclusion criteria.
1.0 Study Synopsis 2.9 Exclusion Criteria	Exclusion #33 list of medications extended to "Liraglutides, Dulaglutides, and Tirzepatides".	To reflect additional medications that are exclusionary.
1.0 Study Synopsis 2.14.9 Rescue Criteria	Added "Development of ASNV".	To clarify the rescue criteria.
1.0 Study Synopsis 2.20 Statistical Considerations	Updated safety summary sections to clarify what will be included in the Safety Analysis Set and summary of TEAEs. Primary estimand clarified and updated.	To clarify the statistical considerations.
2.4 OTT166 Background	Background on OTT166 updated to reflect current knowledge.	To align with current knowledge of background.
2.14.4 Study Intervention Compliance and Adherence 2.15.1 Schedule of Activities	Updated to clarify "compliance" per protocol and "adherence" for the MEMS Cap® use, and to emphasize the importance of continuously monitoring adherence to ensure participants are taking the correct number of prescribed doses. "IP adherence review" added to the SoA to ensure sites are discussing adherence with participants at each study visit and continuously monitoring this activity.	To clarify "compliance" and "adherence".

Section # and Name	Description of Change	Brief Rationale
2.15.1 Schedule of Activities	Visit 8 “continue study treatment” in the SoA removed as this is the end of study visit.	Administrative update.
2.15.1 Schedule of Activities	Footnote “m” added to ensure cornea is assessed at the beginning of the visits before dilation.	To ensure cornea is not impacted by other tests.
2.16.9 Vision-threatening Complications	Updated more than mild PDR as “DRSS 65 and above” and added “CI-DME” to the list.	To clarify the definition of vision-threatening complication.
3.2 Appendix 2	ETDRS table updated to be more specific.	To provide specific details for each level of severity.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
ANCOVA	analysis of covariance
ASNV	anterior segment neovascularization
Anti-VEGF	anti-vascular endothelial growth factor
AUC	area under the curve
BCVA	best corrected visual acuity
BID	twice daily
BL	baseline
BMI	body mass index
CFP	color fundus photography
CI	confidence interval
CI-DME	center-involved diabetic macular edema
CNV	choroidal neovascularization
CST	central subfield thickness
CONSORT	Consolidated Standards of Reporting Trials
DD	disk diameter
DME	diabetic macular edema
DR	diabetic retinopathy
DRIL	disorganization of retinal inner layers
DRSS	Diabetic Retinopathy Severity Scale
ETDRS	Early Treatment Diabetic Retinopathy Study
ETRDR	Early Treatment Report Diabetic Retinopathy
eCRF	Electronic Case Report Form
EOS	end of study
FA	fluorescein angiography
FAS	full analysis set
FAZ	foveal avascular zone
GAT	Goldmann applanation tonometry
GCP	Good Clinical Practice
HbA1c	glycosylated hemoglobin
IB	Investigator's Brochure
IC_{50}	half maximal inhibition concentration
ICF	informed consent form
ICE	intercurrent event
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
IOP	intraocular pressure
IRB	Institutional Review Board

Abbreviation	Definition
IRMAs	intraretinal microvascular anomalies
ITT	intent-to-treat
IVT	intravitreal injection
IWRS	interactive web response system
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
Mg	milligram
MH	Mantel-Haenszel
mmHg	millimeter of mercury
n	sample size
nAMD	neovascular age-related macular degeneration
NPDR	non-proliferative diabetic retinopathy
NVE	neovascularization elsewhere
OCT	optical coherence tomography
OCTA	optical coherence tomography – angiography
OU	both eyes
PK	pharmacokinetic
PDR	proliferative diabetic retinopathy
PRP	pan-retinal photocoagulation
PPS	per-protocol set
PT	preferred term
QID	four times daily
QTL	quality tolerance limit
SAP	statistical analysis plan
SAE	serious adverse event
SD	standard deviation
SD-OCT	spectral-domain optical coherence tomography
SE	study eye
SoA	schedule of activities
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction
TEAE	treatment-emergent adverse event
TESAE	treatment-emergent serious adverse event
MV	macular volume
VEGF	vascular endothelial growth factor
VTC	visually threatening complications
WHO DD	World Health Organization Drug Dictionary
YAG	yttrium aluminum garnet

1 STUDY SYNOPSIS

Name of Sponsor/Company:	OcuTerra Therapeutics, Inc.
Name of Investigational Product:	OTT166 Ophthalmic Solution (5.0%)
Title of Study:	OTT166-201 A Phase 2 Randomized, Double-Masked, Vehicle-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of OTT166 Ophthalmic Solution in the Treatment of Diabetic Retinopathy (DR)
Protocol Number:	OTT166-201
Phase of Development:	2
Study Period:	Up to 24 weeks of follow-up

Methodology:

A prospective, randomized, double-masked, vehicle-controlled Phase 2 study will be conducted to further evaluate safety and efficacy of OTT166 Ophthalmic Solution in diabetic retinopathy patient population and to select an optimum dosing regimen (frequency) for Phase 3 pivotal trials. Approximately 210 participants diagnosed with moderately severe to severe non-proliferative diabetic retinopathy (NPDR) or mild proliferative diabetic retinopathy (PDR) and who are treatment naïve (ie, no prior anti-vascular endothelial growth factor [anti-VEGF] or laser [focal, grid, pan-retinal photocoagulation (PRP)] administered) will be randomized 2:2:1:1 into the following groups: OTT166 5% twice daily (BID), OTT166 5% four times daily (QID), vehicle control BID, vehicle control QID. Randomization will be stratified by baseline Diabetic Retinopathy Severity Scale (DRSS) score (47 or 53 or 61B). Participants with PDR (DRSS score 61B) will be capped at 20% of all randomized participants. Each group will self-administer one 50-µl eye drop of study solution (frequency as assigned) for 24 weeks.

Sample size:

The study will enroll approximately 210 participants randomized as above, approximately 70 participants in each OTT166 treatment group and approximately 35 participants per group into each matching vehicle control group and allowing for around 5% study attrition; this will ensure approximately 66 participants per treatment group and combined vehicle control group.

For the primary endpoint of improvement by at least 2 steps from baseline on the DRSS at Week 24, completing 66 participants per group (active treatment BID, QID, combined vehicle control) through Week 24 will yield 90% power to demonstrate that the active

treatment is superior to vehicle control using a 1-sided alpha of 0.05, provided that the true treatment effect (active treatment efficacy rate minus vehicle control efficacy rate) is at least 0.20 and the vehicle control efficacy rate is at most 0.06.

The two OTT166 dose regimens will cover a 2-fold dose range in total daily dose (5.0 mg/day to 10.0 mg/day).

Population:

The population will be participants with Type 1 or Type 2 diabetes under adequate control as evidenced by glycosylated hemoglobin (HbA1c) less than or equal to 12.0% , who show typical fundus alterations, and diagnosed with moderately severe to severe NPDR or mild PDR, as defined by the DRSS Steps 47, 53, and 61B, with the PDR population capped at no more than 20% of all randomized participants. Baseline Best-Corrected Visual Acuity (BCVA) must be ≥ 69 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (Snellen equivalent 20/40) and central subfield thickness (CST) must be ≤ 325 μm at the screening and baseline examinations. DRSS steps, CST measurements and the presence of a normal foveal contour must be confirmed by the central reading center.

Primary Objectives:	**Primary Endpoints:**
To characterize the safety of topical OTT166 in participants with DR	• Proportion of participants who develop treatment-emergent adverse events (TEAEs) through Week 24
To characterize the efficacy of topical OTT166 in participants with DR	• Proportion of participants who have improved by ≥ 2 steps from baseline at Week 24 as determined by central reading center assessment using the DRSS
Secondary Objectives:	**Secondary Endpoints:**
To determine if topical OTT166 will prevent or delay the occurrence of visually threatening complications (VTC), defined as PDR worse than mild and/or anterior segment neovascularization (ASNV) and/or CI-DME (defined below) To determine if topical OTT166 will prevent or delay the occurrence of CI-DME. (CI-DME	• Proportion of participants developing worse than mild PDR (DRSS 65 and above) at Week 24 • Proportion of participants who develop ASNV determined by the investigator at Week 24 • Time to development of PDR worse than mild (DRSS 65 and above) • Proportion of participants who develop CI-DME at Week 24 • Time to development of CI-DME

is defined as the presence of fluid in the central subfield in participants who have no fluid at baseline or CST > 325 µm)	• Proportion of participants developing VTC at Week 24 • Time to development of PDR worse than mild (DRSS 65 and above) or CI-DME
To determine the effect of OTT166 on DRSS in participants with moderately severe to severe NPDR and mild PDR treated with topical OTT166	• Proportion of participants with change in DRSS steps at Week 24 compared to baseline - Change in DRSS steps is defined as DR worsening or improving by 1, 2, or ≥ 3 steps. • Proportion of participants with mild PDR (DRSS score 61B) at baseline who regress to NPDR (DRSS score ≤ 53) by Week 24
To determine the effect of OTT166 on BCVA in participants with moderately severe to severe NPDR and mild PDR	• Mean and median change in BCVA (ETDRS letters) from baseline to Week 24 • Lines gained/lost in BCVA (± 5, 10, and 15 ETDRS letters) at Week 24 • Mean and median change in area under the curve (AUC) for change in BCVA from baseline to Week 24
To determine the effect of OTT166 on central subfield thickness in participants with moderately severe to severe NPDR and mild PDR	• Mean and median change in CST from baseline to Week 24 • AUC for change in CST from baseline to 24 weeks
To determine the effect of OTT166 on the need for rescue therapy in participants with moderately severe to severe NPDR and mild PDR	• Proportion of participants who met the objective rescue criteria as defined in protocol Section 2.14.9. • Time to meet objective rescue therapy criteria as defined in protocol Section 2.14.9. • Time to administration of rescue therapy

Exploratory Objectives	Exploratory Endpoints
To evaluate the change in DME in participants treated with OTT166	• Change in Macular Volume (MV) from baseline to Week 24 for 1, 3, and 6 mm regions
To assess the effects of treatment on anatomic markers other than DRSS and on retinal non-perfusion	• Change in area of retinal non-perfusion from baseline to Week 24 seen on widefield fluorescein angiography (FA) • Proportion of participants with intact foveal avascular zone (FAZ) seen on widefield FA at Week 24 • Optical coherence tomography angiography (OCTA) endpoints within the 6 mm area: - Change from baseline to Week 24 in FAZ area - Change from baseline to Week 24 in non-perfusion area - Change from baseline to Week 24 in vessel caliber - Ischemic Index at Week 24 compared to baseline - Proportion of participants with new neovascularization at Week 24 - Proportion of participants with new intraretinal microvascular anomalies (IRMA) at Week 24 - Change in vessel density from baseline to Week 24 in the superficial and deep layers
Key Inclusion Criteria: 1. Men or women ≥ 18 years of age with type 1 or 2 diabetes mellitus who have moderately severe to severe NPDR [DRSS levels 47 or 53], or mild PDR [DRSS level 61B] NVE < 0.5 DA in 1 + quadrants], confirmed by the central reading center, in whom PRP and/or anti-VEGF intravitreal injection (IVT) can be safely deferred for at least 6 months	**Key Exclusion Criteria:** 1. CST of > 325 μm a. Fluid in the central subfield is allowed so long as CST is ≤ 325 μm and there is a normal foveal contour as determined by the Central Reading Center 2. Any prior focal or grid laser photocoagulation or any prior PRP in the study eye as it pertains to treatment of DME or DR (peripheral retinal hole treated with laser is allowed)

per the Investigator. (ETDRS Report 1991). 2. BCVA ETDRS letter score in the study eye of ≥ 69 letters (approximate Snellen equivalent of 20/40 or better) 3. Normal foveal contour 4. Treatment -naïve (ie, no previous anti-VEGF or steroid treatment or PRP or laser) 5. Willing and able to return for all study visits and comply with study-related procedures 6. Able to adhere to the study dosing requirements 7. Understands and signs the written informed consent form (ICF)	3. Eyes with DRSS score 61 due to fibrous proliferations at disc or fibrous proliferations elsewhere a. DRSS score 61B with NVE only is allowed. Any sign of fibrosis proliferation is exclusionary 4. Any prior systemic anti-VEGF or IVT anti-VEGF treatment in the study eye 5. Any prior intraocular steroid injection in the study eye, inclusive of Iluvien® and Retisert® a. History of Ozurdex® and triamcinolone use prior to 12 months before study enrollment is allowed 6. Current ASNV, vitreous hemorrhage, or tractional retinal detachment visible at the screening assessments in the study eye 7. Uncontrolled glaucoma or ocular hypertension in the study eye defined as an intraocular pressure (IOP) > 25 millimeter of mercury (mmHg) regardless of concomitant treatment with IOP-lowering medications 8. Hypertension defined as systolic > 180 mmHg or > 160 mmHg on 2 consecutive measurements (during the same visit) or diastolic > 100 mmHg 9. Screening HbA1c blood test > 12.0% 10. Renal failure (stage 4 or end-stage), dialysis, or history of renal transplant 11. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the participant at high risk for treatment complications 12. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to randomization or plans to do so in the next 4 months

