A Phase 2a, Prospective, Multicenter, Randomized, Double-Masked, Sham-Controlled Study to Assess the Safety, Tolerability and Evidence of Activity of a Single Intravitreal Injection of UBX1325 in Patients with Diabetic Macular Edema

Investigational Product(s)	UBX1325
Protocol Number	UBX1325-02
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Sponsor	UNITY Biotechnology, Inc.
Sponsor's Legal Representative	Sharon Klier, MD, MPH VP of Ophthalmology and Medical Director, UNITY Biotechnology, Inc. 285 East Grand Avenue South San Francisco, CA 94080

Dates	
Original Protocol:	12 April 2021 Version 1.0
Amendment 1	27 April 2021 Version 2.0
Amendment 2	07 July 2021 Version 3.0
Amendment 3	03 November 2021 Version 4.0
Amendment 4	14 February 2022 Version 5.0

SUMMARY OF CHANGES: AMENDMENT 1

The following summarizes the principal elements requiring that change be made to the protocol. Other minor modifications for administrative purposes have been made to provide clarity and are captured in the redlined document.

The principal components of change to the study are described in the table below.

Area of Change	**Rationale**
Revisions were made throughout to reflect double-masking, and any reference to single-masking has been replaced accordingly.	The study is considered double-masked because the patient and investigator are masked.
Exclusion Criteria 5: History of IVT, subconjunctival, or periocular steroids in the 3 months prior to screening conflicts with Exclusion Criteria 13: History of systemic and intraocular steroid use for 6 months prior to Day 1. The use of intravitreal nonbiodegradable steroid implants (ex. Iluvien®, Yutiq®, Retisert® is prohibited. Exclusion Criteria #5 has been removed. [Section Number 4.6 Exclusion Criteria]	Retaining Exclusion Criteria #13 (now documented as #12 in this amendment) as a higher standard reflects the scientific rigor and safety precautions taken in this study population.
Post-dilution solutions for clinical administration will be held no more than 1 hour at room temperature prior to administration to the patient. [Section Number 6.1.1 UBX1325]	The original hold times and conditions were inaccurate. This change was made to align with guidance in the Pharmacy Manual.

SUMMARY OF CHANGES: AMENDMENT 2

The following summarizes the principal elements requiring that change be made to the protocol. Other minor modifications for administrative purposes have been made to provide clarity and are captured in the redlined document.

The principal components of change to the study are described in the table below.

Area of Change	Rationale
Screening window was increased from 28 days to 42 days [Section 1.2 Schedule of Events Table 1] and [Section 5 Study Conduct]	Linking the window acceptable for screening period to coincide with the optimal timing post last anti-VEGF injection (see item 3). The number of days permitted for screening is increased to avoid a patient falling outside the screening window due to when they received their last anti-VEGF treatment and having to rescreen.
Removal of reference to dose starting at 5 µg [Section 4.2.2 Dose Rationale]	10 µg was confirmed safe per the Safety Assessment Committee (SAC) in UBX1325-01 study. On 18May2021, the SAC confirmed that 10 µg should be used in all future UBX1325 study enrollments.
Inclusion criteria for last anti-VEGF given was changed from between 3 and 5 weeks prior to day 1, to between 3 and 6 weeks prior to day 1 [Section 4.5 Inclusion Criteria]	Matching the time from last anti-VEGF injection to best capture response sensitivity (i.e., long enough from last anti-VEGF injection to avoid anti-VEGF induced inhibition of activity but short enough to avoid re-emergence of active neovascularization and inflammation). This increases the likelihood of differentiating between UBX1325 and sham treated patients. Furthermore, the window of 3–6 weeks might better capture standard visit timing for patients and clinic.
Revised inclusion criteria to allow patients with PDR DME (ETDRS-DRSS Score at 65C [or DR severity level of 8] or less severe) to participate in this study [Section 4.5 Inclusion Criteria]	Based on preclinical animal studies and impact on neovascularization, there is sufficient data to support the hypothesis that there could be a potential effect of UBX1325 on PDR patients.
Stratification by anti-VEGF treatment agents [Section 6.2 Method for Assigning Patients to the Treatment Group]	To obtain a balance between UBX1325 and sham treatment arms with regards to prior anti-VEGF agents.
Established that a formal interim analysis will occur at 12 weeks [Section 8.7 Planned Interim Analysis]	Revised the plan for analysis to reflect a planned interim analysis at 12 weeks after a certain proportion of patients meet the 12-week timepoint as will be determined in the SAP. Based on UBX1325-01 data, we believe study drug effects may be seen earlier at 8–12 weeks. This is also to ensure the safety and well-being of patients at 12-week timepoint.

Area of Change	Rationale
Changed rescue criteria from "worsening CST by 100 µm from Baseline (Day 1) per SD-OCT or a 10-letter decrease in BCVA compared to peak BCVA from Baseline (Day 1)" to "worsening CST by 75 µm from Baseline (Day 1) per SD-OCT and/or a 10-letter decrease in BCVA compared to peak BCVA from Baseline (Day 1)" [Section 5.1.10 Rescue Criteria]	To better align with the standard practices followed by the treating physician.

SUMMARY OF CHANGES: AMENDMENT 3

The following summarizes the principal elements requiring that change be made to the protocol. Other minor modifications for administrative purposes have been made to provide clarity and are captured in the redlined document.

The principal components of change to the study are described in the table below.

Area of Change	Rationale
Inclusion Criteria 2: clarified DRSS will be determined by a Central Reading Center and re-incorporated the requirement for having at least 3 anti-VEGF treatments in the preceding 6 months Inclusion Criteria 3: CST requirement was reduced from ≥ 350 μm to ≥ 300 μm Inclusion Criteria 5: BCVA requirement in non-study has been removed since safety was established Inclusion Criteria 6: IOP ≤ 23 mmHg requirement applies to Day 1 Exclusion Criteria 1: removed definition of high risk/active PDR as Inclusion Criteria 2 is more explicit Exclusion Criteria 9: patients with glaucoma who are poorly controlled in the opinion of the investigator or on more than 3 meds (new language explicitly excludes patients with unstable glaucoma) Exclusion Criteria 12: prior steroid use applies to study eye only [Section 4.5 Inclusion Criteria] and [Section 4.6 Exclusion Criteria]	Eligibility criteria revised to address 1 or more issues: • Remove redundancies • Remove specific criteria put in place before additional safety data was ascertained in the First-in-Human study • Clarify requirements • Better align with patient population
Reordering of secondary endpoints putting BCVA changes at the top of the list [Section 1 Synopsis] and [Section 3 Objectives and Endpoints] and [Section 8 Statistical Methods]	Change of CST enrollment requirement may limit the magnitude of change from baseline in CST, while BCVA is deemed less sensitive to this change.
PK sampling was removed as a study procedure and subsequently as an endpoint [Section 1 Synopsis] and [Section 1.2 Schedule of Events Table 1] and [Section 5 Study Conduct]	An adequate number of PK samples were collected in the First-in-Human study and analysis confirmed there is no evidence of systemic exposure of UBX0601/UBX1325
Removal of Australia and New Zealand as participating countries [Section 1 Synopsis]	The Sponsor opted to conduct the study within North America only

SUMMARY OF CHANGES: AMENDMENT 4

The following summarizes the principal elements requiring that change be made to the protocol. Other minor modifications for administrative purposes have been made to provide clarity and are captured in the redlined document.

The principal components of change to the study are described in the table below.

Area of Change	Rationale
Inclusion Criteria 2: Revised the requirement for having at least 3 anti-VEGF treatments in the preceding 6 months, to having at least 2 anti-VEGF treatments in the preceding 6 months Inclusion Criteria 4: Upper BCVA limit in study eye changed from 70 letters to 73 letters Exclusion Criteria 20: replaced reference to permit non-facial basal cell carcinoma with reference to permit stable, superficial basal or squamous cell carcinoma of the skin [Section 4.5 Inclusion Criteria] and [Section 4.6 Exclusion Criteria]	Eligibility criteria were revised to clarify requirements and/or better align with patient population
Extended the follow-up period from 24 weeks to 48 weeks, subsequently, the number of visits increased from 9 to 12. The secondary and exploratory endpoints apply to Week 48 [Section 1 Synopsis] and [Section 1.2 Schedule of Events Table 1] and [Section 3 Objectives and Endpoints] and [Section 5 Study Conduct]	To assess the extent of durability of a single IVT injection of UBX1325
As of November 9, 2021, additional data became available supporting the ongoing safety profile of UBX1325 [Section 2.3.5 Benefit: Risk Assessment] [Section 4.2.2 Dose Rationale]	The safety summary was revised to reflect additional non-serious, non-drug related adverse events
Clarified that the pre-dose assessments, specifically Anterior Segment Evaluation, Posterior Segment Evaluation and IOP are done OU, however, only the study eye should be assessed post-dose [Section 1.2 Schedule of Events Table 1] and [Section 5 Study Conduct]	This clarifies required pre- and post-dose assessments
The following data will be collected for both eyes in the preceding 6 months prior to screening: use of anti- VEGF and historical imaging and visual acuity assessments [Section 5.1.3 Medical History and Concomitant Medication Review]	This allows us to have better access to data as to patient's prior treatment history; such prior treatment could be a useful tool in assessing patients who are most likely to respond well to UBX1325

Area of Change	Rationale
Clarified that patients, investigators, visual acuity technicians, photographers, reading center personnel and Sponsor are masked. [Section 6.6 Masked and Unmasked Study Staff]	To clarify who is masked in this double-masked study
The following changes were made to the section on prohibited treatments. Removed this statement: There are no known contraindications to the administration of UBX1325 injection Revised this statement: Patients should not have received any therapy that would preclude an IVT injection or can potentially exhibit retinal toxicity (such as tamoxifen, hydroxychloroquine and trastuzumab). [Section 6.8.2 Prohibited Treatments]	Safety has been established in the First-in-Human study and prohibited medication statement was further clarified with specific examples
Added that interim analyses may be conducted at timepoints outside the initially specified 12 Week [Section 8.7 Planned Interim Analysis]	To allow for assessment of data at other study timepoints

STATEMENT OF COMPLIANCE

The study will be conducted in compliance with this clinical study protocol, Good Clinical Practices (GCPs) as outlined by International Conference on Harmonisation (ICH) E6(R2), and all applicable local and national regulatory requirements. Enrollment at any clinical study site may not begin prior to that site receiving approval from the Institutional Review Board (IRB), Independent Ethics Committee (IEC) or Ethics Committee of Record (ECR) for the protocol and all materials provided to potential patients. Screening at a site may not begin prior to approval from the IRB/IEC and the Sponsor.

Any amendments to the protocol or changes to the consent document will be approved by the IRB/IEC before implementation of that amendment. Reconsent of previously enrolled patientsmay be necessary, depending on the nature of the amendment.

All personnel involved in the conduct of this study have completed Human Subjects Protection and GCPs training, as outlined by their governing institution.

SPONSOR'S APPROVAL

Title	A Phase 2a, Prospective, Multicenter, Randomized, Double-Masked, Sham-Controlled Study to Assess the Safety, Tolerability and Evidence of Activity of a Single Intravitreal Injection of UBX1325 in Patients with Diabetic Macular Edema
Protocol Number	UBX1325-02
Version Number	5.0
Version Date	14 February 2022

The design of this study as outlined by this protocol has been reviewed and approved by the Sponsor's responsible personnel as indicated in the signature table below.