	13. Epiretinal membrane, posterior hyaloidal traction, and/or vitreomacular traction in the study eye as determined to be significant by the Investigator 14. Previous pars plana vitrectomy in the study eye 15. Any intraocular surgery in the study eye within 90 days (3 months) prior to study enrollment 16. Yttrium Aluminum Garnet (YAG) laser treatment in the study eye within 90 days prior to study enrollment 17. Concomitant use of any topical ophthalmic medications in the study eye, including dry eye or glaucoma medications, unless on a stable dose for at least 90 days prior to study enrollment and expected to stay on stable dose throughout study participation. Topical eyedrops are not allowed within ± 10 minutes of study drop application 18. Contact lens use from time of screening throughout the study 19. Central corneal changes from dry eye that are visually significant and/or Sjogren's syndrome 20. Visually significant Fuchs endothelial dystrophy or other diagnosed conditions of corneal compromise including Anterior Basement Membrane Dystrophy, or any corneal dystrophy affecting central vision (peripheral processes are not exclusionary) 21. Chronic or recurrent uveitis in the study eye 22. Ongoing ocular infection or inflammation in either eye 23. A history of cataract surgery complicated by vitreous loss in the study eye 24. Congenital eye malformations in the study eye 25. A history of penetrating ocular trauma in the study eye 26. Cognitive impairment that, in the opinion of the investigator, could compromise compliance with the requirements of the study 27. Females of childbearing potential (ie, who are not postmenopausal for at least 1 year or surgically sterile for at least 6 weeks prior to Visit 1 –

	Screening/Randomization) who are lactating, or who are pregnant as determined by a positive serum pregnancy test at Visit 1 – Screening/ Randomization. Women of childbearing potential must agree to use acceptable methods of birth control throughout the study. a. Women who are breastfeeding or who have a positive serum hCG/urine pregnancy test at the screening or Baseline (BL) Visit 28. Females and males of childbearing potential unwilling or unable to utilize the following acceptable methods of birth control: tubal ligation, transdermal patch, intrauterine devices/systems, oral/implantable/injectable or contraceptives, diaphragm or cervical cap with spermicide, or vasectomized partner for females; condoms with spermicidal agent and vasectomy for males; or sexual abstinence for males and females 29. Participation in any other investigational device or drug clinical research study within 12 weeks of Visit 1 – Screening/ Randomization and during the duration of enrollment 30. Contraindication to the study medications or fluorescein dye 31. Other ocular pathologies that, in the investigator's opinion, would interfere with the participant's vision in the study eye 32. Ocular media of insufficient quality to obtain fundus photographs, fluorescein angiography, and OCT images in the study eye 33. Concomitant use of Semaglutide (Wegovy®, Ozempic®, Rybelsus®),Thiazolidinediones (Actos®, Avandia®), Liraglutides (Victoza®, Saxenda®), Dulaglutide (Trulicity®), or Tirzepatide (Mounjaro®) within 12 months prior to Visit 1(allowed if a stable dose has been established for at least1 year of use) a. Plans to start concomitant use of Semaglutides,Thiazolidinediones, Liraglutides, Dulaglutides, or Tirzepatides during the study duration is exclusionary

Note: The inclusion and exclusion criteria apply through study duration for non-emergency ocular procedures and surgery.

Duration of Treatment:	24 weeks

Designation of Study Eye:

Only one eye will be designated as the study eye in every participant. For participants who meet eligibility criteria in both eyes during the screening phase, the eye with the higher reading center confirmed DRSS should be selected. If both eyes have the same score, the eye with the clearest lens and ocular media will be selected. If there is no objective basis for selecting the study eye, factors such as ocular dominance (better focus ability), other ocular pathology, and participant preference should be considered in making the selection. The final selection will be done by the Investigator in discussion with the participant at baseline and must not be changed during the course of the study.

The non-study eye is eligible for treatment according to the standard of care treatments through the duration of the study.

Study procedures/assessments:

Assessments will be performed monthly during each clinic visit unless indicated otherwise in the Schedule of Activities (SoA), and include the following:

- Adverse event recording
- Review of concomitant medications
- Vital signs
- Physical examination (Screening Visit only)
- Laboratory testing (hematology, blood chemistry, urinalysis, and HbA1c)
- Pregnancy testing (as applicable)
- BCVA (ETDRS)
- Slit-lamp bio-microscopy
- Ocular surface health [fluorescein stain of the cornea]
- IOP
- Indirect ophthalmoscopy/dilated fundus examination
- Spectral domain OCT (SD-OCT) (central subfield thickness, macular volume, foveal contour) at every visit

- 7-field or 4-wide field color fundus photography at every visit [optional wide-field if available]
- DRSS scoring, based on 7-field or 4-wide field photos by the PI at baseline to confirm eligibility
- Fluorescein angiography (FA) (wide field if available) at baseline and end of study
- OCTA if available (for exploratory endpoints) at baseline and end of study
- Rescue therapy initiation, per alignment between Investigator and Medical Monitor
- HbA1c at screening and end of treatment
- Gonioscopy

Visit Timeline:

Screening – Visit 1; Baseline (BL) – Visit 2; Week 4 – Visit 3; Week 8 – Visit 4; Week 12 – Visit 5; Week 16 – Visit 6; Week 20 – Visit 7; Week 24 – Visit 8

Rescue Criteria:

Participants meeting any of the following criteria would qualify for rescue with anti-VEGF therapy and/or PRP laser after consultation with the Medical Monitor:

Objective Rescue Therapy Criteria:

- Worsening DRSS level to 65 or higher as confirmed by the central reading center
- Development of ASNV
- Decrease in BCVA of ≥ 10 ETDRS letters from baseline associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline at any visit
- Decrease in BCVA of 5 to 9 ETDRS letters from baseline at two consecutive visits 4 weeks apart associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline
- Increase in CST of ≥ 75 microns from baseline associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline

Subjective Rescue Criteria:

- Any condition that, in the clinical opinion of the Investigator, warrants rescue therapy after consultation with the Medical Monitor

Statistical Analyses:

Statistical analyses will include tabulations of summary data, inferential analyses, by participant listings, and figures.

All statistical tests will be one-sided and will be performed at the 5% level of significance, unless otherwise stated. The vehicle control group will be combined for all efficacy analyses and two-sided 90% confidence intervals will be presented. Frequency counts (n and percentages) will be made for each qualitative variable. Descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) will be calculated for each quantitative variable, unless otherwise stated. All data will be summarized by treatment group.

The baseline value for analysis and reporting will be based on the last (non-missing) measurement before dosing on Day 1.

Comparison of the primary endpoint will be made between each dose group and the combined vehicle control group using a Mantel-Haenszel (MH) test of the difference in 2 proportions stratified by the randomization stratification factor (BL DRSS score 47 or 53 or 61B). The estimated MH risk difference will be summarized along with the 2-sided 90% CI using MH stratum weights and the Sato variance estimator. The primary efficacy analysis will be performed on the ITT, while the PP set will be used as supplementary analyses.

All secondary endpoint analyses will be performed on the ITT analysis set. The secondary endpoints involving proportions of participants will be analyzed using the same approach (MH test) as described for the primary efficacy analysis. Time to event secondary endpoints will be analyzed using a stratified log-rank test adjusting for the randomization stratification factor. Continuous secondary endpoints involving change from BL will be analyzed using an analysis of covariance (ANCOVA) model with BL measurement of the variable as a covariate and treatment group and the randomization stratification factor as fixed factors. The pair-wise comparisons of each OTT166 dose group versus combined vehicle control will be done in the ANCOVA model. Two-sided 90% confidence intervals for the difference of each OTT166 dose group minus combined vehicle control will be calculated.

All safety summaries and analyses will be based on the Safety Analysis Set by treatment group (i.e., vehicle control doses (5% BID, 5% QID), vehicle control combined, active OTT166 doses (5% BID, 5% QID), and OTT166 combined). and overall. AEs will be coded using MedDRA and summarized by system organ class (SOC) and preferred term (PT). Analysis and reporting for AEs will be based on TEAEs. A TEAE is defined as an AE occurring (onset date/time) at the time of or after dosing on Day 1. Summaries of TEAEs by treatment group, SOC, and PT will include the following: All TEAEs, ocular TEAEs and TESAEs in the study eye, ocular TEAEs and TESAEs in the fellow eye, non-ocular TEAEs and TESAEs, all TESAEs, TEAEs and TESAEs leading to

discontinuation of study drug, TEAEs and TESAEs leading to discontinuation of study, drug-related TEAEs and TESAEs, TEAEs by intensity, AESIs.

2 STUDY PROTOCOL

2.1 Study Rationale

This study is designed to provide proof -of -concept that NPDR and mild PDR can be effectively treated with a topical eye drop formulation of OTT166. Furthermore, this study is designed to determine the dose-exposure response of OTT166 in terms of efficacy and safety in order to support the dose selection for the subsequent pivotal Phase 3 studies with OTT166 in DR.

OTT166 is a representative from a new class of drugs (ie, a topical integrin inhibitor targeted to the back of the eye). It is being developed for treatment of DR. The study is intended to show in particular that OTT166:

- Is overall safe and well tolerated
- Prevents progression of DR
- Prevents VTCs and/or CI-DME
- Reverses retinal changes attributed to DR
- Maintains visual acuity

2.2 Study Objectives

The primary objectives for this study are:

- To characterize the safety of topical OTT166 in participants with DR
- To characterize the efficacy of topical OTT166 in participants with DR

The secondary objectives of this study are:

- To determine if topical OTT166 will prevent or delay the occurrence of VTC, defined as PDR worse than mild and/or ASNV and/or CI_DME
- To determine if topical OTT166 will prevent or delay the occurrence of CI-DME
- To determine the effect of OTT166 on DRSS in participants with moderately severe to severe NPDR and mild PDR treated with topical OTT166
- To determine the effect of OTT166 on BCVA in participants with moderately severe to severe NPDR and mild PDR
- To determine the effect of OTT166 on central subfield thickness in participants with moderately severe to severe NPDR and mild PDR

- To determine the effect of OTT166 on the need for rescue therapy in participants with moderately severe to severe NPDR and mild PDR

The exploratory objectives of this study are:

- To evaluate the change in DME in participants treated with OTT166
- To assess the effects of treatment on anatomic markers other than DRSS and on retinal non-perfusion

2.3 Introduction DR

NPDR has a multifactorial origin and is caused by hyperglycemia-induced oxidative stress, hypoxia, pericyte loss, endothelial cell loss, leukostasis, and inflammation. It leads to a breakdown of the blood-retinal barrier. There is extensive evidence that angiogenic cytokines (eg, VEGF) and inflammatory cytokines are involved in the development of DR. DR is categorized based on the presence of vascular and vascular associated lesions and the absence (in NPDR) or presence (in PDR) of neovascularization. Earlier clinical features of DR include microaneurysm, hemorrhage, cotton wool spots, hard exudates and IRMAs. As the severity of DR progresses, capillary non-perfusion leads to retinal ischemia. This induces an upregulation of pro-angiogenic and pro-inflammatory cytokines, leading to proliferation of aberrant retinal vasculature. When left untreated, these new, fenestrated, brittle and leaky vessels extend and grow and result in vitreous hemorrhage and/or exudation. This is associated with the formation of gliosis and fibrous tissue formation. These scars contract and can result in tractional retinal detachment and loss of vision (Lechner 2017, Cheung 2010, Wang 2018).

Treatment options for moderately severe and severe NPDR and PDR include PRP, anti-VEGF agents, and vitrectomy for PDR (Wang 2018). PRP has been the gold standard for > 40 years for the treatment of PDR. The DR Study has shown that PRP reduces the risk of severe vision loss by over 50% (Aiello et al. 1973). However, PRP is destructive and causes permanent peripheral vision loss that may interfere with driving and impair night vision, color vision, and contrast sensitivity. It may also exacerbate DME (Brucker 2009, Ferris 1987). Anti-VEGF therapies are globally approved for DR, regardless of severity, but they can require monthly intravitreal injections, with the continuous risk of serious side effects, such as endophthalmitis or retinal detachment, and the inconvenience of traveling to a retina specialist office for every injection. Thus, there is an unmet clinical need for non-invasive efficient treatment options.

OTT166 is intended to address the current limitations in the treatment of DR. As outlined above, anti-VEGF treatment requires IVT every 4 to 12 weeks under topical anesthesia. The frequent visits to a retina specialist represent a significant burden to participants, particularly for DR participants, many of whom are of working age. An alternative mode of drug administration, particularly topical eye drops, represents a very attractive treatment option.

The objective of this study is to determine an efficacy and safety profile of OTT166 in the treatment of participants with DR, with the objective to evaluate the potential to improve therapeutic outcomes, while minimizing treatment burden for participants. Preclinical and clinical studies with OTT166 have demonstrated notable anti-angiogenic activity and have shown that the fluorination in the compound has a positive impact on the ability for the treatment to reach the back of the eye. Activity of OTT166 in VEGF-driven models appears similar to anti-VEGF biologics. Moreover, OTT166 also blocks angiogenic signaling driven by other growth factors (platelet-derived and fibroblast growth factor), so clinical efficacy could exceed that of marketed drugs that only target VEGF.

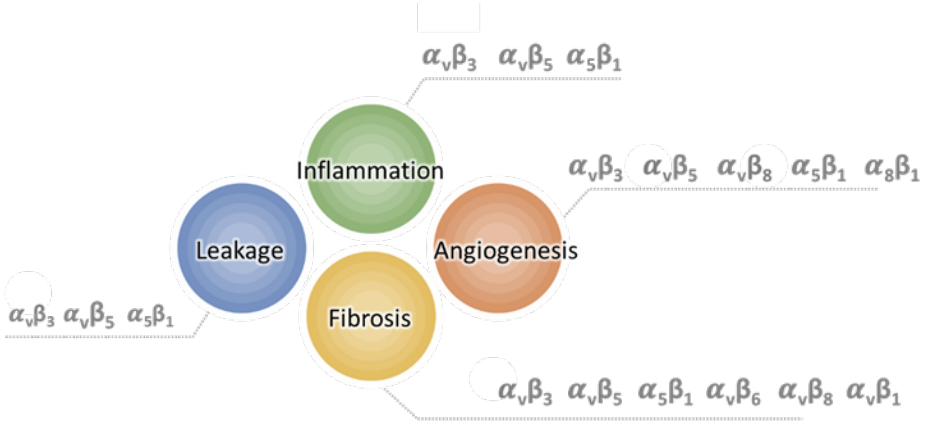
Based on the pre-clinical information, it is expected that the benefits of OTT166 outweigh the risks of its use.

2.4 OTT166 Background

DR is caused by damage to retinal blood vessels in patients with diabetes and is the leading cause of blindness among the United States working-age population. The disease starts with the formation of microaneurysms and progresses to hemorrhages and neovascularization of the retina and vitreous. At any point along this continuum, vessels can leak fluid into the macula, causing diabetic macular edema (DME) and consequently cause loss of eyesight, particularly if the fluid accumulates in the center of the macula. Patients with mild or moderate DR have an approximately 23% chance of progressing to severe disease over 5 years and an approximately 60% chance of developing DME (Cheung 2010, Ferris 1987, Wang 2018).

Integrins are proteins found on the surface of cells that help them attach to, and communicate with nearby cells, and play an important role in cell growth, cell movement, and other cell functions. Integrins are comprised of an α subunit and a β subunit and 24 αβ combinations have been identified. Integrins that recognize ligands with an RGD sequence, called RGD-binding integrins, play key roles in the 4 hallmarks of DR: vascular leakage, neovascularization, inflammation, and fibrosis (Figure 1). Expression of high levels of the integrin $\alpha_v\beta_3$ and $\alpha_v\beta_5$ in human retinal membranes from patients with proliferative DR have been reported.

Figure 1 RGD-Binding Integrins Involved in the Pathogenesis of Diabetic Retinopathy



RGD = arginine-glycine-aspartic acid.

Source: Van Hove I, Hu TT, Beets K, et al. Targeting RGD-binding integrins as an integrative therapy for diabetic retinopathy and neovascular age-related macular degeneration. *Prog Retin Eye Res.* 2021;85:100966.

Small molecule inhibitors (antagonists) of RGD-binding integrins have shown efficacy in animal models of retinal neovascularization when administered by subcutaneous injection, intraperitoneal injection, periocular injection, or topically. A reported RGD-binding integrin inhibitor, risuteganib (Allegro Ophthalmics), that is delivered by intravitreal injection (IVT) has completed a Phase 2b study for the treatment of DME (ClinicalTrials.gov: NCT02348918). This integrin inhibitor has been studied in more than 200 participants and has shown efficacy similar to anti-VEGF (vascular endothelial growth factor) with a reduced number of injections. Another small molecule RGD-binding integrin inhibitor, THR-687 (Oxurion NV), administered by IVT, has recently been studied in a single-dose-escalation Phase 1 trial. The Phase 2 INTEGRAL trial data showed THR-687 to be safe and well tolerated with no serious adverse events (SAEs) and none of the patients required rescue medication through Month 3, however, there was insufficient evidence of efficacy on the key endpoints (best corrected visual acuity [BCVA] and central subfield thickness). As a result, Oxurion NV has decided not to continue the clinical development of THR-687. No topically administered RGD-binding integrin inhibitor, other than OTT166, has advanced into clinical testing to date.

OTT166 is a potent and selective small molecule RGD-binding integrin inhibitor with a balance of physiochemical properties to allow it to distribute to the retina in high concentrations after topical administration. The company discovered OTT166 by applying in-depth knowledge of the impact of incorporating fluorine substituents into small molecules drugs to alter their chemical and pharmacological properties. MK-0429, the predicate molecule to OTT166, was tested in clinical trials for the treatment of osteoporosis in high oral doses and was found to be safe and well tolerated but was not further developed for commercial reasons. OcuTerra demonstrated that while MK-0429 does not distribute to the back of the eye after topical administration, addition of fluorine substituents in specific positions on MK-0429 resulted in OTT166 with high potency and selectivity for

RGD-binding integrins and OTT166 does distribute to the back of the eye after administration as an eye drop.

2.5 Previous Clinical Studies

OTT166 topical ophthalmic solution has been studied in 2 Phase 1b clinical trials, 1 in participants with center-involving (CI) DME and the other in participants with nAMD. Each study enrolled 44 participants who were treated with OTT166 eye drops twice daily (BID) for 28 days and then followed for an additional 28 days after concluding treatment. There were 2 dose groups in each study: 2.5% (1.25 mg/eye) BID and 5% (2.5 mg/eye) BID; participants were randomized 1:1 to the 2 groups. The primary objective of each study was to determine safety and tolerability of the study treatments. The safety evaluations included adverse event (AE) reporting, intraocular pressure (IOP) and BCVA measurements, slit-lamp and fundus examinations, and fluorescein angiography (FA). Exploratory efficacy (ie, evidence of biological activity) was assessed by change from baseline (BL) in retinal thickness parameters measured by spectral-domain optical coherence tomography and change from BL in BCVA.

2.5.1 OTT166 in DME

The results of the first study indicate that both doses of OTT166 (1.25 and 2.5 mg/eye BID) are safe and well tolerated in adult participants with CI-DME. Six participants, 5 (22.7%) in the OTT166 1.25 mg/eye group and 1 (4.5%) in the OTT166 2.5 mg/eye group, had a serious TEAE, but all were non-ocular in nature and unrelated to study drug. Six participants experienced ocular TEAEs in the study eye, 2 (9.1%) in the OTT166 1.25 mg/eye group and 4 (18.2%) in the OTT166 2.5 mg/eye group. All the ocular TEAEs in the study eye were mild in intensity. One event, ocular hyperemia, was considered possibly drug related.

One participant (OTT166 1.25 mg/eye) was discontinued from study drug due to an AE (hypersensitivity [verbatim: whole body allergic reaction/itching]). Given the onset and resolution pattern of the event, the investigator reported the event as probably related to study drug.

Mean changes from BL in IOP were nominal in both treatment groups throughout the study. There were no TEAEs of ocular hypertension or increased IOP. None of the participants had a clinically relevant change from BL in the study eye in the slit-lamp, dilated fundoscopy, or FA results.

Four participants received rescue treatment during the 28-day follow-up period, none during the treatment phase. None of the 4 participants rescued during the recovery phase met the rescue criteria.

There were insignificant changes in the exploratory efficacy measures, change in central retinal thickness from BL, and change in BCVA from BL, on a group mean basis; however, there was a subset of participants that showed significant improvement by OCT as judged by a panel of 3 retina specialists who reviewed all the OCT scans. Applying their expert assessment to the review of the scans of every participant, they determined there were

14 participants who showed evidence of response (ie, biological activity), constituting 37% of the completed participants in the study.

2.5.2 OTT166 in nAMD

The results of the second study indicate that both doses of OTT166 (1.25 and 2.5 mg/eye BID) are safe and well tolerated in adult participants with nAMD. One participant (OTT166 2.5 mg/eye group) had a serious TEAE; the event was non-ocular in nature (peripheral artery thrombosis) and not considered related to study drug. In terms of ocular events in the study eye, 4 participants, 1 (4.3%) in the OTT166 1.25 mg/eye group and 3 (14.3%) in the OTT166 2.5 mg/eye group, had a total of 1 and 3 ocular TEAEs, respectively. None of the participants had > 1 ocular TEAE in the study eye during the study. All the ocular TEAEs in the study eye were mild or moderate in intensity. One participant (OTT166 2.5 mg/eye group) had an ocular TEAE, dry eye, that was possibly related to study drug.

Two participants (both in the OTT166 2.5 mg/eye group) were discontinued from study drug due to an AE; these events were headache and retinal hemorrhage in the study eye (1 participant each). The retinal hemorrhage in the study eye was considered by the investigator to be unrelated to study drug.

Throughout the study, mean changes from BL in IOP were nominal in each treatment group. Few slit lamp abnormalities were observed in the study eye during the study, regardless of treatment group. Two participants had a worsening in opacity status in both the study and non-study eyes. There were no clinically relevant shifts from normal to abnormal in the fundus examination findings.

Ten participants were rescued by administration of an anti-VEGF drug, 2 during the treatment phase and 8 during the follow-up phase of the study, although the criteria for rescue were not met in any of the cases.

There were insignificant changes in the exploratory efficacy measures, change in central retinal thickness from BL, and change in BCVA from BL, on a group mean basis; however, there was a subset of participants that showed significant improvement by OCT as judged by a panel of 3 retina specialists who reviewed all the OCT scans. The retina specialists determined that there were 8 participants (19.5%) in the PP population who showed evidence of response (ie, biological activity).

Additional details about the clinical data are provided in the IB.

2.6 Study Design

The OTT166 201 Phase 2 will be conducted to select an optimum dosing regimen (frequency) for Phase 3 pivotal trials. Approximately 210 participants diagnosed with moderately severe to severe NPDR or mild PDR and who are treatment naïve (ie, no prior anti-VEGF or laser [focal, grid, PRP] administered) will be randomized 2:2:1:1 into the following groups: OTT166 5% BID, OTT166 5% QID, vehicle control BID, or vehicle control QID. Randomization will be stratified by BL DRSS score (47 or 53 or 61B). Participants with PDR (DRSS score 61B) will be capped at 20% of all randomized

participants. Each group will self-administer one 50-microliter (µL) eye drop of study solution (frequency as assigned) for 24 weeks.

2.7 Study Population

Participants should show typical fundus alterations with moderately severe to severe NPDR or mild PDR (participants with mild PDR will be capped at 20% of all randomized participants), as defined by the DRSS Steps 47, 53, and 61B. Participants may have fluid in the macula with BCVA of ≥ 69 ETDRS letters (Snellen equivalent 20/40) and CST of ≤ 325 µm, and a normal foveal contour, which need to be confirmed by an independent central reading center, in at least 1 eye.