Sponsor's Legal Representative			
Name	**Title**	**Signature**	**Date**
Sharon Klier, MD, MPH	VP of Ophthalmology and Medical Director	Esigned by: Sharon Klier, MD, MPH 2022.02.15 16:46:27 -0600 I approve this document Sharon Klier, MD, MPH Vp, Clinical Development, UNITY Biotechnology	

INVESTIGATOR'S AGREEMENT

I have read the protocol, appendices, and accessory materials related to Study UBX1325-02 and agree to the following:

- To conduct this study as described by the protocol and any accessory materials
- To protect the rights, safety, and welfare of the participants under my care
- To provide oversight to all personnel to whom study activities have been delegated
- To control all Study Drug provided by the Sponsor and maintain records of thedisposition of those products
- To conduct the study in accordance with all applicable local and national regulations, the requirements of the ethics committee of record for my clinical site, and Good Clinical Practices as outlined by ICH E6(R2)
- To obtain approval for the protocol and all written materials provided to participants prior to initiating the study at my site
- To obtain informed consent – and updated consent in the event of new information or amendments – from all participants enrolled at my study site prior to initiating any study-specific procedures or administering investigational products to those participants
- To maintain records of each patient's participation and all data required by the protocol

Name	**Title**	**Institution**
Signature		**Date**

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LIST OF ABBREVIATIONS

Abbreviation	Definition
aAMD	atrophic age-related macular degeneration
AMD	age-related macular degeneration
AE	adverse event
AUC	area under the curve
AUC_{0-672}	area under the curve from t=0 to t=672
AUC_{0-t}	area under the curve from t=0 to t
AUC_{0-inf}	area under the curve from t=0 to infinity
$AUC_{\%extrap}$	percent of the area under the curve extrapolated from AUC_{0-t} to AUC_{0-inf}
AR	adverse reaction
Bcl-2	B-cell lymphoma 2
BCVA	best corrected visual acuity
BRB	blood-retinal barrier
CFP	color fundus photography
CL/F	apparent total clearance of the drug from plasma after intravitrealinjection
C_{max}	maximum concentration
CRA	clinical research associate
CST	central subfield thickness
CV	coefficient of variation
DLT	dose-limiting toxicity
DME	diabetic macular edema
DNA	deoxyribonucleic acid
DR	diabetic retinopathy
DRCR	Diabetic Retinopathy Clinical Research Network
DRSS	diabetic retinopathy severity scale
EC	European Commission
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EMR	electronic medical record
ERG	electroretinogram
ERM	epiretinal membrane
ET	early termination
ETDRS	Early Treatment Diabetic Retinopathy Study
FA	fluorescein angiography
FDA	Food and Drug Administration
FIH	first-in-human
GCP	good clinical practice
GLP	good laboratory practice
HbA1C	hemoglobin A1C

Abbreviation	Definition
hERG	human ether-à-go-go-related gene
HRMEC	human retinal microvascular endothelial cells
IB	investigator's brochure
ICAM-1	intercellular adhesion molecule-1
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IHC	immunohistochemistry
IL	interleukin
IND	investigational new drug (application)
IOP	intraocular pressure
IRB	institutional review board
IVT	intravitreal
LAR	legally authorized representative
LLOQ	lower limit of quantification
MedDRA	Medical Dictionary for Regulatory Activities
MMPs	matrix metalloproteinases
MRI	magnetic resonance imaging
MTD	maximally tolerated dose
nAMD	neovascular age-related macular degeneration
NCA	non-compartmental analyses
NCI CTCAE v4.03	National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03
NOAEL	no-observed-adverse-effect-level
NOEL	no-observed-effect-level
NV	neovascularization
NZW	New Zealand White
OCT	optical coherence tomography
OCT-A	optical coherence tomography angiography
OIR	oxygen-induced retinopathy
OU	both eyes
PAD	pharmacologically active dose
PDR	proliferative diabetic retinopathy
PDGF	platelet-derived growth factor
PEDF	pigment epithelium-derived growth factor
PK	pharmacokinetic
POC	proof-of-concept
PS 80	polysorbate 80
RPE	retinal pigment epithelium

Abbreviation	Definition
SAC	safety assessment committee
SAD	single-ascending dose
SAE	serious adverse event
SAP	statistical analysis plan
SAR	suspected adverse reaction
SASP	senescence-associated secretory phenotype
SD-OCT	spectral domain optical coherence tomography
SDM	subthreshold diode micropulse laser therapy
SE	study eye
SLE	slit lamp exam
SnCs	senescent cells
SRT	selective retinal therapy
SUSAR	suspected unexpected serious adverse reaction
T1D	Type 1 diabetes
T2D	Type 2 diabetes
$T_{1/2}$	half-life
T_{max}	time at which the maximum concentration is observed
TA	triamcinolone acetonide
TGF	tumor growth factor
TNF	tumor necrosis factor
US	United States
VA	visual acuity
Vd/F	apparent volume of distribution after non-intravenous administration
VEGF	vascular endothelial growth factor
VO	vaso-obliteration
WHO	World Health Organization

1 SYNOPSIS

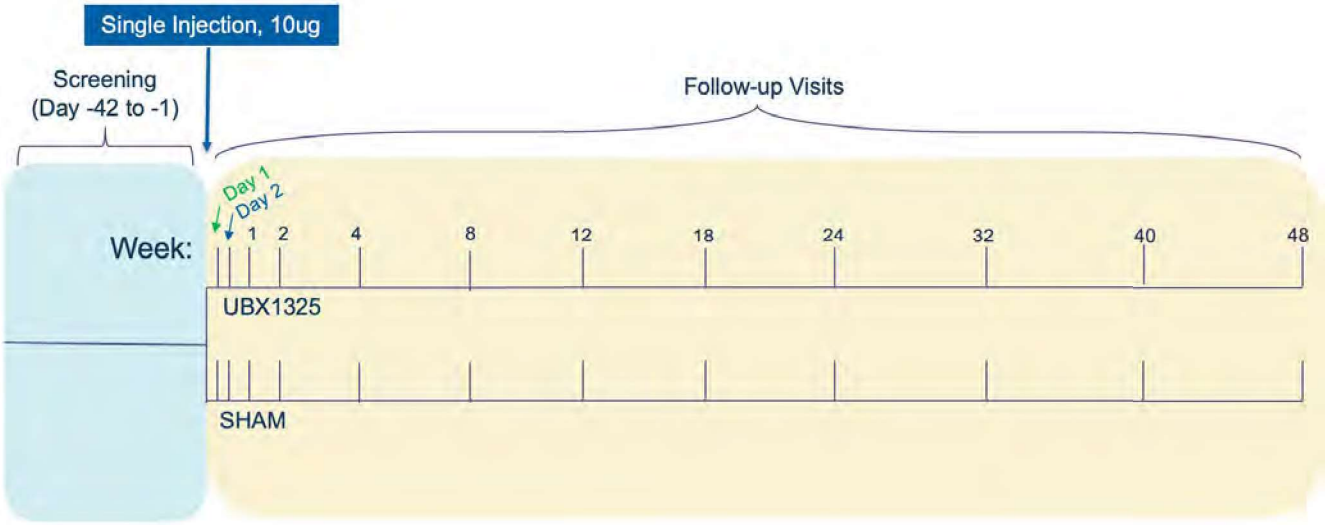
Title	A Phase 2a, Prospective, Multicenter, Randomized, Double- Masked, Sham-Controlled Study to Assess the Safety, Tolerabilityand Evidence of Activity of a Single Intravitreal Injection of UBX1325 in Patients with Diabetic Macular Edema (DME)
Phase	Phase 2a
Study Design	This is a Phase 2a Proof-of-Concept (POC) study. The total number of patients will be approximately 62 patients who will be enrolled and randomized 1:1 into either the UBX1325 or sham study arms, in order to assess the primary objective. All patients will be followed for approximately 48 weeks The injector will be unmasked but the evaluator is masked.
Rationale	This study is intended to assess the exposure, safety, biological activity and durability of UBX1325, a phosphate pro-drug, and its active parent molecule (UBX0601) following a single intravitreal (IVT) injection of UBX1325 in patients with DME.
Target Population	This study will enroll patients ≥ 18 years of age with DME with best corrected visual acuity (BCVA) between 73 to 20 Early Treatment Diabetic Retinopathy Study (ETDRS) letters (equivalent to 20/40 to 20/400 on the Snellen chart) at Screening and at Day 1.
Number of Patients	Approximately 62 patients
Length of Participation	**On treatment:** 1 day. Each patient will receive UBX1325 IVT injection or sham on Day 1. **On study:** Up to 54 weeks (6-week screening period + 1 dose day + 48-week follow-up period)
Intervention	Patients will be administered a single 50 µL UBX1325 IVT injection (10 µg) or sham.
Primary Objective and Primary Endpoint	**Objective:** Assess the local and systemic safety and tolerability following a single IVT injection of UBX1325 compared to sham **Endpoint:** • Ocular and systemic safety and tolerability of a single IVT injection of UBX1325 evaluated by treatment emergent adverse events (TEAEs)

Secondary Objectives and Corresponding Endpoints	**Objectives:** Assess the biological activity following a single UBX1325 injection in patients with DME. Also, assess efficacy parameters and retinal structure improvement of patients following a single IVT injection of UBX1325 compared to sham. **Endpoints**: • Changes in BCVA from Baseline to Weeks 12, 24 and 48 • Proportion of patients who require 2 or more anti-VEGF rescue over 12, 24 and 48 Weeks • Change in CST from Baseline to each study visit as assessed by SD-OCT and read by a Reading Center • Change in CST area under the curve (AUC) from baseline to each study visit • Proportion of patients without macular fluid at Weeks 12, 24 and 48 as assessed by SD-OCT
Exploratory Objectives and Corresponding Endpoints	**Objective:** Assess efficacy parameters and retinal structure improvement of patients following a single IVT injection of UBX1325 compared to sham. **Endpoints:** • Change from Baseline in Diabetic Retinopathy Severity Scale (DRSS) score at Weeks 24 and 48 • Change in capillary perfusion from Baseline to Weeks 12, 24 and 48 as assessed by OCT-A and FA
Number of Sites	Approximately 32 sites in the US and Canada
Study Duration	Approximately 24 months including start up
Safety Assessment Committee	A Safety Assessment Committee (SAC) will be established for adjudication of adverse events (AEs) or possible safety signals. This committee will meet on an ad hoc basis.

1.1 Study Schematic

The study schematic is presented in Figure 1.

Figure 1 Schematic of Phase 2a Proof-of-Concept Study



1.2 Schedule of Events

The schedule of events (SOE) is presented in Table 1.

Table 1 Schedule of Events

Test/Procedure	Screening Day -42 to Day -1	Visit 1 Week 0 Day 1	Visit 2 24 ± 4 hours Observation[a] Day 2	Visit 3 Week 1 Day 8 ±1	Visit 4 Week 2 Day 15 ±2	Visit 5 Week 4 Day 29 ±7	Visit 6 Week 8 Day 57 ±7	Visit 7 Week 12 Day 85 ±7	Visit 8 Week 18 Day 127 ±7	Visit 9 Week 24 Day 169 ±7
Informed Consent	X									
Demographics	X									
Medical/ Ophthalmic History	X	X	X	X	X	X	X	X	X	X
Medication History/ Concomitant Medications	X	X	X	X	X	X	X	X	X	X
Physical Examination	X									X
Vital Signs and Weight	X[b]	X	X	X	X	X	X	X	X	X
Laboratory Tests: Hematology and Chemistry	X									X
Pregnancy Test	X (serum)	X (urine)[c]								X (urine)[c]
12-Lead ECG	X									X
BCVA	X	X	X	X	X	X	X	X	X	X
Anterior Segment Evaluation	X	X[d]	X	X	X	X	X	X		X
Posterior Segment Evaluation	X	X[d]	X	X	X	X	X	X		X
IOP	X	X[d]	X	X	X	X	X	X		X
SD-OCT[e]	X	X		X	X	X	X	X	X	X
OCT-A[e]	X							X		X
FA[e]	X					X		X		X

Test/Procedure	Screening Day -42 to Day -1	Visit 1 Week 0 Day 1	Visit 2 24 ± 4 hours Observation[a] Day 2	Visit 3 Week 1 Day 8 ±1	Visit 4 Week 2 Day 15 ±2	Visit 5 Week 4 Day 29 ±7	Visit 6 Week 8 Day 57 ±7	Visit 7 Week 12 Day 85 ±7	Visit 8 Week 18 Day 127 ±7	Visit 9 Week 24 Day 169 ±7
CFP[e]	X							X		X
DRSS score	X							X		X
Eligibility Criteria	X	X								
Study Drug Administration[g]		X								
AE assessment		X	X	X	X	X	X	X	X	X

Table 1 Schedule of Events (Continued)

Test/Procedure	Visit 10 Week 32 Day 225 ± 7	Visit 11 Week 40 Day 281 ± 7	Visit 12 Week 48 Day 337 ± 7	Unscheduled Visit/ET
Medical/Ophthalmic History	X	X	X	X
Medication History/Concomitant Medications	X	X	X	X
Physical Examination			X	X
Vital Signs and Weight	X	X	X	X
Laboratory Tests: Hematology and Chemistry			X	X
Pregnancy Test			X (urine)	
12-Lead ECG			X	X
BCVA	X	X	X	X
Anterior Segment Evaluation	X	X	X	X
Posterior Segment Evaluation	X	X	X	X
IOP	X	X	X	X
SD-OCT	X	X	X	X
OCT-A			X	X
FA			X	X[f]
CFP			X	X
DRSS score			X	X
AE assessment	X	X	X	X

AE = adverse event; BCVA = best corrected visual acuity; CFP = color fundus photography; DRSS = diabetic retinopathy severity scores; ECG = electrocardiogram; ET = early termination; FA = fluorescein angiography; IOP = intraocular pressure; OCT-A = optical coherence tomography angiography; SD-OCT = spectral domain optical coherence tomography; rescue treatment administered if needed

[a] Procedures to be performed at the end of the ~24 hours (± 4 hours) of observation.