Treatment of DR with OTT166 targets the retina and is being investigated for signs of efficacy in this clinical trial. To be able to examine the retina appropriately at screening and during the study, participants whose eyes have anomalies that interfere with protocol -required examinations are excluded.

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

2.8 Inclusion Criteria

Participants are eligible to be included in the study only if all the following criteria apply:

1. Men or women ≥ 18 years of age with type 1 or 2 diabetes mellitus who have moderately severe to severe NPDR [DRSS levels 47 or 53], or mild PDR [DRSS level 61B] NVE < 0.5 DA in 1 + quadrants], in whom PRP and/or anti-VEGF IVT can be safely deferred for at least 6 months per the Investigator (ETDRS report 1991).
2. BCVA ETDRS letter score in the study eye of ≥ 69 letters (approximate Snellen equivalent of 20/40 or better)
3. Normal foveal contour
4. Treatment naïve (ie, no previous anti-VEGF or steroid treatment or PRP or laser)
5. Willing and able to return for all study visits and comply with study-related procedures
6. Able to adhere to the study dosing requirements
7. Understands and signs the written ICF

2.9 Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

1. CST of > 325 μm
    a. Fluid in the central subfield is allowed so long as CST is ≤325 μm and there is a normal foveal contour as determined by the Central Reading Center
2. Any prior focal or grid laser photocoagulation or any prior PRP in the study eye as it pertains to treatment of DME or DR (peripheral retinal hole treated with laser is allowed)
3. Eyes with DRSS score 61 with fibrous proliferations at disc or fibrous proliferations elsewhere
    a. DRSS score 61B with NVE only is allowed. Any sign of fibrosis proliferation is exclusionary
4. Any prior systemic anti-VEGF treatment or IVT anti-VEGF treatment in the study eye
5. Any prior intraocular steroid injection in the study eye, inclusive of Iluvien® and Retisert®
    a. History of Ozurdex® and triamcinolone use prior to 12 months before study enrollment is allowed
6. Current ASNV, vitreous hemorrhage, or tractional retinal detachment visible at the screening assessments in the study eye
7. Uncontrolled glaucoma or ocular hypertension in the study eye defined as an IOP > 25 mmHg regardless of concomitant treatment with IOP-lowering medications
8. Hypertension defined as systolic > 180 mmHg or > 160 mmHg on 2 consecutive measurements (during the same visit) or diastolic > 100 mmHg
9. Screening HbA1c blood test > 12.0%
10. Renal failure (stage 4 or end-stage), dialysis, or history of renal transplant
11. History of other disease, metabolic dysfunction, physical examination finding, or clinical laboratory finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or render the participant at high risk for treatment complications
12. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to randomization or plans to do so in the next 4 months
13. Epiretinal membrane, posterior hyaloidal traction, and/or vitreomacular traction in the study eye as determined to be significant by the Investigator
14. Previous pars plana vitrectomy in the study eye

15. Any intraocular surgery in the study eye within 90 days (3 months) prior to study enrollment
16. YAG laser treatment in the study eye within 90 days prior to study enrollment
17. Concomitant use of any topical ophthalmic medications in the study eye, including dry eye or glaucoma medications, unless on a stable dose for at least 90 days prior to study enrollment and expected to stay on stable dose throughout study participation. Topical eyedrops are allowed but not within ± 10 minutes of study drop application
18. Contact lens use from time of screening throughout the study
19. Central corneal changes from dry eye that are visually significant and/or Sjogren's syndrome
20. Visually significant Fuchs endothelial dystrophy or other diagnosed conditions of corneal compromise including Anterior Basement Membrane Dystrophy, or any corneal dystrophy affecting central vision (peripheral processes are not exclusionary)
21. Chronic or recurrent uveitis in the study eye
22. Ongoing ocular infection or inflammation in either eye
23. A history of cataract surgery complicated by vitreous loss in the study eye
24. Congenital eye malformations in the study eye
25. A history of penetrating ocular trauma in the study eye
26. Cognitive impairment that, in the opinion of the investigator, could compromise compliance with the requirements of the study
27. Females of childbearing potential (ie, who are not postmenopausal for at least 1 year or surgically sterile for at least 6 weeks prior to Visit 1 – Screening/Randomization) who are lactating, or who are pregnant as determined by a positive serum pregnancy test at Visit 1 – Screening/Randomization. Women of childbearing potential must agree to use acceptable methods of birth control throughout the study
    a. Women who are breastfeeding or who have a positive serum hCG/urine pregnancy test at the screening or BL Visit
28. Females and males of childbearing potential unwilling or unable to utilize the following acceptable methods of birth control: tubal ligation, transdermal patch, intrauterine devices/systems, oral/implantable/injectable or contraceptives, diaphragm or cervical cap with spermicide, or vasectomized partner for females; condoms with spermicidal agent and vasectomy for males; or sexual abstinence for males and females
29. Participation in any other investigational device or drug clinical research study within 12 weeks of Visit 1 – Screening/Randomization and during the duration of enrollment
30. Contraindication to the study medications or fluorescein dye
31. Other ocular pathologies that, in the investigator's opinion, would interfere with the participant's vision in the study eye

32. Ocular media of insufficient quality to obtain fundus photographs, fluorescein angiography, and OCT images in the study eye
33. Concomitant use of Semaglutide (Wegovy®, Ozempic®, Rybelsus®), Thiazolidinediones (Actos®, Avandia®), Liraglutides (Victoza®, Saxenda®), Dulaglutide (Trulicity®), or Tirzepatide (Mounjaro®) within 12 months prior to Visit 1 (allowed if a stable dose has been established for at least 1 year of use)
    a. Plans to start concomitant use of Semaglutide, Thiazolidinediones, Liraglutides, Dulaglutides, or Tirzepatides during the study duration is exclusionary

2.10 Lifestyle Considerations

No study specific lifestyle restrictions are required. Participation in this study does not require modifications of lifestyle. Participants should follow their usual lifestyle, as part of their diabetes management plan.

2.11 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently assigned to study intervention. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, and eligibility criteria.

Individuals who do not meet the criteria for participation in this study (screen failure) may only be rescreened once. In any case, the Investigator must ensure that the repeated screening procedures do not expose the participant to an unjustifiable health risk. For re-screening, the participant must re-sign the ICF, even if it was not changed after the participant's previous screening.

Rescreened participants will be assigned a new participant number.

In case of abnormal or implausible results, which may be caused by intercurrent diseases, short-term treatable conditions, other temporary health disorders (eg, acute infection, laboratory changes, blood pressure outside defined range), or inappropriate circumstances (eg, inadequate rest when required, hemolysis), the investigator may decide to repeat the respective screening parameter(s) twice without rescreening (within 21 days).

2.12 Criteria for Temporarily Delaying Randomization / Study Intervention Administration

If a participant is otherwise eligible, the participant’s study intervention may be postponed to allow acute intercurrent conditions to resolve. Under these circumstances, the screening period may be extended to 6 weeks upon discussion with the medical monitor.

2.13 Selection of the Study Eye

Only one eye will be designated as the study eye in each participant. For participants who meet eligibility criteria in both eyes during the screening phase, the eye with the higher reading center confirmed DRSS should be selected. If both eyes have the same score, the eye with the clearest lens and ocular media will be selected. If there is no objective basis for selecting the study eye, factors such as ocular dominance (better focus ability), other ocular pathology, and participant preference should be considered in making the selection. The final selection will be performed and documented by the investigator in discussion with the participant at BL and must not be changed during the study.

The non-study eye is eligible for treatment according to the standard of care treatments through the duration of the study.

2.14 Study Intervention(s) and Concomitant Therapy

Study intervention is defined as any investigational intervention(s), marketed product(s), vehicle control, or medical device(s) intended to be administered to a study participant, according to the study protocol.

2.14.1 Study Intervention

In this study, 5% OTT166 ophthalmic solution or vehicle control, will be given to the participants who will self-administer one 50-µL eye drop of study solution at the prescribed frequency based on randomization, for 24 weeks. Approximately 210 participants diagnosed with moderately severe to severe NPDR or mild PDR (participants with mild PDR will be capped at 20% of all randomized participants) and who are treatment naïve (ie, no prior anti-VEGF or laser [focal, grid, PRP] administered) will be randomized 2:2:1:1 into the following groups: OTT166 5% BID (5.0 mg/day),OTT166 5% QID (10 mg/day), vehicle control BID, or vehicle control QID. Randomization will be stratified by baseline DRSS score (47 or 53 or 61B).

Eye dropper bottles containing active treatment of OTT166, and corresponding vehicle control, are identical in appearance (size, color, shape) and associated with a unique number.

Eye dropper bottles will be dispensed at the study visits summarized in the SoA (Section 2.15.1). Returned eye dropper bottles should not be re-dispensed to the participants.

2.14.2 Preparation / Handling / Storage / Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all eye dropper bottles received and any discrepancies are reported and resolved before use of the eye dropper bottle.

Only participants randomized in the study may receive eye dropper bottles, and only authorized site staff may supply the eye dropper bottles. All eye dropper bottles must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in

accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator or the head of the institution (where applicable) is responsible for eye dropper bottle accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records). Drug receipt, reconciliation, and destruction information on the study sites will be captured in the interactive web response system (IWRS).

Further guidance and information for the final disposition of unused eye dropper bottles are provided in the Investigator Site File.

2.14.3 Measures to Minimize Bias: Randomization and Masking

All participants will be centrally assigned to randomized study intervention using IWRS.

Participants will be randomized 2:2:1:1 into the following groups: OTT166 5% twice daily (BID), OTT166 5% four times daily (QID), vehicle control BID, vehicle control QID. Randomization will be stratified by BL DRSS score (47 or 53 or 61B). Participants with PDR (DRSS score 61B) will be capped at 20% of all randomized participants.

The participant and the clinical assessor will be masked to the treatment group / dose.

The IWRS will be programmed with instructions on how to break/unmask a participant's randomized treatment. In case of an emergency, the investigator has the responsibility for determining if unmasking of a participant's intervention assignment is warranted. If the investigator is unavailable, and a treating physician not associated with the study requests emergency unmasking, the emergency unmasking requests are forwarded to the emergency medical advice 24-hour/7-day service. Participant safety must always be the first consideration in making such a determination. If the investigator decides that unmasking is warranted, the investigator should make every effort to contact the medical monitor prior to unmasking a participant's intervention assignment, unless this could delay emergency treatment of the participant. If a participant's intervention assignment is unmasked, the sponsor must be notified within 24 hours after the unmasking. Date and reason for the unmasking must be recorded in the source documentation and case report form, as applicable.

2.14.4 Study Intervention Compliance and Adherence

The clinical investigation uses the Medication Event Monitoring System (MEMS®, AARDEX Group, Belgium) during the complete study follow-up period to automatically compile dosing history data in participants remotely. Study medication will be provided to the participants in dropper bottles which should be placed in the MEMS® Cap and bottle system when in use for the MEMS Cap ® to electronically record the date and time of each opening of the medication container. Site personnel will dispense at the baseline visit and then read the MEMS® Caps at each clinic visit using the MEMS Adherence Software (MEMS AS®). The purpose of using the MEMS® is to monitor the participant's medication intake behavior and related adherence metrics longitudinally in the participant's home

environment during the clinical investigation. Adherence is calculated in the MEMS AS® dashboard and is a feedback tool for sites to intervene when participants deviate from the dosing regimen. When a participant deviates from the dosing regimen (i.e., overdoses, underdoses, missed data transfer), the dashboard displays suboptimal adherence or absence of data transfer, and an email is sent to notify the applicable site to intervene. This intervention should be properly documented in the patient dashboard to indicate what type of intervention was needed (e.g., retraining on dosing adherence, retraining on MEMS ® Cap use, retraining on MEMS ® app, etc.). Adherence to study medication should be monitored continuously by sites between visits to ensure participants are taking the correct number of prescribed doses.

Compliance is calculated per protocol and is the number of doses taken divided by the number of expected doses. Compliance will be calculated for the final analysis and will be determined monthly and overall, through W24. Non-compliance is defined as a dosing record of < 75% of the expected number of dosing events, based on the participants' dosing schedule prescribed at randomization.

A record of the quantity of bottles dispensed to and administered by each participant must be maintained and reconciled with study intervention and compliance records. Intervention start and stop dates, including dates for intervention delays and/or missed doses, will also be recorded.

IWRS will be used for drug accountability on a participant basis. Drug returns, reconciliation, and destruction information will be captured in the IWRS.

Taken together, these measures are deemed appropriate to support participants' adherence and to detect participants who are systematically non-adherent in the opinion of the investigator.

2.14.5 Dose Modification

The doses to be administered are 5% OTT166 (or vehicle control) either BID or QID.

No dose modification is planned.

2.14.6 Stopping Rules (Study Level)

The following criteria result in an immediate stop of dosing and will result in a temporary halt of the study:

Any relevant information, from this trial or from outside of this trial, indicating a relevant deterioration of the benefit-risk ratio. In particular, consistently observed serious adverse events (SAEs) or severe drug-related AEs will be thoroughly assessed by the medical monitor and Study Steering Committee.

Resuming the trial after a temporary halt requires an approved substantial amendment. For discontinuation of study intervention for individual study participants, see Section 2.14.10.

2.14.7 Continued Access to Study Intervention After the End of the Study

No treatment with study medication will be provided after the end of the planned 24-week treatment period or after early withdrawal of treatment.

2.14.8 Concomitant and Prohibited Therapy in the Study Eye

No other ocular topical drugs (eye drops) should be taken during the study unless prescribed by the treating study physician and after discussion with the sponsor and medical monitor. If topical therapy (eye drops) is prescribed, the application of such therapy should not occur within 10 minutes (before or after) of instillation of study therapy. Other approved or investigational therapies for retinal disease (ie, oral, topical, and/or intravitreal injected drugs like steroids or anti-VEGFs) are not permitted, unless applied as rescue therapy (Section 2.14.9). Such intake is to be recorded (see below). Contact lens wear is strictly prohibited from the time of screening throughout the study. If participant has current contact lens use at screening, contact lens use should be discontinued immediately, and a 14 day ocular surface stabilization period is needed between screening and baseline.

Concomitant medications are to be recorded in the eCRF.

The medical monitor should be contacted if there are any questions regarding concomitant or prior therapy.

2.14.9 Rescue Therapy

Participants meeting any of the following criteria would qualify for rescue with anti-VEGF therapy and/or PRP laser after consultation with the medical monitor:

Objective Rescue Therapy Criteria:

- Worsening DRSS level to 65 or higher as confirmed by the central reading center
- Development of ASNV
- Decrease in BCVA of ≥ 10 ETDRS letters from BL associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline at any visit
- Decrease in BCVA of 5 to 9 ETDRS letters from BL at two consecutive visits 4 weeks apart associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline
- Increase in CST of ≥ 75 microns from BL associated with presence of new fluid or an increase in fluid in the central subfield compared to baseline

Subjective Rescue Criteria:

- Any other condition that, in the clinical opinion of the investigator, warrants rescue therapy after consultation with the medical monitor

2.14.10 Discontinuation of Study Intervention and Participant Discontinuation / Withdrawal

2.14.10.1 Permanent Discontinuation

In rare instances, it may be necessary for a participant to permanently discontinue (definitive discontinuation) study intervention. If study intervention is permanently discontinued, the participant may remain in the study for continued assessment of safety and efficacy outcomes.

Unscheduled visits may be performed as deemed appropriate by the investigator, in particular, in case of withdrawal due to an AE.

Participants *must* be withdrawn from the *study intervention* for any of the following reasons:

- If rescue therapy has been initiated
- Safety concerns for a participant by the investigator or the sponsor
- Vision threatening complication meeting rescue criteria and/or progression to worse than mild PDR with DRSS > 61
- Pregnancy (see Section 2.17.4 for details)

Necessity to withdraw the participant from the study will be assessed case-by-case by the investigator in consultation with the medical monitor.

2.14.10.2 Temporary Discontinuation

Treatment should be withheld temporarily if any of the following conditions apply:

- Occurrence of hypersensitivity after instillation of the eye drops
- Rechallenge can be considered at the discretion of the investigator in consultation with the medical monitor

The investigator may decide to temporarily discontinue treatment for other reasons if deemed appropriate for the safety and well-being of the participant. The medical monitor should be informed.

2.14.11 Participant Discontinuation / Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the investigator for safety, behavioral, or compliance reasons. This includes cases in which, in the investigator's opinion, continued participation in the study would be harmful to the participant's well-being. This is expected to be uncommon.

- At the time of discontinuing from the study, if possible, the final study visit should be conducted as early discontinuation visit, followed by safety follow-up as determined by the investigator in keeping with standard of care. See SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.
- The participant will be permanently discontinued both from the study intervention and from the study at that time.
- If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.

2.14.12 Lost to Follow-up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible, counsel the participant on the importance of maintaining the assigned visit schedule, and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study.

Discontinuation of specific sites or of the study as a whole are handled separately.

2.15 Study Assessments and Procedures

Study procedures and their timing are summarized in the SoA (Table 1). Protocol waivers or exemptions are not allowed.

Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study intervention.

Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.

All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or BL purposes, provided the procedures met the protocol-specified criteria and were performed within the time frame defined in the SoA.

2.15.1 Schedule of Activities

Table 1 OTT166 201 Phase 2 DR Study: Schedule of Activities (Through Week 24 – Primary Endpoint)

Study Procedure	**Screening Visit 1**	**Baseline (BL) Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8[a]**
Week	**--**	**0**	**4**	**8**	**12**	**16**	**20**	**24**
Day (visit window)	**-21 to -1**	**1**	**29 ± 7 days**	**57 ± 7 days**	**85 ± 7 days**	**113 ± 7 days**	**141 ± 7 days**	**169 ± 7 days**
Screening/Baseline (BL):								
Informed consent	X							
Inclusion/exclusion	X	X						
Medical and ophthalmic history	X							
Demographics	X							
Randomization		X						
Interval Medical Review:								
Review of concomitant medications	X	X	X	X	X	X	X	X
Continue study treatment[b]		X	X	X	X	X	X	
Ocular Assessments[n]:								
Refraction and BCVA (ETDRS)	OU	OU	OU	OU	OU	OU	OU	OU
Slit lamp examination	OU	OU	OU	OU	OU	OU	OU	OU
Assessment of cornea[c]	OU	OU	OU	OU	OU	OU	OU	OU
Intraocular pressure[d]	OU	OU	OU	OU	OU	OU	OU	OU
Indirect ophthalmoscopy	OU	OU	OU	OU	OU	OU	OU	OU
4-wide or 7-field color fundus photography (mandatory)[e]	OU	OU	SE	SE	OU	SE	SE	OU
SD-OCT (widefield if available)	OU	OU	SE	SE	OU	SE	SE	OU
OCTA (if available)	OU							OU
Wide field Fluorescein Angiography[m]	OU							OU
Gonioscopy	OU							OU

Study Procedure	**Screening Visit 1**	**Baseline (BL) Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8[a]**
Week	**--**	**0**	**4**	**8**	**12**	**16**	**20**	**24**
Day (visit window)	**-21 to -1**	**1**	**29 ± 7 days**	**57 ± 7 days**	**85 ± 7 days**	**113 ± 7 days**	**141 ± 7 days**	**169 ± 7 days**
Non-Ocular Assessments:								
Physical examination[f]	X							
Vital signs[g]	X	X	X	X	X	X	X	X
Adverse events[h]	X	X	X	X	X	X	X	X
IP adherence review[o]			X	X	X	X	X	X
Laboratory Testing:[i]								
Hematology, blood chemistry, urinalysis, and HbA1c[j,l]	X							X
Pregnancy test, women of childbearing potential[k]	serum	urine	urine	urine	urine	urine	urine	urine

Abbreviations: BCVA: best corrected visual acuity; BL: baseline; ETDRS: Early Treatment Diabetic Retinopathy Study; HbA1c: glycosylated hemoglobin; OCTA: optical coherence tomography – angiography; OU: both eyes; SD-OCT: spectral-domain optical coherence tomography; SE: study eye.

[a] Participants who are withdrawn from the study before Week 24/Visit 8 will be asked to return to the clinic to complete the end of study assessments.

[b] Participants will continue to receive study treatment based on exam findings, AE review, and verification that rescue criteria are not met.

[c] Cornea health assessment following instillation of fluorescein stain, including epithelium and endothelium, for signs of toxicity.

[d] Intraocular pressure should be measured at approximately the same time of day from visit to visit, if possible.

[e] An FP and FA must be performed once a participant has been diagnosed with worse than mild PDR, ASNV, or new onset or worsening CI-DME in the study eye, and before rescue treatment is given.

[f] May be performed at Visit 1 or Visit 2, Including height, weight, and body temperature.

[g] Vital signs (blood pressure and heart rate) will be measured after the participant has been sitting for 5 minutes at the screening visit. From baseline through end of study only blood pressure and heart rate will be measured.

[h] AEs will be collected from the time the ICF is signed until early termination or the end of study visit. If a participant withdraws from the study, ongoing AEs will be followed to the end of study visit or until the participant withdraws consent.

[i] All samples collected for laboratory assessments will be obtained prior to administration of study drug.

[j] At visits at which FA is performed, urinalysis samples will be collected before FA in order to avoid false elevations in urine protein values.

[k] For women of childbearing potential, a negative screening serum pregnancy test at Visit 1 is required before randomization. All women of childbearing potential will have a urine pregnancy test at each treatment visit starting at Visit 2 (Day 1); a negative urine pregnancy test is required before treatment is continued (randomized treatment, rescue treatment).

[l] Refer to Appendix 3 for clinical laboratory test details.

[m] Screening Fluorescein angiography is valid for 3 months. Participants undergoing rescreen do not need IVFA repeated within 3 months of original angiogram.

[n] Cornea should be assessed at the beginning before dilation.

[o] Adherence to the dosing regimen should be monitored continuously and discussed with the participant at each visit. Sites are expected to intervene if there is suboptimal adherence or absence of data transfer and document the intervention in the patient dashboard.

2.15.2 Study Activities

After signing an informed consent, participants will be invited to a screening visit, Visit 1, to evaluate their eligibility for this study. Eligible participants with DR who meet all the inclusion criteria and none of the exclusion criteria will be randomized to treatment with one of two dosing frequencies (BID/QID) of 5% OTT166 or vehicle control. During the treatment period, participants will be monitored for AEs and concomitant medication, and undergo the assessments outlined in the SoA (Section 2.15.1).

The treatment period comprises Visits 2 to 8 (BL/Day 1 to End of Treatment/Study/Week 24), at which the primary endpoint will be assessed.

2.15.3 Blood Sampling

Blood will be taken at Visits 1 and 8 for laboratory assessments.

- The amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will be approximately 30 mL.
- Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

2.16 Efficacy Assessments

Planned time points for all efficacy assessments are provided in the SoA (Section 2.15.1).

2.16.1 Ophthalmology Assessments

Ophthalmology examinations will be done for the study eye and/or both eyes as described in the SoA (Section 2.15.1).

2.16.2 Color Fundus Photography (CFP)

The anatomical state of the retinal vasculature will be evaluated by an ophthalmologist from posterior segment examination and CFP. All digital CFP images need to be taken by a certified technician following an acquisition protocol for modified 7-field stereoscopic color fundus photographs or 4-wide field imaging of the central reading center at the visits indicated in the SoA (Section 2.15.1).

All CFPs will be transmitted to the central reading center for ETDRS DRSS (see Appendix 2) grading and storage. The images from the screening visit of both eyes will be used as part of the eligibility assessment regarding the severity of diabetic retinopathy.

CFPs may, in addition, be evaluated for additional, exploratory, endpoints. This may include assessment of microaneurysm turnover (Nunes 2009) or measurement of diameters of retinal vessels as described previously (Lundberg 2013). This may involve transmission of CFP to additional central reading centers.

2.16.3 Best Corrected Visual Acuity (BCVA)

Visual function will be assessed using a modified ETDRS protocol starting at 4 meters (AREDS 1999). Visual Acuity examiners must be qualified to ensure consistent measurement of BCVA and will be masked to study treatment assignment.

2.16.4 Fluorescein Angiography (FA)

The anatomical state of the retinal vasculature of the study eye and the fellow eye (only at BL and end of study [EOS]) will be evaluated by FA (widefield preferred). All FAs will be conducted by a qualified technician following an acquisition protocol of the central reading center. The angiographic images will be evaluated by a study ophthalmologist for individual safety decisions. All FA images will be archived electronically at the site as part of the source documentation and sent to the central reading center for evaluation and storage on visits stated in the SoA (Section 2.15.1).