[b] Height should also be measured at Screening.

[c] Pre-dose urine dipstick for females of childbearing potential. Urine dipstick should also be done at the last study visit, Visit 9 Week 24.

[d] Procedure to be performed both pre-dose and post-dose. Post-dose procedures will be done in the study eye only by the unmasked injector, 30 minutes ± 15 minutes post dose.

[e] CFP, FA, SD-OCT, and OCT-A images should be transmitted to the Central Reading Center at each applicable visit. Screening images should be transmitted to Central Reading Center for eligibility verification. OCT-A to be conducted at sites with the proper equipment available.

[f] To be completed only if assessment has not been performed within the preceding 30 days.

[g] After all other study procedures have been completed. Optional post-injection prophylactic antibiotics may be administered. Sites should follow their standard practice and document all medication given.

2 INTRODUCTION

UBX1325 is a new molecular entity that is being investigated by UNITY Biotechnology, Inc. (theSponsor) for the treatment of diabetic macular edema (DME).

DME is a complication of diabetic retinopathy (DR) following chronic, poorly controlled diabetes, and is the most common form of sight-threatening retinopathy in people with diabetes (Tan et al., 2016, IDF 2019). Approximately one in 14 patients with diabetes has some degree of DME (Coney 2019).

The overall prevalence of DR in patients with diabetes using retinal images was estimated to be 35%, with vision-threatening DR present in 12% (WHO 2015). Prevalence depends on the type of diabetes and the duration of the disease. For both types of diabetes, type 1 diabetes (T1D) and type 2 diabetes (T2D), after 25 years duration, prevalence approximates 30% (Browning et al., 2018).

In the US, at least 5.5 million individuals over the age of 40 are estimated to have DR in the absence of DME, and an additional 800,000 to 1 million patients have DME. According to some estimates, only 40% of them diagnosed and treated, and about 5% are diagnosed and observed (IDF 2019, VanderBeek et al., 2016).

Fewer than half of all US adults with diabetes adhere to guideline-recommended eye-screeningschedules. Patients with DR typically have no symptoms in the early stage of the disease and may not seek medical evaluation until DR advances and results in vision impairment. These delays in diagnosis and treatment may result in visual impairment that is permanent and irreversible (Coney 2019).

Despite advances in diagnosis and management of ocular disease in patients with diabetes, eye complications from diabetes continue to be a leading cause of vision loss and new onset blindness in working-age individuals in the United States (Aiello 2005, Antonetti et al., 2012).

2.1 Overview of DME

The most common early clinically visible manifestations of DR include microaneurysm formation and intraretinal hemorrhages. Microvascular damage (e.g., retinal capillary non-perfusion, cotton wool spots, increased numbers of hemorrhages, venous abnormalities, and intraretinal microvascular abnormalities) leads to increased vasopermeability, which can subsequently result in retinal thickening and/or exudates that may lead to loss in central visual acuity (VA).

When blood-retinal barrier (BRB) is further compromised, pooling of fluid within the retina's central area, the macula, may ensue and cause DME. Capillary leakage causes diffuse edema, whereas focal or multifocal leakage from grouped microaneurysms leads to localized edema (IDF 2019).

The natural history of DME is characterized by a slow progression of retinal thickening until the center of the macula is involved, causing VA deterioration. Spontaneous resolution of

DME is rare and usually secondary to improvement in systemic risk factors, such as glycemic control, hypertension, or hypercholesterolemia. If untreated, 29% of eyes with DME and foveal involvement experience moderate visual loss (doubling of the visual angle) after 3 years.

Spontaneous visual recovery is also unusual, with improvement of at least 3 Early Treatment Diabetic Retinopathy Study (ETDRS) lines occurring in 5% of cases (Bandello et al., 2017).

No difference in the prevalence of DME was observed when subjects were analyzed by age orgender; however, non-Hispanic black subjects had a greater odd of having DME compared with non-Hispanic white subjects (Varma et al. 2014). Systemic risk factors associated with DME include longer duration of diabetes, high systolic blood pressure, and elevated hemoglobinA1C (HbA1C) levels. Other studies found that lipid and triglyceride levels, advanced diabetic nephropathy, and pregnancy are also associated risk factors. The sole ocular factor associated with DME is DR severity as increasing severity is associated with increasing prevalence of DME (Browning et al., 2018, Kim et al., 2019).

The macula in DME is thickened due to increased extracellular fluid derived from hyperpermeable retinal capillaries. Prolonged hyperglycemia in uncontrolled diabetes leads to reduced inner retinal oxygen tension, venous dilation, increased vascular endothelial growth factor (VEGF) concentration within the retina, leukocyte stasis, and dysregulated growth factor levels, which together are associated with increased exudation of serum out of the retinal vasculature and into the extracellular space. The retinal pigment epithelium (RPE) pump is overwhelmed by the exudation of serum and macular swelling results (Browning et al., 2018).

While the underlying pathophysiology of DME is believed to be VEGF-mediated, there is a growing body of evidence suggesting that inflammatory mediators and cytokines contribute to the vascular permeability and edema in DME (Ascaso et al., 2014, Owen and Hartnett 2013, Browning et al., 2018). Increased inflammation, characterized by leukostasis, accumulation of macrophages, intercellular adhesion molecule-1 (ICAM-1), and prostacyclin upregulation is associated with capillary non-perfusion and breakdown of BRB (Browning et al., 2018). Patientswith DME have elevated vitreous levels of VEGF, ICAM-1, interleukin (IL)-6, and monocyte chemoattractant protein-1 compared with non-diabetic patients (Funatsu et al., 2009).

Inflammatory cytokines such as tumor necrosis factors alpha and beta, alpha 4 integrin, nitric oxide, and IL-1β mediate vascular permeability. High lipid levels may cause endothelial dysfunction and increased vascular permeability through a local inflammatory response and higher levels of advanced glycation end products (Miljanovic et al., 2004). Many other small molecules and growth factors may contribute to the development of DME, although the details of the pertinent pathways are incompletely understood (Kent et al., 2000; Patel et al., 2006, Browning et al., 2018).

2.2 Standard of Care for DME

Photocoagulation has been the standard of care for DME for decades. However, a substantial group of patients are unresponsive to laser therapy and fail to improve after photocoagulation (Bandello et al., 2017). Some studies show that conventional photocoagulation is effective in reducing macular thickness in patients with DME; however, it causes visible laser scars that may enlarge once treatment is finished. In addition, the thermal effects of photocoagulation cantrigger complications, including choroidal neovascularization, subretinal fibrosis, and visual fieldloss. Subthreshold diode micropulse laser therapy (SDM) and selective retinal therapy (SRT) may be valuable for treating sub-clinically significant DME that is diagnosed early (Park et al., 2014). However, given the risk of progression and general lack of early detection, in addition toAEs, other treatment modalities for DME have been investigated.

Intravitreal (IVT) administration of steroids has provided promising results for the treatment of DME (Sarao et al., 2014). The anti-inflammatory, angiostatic, and antipermeability properties ofthese compounds have gained interest in chronic retinal conditions such as DME. A complete understanding of the mechanism of action of corticosteroids has not been fully clarified.

However, corticosteroids have been shown to interfere with many regulatory components of gene expression inhibiting the expression of VEGF and key proinflammatory genes (tumor necrosis factor α and other inflammatory chemokines), while inducing gene functioning as anti-inflammatory factors (pigment epithelium-derived growth factor [PEDF]) (Tsaprouni et al., 2002; Tong et al., 2006; Kim et al., 2019; Zhang et al., 2006). The anti-inflammatory activity of steroids is also related to the inhibition of the phospholipase A 2 pathway, to the lower release of inflammatory cell mediators, and to the reduced leukocyte chemotaxis (Bandello et al. 2017). Additionally, triamcinolone acetonide (TA) seems to reduce the expression of matrix metalloproteinases (MMPs) and down-regulates ICAM-1 on choroidal endothelial cells (Mizuno et al., 2007). IVT and peribulbar injections of TA must be repeated every 2 to 4 months to maintain effect. Several steroid applications have been studied, for example, biodegradable dexamethasone extended release implant (Ozurdex®), fluocinolone acetonide (Iluvien®) and TA implants to prolong the intervals between treatments (Bandello et al., 2017).

Side effects of steroids are a concern, especially considering the availability of anti-VEGF medications that have minimal ocular side effects. The primary risks associated with IVT administration of corticosteroids for DME are cataract progression and development of glaucoma, which is why it is sometimes contraindicated in patients with glaucoma or ocular hypertension. Nonetheless, steroid treatment can be considered first line in special populations such as, patients who have had a recent history of cardiovascular event, pregnant women, patients unable to adhere to monthly treatments, or DME in eyes undergoing cataract surgery (Zur et al., 2019).

Surgical vitrectomy was proposed as a treatment option for DME in the early 1990s. Eyes with DME were observed to have lower prevalence of posterior vitreous detachment than eyes without DME, and it was thought that vitreomacular adhesion might promote DME. This was further supported by the observation that resolution of DME could occur after

posterior vitreous detachment and surgical induction of a vitreomacular separation might improve DME. With the advent of optical coherence tomography (OCT), vitreomacular adhesion was shown to be a riskfactor for DME (Browning et al., 2018). A controversy exists regarding the effects of vitrectomy for DME. Some reports suggest that vitrectomy reduces macular thickening but does not improve VA, yet others report improved VA and decrease in macular thickening. Prospective, randomized clinical studies have not been conducted to evaluate the effect of vitrectomy on diverse DME patient population. Nonetheless, there is a general acceptance that vitrectomy hasa role in the management of at least some cases of DME (Browning et al., 2018).

Studies have shown the safety and efficacy of IVT anti-VEGF treatments in patients with DME, some of which were the basis for Food and Drug Administration (FDA) approval of anti-VEGF therapies for the treatment of DME (Elman et al., 2010; Nguyen et al. 2012; Brown et al., 2015).The Diabetic Retinopathy Clinical Research Network (DRCR) net trial, Protocol T, was a 660-patient study comparing 3 IVT anti-VEGF treatments in patients with DME, followed for 2 years of treatment. Focal/grid laser was added if DME persisted and was not improving at 6 months or later. Results showed that focal/grid laser was administered as a supplement in 41%, 64%, and 52% of aflibercept, bevacizumab, and ranibizumab groups, respectively. From Baseline to 2 years, mean VA letter score improved by 12.8 with aflibercept, 10.0 with bevacizumab, and 12.3 with ranibizumab. Treatment group differences varied by baseline VA (Wells et al. 2016).

A meta-analysis published by Virgili et al., 2018 concluded that anti-VEGF treatments are effective at improving vision in patients with DME with 3 to 4 in every 10 patients likely to experience an improvement of 3 or more lines VA at 1 year. Aflibercept may confer some advantage over ranibizumab and bevacizumab in patients with DME at one year in visual and anatomic terms, but it is unclear whether this applied to the long-term. Overall, the safety profile between the 3 anti-VEGF drugs, aflibercept, ranibizumab, or bevacizumab is similar (Virgili et al., 2018).

Evidence from randomized controlled trials may not apply to real-world practice, where patients in need of anti-VEGF treatment are often under treated and under monitored. In a number of studies, the full effect of anti-VEGF agents are not even experienced for up to 12 months, and inmany patients, even after 12 months, significant retinal fluid remains. This suggests that other therapeutic options with more rapid and complete effect could have an important role in the treatment of this disease.

Despite the success achieved with anti-VEGF treatment for DME, patients' poor compliance with the regimen (monthly and/or bimonthly IVT injections or a treat-and-extend regimen), refractory cases to anti-VEGF treatment (50% of DME patients (AAO PPP for AMD 2019; AAO PPP for DR 2017), the long-term complication of retinal fibrosis and the prolonged time it takes to achieve maximal response account for the unmet need that remains in DME. Although VEGFhas been identified as a primary biomarker for the neovascular disease, other biomarkers are present in DR (including IL-1β, tumor necrosis factor [TNF]-α, IL-6, tumor growth factor [TGF-β], among others) and it could be argued that anti-VEGF treatment alone does not address the multifactorial nature of the disease.