At screening, a standardized set of FA images will be sent to the central reading center for evaluation and eligibility assessment.

2.16.5 Optical Coherence Tomography (OCT)

Structural OCT (widefield preferred) will be performed by using spectral domain devices. The specific device models accepted for the study will be defined by the central reading center. OCTs will be performed at every visit by qualified technicians following an acquisition protocol of the central reading center. OCTs will also be used for local safety assessment. Newly found clinically significant pathologies should be reported as AEs. All obtained images will be sent to the central reading center for evaluation, and central reading center data will be used for the final analysis.

At screening, a standardized set of OCT images will be sent to the central reading center for evaluation and eligibility assessment. Beside central subfield thickness within the central 1 mm (CST) and whole macular volume also the presence of center involving and non-center involving macular edema will be assessed. SD-OCT will be also assessed for foveal contour and several exploratory outcome parameters and morphologic predictive biomarkers.

2.16.6 Optical Coherence Tomography Angiography (OCTA)

OCTA is optional at sites that have an OCTA. The OCTA needs to be performed by a qualified technician following an acquisition protocol of the central reading center at Visits 1 and 8, as scheduled in the SoA (Section 2.15.1). The specifications of this OCTA can be found in the acquisition protocol of the central reading center. Images obtained via OCTA will be sent to the Central Reading Center for evaluation. Several quantitative and qualitative parameters, including the FAZ and area of nonperfusion, which have been shown to be associated with DR severity, will be assessed.

2.16.7 Slit Lamp Biomicroscopy (Anterior and Posterior Segment)

The slit lamp examination (anterior and posterior segment) will be performed according to local medical practice and applicable medical standards at the site at every visit, as stated in the SoA (Section 2.15.1). Abnormal findings are to be recorded in the eCRF as either medical history or AE, as applicable.

2.16.8 Tonometry (Intraocular Pressure (IOP) Measurement)

IOP is to be measured at every visit with any locally approved non-contact tonometer.

If there is no non-contact tonometer available at the site, applanation tonometry (Goldmann, Tonopen, or other locally approved alternatives) may be used. In any case, where both methods are available, the non-contact method is to be used. In cases in which Goldmann applanation tonometry (GAT) is used, assessment of corneal health using fluorescein stain should be performed prior to tonometer applanation.

The same method of IOP measurement must be used throughout the study for each individual participant and should be performed at approximately the same time of day, if possible. Values, including time of day and measuring type, are to be recorded in the eCRF.

2.16.9 Vision-threatening Complications

VTC is defined as the composite outcome of PDR (inclusive of participants who have vitreous hemorrhage or tractional retinal detachment believed to be due to PDR) and/or ASNV identified by Investigators (participants with neovascularization of the iris [at least 2 cumulative clock hours], and/or definitive neovascularization of the iridocorneal angle) and/or CI-DME as defined in Section 2.16.10 below.

VTCs are defined as occurrence of any of the following AEs:

- More than mild PDR (DRSS 65 and above)
- Iris neo-vascularization
- CI-DME

2.16.10 DME and CI-DME

DME is defined as fluid in the macula.

CI-DME is defined as the presence of fluid in the central subfield in participants who have no fluid at baseline or CST > 325 µm.

The assessment will be continued after treatment discontinuation until end of study.

2.17 Safety Assessments

Safety will be assessed, and TEAEs recorded (as indicated) at every visit.

2.17.1 Physical Examination

A physical examination (by means of inspection, palpation, auscultation) will be performed at Screening (Visit 1) as described in the SoA (Section 2.15.1) by a physician at the study site and will cover at least the organs of the cardiovascular, respiratory, and abdominal systems; measuring body temperature will be included. Abnormal physical examination findings are recorded either as medical history or as AEs in the eCRF. Body weight will be measured by a member of the investigator's team. The body mass index (BMI) will be calculated by Data Management or the eCRF based on weight and height.

2.17.2 Vital Signs

Pulse rate and systolic / diastolic blood pressure will be measured repeatedly as outlined in the SoA (Section 2.15.1). Blood pressure and heart rate will be measured in a supine or semi supine position after ≥ 5 minutes rest. Blood pressure and heart rate should always be measured before blood draws when scheduled at the same time point.

2.17.3 Clinical Safety Laboratory Assessments

A lab assessment (hematology, blood chemistry, urinalysis, and HbA1c) will be performed at Screening (Visit 1) and at Week 24 (Visit 8) as described in the SoA (Section 2.15.1). Blood and urine samples will be taken. See Section 3.3 for a table of clinical laboratory tests to be performed.

The investigator must review the laboratory report, document this review, and record any clinically significant changes, including any lab test that was in the age-adjusted normal range and became abnormal at EOS, occurring during the study as an AE. The laboratory reports must be filed with the source documents.

Abnormal laboratory findings associated with the underlying disease are not considered clinically significant unless judged by the investigator to be more severe than expected for the participant's condition.

All laboratory tests with values considered clinically significantly abnormal during participation in the study or within one month after the last dose of study intervention should be repeated until the values return to normal or BL or are no longer considered clinically significant by the investigator or the medical monitor.

If such values do not return to normal/BL within a period of time judged reasonable by the investigator, the etiology should be identified, and the sponsor notified.

All protocol-required laboratory tests must be conducted in accordance with the laboratory manual and the SoA (Section 2.15.1). If laboratory values from non-protocol specified laboratory tests performed at the institution's local laboratory require a change in participant

management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded.

2.17.4 Pregnancy Testing

Both urine and serum ß-hCG pregnancy tests are planned to be conducted in woman of childbearing potential enrolled in this clinical study. A serum test is planned at Screening (Visit 1); during all other visits, urine tests will be performed. Dip stick urine pregnancy tests allow the investigator to take action while the participant is at site in case of a positive test.

If the urine pregnancy test is positive, the study medication should be discontinued immediately. The urine pregnancy test results should be however confirmed using a serum pregnancy test at a local lab before permanently discontinuing the participant from the study.

2.18 Adverse Events (AEs), Serious Adverse Events (SAEs), and Other Safety Reporting

The definitions of AEs and SAEs can be found in Appendix 3 and will conform to the current Medical Dictionary for Regulatory Activities (MedDRA) standard.

AEs will be reported by the participant (or, when appropriate, by a caregiver, surrogate, or the participant's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up all AEs reported during the study until resolution or until the end of the study.

2.18.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs/SAEs will be collected from the signing of the ICF until end of study.

All SAEs will be recorded and reported to the sponsor or designee immediately and under no circumstance should this exceed 24 hours of learning of the event, as indicated in Section 2.21.5. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.

TEAEs will be defined as those AEs that occurred after first dosing and those existing pre-dose AEs that worsened in severity post-dose during the main treatment period until the end of study.

Investigators are not obligated to actively seek information on AEs or SAEs after conclusion of the study participation/the End-of-Study visit. However, if the investigator learns of any AE/SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study intervention or study participation, the investigator must promptly notify the sponsor.

2.18.2 Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences.

2.18.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs, and AEs of special interest (as defined in Section 2.18.6), will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up (as defined in Section 2.14.12). Further information on follow-up procedures is provided in Appendix 4.

2.18.4 Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of an SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Board (IRB)/ Independent Ethics Committee (IEC), and Investigators.
- An investigator who receives an investigator safety report describing an SAE or other specific safety information (eg, summary or listing of SAEs) from the sponsor will review and then file it and will notify the IRB/IEC, if appropriate according to local requirements.
- For all studies, investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.

2.18.5 Pregnancy

- Details of all pregnancies in female participants and, female partners of male participants will be collected after the start of study intervention and until the end-of study visit.
- If a pregnancy is reported, the investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours after obtaining the necessary signed informed consents from the pregnant participant. Study medication should be discontinued immediately upon receipt of a positive pregnancy test.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs, and will be reported as such.
- The participant /pregnant female partner will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant/pregnant female partner and the neonate, and the information will be forwarded to the sponsor.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention.

2.18.6 Adverse Events of Special Interest (AESI)

AESI must be reported to the Sponsor along the timelines set for SAEs (even though they may not be classified as serious), ie, within 24 hours of the investigator´s awareness, as described in Appendix 4.

AESI are:

- Hypersensitivity reactions. Symptoms of hypersensitivity reactions (allergic reactions) include:
  - Rash
  - Wheezing and difficulty breathing
  - Dizziness and fainting
  - Swelling around the mouth, throat or eyes
  - A fast pulse
  - Sweating

2.19 Pharmacokinetics

In this study, no PK analysis will be done.

2.20 Statistical Considerations

2.20.1 Statistical Hypotheses / Sample Size Determination

This study is designed to evaluate the superiority of two doses of OTT166 (5 mg/day and 10 mg/day) compared to vehicle control for the treatment of DR. For each dose within each endpoint, the null hypothesis is that there is no difference between OTT166 and vehicle control or the difference is in the direction of inferiority of OTT166, and the alternative hypothesis is that there is a difference between OTT166 and vehicle control in the direction of superiority of OTT166. The vehicle control groups will be combined for all statistical analyses. No multiplicity adjustment is planned for this study.

The study will enroll approximately 210 participants randomized as described in Section 2.14.3, 70 participants per group into each treatment arm and 35 participants per group into each matching vehicle control group and allowing for around 5% study attrition, aiming for at least 66 participants per treatment group and combined vehicle control group.

For the primary endpoint of improvement by at least 2 steps from BL on the DRSS at Week 24, completing 66 participants per group (active treatment BID, active treatment QID, combined vehicle control) through Week 24 will yield at least 90% power to demonstrate that the active treatment is superior to vehicle control using a 1-sided alpha of 0.05, provided that the true treatment effect (active treatment efficacy rate minus vehicle control efficacy rate) is at least 0.20 and the vehicle control efficacy rate is at most 0.06.

The sample size calculation was computed in nQuery 8.7 using a Chi square test for two proportions (continuity corrected).

2.20.2 Outcome Measures

The primary safety outcome measure of the study will be the proportion of participants who develop TEAEs through Week 24.

The primary efficacy outcome measure of the study will be the proportion of participants who have improved by ≥ 2 steps from baseline at Week 24 as determined by central reading center assessment using DRSS.

The secondary outcome measures are as follows:

- Proportion of participants developing worse than mild PDR (DRSS 65 and above) at Week 24
- Proportion of participants who develop ASNV at Week 24
- Time to development of PDR worse than mild (DRSS 65 and above)
- Proportion of participants who develop CI-DME at Week 24
- Time to development of CI-DME

- Proportion of participants developing VTC at Week 24
- Time to development of PDR worse than mild (DRSS 65 and above) or CI-DME
- Proportion of participants with change in DRSS steps at Week 24 compared to baseline
  - Change in DRSS steps is defined as DR worsening or improving by 1, 2, or ≥ 3 steps
- Proportion of participants with mild PDR (DRSS score 61B) at baseline who regress to NPDR (DRSS score ≤ 53) by Week 24
- Mean and median change in BCVA (ETDRS letters) from baseline to Week 24
- Lines gained/lost in BCVA (± 5, 10, and 15 ETDRS letters) at Week 24
- Mean and median change in AUC for change in BCVA from baseline to Week 24
- Mean and median change in CST from baseline to Week 24
- AUC for change in CST from baseline to 24 weeks
- Proportion of participants who met the objective rescue criteria as defined in Section 2.14.9.
- Time to meet objective rescue therapy criteria as defined in Section 2.14.9.
- Time to administration of rescue therapy.

Exploratory outcomes measures will include:

- Change in Macular Volume (MV) from baseline to Week 24 for 1, 3, and 6 mm regions
  - MV will be defined as the sum of the 9 subfield measurements as provided by the imaging center
- Change in area of retinal non-perfusion from baseline to Week 24 seen on widefield fluorescein angiography (FA)
- Proportion of participants with intact foveal avascular zone (FAZ) seen on widefield FA at Week 24
- Optical coherence tomography angiography (OCTA) endpoints within the 6 mm area:
  - Change from baseline to Week 24 in FAZ area
  - Change from baseline to Week 24 in non-perfusion area
  - Change from baseline to Week 24 in vessel caliber
  - Change from baseline in ischemic Index at Week 24 defined as total area of non-perfusion divided by total area of retina visualized (28.3 mm^2)
  - Proportion of participants with new neovascularization at Week 24

- Proportion of participants with new IRMA at Week 24
- Change in vessel density from baseline to Week 24 in the superficial and deep layers

2.20.3 Analysis Sets

For purposes of analysis, the following analysis sets are defined:

Table 2 Analysis Sets

Analysis Set	Description
Enrolled Set (ES)	All participants who signed informed consent.
Intent-to-Treat (ITT) Analysis Set	All randomized participants who received at least one dose of study drug. Participants will be included into the analysis as randomized. This corresponds to what is referred to in ICH E9 as the Full Analysis Set.
Per-Protocol Set (PPS)	All ITT participants without any major protocol deviations that could impact the primary endpoint.
Safety Analysis Set (SS)	All participants who receive at least one dose of study drug. Participants will be included into the analysis based on treatment actually received, regardless of the treatment randomized.