Analysis of electronic medical records (EMRs) from a geographically and demographically diverse sample of US retina specialists revealed that real-world outcomes of anti-VEGF therapies in DME are meaningfully poorer than those shown in anti-VEGF randomized control trials (Ciulla et al., 2018). Overall, this EMR study demonstrated that best corrected visual acuity (BCVA) outcomes in DME patients treated with anti-VEGF agents in the real world are inferior tothose in randomized control trials (RCTs) by approximately 1 line of VA at 1 year (Ciulla et al., 2018). A subsequent study by the same group confirmed that real world patients with DME undergo fewer anti-VEGF injections and exhibit worse 1-year VA gains compared with RCTs, regardless of anti-VEGF medication (Ciulla et al., 2020). This underscores the importance of asafe drug that has long durability of effect on DME patients.

2.2.1 Target Indication and Population

This study will enroll patients ≥ 18 years of age with DME with BCVA of 73 to 20 ETDRS letters (equivalent to 20/40 to 20/400 on the Snellen chart).

2.3 Scientific Rationale for UBX1325 as a Treatment for DME

The Sponsor hypothesizes that the accumulation of senescent cells (SnCs) in the retina contributes to retinal disease. UBX1325 is a phosphate pro-drug of the active parent molecule, UBX0601, an inhibitor of the anti-apoptotic protein Bcl-xL in the retina. Inhibition of Bcl-xL leads to induction of apoptosis, ultimately leading to cell death. In preclinical studies, activation of the apoptotic cascade was selective for animals with a senescent cell burden. IVT administration of UBX1325 in diabetic animals led to improvements in retinal vascular leakage, providing support for the treatment of DME.

2.3.1 Role of Senescence in Retinal Diseases

The principal feature of senescence, a regulated, stress-induced, cellular program, is to induce a cell to enter a permanent state of cell cycle arrest. The first observation that human cells do not divide indefinitely was proposed in 1961 by the seminal work by Leonard Hayflick (Hayflick and Moorhead 1961). Several important features of the senescence program have since been elucidated. Exposure to damage-inducing stimuli such as oxidative stress, radiation, and ischemia are among factors associated with the induction of senescence. Several studies (Marazita et al., 2016; Lamoke et al., 2015; Ma and Wong 2016) have confirmed the correlation between these stressors and angiogenic retinal disease.

SnCs produce a set of proinflammatory factors, cytokines, chemokines, and others that are called the senescence-associated secretory phenotype (SASP). Although many factors of SASP have been identified in neovascular age-related macular degeneration (nAMD) and DR, a list of the most relevant ones associated with each disease have been studied and identified with many of them having a proinflammatory role (Funk et al., 2009; Sato et al., 2018; Schori et al., 2018).

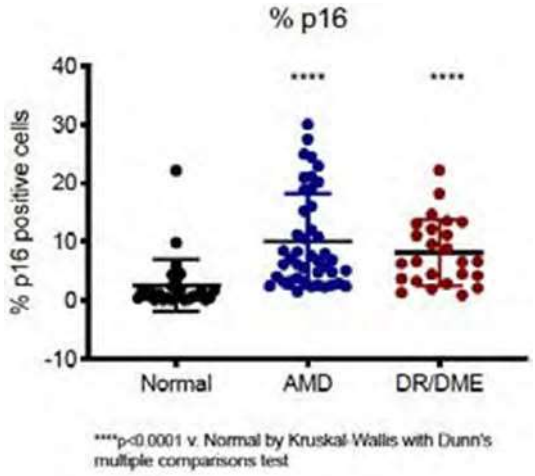
Blasiak et al., 2017 demonstrated the role of SASP in RPE cells, photoreceptors, and choriocapillaris and postulated that in the central retina, RPE cells cannot be easily replaced by peripheral RPE cells leading to DNA damage response, autophagy, photoreceptor degeneration, and eventually, vision loss.

Evidence of SnCs in human retinopathies has focused on SASP and in vitro culture systems, but reports of $p16^+$ immunohistochemistry (IHC) and cell-type identification are limited.

Literature reports suggest presence of SnCs in aged primates (Mishima et al., 1999), and the proliferation potential of human RPE cells in vitro is suggested to be influenced by donor age (Flood et al., 1980). Recently, Lopez-Luppo et al., 2017 reported the presence of $p16^+$ cells in the retinal vasculature of aged human donors and vessels associated with microaneurysms.

Immunohistochemical (IHC) staining performed by the Sponsor of retinal sections from patients with DR/DME and age-related macular degeneration (AMD) indicates the presence of $p16^+$ cells (Figure 2). When quantified and compared to age-matched normal tissue, a significant SnC burden was observed in both DR/DME and AMD globes.

Figure 2 Immunohistochemical Staining of $p16^+$ Cells in Human Donors



AMD = age-related macular degeneration; DME = diabetic macular edema; DR = diabetic retinopathy; N = number of patients; p16 = p16 cellular biomarker.
Retinal sections from human donor tissue were subject to immunohistochemical staining for p16. Cell nuclei were stained with DAPI, and % cells positive for p16 were calculated for each donor globe.
N = 27–43 per group.

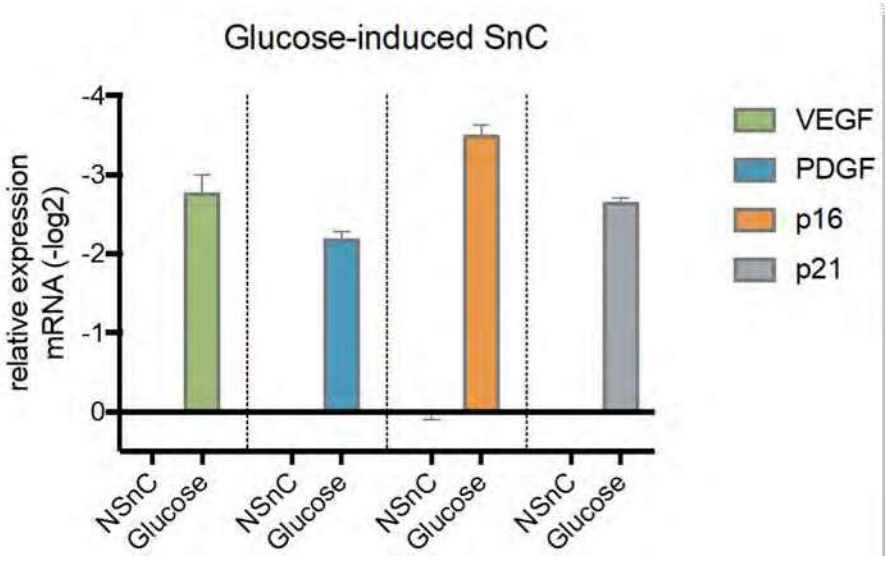
The Sponsor hypothesizes that there is a close relationship between senescence burden, measured by p16 IHC in the retina, the presence of SASP in the tissue, and clinical progression in patients with DR and DME. UBX1325 has the potential to remove SnCs from a tissue without altering the healthy resident cells in the eye and slow down the progression of the disease or even provide structural and functional recovery by addressing not just one target factor like VEGF, but a set of proinflammatory or growth factors present in the SASP. This approach would address more effectively the multifactorial character of the disease and may decrease the proportion of patients that respond either poorly or partially to anti-VEGF, the need for repeated treatments, and the long-term complications like fibrosis and macular atrophy.

2.3.2 Disease-relevant SASP from Senescent Retinal Cells

In addition to the cellular marker of senescence, elevated expression of several secreted factors has been associated with retinal diseases, which have been detected in the vitreous humor and aqueous humor from patients with ocular disease. Levels of mediators such as VEGF, TNF, IL-1β, platelet-derived growth factor (PDGF), and IL-6 are significantly increased in the ocular fluids of DR and AMD globes (Bromberg-White et al. 2013; Boss et al., 2017; Ghodasra et al., 2016; Sato et al., 2018). In addition to p16, the Sponsor is focused on the association of disease-relevant SASP to our senescent cellular and in vivo animal models. Elimination of SnCs by UBX1325 may lead to a reduction in SASP by targeting the source, resulting in the restoration of homeostasis and retinal function.

Human ocular cell types can be cultured and rendered senescent by a variety of different insults, including deoxyribonucleic acid (DNA)-damaging treatment (e.g., irradiation) and disease-relevant conditions such as incubation in elevated glucose media. Human retinal microvascular endothelial cells (HRMEC) upregulate the production of important mediators such as VEGF and PDGF upon induction of senescence by glucose (Figure 3). As these molecules have been shown to be elevated in DR patients (Ghodasra et al. 2016; Klaassen et al., 2017), it stands to reason that senescent HRMEC in the retinas of patients may be the source of the elevated VEGF/PDGF that contribute to disease pathogenesis.

Figure 3 Induction of Senescence and Diabetic Retinopathy-relevant Genes AfterGlucose Treatment



DR = diabetic retinopathy; SnC = senescent cells; mRNA = messenger ribonucleic acid; VEGF = vascular endothelial growth factor; PDGF = platelet-derived growth factor; p16 = p16 cellular biomarker; p21 = p21 cellular biomarker; NSnC = non-senescent cells. Increased expression of several genes was observed by qRT-PCR in non-senescent HRMEC (NsnC) or HRMEC treated with elevated glucose (200 mM) in culture media for 2 weeks.

2.3.3 Description of UBX1325

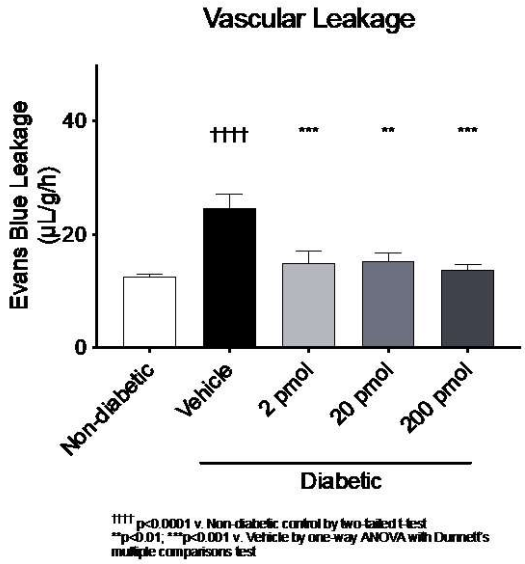
UBX1325 is a soluble, small-molecule phosphate pro-drug, which is cleaved rapidly in tissues by ubiquitous phosphatases to yield the active parent molecule UBX0601. UBX1325 was designed to improve the solubility of UBX0601 as an ophthalmic formulation. In this protocol, the phrase "Study Drug" refers to the administered phosphate form (UBX1325), not the active inhibitor UBX0601. In all nonclinical studies, Study Drug is administered as UBX1325, and converted rapidly to UBX0601, which potently inhibits subtypes within the B-cell lymphoma 2 (Bcl-2) family of apoptosis regulatory proteins, including Bcl-2, Bcl-xL, and Bcl-w.

The Bcl-2 gene family encodes more than 20 proteins that regulate the intrinsic apoptosis pathway and are fundamental to the balance between cell survival and programmed cell death. Inhibition of particular members of the Bcl-2 family of apoptosis regulatory proteins disrupts the interactions with pro-apoptotic activators and effectors, resulting in cell death.

SnCs utilize pro-survival mechanisms to remain viable and rely on particular members of the Bcl-2 family of apoptosis regulatory proteins members to persist and accumulate in tissues (Yosef et al., 2016). Binding of retinal Bcl-xL by UBX0601 in senescent HRMEC acts to promotecaspase activation and the apoptotic cascade, ultimately leading to cell death. In mouse modelsof retinal dysfunction, UBX1325 demonstrated inhibition of disease-relevant endpoints suggesting the potential for efficacy.

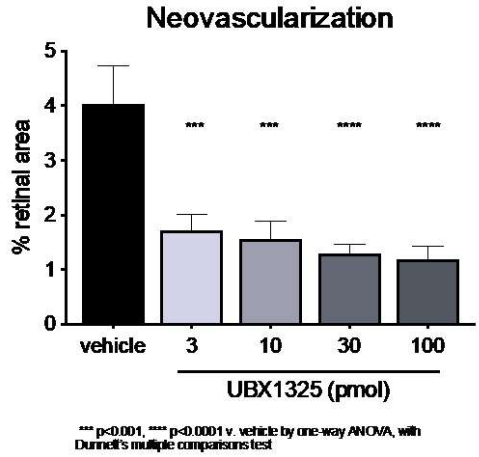
In a streptozotocin-induced diabetic retinopathy mouse model, IVT administration of UBX1325 resulted in decreased vascular leakage at all doses tested (2–200 pmol) (Figure 4). In a mouse model of retinal neovascularization (oxygen-induced retinopathy (OIR) model), an IVT administration of UBX1325 led to a decrease in the total area of neovascularization at all doses tested (3–100 pmol) (Figure 5). Refer to the Investigator's Brochure (IB) for additional details.

Figure 4 Improved Vascular Leakage in the Diabetic Mouse



Increased retinal vascular leakage was measured by Evans Blue extravasation in diabetic mice (Vehicle) as compared to non-diabetic animals. All 3 dose levels of UBX1325 (2–200 pmol, IVT) significantly reduced vascular leakage.

Figure 5 Decreased Neovascularization in the OIR Mouse



Neovascular area was determined from isolectin B4-stained images of OIR retinas. All 4 dose levels of UBX1325 (3–100 pmol, IVT) significantly reduced retinal neovascularization as compared to vehicle control animals.

2.3.3.1 Administration Regimen

On Day 1, patients will be administered a single 50 µL IVT treatment of 10 µg of UBX1325 or a sham procedure. The UBX1325 dose is based on the safe dose established in UBX1325-01, the Phase 1 SAD study (NCT04537884).

2.3.3.2 Justification for Dosing Strategy

The UBX1325 dose regimen planned for evaluation in this study was selected based on the safe dose established in the Phase 1 SAD study.

In the Phase 1 SAD study, single IVT doses of 0.5, 1, 5 μg, and 10 μg are studied. For this study, the dose is 10 μg.

2.3.4 Supportive Nonclinical Data

All nonclinical pharmacology, toxicology, and PK studies briefly described in this section were conducted by or for the Sponsor. More detailed information is provided in the current version of the UBX1325 Investigator's Brochure (IB).

2.3.4.1 Pharmacology

UBX1325 (pro-drug) and UBX0601 (active parent molecule) were studied in cellular and animal models of ocular disease to demonstrate activity in the retina. UBX1325 is a potent inhibitor of Bcl-2 family of apoptosis regulatory proteins, which results in initiation of apoptosis in HRMEC, in vitro. In 2 different mouse models of retinal dysfunction, UBX1325 demonstrated inhibition ofdisease-relevant endpoints such as neovascularization (NV) and vascular leakage.

2.3.4.2 Toxicology

UBX1325 has been evaluated in Good Laboratory Practice (GLP) ocular toxicity studies in New Zealand White (NZW) rabbits and monkeys with observation periods of up to 2 months following a single IVT dose. In order to evaluate the potential for systemic toxicity, one-month GLP studies were conducted in which UBX1325 was given as a once weekly IV dose to rats and dogs. The no-observed-effect-level (NOEL) following IVT injection in rabbits at which there were no findings was 5 μg/eye; the no-observed-adverse-effect-level (NOAEL) in monkeys was 25 μg/eye. In the systemic repeat-dose toxicity studies, NOAELs were 0.3 mg/kg and 0.1 mg/kg for rats and dogs, respectively. The systemic levels noted at these NOAELs (NOAEL = 0.3 mg/kg, C_{max} = 1260 ng/mL and AUC_{last} = 649 hr·ng/mL; NOAEL = 0.1 mg/kg, C_{max} = 568 on Day 29; AUC could not be determined) are not anticipated to be observed following IVT dosing.

Cardiovascular, respiratory, and neurobehavioral safety pharmacology studies following IV dosing of UBX1325 up to 0.3 mg/kg UBX1325 did not result in any adverse findings. Of note, the cardiovascular study in dogs did not show any effect on QT prolongation, consistent with in vitro results evaluating the effect of UBX1325 and UBX0601 on the human ether-à-go-go- related gene (hERG) channel. In addition, UBX1325 and UBX0601 were not genotoxic in in vitroand in vivo studies. Further development of UBX1325 is supported by the available data.

2.3.4.3 Pharmacokinetics

Ocular tissue exposures of UBX1325 and UBX0601 following a single IVT injection of 50 ug of UBX1325 were well-characterized in rabbits following serial necropsy at 2, 24, 72, 168, 240, and 336 hours, post-dose. UBX601 half-lives in the vitreous humor, retina, choroid, and iris-ciliary body of rabbits were 48 hours, 34 hours, 47 hours, and 80 hours, respectively. The concentration of UBX601 in the lens showed a slower rate of decline with an estimated half-life of 173 hours. Aqueous humor concentrations, which did not allow for PK estimates, appeared to remain at a very low level of 0.02 µg/mL. Systemic exposure to UBX1325/UBX0601 following intraocular dose was minimal, usually below the lower limit of quantification (LLOQ) in the assay (1 ng/mL), suggesting rapid clearance once UBX0601 reaches systemic circulation.

2.3.5 Benefit: Risk Assessment

2.3.5.1 Previous Human Experience with Bcl-2 Inhibitors

UBX1325 is in a class of agents known to inhibit certain members of the Bcl-2 family that are apoptosis regulatory proteins. Targeting this pathway has been extensively studied in connection with the search for new oncology medicines. Some tumor cells can become dependent on Bcl-2 for survival, which allows for specific targeting with inhibitors of Bcl-2. Bcl-2inhibitors have been investigated in the oncology setting indicating safety and tolerability when administered systemically in humans (Tse et al. 2008; Touzeau et al., 2014; Souers et al., 2013). To the best of the Sponsor's knowledge, Bcl-2 inhibitors have not previously been investigated in an ocular setting.

UBX1325 was administered to patients with advanced DME and wet AMD in the Phase 1 single ascending dose (SAD) study, UBX1325-01. UBX1325 was well tolerated with favorable safety profile supporting further development. As of November 9, 2021 there were a total of 14 adverse events unrelated to study drug. Of those 14, 4 were non-ocular, non-serious, non-drug related adverse events in 2 wet AMD patients (1 patient contracted COVID-19 and 1 patient had a tooth extraction, blister on gums and constipation). The remaining 10 ocular, non-serious, non-drug related adverse events occurred in 9 patients (2 DME and 7 wet AMD). Nine were study eye related (progression of disease in 8 patients, 1 DME and 7 wet AMD) and 1 was non-study eye related (retinal detachment in 1 DME patient). There were no serious adverse events, dose-limiting toxicities, inflammation, or AEs that would preclude advancement into later-stage clinical development.

The Sponsor is aware of the systemic toxicities associated with venetoclax, an FDA-approved oral Bcl-2 selective inhibitor for chronic lymphocytic leukemia/small lymphocytic lymphoma and acute myeloid leukemia. As a consequence of being in the same class of molecules, and in view of similar systemic toxicities noted in UBX1325 IV nonclinical studies, the Sponsor is planning tomonitor for potential Bcl-2 inhibitor related toxicities (e.g., cytopenia) that may emerge from UBX1325 IVT treatment (Section 7). However, based on currently available exposure data from nonclinical studies following IVT administration (Section 2.3.4.3), and the lack of measurable systemic exposure of UBX1325 and UBX0601 after a single IVT dose at all doses tested in patients in the Phase 1 SAD

study, the Sponsor expects systemic exposure of UBX1325 following IVT treatment to be low in patients and, therefore, the potential to observe such systemic events is also low.

3 OBJECTIVES AND ENDPOINTS

Tier	Objective	Endpoints
Primary	Assess the local and systemic safety and tolerability following a single-dose IVT injection of UBX1325 in patients with DME	• Ocular and systemic safety and tolerability of a single IVT injection of UBX1325 evaluated by treatment emergent adverse events (TEAEs)
Secondary	Assess the biological activity following a single UBX1325 injection in patients with DME Assess further efficacy parameters and retinal structure improvement of patients following a single UBX1325 injection compared to sham	• Changes in BCVA from Baseline to Weeks 12, 24 and 48 • Proportion of patients who require 2 or more anti-VEGF rescue over 12, 24 and 48 Weeks • Change in CST from Baseline to each study visit as assessed by SD-OCT and read by a Reading Center • Change in CST area under the curve (AUC) from baseline to each study visit • Proportion of patients without macular fluid at Weeks 12, 24 and 48 as assessed by SD-OCT
Exploratory	Assess further efficacy parameters and retinal structure improvement of patients followinga single UBX1325 injection compared to sham	• Change from Baseline in Diabetic Retinopathy Severity Scale (DRSS) score at Weeks 24 and 48 • Change in capillary perfusion from Baseline to Weeks 12, 24 and 48 as assessed by OCT-A and FA

4 STUDY PLAN

4.1 Study Design

This is a Phase 2a, Proof-of-Concept (POC), prospective, multicenter, randomized, double-masked, sham-controlled study to assess the safety, tolerability, evidence of activity and durability of a single IVT injection of UBX1325 in subjects with DME. Approximately 62 total patients will be enrolled 1:1 in the UBX1325 or sham study arms, in order to assess the primary objective. All patients will be followed for approximately 48 weeks.

See Section 6.6 for details on double-masking and site requirements. A schematic representation of the study design is presented in Figure 1.

4.2 Design Rationale

4.2.1 Patient Population Selection

Based on the in vitro and in vivo pharmacology and toxicology data (UBX1325 Investigator Brochure), scientific rationale (see Section 2.3), and safety data from the UBX1325-01 First-in-Human study, the Sponsor asserts that UBX1325 can be tested for safety and efficacy in DME patients.

Patients will be those diagnosed as having DME (as defined by the AAO PPP DME Guidelines 2019) with BCVA in the study eye of 73 to 20 ETDRS letters (equivalent to 20/40 to 20/400 on the Snellen chart).

4.2.2 Dose Rationale

The planned dose is 10 µg based on favorable safety data in the Phase 1 SAD study. As of November 9, 2021, in patients with advanced DME and wet AMD in the SAD Phase 1 study, UBX1325 was well tolerated with favorable safety profile supporting further clinical development; no dose-limiting toxicities in any of the dose cohorts, including the high dose of 10 µg. The planned dose is 10 µg based on favorable safety data in the Phase 1 SAD study.

4.3 Recruitment

Approximately 62 patients are planned to enroll in this study.

4.4 Definitions

Patients officially enter the Screening Period following provision of informed consent directly.

A screen failure is a consented patient who has been deemed ineligible on the basis of 1 or more eligibility criteria or who has withdrawn consent prior to randomization. Screen failures may be rescreened. Patients will be permitted to re-screen once, and in consultation with the Sponsor's Medical Monitor.

An enrolled patient is one who has been deemed eligible and has been randomized.

4.5 Inclusion Criteria

To be included in this study, each individual must satisfy all of the following criteria:

1. Patients aged ≥ 18 years.
2. DR (PDR and NPDR) patients with DME (ETDRS-DRSS Score at 65C [or DR severity level of 8] or less severe as determined by a Central Reading Center), who had at least 2 anti-VEGF intravitreal (IVT) injections (one or more of the following agents: aflibercept, bevacizumab or ranibizumab) in the preceding 6 months prior to Day 1, with the last anti-VEGF given between 3 and 6 weeks prior to Day 1.
3. Center-involved DME with central subfield thickness (CST) ≥ 300 μm on SD-OCT at Screening as determined by a Central Reading Center.
4. BCVA in the study eye (most affected) of 73 to 20 ETDRS letters (equivalent to 20/40 to 20/400 on the Snellen chart) at Screening and at Day 1. If both eyes are equal, then it's Investigator discretion.
5. IOP ≤ 23 mmHg in the study eye on Day 1.
6. Clear ocular media and adequate pupillary dilation to permit CFP and adequate BCVA evaluation.
7. Patients who have the capacity to give informed consent and who are willing and able to comply with all study-related procedures and assessments.