The ITT Analysis Set will be used for all efficacy and BL analyses except participant disposition which will be shown for the ITT Analysis Set and for the Safety Analysis Set if the two analysis sets differ. All randomized participants are expected to be dosed and any exceptions will be described in the CSR. The PP set will be determined prior to database lock and will be used as supplementary analyses of the primary endpoint. All safety analyses will be based on the Safety Analysis Set (SS).

2.21 Statistical Analyses

2.21.1 General Considerations

Statistical analyses will include tabulations of summary data, inferential analyses, by participant listings, and figures.

All statistical tests will be one-sided and will be performed at the 5% level of significance, unless otherwise stated. The vehicle control group will be combined for all efficacy analyses, and two-sided 90% confidence intervals will be presented. Frequency counts (n and percentages) will be made for each qualitative variable. Descriptive statistics (n, mean, standard deviation [SD], median, minimum, and maximum) will be calculated for each quantitative variable, unless otherwise stated. All data will be summarized by treatment group. All safety summaries and analyses will be based upon the Safety Analysis Set by treatment group (i.e., vehicle control doses (5% BID, 5% QID), vehicle control combined, active OTT166 doses (5% BID, 5% QID), and OTT166 combined).

The BL value for analysis and reporting will be based on the last (non-missing) measurement before dosing on Day 1. If any safety measurements are repeated after dosing on Day 1, then the last (non-missing) value of any repeated measurements will be used in the descriptive statistics and in the calculation of changes from BL.

2.21.2 Efficacy Analyses

2.21.2.1 Analysis of the Primary Endpoint

The primary estimand for the primary endpoint analysis is as follows:

	Primary Estimand
Treatment conditions of interest	OTT166 5 mg/day, OTT166 10 mg/day and matching vehicle control
Participant Population	DR participants with Type 1 or Type 2 diabetes under adequate control (HbA1c ≤12.0%) who show typical fundus alterations and have been diagnosed with moderately severe to severe NPDR or mild PDR as defined by the DRSS Steps 47, 53, and 61B, with BCVA ≥ 69 ETDRS letters, CST of ≤ 325 μm, and a normal foveal contour.
Endpoint	≥ 2 steps improvement from BL in DRSS score at Week 24.
Population level summary	Difference in proportion of participants achieving ≥ 2 steps improvement from BL in DRSS score at Week 24 between each OTT166 treatment group and the combined vehicle control group.
Intercurrent events (ICEs) and strategies to handle ICEs	a. Rescue treatment due to worsening DRSS level to 65 or higher prior to assessment of DRSS at Week 24 *Composite strategy (non-response)* Death prior to DRSS assessment at Week 24 *Hypothetical strategy* Use of rescue treatment for any other reason prior to DRSS assessment at Week 24 *Composite strategy (non-response)* Premature discontinuation of study drug for any reason prior to DRSS assessment at Week 24 *Hypothetical strategy* Study drug compliance < 75% through Week 24 *Treatment policy strategy* b. Study drug compliance between 75% to < 90% through Week 24 *Treatment policy strategy*
	ICE(s) with a composite variable strategy take priority over ICEs with other strategies. And ICEs with a hypothetical strategy take priority over ICEs with treatment policy strategy.

Handling of missing DRSS response data at Week 24 in participants without ICEs:

- multiple imputation assuming missing at random (MAR)

Handling of DRSS response data at Week 24 missing after ICEs:

- single imputation (non-response) when applying composite variable strategy
- multiple imputation assuming MAR when applying hypothetical and treatment policy strategy

Handling of DRSS response data at Week 24 observed after ICEs:

- replaced by non-response (single imputation) in the analysis when applying composite variable strategy
- replaced by multiple imputation algorithm based on data from participants not affected by the respective ICE when applying hypothetical strategy
- kept in the analysis when applying treatment policy strategy

Comparison of the primary endpoint will be made between each dose group and the combined vehicle control group using a Mantel-Haenszel (MH) test of the difference in 2 proportions stratified by the randomization stratification factor (BL DRSS score 47 or 53 or 61B). The estimated MH risk difference will be summarized along with the 2-sided 90% CI using MH stratum weights and the Sato variance estimator. The primary efficacy analysis will be performed on the ITT Analysis Set, while the PP set will be used as supplementary analyses. ICEs and missing data will be handled as described above.

The primary efficacy analysis will also be performed by demographic subgroups including age, gender, and race to assess the consistency of the treatment effect. Additional subgroup analyses may also be conducted and will be described in the Statistical Analysis Plan (SAP).

2.21.2.2 Analyses of Secondary and Other Efficacy Endpoints

Estimands for the secondary endpoint analyses will be described in the SAP.

The secondary endpoints involving proportions of participants will be analyzed using the same approach (MH test) as described for the primary efficacy analysis and based on the ITT.

Time to event secondary endpoints will be analyzed using a stratified log-rank test adjusting for the randomization stratification factor.

Continuous secondary endpoints involving change from BL will be analyzed using an analysis of covariance (ANCOVA) model with BL measurement of the variable as a covariate and treatment group and the randomization stratification factor as fixed factors. The pair-wise comparisons of each OTT166 dose group versus combined vehicle control will be done in the ANCOVA model. Two-sided 90% confidence intervals for the difference of each OTT166 dose group minus combined vehicle control will be calculated. Additional details on

sensitivity analyses and missing data imputation of the secondary endpoints will be provided in the SAP.

Analyses of exploratory imaging outcome measures will be described in the SAP.

2.21.3 Safety Analyses

All safety analyses will be performed on the Safety Analysis Set. No inferential statistics will be performed for safety variables.

2.21.4 Study Drug Exposure

Exposure to study drug will be summarized using the ITT analysis Set and also Safety Analysis Set. The total number of doses administered and the duration of treatment for each participant will be summarized using descriptive statistics by treatment group.

2.21.5 Adverse Events

AEs will be coded using MedDRA version 24.1 or higher and summarized by system organ class (SOC) and preferred term (PT). Analysis and reporting for AEs will be based on TEAEs. A TEAE is defined as an AE occurring (onset date/time) at the time of or after dosing on Day 1. AEs with missing start and/or end dates and/or times (if applicable) will be handled as described in the SAP.

All TEAEs will be listed for each participant. Separate listings will be presented for TEAEs leading to discontinuation of study drug and treatment-emergent serious adverse events (TESAEs). Any AEs occurring between signing of ICF and dosing on Day 1 will be presented in a separate listing. All listings will be done by treatment group and participant, detailing verbatim, SOC, PT, start date, stop date (if resolved), intensity, seriousness, outcome, action taken with respect to study drug, and relationship to study drug. The AE onset will also be shown relative (in number of days) to the day of the first study drug administration.

An overview of all TEAEs will be presented by treatment group. The overview will include the following:

- Number and percentage of participants with TEAEs
- Number and percentage of participants with drug-related TEAEs
- Number and percentage of participants with TEAEs leading to discontinuation of study drug
- Number and percentage of participants with TEAEs leading to study discontinuation
- Number and percentage of participants with TESAEs
- Number and percentage of participants with drug-related TESAEs

- Number and percentage of participants with TESAEs leading to discontinuation of study drug
- Number and percentage of participants with TESAEs leading to study discontinuation
- Number and percentage of participants by intensity of TEAEs (mild, moderate, severe)
- Number and percentage of participants who experienced death from any cause
- Number and percentage of participants with AESIs

Summaries of TEAEs by treatment group, SOC, and PT will be presented for the following:

- All TEAEs
- Ocular TEAEs in the Study Eye
- Ocular TEAEs in the Fellow Eye
- Non-Ocular TEAEs
- TEAEs leading to discontinuation of study
- TEAEs leading to discontinuation of study drug
- Drug-related TEAEs
- All TESAEs
- Ocular TESAEs in the Study Eye
- Ocular TESAEs in the Fellow Eye
- Non-Ocular TESAEs
- TESAEs leading to discontinuation of study
- TESAEs leading to discontinuation of study drug
- Drug-related TESAEs
- TEAEs by intensity
- AESIs
- AESIs leading to discontinuation of study
- AESIs leading to discontinuation of study drug

Additional details on AE summaries will be provided in the SAP.

2.21.6 Vital Signs

Vital signs measurements will be listed by participant and time point including changes from BL and any repeated/unscheduled measurements. Descriptive statistics (n, mean, SD, minimum, median, maximum) will be provided by treatment group and time point for both absolute values and changes from BL.

2.21.7 Safety Laboratory Tests

Laboratory safety assessments will be listed by participant and time point including changes from BL, flags for any measurements that are outside the reference ranges, and any repeated/unscheduled assessments. Descriptive statistics (n, mean, SD, minimum, median, maximum) will be provided by treatment group and time point for both absolute values and changes from BL. A shift from BL table describing shifts to out-of-normal range will be provided by treatment group.

Urinalysis results will be listed by participant and time point including changes from BL for numeric variables, flags for any measurements that are outside the reference ranges, and any repeated/unscheduled assessments.

The results of pregnancy and HbA1c testing will be listed for each participant.

2.21.8 Other Analyses

Descriptive summaries of participant disposition and BL characteristics (including demographic data and medical and ophthalmic history) will be presented by treatment group. In addition, a listing of participants who discontinued from the study along with discontinuation reason will be provided. Listings of BL characteristics and prohibited medications will also be provided.

Protocol deviations will be listed and summarized by treatment group and category.

Medications will be coded using the World Health Organization Drug Dictionary (WHO DD) and listed by participant. A summary of concomitant medications by treatment group and medication class will also be tabulated.

2.21.9 Interim Analysis

There are no interim analyses planned.

3 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

3.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

3.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
  - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
  - Applicable International Council for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines
  - Applicable laws and regulations
- The protocol, protocol amendments, ICF, IB, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants. Any substantial modification of the protocol will be submitted to the competent authorities as substantial amendments for approval, in accordance with ICH GCP and national and international regulations.
- Protocols and any substantial amendments to the protocol will require health authority approval prior to initiation except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
  - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
  - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
  - Providing oversight of the conduct of the study at the site and adherence to requirements of ICH guidelines, the IRB/IEC, and all other applicable local regulations

3.1.2 Financial Disclosure

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the study and for 1 year after completion of the study.

3.1.3 Informed Consent Process

- The Investigator or his/her representative will explain the nature of the study to the participants and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participants.
- Participants who are rescreened are required to sign a new ICF.

3.1.4 Data Protection

- Participants will be assigned a unique identifier by the Sponsor. Any participant records, datasets or biological samples that are transferred to the Sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant who will be required to give consent for their data to be used as described in the informed consent.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

3.1.5 Committees Structure

3.1.5.1 Central Reading Center

The digital retinal images, OCT scans, fluorescence angiographies, and other images as applicable will be assessed by independent central reading centers. Details pertaining to each imaging technique are as follows:

- FP: DRS 4W/7M standard 2-step plus adjudication
- OCT: CI-DME, SRF/IRF, DRIL, TMV (1, 3, and 6mm)
- FA: We will grade ONLY study eye
- OCTA: vessel density, FAZ area, and nonperfusion

3.1.5.2 Steering Committee

A steering committee consisting of experts in the field is created to provide scientific and operational recommendations to the study protocol and to potential day-to-day decisions in study conduct. The OcuTerra team and Steering Committee members will collaborate to ensure that the scope for the study is constrained to what is relevant with regard to the clinical indication, outcomes, and product under investigation, and most importantly, study participant safety. The composition of the committee, the functional roles, and responsibilities can be found in the Steering Committee charter.

3.1.6 Data quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- All data objective and subjective will be captured in the EDC and reported on.
- Guidance on completion of CRFs will be provided in eCRF completion guidelines.
- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Quality tolerance limits (QTLs) will be pre-defined in the Integrated Data Review Plan to identify systematic issues that can impact participant safety and/or reliability of study results. These pre-defined parameters will be monitored during the study and important deviations from the QTLs and remedial actions taken will be summarized in the clinical study report.

- Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based

Monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.

- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (eg, Contract Research Organizations).
- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the investigator for 15 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

3.1.7 Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the Source Data Location List.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

3.1.8 Study and Site Start and Closure

3.1.8.1 First Act of Recruitment

The first act of recruitment is the first participant visit and will be the study start date.

3.1.8.2 Study / Site Termination

The sponsor or designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

For study termination:

- Discontinuation of further study intervention development

For site termination:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the investigator
- Total number of participants included earlier than expected.

If the study is prematurely terminated or suspended, the sponsor shall promptly inform the investigators, the IECs/IRBs, the regulatory authorities, and any contract research organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The investigator shall promptly inform the participant and should assure appropriate participant therapy and/or follow-up.

3.1.9 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.

- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

3.2 Appendix 2: ETDRS Final Retinopathy Severity Scale

Table 3 ETDRS Final Retinopathy Severity Scale (for Individual Eyes)

Level	Severity	Definition
10	DR absent	Microaneurysms and other characteristics absent
12*	Non-DR abnormalities	
14*	DR questionable	14A HE definite; microaneurysms absent
		14B SE definite; microaneurysms absent
		14C IRMA definite; microaneurysms absent
		14Z Venous loops > D/1; microaneurysms absent
15*	DR questionable	Hemorrhage(s) definite; microaneurysms absent
20	Microaneurysms only	Microaneurysms definite, other characteristics absent
35†	Mild NPDR	35A Venous loops ≥ D/1
		35B SE, IRMA, or VB = Q
		35C Retinal hemorrhages present
		35D HE ≥ D/1 (< STD 3 in any field)
		35E HE ≥ M/1 (≥ STD 3 in any field)
		35F SE ≥ D/1 (any definite)
43	Moderate NPDR	43A H/Ma = M/4-5 (> STD 1(4+ fields) -OR- ≥ STD 2A (1 field))
		43B IRMA = D1-3 (any definite < STD 8A)
47	Moderately Severe NPDR	47A Both L43 characteristics
		47B IRMA = D/4-5 (< STD 8A)
		47C H/Ma = S/2-3 (< STD 2B)
		47D VB = D/1
53	Severe NPDR	53A ≥ 2 of the L47 characteristics
		53B H/Ma ≥ S/4-5 (≥ STD 2A in 4+ fields or ≥ 2B in any field)
		53C IRMA ≥ M/1 (≥ STD 8A)
		53D VB ≥ D/2-3 or S1 (≥ STD 6 in 2+ fields or ≥ 6B in any field)
53E	Very Severe NPDR	53E ≥ 2 of 53B, 53C, and 53D
60	Inactive PDR	60 Scatter or Local Rx
61	Mild PDR	61A FPD and/or FPE only (regressed PDR)
		61B NVE < ½ DA in ≥ 1 field
65	Moderate PDR	65A NVE ≥ M/1 (≥ ½ DA in ≥ 1 field)
		65B NVD = D (< STD 10A) ; and VH and PRH = A or Q
		65C VH or PRH = D (< 1 DA) and NVE < M (< ½ DA) and NVD absent

71/75	High-risk PDR	71A VH or PRH ≥ M/1 (≥ 1 DA)
		71B NVE ≥ M/1 (≥ ½ DA 1 field) and VH or PRH ≥ D/1 (any)
		71C NVD = D (< STD 10A) and VH or PRH ≥ D/1 (any)
		71D NVD ≥ M (≥ STD 10A)
		75 NVD ≥ M (≥ STD 10A) and VH or PRH ≥ D/1 (any)
81	Advanced PDR: fundus partially obscured, center of macula attached	NVD = cannot grade, or NVD < D and NVE = cannot grade in ≥ 1 field and absent in all others; and retinal detachment at center of macula < D
85	Advanced PDR: posterior fundus obscured, or center of macula detached	85A VH = VS (obscuring) in Field 1 or 2 85B Retinal detachment at center of macula = D (present)
90	Cannot grade, even for 81 or 85	

Abbreviations: DR = diabetic retinopathy; NPDR = nonproliferative DR; PDR = proliferative DR; HE = hard exudates; SE = soft exudates; IRMA = intraretinal microvascular abnormalities; VB = venous beading; H/Ma = hemorrhages/microaneurysms; NVE = new vessels elsewhere; NVD = new vessels on or adjacent to optic disc; VH = vitreous hemorrhage; PRH = preretinal hemorrhage

Severity categories are of the form (maximum severity/extent), where maximum severity can be absent (A), questionable (Q), definitely present (D), moderate (M), severe (S), or very severe (VS), and extent is the number of photographic fields at that severity level. For example, M/2-3 means there are two or three fields from fields 3 to 7 with moderate severity, and none with higher severity.

* Levels 12, 14, and 15 are not considered separate steps in the scale, but are pooled with level 10 or 20 (or excluded).

† NPDR levels 35 and above all require presence of microaneurysms.

3.3 Appendix 3: Clinical Laboratory Tests

- The tests detailed in Section 2.15.1 and the table below (Table 4) will be performed locally at the study site and sent to the central laboratory for analysis with the exception of urine pregnancy tests, which will be performed and analyzed locally at the site.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Investigators must document their review of each laboratory safety report.

The laboratory parameters to be analyzed are defined below in Table 4. The time points of analysis are at Screening and EOS as defined in the SoA (Section 2.15.1). All labs are expected to be random (non-fasting).

Additional local laboratory tests may be performed as deemed appropriate by the Investigator (eg, in case of signs of any toxicity).

Table 4 Clinical Laboratory Tests

CHEM 20	CBC	Urinalysis
Glucose	Hemoglobin	Sp. Gravity (SG)
Sodium	Hematocrit	pH
Potassium	MCV	Leukocyte esterase
Carbon dioxide	MCH	Nitrites
Chloride	Red Cell Count	Hemoglobin
BUN	White Cell Count	Protein (Total)
Creatinine	Neutrophils (%)	Glucose
Calcium	Total Lymphs (%)	Ketones
Iron	Monocytes (%)	Urobilinogen
Albumin	Eosinophils(%)	Bilirubin
Total protein	Basophils (%)	
ALP	Neutrophils (Abs)	
ALT (SGPT)	Total Lymphs (Abs)	
AST (SGOT)	Monocytes (Abs)	
LDH	Eosinophils(Abs)	
GGT	Basophils (Abs)	
Bilirubin (total)	Platelets	
Uric acid		
Amylase		
Lipase		

3.4 Appendix 4: AEs and SAEs: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

3.4.1 AE Definition

- An AE is any untoward medical occurrence in a clinical study participant, associated with the use of study intervention, whether or not considered related to the study intervention.
- NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) associated with the use of study intervention.

3.4.2 Events Meeting the AE Definition

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, radiological scans, vital signs measurements), including those that worsen from BL, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease).
- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- This includes progression of DR New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected intervention-intervention interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

3.4.3 Events NOT Meeting the AE Definition

- Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant’s condition.
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant’s condition.
- Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE.

- Situations in which an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

An SAE is defined as any AE that, at any dose:

a. Results in death

b. Is life-threatening

- The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

- In general, hospitalization signifies that the participant has been admitted (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.
- Hospitalization for elective treatment of a pre-existing condition that did not worsen from BL is not considered an AE.

d. Results in persistent or significant disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised by the Investigator in deciding whether SAE reporting is appropriate in other situations such as significant medical events that may jeopardize the participant or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious.
- Examples of such events include invasive or malignant cancers, intensive treatment for allergic bronchospasm, blood dyscrasias, convulsions, or development of intervention dependency or intervention abuse.

3.4.4 Recording and Follow-up of AE and / or SAE

AE and SAE recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- The Investigator will then record all relevant AE/SAE information.
- It is not acceptable for the Investigator to send photocopies of the participant's medical records to Sponsor in lieu of completion of the AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by Sponsor. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to Sponsor.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

3.4.5 Assessment of Intensity

- The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:
- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficient discomfort to interfere with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe.
- An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

3.4.6 Assessment of Causality

- The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE.
- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.

- The Investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study
- intervention administration will be considered and investigated.
- The Investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the Investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred, and the Investigator has minimal information to include in the initial report to Sponsor. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission** of the SAE data to Sponsor.
- The Investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

3.4.7 Follow-up of AEs and SAEs

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- New or updated information will be recorded in the originally submitted documents.
- The Investigator will submit any updated SAE data to the Sponsor within 24 hours of receipt of the information.

3.4.8 Reporting of SAEs

SAE reporting to Sponsor via an electronic data collection tool

The primary mechanism for reporting an SAE to Sponsor will be the electronic data collection tool.

- If the electronic system is unavailable, then the site will use the paper SAE data transmission (see next section) to report the event within 24 hours.

- The site will enter the SAE data into the electronic system as soon as it becomes available.
- After the study is completed at a given site, the electronic data collection tool.
- will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the Sponsor's Medical Monitor by telephone.
- Contacts for SAE reporting can be found in the Investigator Site File.

SAE reporting to Sponsor via paper data collection tool

- Email transmission of the SAE paper data collection tool is the preferred method to transmit this information to the Sponsor.
- In rare circumstances and if email transmission is not feasible, notification by telephone is acceptable with a copy of the SAE data collection tool sent by overnight mail or courier service.
- Initial notification via telephone does not replace the need for the Investigator to complete and sign the SAE data collection tool within the designated reporting time frames.
- Contacts for SAE reporting can be found in the Investigator Site File.

3.5 Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

3.5.1 Definitions

Woman of childbearing potential

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered women of childbearing potential

a. Premenarchal
b. Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, Mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's: review of the participant's medical records, medical examination, or medical history interview.

Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

3.5.2 Contraception Guidance

Female participants of childbearing potential must use a highly effective method of contraception, as shown in the table below. Male participants must agree to use condoms. This applies from the time of ICF signature until the end of the study period.

Table 5 Highly Effective Contraception Methods

- Implantable hormonal contraception associated with inhibition of ovulation
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal occlusion
- Hormonal contraception with inhibition of ovulation, either estrogen and progestogen containing or progesterone-only
- Vasectomized partner if sole sexual partner
- Sexual abstinence

Modified from: Clinical Trial Facilitation Group 2014: Recommendations related to contraception and pregnancy testing in clinical trials (CTFG 2014).

3.5.3 Collection of Pregnancy Information

Male participants with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male participant's female partner who becomes pregnant while the male participant is in this study. This applies only to male participants who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hours of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female participants who become pregnant

- The Investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. The initial information will be recorded on the appropriate form and submitted to the Sponsor within 24 hours of learning of a participant's pregnancy.
- The participant will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of

pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be reported as an AE or SAE.
- A spontaneous abortion (occurring at < 22 weeks gestational age) or still birth (occurring at > 22 weeks gestational age) is always considered to be an SAE and will be reported as such.

- Any post-study pregnancy related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Appendix 4. While the Investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.
- Any female participant who becomes pregnant while participating in the study will discontinue study intervention.

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SPONSOR SIGNATURES

Study Title:	**OTT166-201 A Phase 2 Randomized, Double-Masked, Vehicle-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of OTT166 Ophthalmic Solution in the Treatment of Diabetic Retinopathy (DR)**
Study Number:	**OTT166-201**
Version Number:	**V5.0**

This clinical study protocol was subject to critical review and has been approved by the sponsor. The following personnel contributed to writing and/or approving this protocol:

Signed:  Date: October 11, 2023

David J. Tanzer, MD
OcuTerra Therapeutics
Chief Medical Officer

INVESTIGATOR'S SIGNATURE

Study Title:	**OTT166-201 A Phase 2 Randomized, Double-Masked, Vehicle-Controlled, Multicenter Study to Evaluate the Safety and Efficacy of OTT166 Ophthalmic Solution in the Treatment of Diabetic Retinopathy (DR)**
Study Number:	**OTT166-201**

I have read the clinical study described herein, recognize its confidentiality, and agree to conduct the described study in compliance with Good Clinical Practices (GCP), the ethical principles contained within the Declaration of Helsinki, this protocol, and all applicable regulatory requirements, including United States (US) Food and Drug Administration (FDA) 21 Code of Federal Regulations (CFR) 312

Study Center: _____

Principal Investigator: _____
(Print Name)

Signature: _____ Date: _____