4.6 Exclusion Criteria

If an individual meets any of the following criteria, he or she is ineligible for this study:

1. Concurrent disease in the study eye or structural damage, other than DME, that could compromise BCVA, prevent BCVA improvement, require medical or surgical intervention during the study period, confound interpretation of the results, or interfere with assessment of toxicity or CFP in the study eye. This includes, but is not limited to, the following:
    - Macular edema of etiologies other than diabetes
    - Clinically significant subretinal fibrosis
    - Subfoveal lipid
    - Cataract requiring surgery during the study period
    - RPE atrophy or tear or rips involving the macula
    - Clinically significant macular hole
    - Clinically significant noninfectious uveitis
    - Vitreomacular traction
    - Clinically significant epiretinal membrane (ERM)

- Aphakia
- Pseudophakia with anterior chamber intraocular lens (A/C IOL)

2. HbA1c ≥ 12 and/or recent signs of uncontrolled diabetes
3. Any ocular/intraocular/periocular infection or inflammation in either eye in the past 4 weeksprior to Screening (mild blepharitis is acceptable)
4. History of vitreous hemorrhage in the study eye within 2 months prior to Screening
5. History of vitrectomy in the study eye
6. History of intraocular, periocular, or corneal surgery in the study eye within 3 months prior to Screening, or anticipated need for such surgery during the study
7. History of panretinal photocoagulation (within 3 months) or macular laser photocoagulation (within 3 months) in the study eye prior to dosing
8. History of corneal transplant in the study eye
9. Patients with glaucoma who are poorly controlled in the opinion of the investigator or on more than 3 meds
10. Any condition, including laboratory findings and findings in the medical history or in the pre-study assessments, that, in the opinion of the Investigator, constitutes a risk or contraindication for participation in the study or that could interfere with the study objectives, conduct, or evaluation or prevent the patient from fully participating in all aspects of the study
11. Presence of severe myopia (-8 diopters or greater) in the study eye
12. History of systemic or intraocular steroid use for or in the study eye for 6 months prior to Day 1. The use of intravitreal nonbiodegradable steroid implants (ex. Iluvien®, Yutiq®, Retisert®) is prohibited
13. Significant media opacities, including cataract, which might interfere with VA, assessment of toxicity, or fundus imaging
14. Intraocular surgery, including cataract surgery, in the study eye ≤ 3 months of screening
15. Known allergy to dye injected during FA
16. Known allergy to any component (phosphate buffered saline and polysorbate 80) in the Study Drug. Refer to the Investigator Brochure for more details.
17. Female patients who are pregnant, lactating, or of childbearing potential who do not agree to use highly effective methods of birth control (e.g., progesterone-only hormonal contraception, double barrier, or intrauterine device) during the study and for 3 months following the last dose of Study Drug. Postmenopausal females (> 45 years old and without menses for more than 1 year) and surgically sterilized females are exempt from these requirements.
18. Male patients who do not agree to use a highly effective method of contraception during the study and for 3 months following the last dose of Study Drug, if sexually active with a female partner of childbearing potential.

19. Patients who within 3 months of screening received or are concurrently on another investigational drug or vaccine study, including patients who previously received treatmentin a UBX1325 study. COVID vaccinations are permitted prior to or during the course of the study.
20. Any uncontrolled medical condition, in the opinion of the Investigator, would preclude participation in this study, including, but not limited to, history of malignancy within the last 5 years (not including stable, superficial basal or squamous cell carcinoma of the skin), history of myocardial infarction within the last 6 months, or concomitant therapy.

5 STUDY CONDUCT

The expected duration of study participation for patients will be approximately 54 weeks, which includes a 6-week screening period, a single dose at Visit 1, and 48 weeks of follow-up.

The study procedures are outlined below and in the SoE (Table 1).

5.1 Study Procedures

5.1.1 Study Screening and Treatment Periods

5.1.1.1 Screening Period (Day -42 to Day -1)

- Informed Consent
- Demographics
- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Physical Examination
- Vital Signs and Weight
- Height
- Laboratory Tests: Hematology and Chemistry
- Pregnancy Test (serum)
- 12-lead ECG
- BCVA
- Anterior Segment Evaluation
- Posterior Segment Evaluation
- IOP
- SD-OCT*
- OCT-A (to be conducted at sites with the proper equipment available)*
- FA*
- CFP*
- DRSS score*
- Eligibility Criteria

*SD-OCT, OCT-A (if applicable), FA and CFP will be submitted to the Central Reading Center for eligibility confirmation. A report for SD-OCT will be returned to the site within 48 hours and a separate report with DRSS score will be returned to the site within 72 hours to support eligibility and subsequent randomization

5.1.1.2 Visit 1

The following procedures will be done:

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Vital signs and Weight
- Pregnancy Test (pre-dose urine dipstick)
- BCVA
- Anterior Segment Evaluation (conduct pre-dose OU and post-dose SE)
- Posterior Segment Evaluation (conduct pre-dose OU and post-dose SE)
- IOP (conduct pre-dose OU and 30 minutes ± 15 minutes post-dose SE)
- SD-OCT (conduct pre-dose)
- Eligibility Criteria
- Conduct randomization
- Study Drug Administration
- AE Assessment

5.1.1.3 Visits 2, 10 and 11

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Vital Signs and Weight
- BCVA
- Anterior Segment Evaluation
- Posterior Segment Evaluation

- IOP
- SD-OCT for Visits 10 and 11 only
- AE Assessment

5.1.1.4 Visits 3–7

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Vital Signs and Weight
- BCVA
- Anterior Segment Evaluation
- Posterior Segment Evaluation
- IOP
- SD-OCT
- OCT-A (to be conducted at sites with the proper equipment available) for Visit 7 only
- FA for Visit 5 and 7 only
- DRSS score at Visit 7 only
- AE Assessment

5.1.1.5 Visit 8

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Vital Signs and Weight
- BCVA
- SD-OCT
- AE Assessment

5.1.1.6 Visits 9 and 12

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Physical Examination
- Vital Signs and Weight
- Pregnancy Test (urine dipstick)
- Laboratory Tests: Hematology and Chemistry
- 12-lead ECG
- BCVA
- Anterior Segment Evaluation
- Posterior Segment Evaluation
- IOP
- SD-OCT
- OCT-A (to be conducted at sites with the proper equipment available)
- FA
- CFP
- DRSS score
- AE Assessment

5.1.1.7 Unscheduled Visit/Early Termination

- Medical and Ophthalmic History
- Medication History/Concomitant Medications
- Physical Examination
- Vital Signs and Weight
- Laboratory Tests: Hematology and Chemistry
- 12-lead ECG
- BCVA

- Anterior Segment Evaluation
- Posterior Segment Evaluation
- IOP
- SD-OCT
- OCT-A (to be conducted at sites with the proper equipment available)
- FA (only if assessment has not been performed within the preceding 30 days)
- CFP
- DRSS score
- AE Assessment

5.1.2 Informed Consent

It is the responsibility of the Investigator to obtain signed written consent for the study from each patient prior to participating in the study to provide for the protection of the patients by following applicable regulations and institutional policies and procedures. The Informed Consent Form (ICF) used during the informed consent process must be reviewed by the Sponsor or designee and approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

5.1.3 Medical History and Concomitant Medication Review

A detailed medical history will be obtained by the Investigator or qualified designee. This will include a comprehensive medical history and a complete review of systems, with specific attention to inclusion and exclusion criteria. The use of anti-VEGF for both eyes in the preceding 6 months prior to Screening should be documented in the concomitant medication log. All historical imaging and visual acuity assessments when available for both eyes in the preceding 6 months prior to Screening should be entered into the designated eCRF. Past medical history deemed by the Investigator as not clinically relevant to the patient's overall health status or to his or her DME will not be captured. Relevant medical history will be recorded in the electronic case report form (eCRF).

All medications taken will be recorded in the eCRF Concomitant Medication Log. Medical History and Concomitant Medication Review should log should be performed at each study visit.

5.1.4 Physical Examination

Physical examination includes assessments of the skin, head and neck, lungs, heart, abdomen, lymph nodes, extremities, and body weight. Height will be measured at Screening only.

Complete physical examinations will be performed by a licensed physician (or a physician's assistant or nurse practitioner) at Screening, Visit 9 (Week 24), Visit 12 (Week 48) or Unscheduled Visits/Early Termination (ET). After Screening, symptom-directed physical examinations are required to be performed as clinically indicated.

5.1.5 Vital Signs and Body Weight

Vital signs (blood pressure, respiratory rate, pulse rate, and oral temperature) will be obtained in the sitting position. The patient must be in the sitting position for 5 minutes prior to obtaining vital signs.

Vital signs and body weight assessment will be performed at all study visits.

5.1.6 Laboratory Tests

The Laboratory Manual contains detailed instructions for the collection and preparation of samples, directions with respect to the utilization of specialized tubes and requirements for dispensing of aliquots, and storage and shipment of samples to the Sponsor's designated central laboratory.

5.1.7 Pregnancy Test

Serum pregnancy tests will be performed at Screening, and urine pregnancy tests will be performed pre-dose on the dosing day (Day 1), Visit 9 (Week 24) and at the last study visit, Visit 12 Week 48 for all females of childbearing potential.

5.1.8 Electrocardiography

Evaluation will be performed at Screening for baseline reference, Visit 9 (Week 24) and at the last study visit, Visit 12 (Week 48).

5.1.9 Ocular Evaluations (must be conducted on both eyes)

- BCVA: should be assessed using the ETDRS chart starting at 4 meters at Screening, on Day 1 (pre-dose), Day 2, and at Weeks 1, 2, 4, 8, 12, 18, 24, 32 and 48, and Unscheduled/ET. See the Ophthalmic Manual for details.
- Anterior Segment Evaluation: should be performed prior to pupillary dilation, or after, depending on the site Standard of Care. Any abnormalities of the anterior chamber, eyelids, conjunctivae, pupil, iris, lens and cornea should be documented at Screening, on Day 1 pre-dose and post-dose, on Day 2, and at Weeks 1, 2, 4, 8, 12, 24, 32 and 48 and Unscheduled/ET. Any anterior chamber inflammation (cells and flare), phakic status, and posterior lens capsule status should also be noted. For post-dose, only the study eye should be assessed
- Posterior Segment Evaluation/Ophthalmoscope Examination: should be performed after pupillary dilation to examine the vitreous body, optic nerve head, macula, posterior pole, and peripheral retina at Screening, on Day 1 pre-dose and post-dose, on Day 2, and at

Weeks 1, 2, 4, 8, 12, 24, 32 and 48/ET. Any vitreous inflammation (haze and cells) should also be noted. For post-dose, only the study eye should be assessed

- IOP: should be measured using Goldmann applanation tonometry at Screening, on Day 1 pre-dose and 30 minutes ± 15 minutes post-dose, on Day 2, and at Weeks 1, 2, 4, 8, 12, 24, 32 and 48 and Unscheduled/ET. Tonometry should be performed prior to pupillary dilation or as per site's standard procedures. For post-dose, only the study eye should be assessed
- CFP: should be obtained at Screening, Visit 9 (Week 24), Visit 12 (Week 48) and Unscheduled/ET.
- FA: should be obtained at Screening (or on Day 1 pre-dose) and at Weeks 4, 12, 24 and 48, and Unscheduled/ET (if it had not been done within 30 days prior).
- SD-OCT: should be obtained at Screening, on Day 1 (pre-dose), and at Weeks 1, 2, 4,8, 12, 18, 24, 32, 40 and 48 and Unscheduled/ET.
- OCT-A: to be conducted at sites with the proper equipment available at Screening, Weeks 12, 24 and 48 and Unscheduled/ET.

CFP, FA, SD-OCT, and OCT-A (if done) images should be transmitted to the Central Reading Center at each applicable visit.

5.1.10 Rescue Criteria

If at any visit between Visit 4 (Week 2) to Visit 12 (Week 48) or Unscheduled, patients exhibit worsening CST by 75 µm from Baseline (Day 1) per SD-OCT and/or a 10-letter decrease in BCVA compared to peak BCVA from Baseline (Day 1), they can be rescued with standard of care. For this study, standard of care is defined as the last anti-VEGF a patient was administered prior to enrolling into the study. Rescued patients will continue their visit schedule as safety follow-up through Week 48. For any questions on rescue criteria, and prior to administering rescue medication, when possible, please contact the Medical Monitor or designee. Rescued patients continue with study visits per protocol. If additional visits are required outside of study visit windows, these will be considered unscheduled visits.

5.1.11 End of Study

The study will be considered complete when the last patient has completed the last study visit.

5.2 Discontinuation or Withdrawal

5.2.1 Individual Patients

5.2.1.1 Withdrawal from Study

Patients can voluntarily withdraw from the study for any reason at any time. They are to be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow-up for any reason. Patients withdrawing from the study because of an AE should be followed for at least 30 days, until resolution of the AE, or until no further improvement is expected, whichever comes first. Patients withdrawing from the study should be encouraged tocomplete all assessments under the Unscheduled/ET visit described in Table 1.

The Investigator may withdraw a patient from the study for any of the following reasons:

- A protocol violation occurs,
- Lack of efficacy,
- A serious or intolerable AE occurs,
- The Sponsor terminates the study,
- The Investigator suspends or terminates that site's participation in the study, or
- The patient requests to be discontinued from the study.

5.3 Study Termination

The Sponsor may suspend or terminate the study or any part of the study at any time for any reason. If the Investigator suspends or terminates that site's participation in the study, the Investigator will promptly inform the Sponsor and the IRB/IEC and provide a detailed written explanation. Upon study completion, the Investigator will provide the Sponsor with final reportsand summaries as required by regulations. Upon study suspension, completion, or termination,the Investigator will return all Study Drug and other study materials to the Sponsor or designeeor destroy the materials at the investigative site per the Sponsor's instructions.

6 STUDY INTERVENTIONS

6.1 Description of Product

The Study Drug (UBX1325) to be provided to the clinical sites will be bulk packaged (sites will be supplied with adequate overage, which will be accounted for as part of Study Drug accountability). The term "Study Drug" when used throughout this protocol means UBX1325 in solution. Each vial and carton will be individually labeled. Study Drug labeling will include identification required by local law, drug identification, and dosage. The packaging and labeling of the Study Drug will be in accordance with the Sponsor's standards and local regulations. TheStudy Drug was manufactured at Vetter Pharma Development Services USA, Skokie, Illinois.

Sham kits will be supplied by the central lab and will be included in the bulk ancillary supply shipment. There will be a needleless syringe to complete the sham procedure. These will also be accounted for as part of Study Drug accountability.

6.1.1 UBX1325

UBX1325 is formulated as a sterile, clear, colorless to pale yellow solution at a concentration of 1 mg/mL in pH 7.4 phosphate buffered saline that contains 0.3% polysorbate 80 (PS 80), and will be provided in a glass vial, stoppered and crimp-sealed with a teal flip cap for IVT administration. Each vial contains 1 mg/mL of UBX1325 and is *single-use only*. UBX1325 *must be stored at -20°C* upon receipt.

UBX1325 will be provided to the investigational site along with a Pharmacy Manual, which details clinical product presentation and dilutions to be prepared using aseptic technique. Sterile diluent (pH 7.4 phosphate buffered saline) will be provided in a glass vial with a white flip cap along with sterile syringes and sterile needles for the preparation of the Study Drug. Each vial ofsterile diluent is *single use only*.

Post-dilution solutions for clinical administration will be held no more than 1 hour at room temperature prior to administration to the patient.

Storage and dose preparation of Study Drug will be conducted in accordance with instructions in the Pharmacy Manual and under aseptic conditions.

The Investigator is responsible for drug accountability at the investigational site, appointing a qualified individual to oversee the storage, preparation, and dispensing of Study Drug, and keeping records of such activity in accordance with the requirements of the Sponsor.

All used and unused Study Drug must be stored at site and stored in accordance with the directions given in the Pharmacy Manual.

6.2 Method for Assigning Patients to the Treatment Group

After a patient has been identified as potentially eligible for the study, the patient will be invited to participate. If the patient agrees to study participation, written informed consent

will be obtained before any study-specific procedures are performed. Patients who have consented to participate in the study will be assigned a unique number.

After the patient has been consented and completed all screening procedures confirming eligibility, they may proceed to Day 1, where they will be randomized in a 1:1 ratio to UBX1325 or sham arm, stratified by most recent anti-VEGF treatment that patients received prior to Day 1. There will be two strata: 1) aflibercept vs 2) ranibizumab or bevacizumab. Patients will be randomly allocated to receive treatment through the randomization system, which administers the randomization code generated by the Sponsor's biostatistician or designee. Following receipt of the treatment assignment, the site staff will prepare the Study Drug as instructed by the Pharmacy Manual.

Patients who are randomized but do not receive treatment may be replaced.

6.3 Randomization Code Creation and Storage

Randomization personnel of the Sponsor or designee will generate the randomization schedule prior to the start of the study. An interactive web response system will be used for subject randomization. All randomization information will be stored in a secured area, accessible only by authorized personnel.

6.4 Administration of UBX1325

UBX1325 is administered via IVT injection. Following the IVT injection, patients will be monitored for elevation of IOP, decreased optic nerve head perfusion, and for possible injection complications (e.g., vitreous hemorrhage, retinal tears, etc.). Additionally, patients should immediately report any symptoms suggestive of endophthalmitis, such as ocular pain, swelling, redness, haze and gradual loss of vision.

Patients may be prescribed prophylactic antibiotic eye drops following administration of UBX1325 per the site's preferred practice patterns. Such drops, if prescribed, should be documented in the patient's eCRF.

6.5 Administration of Sham Procedure

Control will be a sham procedure (i.e., needleless, empty sterile syringe) touched to the surface of the study eye to mimic an IVT injection. Please refer to the current Pharmacy Manual for details on how to prepare and administer the sham procedure.

Patients may be prescribed prophylactic antibiotic eye drops following a sham procedure per the site's preferred practice patterns. Such drops, if prescribed, should be documented in the patient's eCRF.

6.6 Masked and Unmasked Study Staff

This study will be double-masked. Patients, Investigators, visual acuity technicians, photographers, reading center personnel and the Sponsor are masked. Sites will have a qualified injector who is unmasked in order to perform the study treatment injection or sham

procedure, as well as certain post-injection assessments on Day 1 only. Specifically, the unmasked injector will do the anterior and posterior segment exams, IOP and AE assessment. Other supporting activities such as study drug preparation, study drug accountability, as well as screening assessments as needed, will be performed by designated unmasked personnel. Unmasked personnel should not be involved in any other study procedures outside of Day 1. Subsequent visits (Day 2 and onward) should be conducted by masked staff. It is important the randomization code is not revealed to anyone in order to maintain the integrity of the double-masking.

6.7 Extent and Maintenance of Double-Masking

In addition to the patients and Investigators being masked, any Sponsor or designee team members who are actively engaged with the site will be masked.

There will be no planned unmasking. If there is an unplanned or unintentional unmasking, the randomization system will be used to manage the roles and permissions with respect to the ability to break the masking. The Investigator must contact the Medical Monitor to discuss any need to unmask, unless medical emergency dictates otherwise. The randomization code and records related to the unmasking will be filed as essential study documents.

A patient who has unplanned or unintentional unmasking during the study will be asked to continue in the study through Week 48 for safety follow-up (AE and concomitant medication reporting, vital signs, physical examinations and laboratory testing).

6.8 Permitted and Prohibited Concomitant Treatments

6.8.1 Permitted Treatments

Permitted concomitant treatments in/for the **study eye and non-study eye** include:

- Topical antibiotics administered prophylactically with IVT injection and topical/systemic antibiotics used for AE treatment.
- Artificial tears
- Steroid use is permitted only for the following conditions:
    - Topical (e.g., for atopic dermatitis treatment), inhaled (e.g., for asthma treatment), or locally injected (e.g., for epidural or joint injection)
    - Topical use of steroids in the fellow eye
    - Topical use of steroids for treatment of inflammation in the study eye (e.g., uveitis).

Concomitant intravitreal therapy in the **non-study eye** is permitted but cannot be administered within 7 days of treatment with masked study drug.

6.8.2 Prohibited Treatments

Patients will not be permitted to received treatments for the management of DME in the study eye, once randomized to this study, unless it is a rescue treatment (see Section 5.1.11).

Systemic (oral, intramuscular, and intravenous) steroids are not allowed unless for an AE treatment. All systemic steroids should be discontinued before the study drug administration.

Patients should not have received any therapy that would preclude an IVT injection or can potentially exhibit retinal toxicity (such as tamoxifen, hydroxychloroquine and trastuzumab).

Patients are prohibited from receiving any medication for the study eye that, in the opinion of the Investigator and/or the Medical Monitor, may have an effect on the study results.

Patients should not have received brolucizumab as their most recent anti-VEGF treatment. Historical use of brolucizumab is not excluded.

7 SAFETY MONITORING

At each visit, patients will undergo ophthalmologic evaluation and assessment for signs of potential toxicity. Safety monitoring activities will be an ongoing activity done by the Sponsor. The SAC will convene on an ad hoc basis to assess the ongoing safety of the clinical trial.

For all patients, all acquired data may be reviewed by the SAC. This process will be detailed in the SAC Charter.

7.1 Definitions

- **AE**: An AE is any untoward medical occurrence associated with the use of an intervention in humans whether or not it is considered intervention related.
- **Suspected unexpected serious adverse reaction** (SUSAR): A serious adverse reaction (AR) that is unexpected based on current product information.
- **Serious adverse event (SAE)**: An event is considered "serious" if, in the view of either the Investigator or the Sponsor, it results in any of the following outcomes:
    - Death
    - A life-threatening AE (An event is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected AR that, had it occurred in a more severe form, might have caused death)
    - Inpatient hospitalization or prolongation of existing hospitalization
    - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
    - A congenital anomaly/birth defect
    - Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.
- **Causality or relatedness**: AEs should be considered probably or possibly treatment-related, unless they fulfill the following criteria (in which circumstances it should be considered unlikely related or unrelated):
    - Evidence exists that the AE has an etiology other than the Study Drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication), and/or

- The AE has no plausible temporal relationship to administration of the Study Drug (e.g., a new cancer diagnosed 2 days after first dose of Study Drug).

  Relatedness to Study Drug will be graded as "probably," "possibly," "unlikely," or "unrelated" as follows:

Probably related: The AE

- Follows a reasonable temporal sequence from drug administration
- Abates upon discontinuation of the drug
- Cannot be reasonably explained by the known characteristics of the patient's clinical state

Possibly related: The AE

- Follows a reasonable temporal sequence from drug administration
- Could have been produced by the patient's clinical state or by other modes of therapy administered to the patient

Unlikely related: The AE

- Is most likely to be explained by the patient's clinical state or by other modes of therapy administered to the patient

Unrelated: The AE

- Does not follow a reasonable sequence from drug administration
- Is readily explained by and considered by the Investigator to be an expected complication of the patient's clinical state, concurrent medical conditions, or by other modes of therapy administered to the patient

- **AR**: An AR is any AE caused by a drug.
- **Suspected adverse reaction** (SAR): An SAR is any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of the investigational new drug (IND) safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the AE. SAR implies a lesser degree of certainty about causality than AR.
- **Unexpected**: An event is considered unexpected if it is not listed in the IB, is not listed at the specificity or severity that has been observed, or, if an IB is not required or available, is not consistent with the risk information described in the General Investigational Plan or elsewhere in the IND. Unexpected also refers to events that are mentioned in the IB as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

- **Severity or intensity**: The severity of an event describes the degree of impact upon the patient and/or the need for medical care necessary to treat the event. AEs reported for patients participating in this study will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (NCI CTCAE v4.03). The Investigator will grade the severity of each AE using, when applicable, the NCI CTCAE v4.03. For AEs not included in the NCI CTCAE v4.03, the criteria outlined in Table 2 shouldbe used as a general guideline.

Table 2 Grading for Adverse Events Not Covered in the NCI CTCAE

Severity	Description
Grade 1 – Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2 – Moderate	Minimal, local or noninvasive intervention indicated; limited age-appropriate instrumental ADL
Grade 3 – Severe	Medically significant but not life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4 – Life-threatening	Life-threatening consequences; urgent intervention indicated
Grade 5 – Fatal	Death

ADL = activities of daily living; NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

7.2 Documenting Adverse Events

7.2.1 Timeframe for Collection

AEs and SAEs will be collected from the time of treatment through the last follow-up visit at Week 48. All events prior to this will be documented as medical history.

The Investigator must follow up on all AEs through Week 48. Non-serious AEs may be followed to resolution past the patient's last study visit at the discretion of the Investigator and/or MedicalMonitor if in the best interest of the patient and the assessment of safety of UBX1325. The Investigator must follow up on all SAEs until the events have subsided, returned to Baseline, or,in case of permanent impairment, the condition stabilizes.

7.2.2 Classification of Events

Although AEs should be based on the signs or symptoms detected during the physical examination and on the clinical evaluation of the patient, a specific diagnosis should be reportedas the AE whenever feasible. In addition to the information obtained from those sources, the patient should be asked the following nonspecific question: "How have you been feeling since your last visit?" Signs and symptoms should be recorded using standard medical terminology.

7.3 Reporting Adverse Events

All AEs and SAEs must be recorded on source documents and collected in the electronic data capture (EDC) system. Any unanticipated risks to the patients must be reported by the Investigator promptly to the Sponsor and IRB/IEC.

All SAEs, whether or not deemed drug-related or expected, must be reported by the Investigator or qualified designee within 24 hours of first becoming aware of the event. The Investigator or qualified designee will enter the required information regarding the SAE into the appropriate module of the eCRF, which will automatically result in distribution of the information to the Sponsors Pharmacovigilance provider. If the eCRF system is temporarily unavailable, the event,including the Investigator-determined causality to Study Drug, should be reported via fax using an SAE form to the appropriate Sponsor or Pharmacovigilance contact. Upon return of the availability of EDC system, the SAE information must be entered on the SAE eCRF.

The Sponsor (or designee) will process and evaluate all SAEs as soon as the reports are received. For each SAE received, the Sponsor will make a determination as to whether the criteria for expedited reporting have been met. The Medical Monitor should also be contacted immediately for any fatal or life-threatening SAE that is considered possibly or probably related to Study Drug.

The Sponsor (or designee) is responsible for reporting relevant SAEs to the relevant regulatory authorities and participating Investigators, in accordance with FDA regulations 21 Code of Federal Regulations 312.32, International Conference on Harmonisation (ICH) Guidelines, European Clinical Trials Directive (Directive 2001/20/European Commission (EC), and/or local regulatory requirements and monitoring the safety profile of the Study Drug. To meet this requirement, the Sponsor (or designee) may request additional information from the sites including, but not limited to, hospitalization records, discharge summaries, or autopsy reports. Any requests for such information should be addressed in a timely manner. Additionally, any SAE considered by an Investigator to be possibly or probably related to the Study Drug that is brought to the attention of the Investigator at any time outside of the time period specified for SAE reporting also must be reported immediately to one of the individuals listed on the Sponsorcontact information page.

Reporting of SAEs by the Investigator to the IRB or IEC will be done in accordance with the standard operation procedures and policies of the IRB/IEC. Adequate documentation must be maintained showing that the IRB/IEC was properly notified.

7.4 Adverse Events of Special Interest

No AEs of special interest have been identified for UBX1325 to date.

7.5 Clinical Laboratory Findings

Clinical laboratory tests will include the analytes in Table 3. Patients should be in a seated or supine position during blood collection.

Table 3 Laboratory Parameters

Hematology:	Serum Chemistry:
• Hematocrit • Hemoglobin • Platelet count • RBC count • Mean corpuscular volume • WBC count with differential	• Albumin • Alkaline phosphatase • Alanine aminotransferase • Aspartate aminotransferase • Blood urea nitrogen • Calcium • Carbon dioxide • Chloride • Creatinine[a] • Globulin • Glucose • HbA1c (Screening)[b] • Human chorionic gonadotropin[c] • Lactate dehydrogenase • Phosphorus • Potassium • Sodium • Total and direct bilirubin • Total cholesterol • Total protein

HbA1c = hemoglobin A1c; RBC = red blood cell; WBC = white blood cell

[a] Creatinine clearance will be calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method.

[b] All patients will be tested for HbA1c; fasting is not required.

[c] Serum human chorionic gonadotropin is required only for females who are of childbearing potential.

7.6 Pregnancy

Although not considered an SAE, cases of pregnancy exposure by parent to the Study Drug must be recorded, reported, and followed up as indicated for an SAE. After the patient has been enrolled in the study and received Study Drug by IVT injection, the Investigator must report immediately (within 24 hours or next business day whichever is the shorter) any drug exposure during pregnancy to the Sponsor using the Sponsor-supplied Pregnancy Reporting Forms, using the same contact method for SAE reporting. Information about exposure in pregnancy encompasses the entire course of pregnancy and delivery and perinatal and neonatal outcomes, even if there were no abnormal findings. All reports of pregnancy must be followed for information about the course of the pregnancy and delivery, as well as the condition of the newborn. When the newborn is healthy, additional follow-up is not needed. Pregnancies occurring up to 12 weeks after administration of the Study Drug must be reported to the Investigator.

7.7 Overdose or Misuse

Although not considered an SAE, cases of overdose (e.g., a dose higher than that indicated in the protocol, with or without an AE) must be recorded, reported, and followed up as indicated foran SAE.

8 STATISTICAL METHODS

8.1 General Considerations

All study parameters will be listed, and a selected list of parameters will be summarized descriptively by dose cohort and overall. The descriptive statistics will include number of patients, mean, standard deviation, median, minimum, and maximum for continuous parameters and frequency and percent for categorical parameters. No imputation of values for missing data will be performed. Details about the statistical analyses for this study will be provided in the Statistical Analysis Plan (SAP), which will be developed and finalized before database lock.

8.2 Determination of Sample Size

Assuming the difference of change from Baseline in BCVA between UBX1325 and sham is 8 letters, the standard deviation is 13 letters, and the drop-out rate is 5%, a sample size of 62 subjects (31 per group) provides 80% power to detect the difference at a two-sided significance level of 0.15.

8.3 Analysis Populations

8.3.1 Full Analysis Set

The Full Analysis Set will include all randomized subjects who received a single IVT treatment of UBX1325 or sham, have a baseline CST assessment, and at least one post-baseline CST assessment. The Full Analysis Set will be the primary population for evaluating all efficacy variables.

8.3.2 Safety Analysis Set

The Safety Analysis Set will include all subjects who received a single IVT treatment of UBX1325 or sham. The Safety Analysis Set will be the primary population for evaluating all safety variables and subject characteristics.

8.3.3 PK Analysis Set

The PK Analysis Set will include subjects who received a single IVT treatment of UBX1325 and have at least one evaluable PK concentration after injection. PK blood draws were done at a subset of sites in the United States and all patients enrolled at these sites up until the approval of the Protocol Amendment V4.0had their blood drawn for PK analysis.

8.4 Demographics and Baseline Characteristics

Demographics and baseline characteristics include sex, age, race, ethnicity, weight, height, and other parameters as appropriate. These variables will be listed and summarized using descriptive statistics for each dose cohort and overall, based on the Safety Analysis Set.

Medical history will be summarized using Medical Dictionary for Regulatory Activities (MedDRA) terms. Prior medications will be tabulated using World Health Organization (WHO) Drug Dictionary terms.

8.5 Efficacy Analysis

The Full Analysis Set will be used for all efficacy analyses.

The BCVA will be assessed using the ETDRS chart starting at 4 meters at each study visit. Change in BCVA from baseline to each visit will be analyzed by mixed model for repeated measures (MMRM). The details of MMRM and the analysis methodsfor other efficacy endpoints described in Section 3 will be provided in the SAP.

8.6 Safety Analysis

The Safety Analysis Set will be used for all safety analyses. The safety variables include the incidence of ocular and systemic safety and tolerability and will be summarized descriptively.

AEs will be coded using the MedDRA. Subjects with any AEs will be tabulated by system organ classification and preferred term specified in the MedDRA. AEs will also be summarized by relationship to treatment and maximum severity. In addition, SAEs and discontinuations due to AEs will be summarized.

8.6.1 Exploratory Analysis

Additional exploratory analyses may be carried out to better inform interpretation of treatment effects depending on examination of the summary statistics originally planned as mentioned above and in the SAP.

8.6.2 Pharmacokinetics Analysis

Analysis of PK parameters will be based on the PK Analysis Set. PK of UBX1325 and UBX0601in plasma following IVT injection of UBX1325 will be obtained at the time points indicated in the Schedule of Events effective through the Protocol Amendment Version 4.0. Mean (SD) plasma concentrations of UBX1325 and/or UBX0601 will be summarized graphically and PK parameters will be estimated when data allow. Individual PK parameters (e.g., time at which the maximum concentration is observed [T_{max}], maximum concentration observed [C_{max}], area under the plasma concentration-time curve extrapolated to the last observed concentration [$AUC_{0\text{-}t}$], area under the plasma concentration-time curve extrapolated to infinity [$AUC_{0\text{-}inf}$], percentage of area under the plasma concentration-time curve extrapolated after last observed concentration [$AUC_{\%extrap}$], apparent total clearance of the drug from plasma after IVT injection [CL/F], apparent volume of distribution after non-intravenous administration [Vd/F], half-life [$t_{1/2}$]) will be calculated using non-compartmental analyses (NCA) when available data supports such calculations. Additional PK parameters may be derived, if deemed appropriate. Descriptive statistics will be used to characterize the inter-patient variability of the PK parameters by cohort. Descriptive statistics will include patients per cohort, arithmetic mean, geometric mean, mean, median, standard deviation, %CV, value range (minimum and maximum), as

appropriate. The PK analysis plan will be detailed in the SAP and more specifically, applied to patient samples collected at the subset of participating sites through the approval of Protocol Amendment Version 4.0.

8.7 Planned Interim Analysis

Formal interim analyses will be performed at 12 weeks and other possible timepoints in the study. The SAP will specify what proportion of patients data is needed for the interim analyses.

9 ETHICAL CONSIDERATIONS

9.1 Good Clinical Practice

This study will be conducted in compliance with the protocol approved by the IRB/IEC, and in accordance with ICH Good Clinical Practice (GCP) standards. Any amendments to the protocolor changes to the consent document will be approved by the IRB/IEC before implementation of that amendment. The study will be conducted in accordance with the ethical principles, which have their origins in the Declaration of Helsinki.

9.2 Ethics Review

The study and any amendments will be reviewed by an appropriately constituted and composed IRB/IEC. Written IRB approval for the Protocol, amendments, ICF, and Investigator(s) will be obtained in accordance with GCP. The IRB/IEC will be notified of SAEs in accordance with IRB/IEC Policy.

9.3 Informed Consent

An initial sample ICF is provided for the Investigator and IRB/IEC to prepare the informed consent document to be used at his or her site. The site-specific informed consent document will be submitted for review to the central IRB and the IRB-approved informed consent document will be held in the site study file and in the Sponsor's Trial Master File.

The ICF is to be prepared in the language(s) of the potential patient population for this study. The languages under consideration are English, French and Spanish.

Before a patient's participation in the clinical study, the Investigator is responsible for obtaining written informed consent from the patient after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study and before any protocol specific screening procedures or any Study Drug is administered.

The Investigator is also responsible for asking the patient if the patient has a primary care physician and if the patient agrees to have his/her primary care physician informed of the patient's participation in the clinical study. If the patient agrees to such notification, the Investigator is to inform the patient's primary care physician of the patient's participation in the clinical study. If the patient does not have a primary care physician and the Investigator will be acting in that capacity, the Investigator is to document such in the patient's medical record. The acquisition of informed consent and the patient's agreement or refusal of his/her notification of the primary care physician, if relevant, is to be documented in the patient's medical records, andthe ICF is to be signed and personally dated by the patient and by the person who conducted the informed consent discussion. The original signed ICF is to be retained in accordance with institutional policy, and a copy of the signed consent form is to be provided to the patient.

9.4 Data Privacy

All study-related laboratory and clinical data gathered in this protocol will be stored in a password-protected database. All patient information will be handled using anonymous identifiers. Linkage to patients' study data is only possible after accessing a password-protected database. Access to the database is only available to individuals directly involved in the study.

Patient personal health information that is accessed for this study will not be reused or disclosed to any other person or entity, or for other research.

9.5 Financial Disclosure

In connection with the clinical study described in the protocol, the Investigator certifies that the Investigator will read and answer the Clinical Investigator Financial Disclosure Form truthfully and to the best of Investigator's ability. The Investigator also certifies that, if asked, the Investigator will have any other applicable parties (e.g., Sub-Investigators) read and answer the Clinical Investigator Financial Disclosure Form as a condition of their participation in the study. Ifthe financial interests reported on the Clinical Investigator Financial Disclosure Form change during the course of the study or within 1 year after the last patient has completed the study as specified in the protocol, the Investigator and the other applicable parties are obligated to update the Sponsor of financial disclosure in accordance with the Sponsor's standard procedures.

9.6 Biological Specimens and Data

Biological samples should not be destroyed at the end of the study. Samples should be sent to the Sponsor's designated laboratory as detailed in the region-specific Laboratory Manual. Samples may be tested for exploratory endpoints or additional further research after consent has been obtained by patients participating at US based sites.

10 OVERSIGHT

10.1 Safety Assessment Committee

A SAC will be established and available ad hoc to support pharmacovigilance activities for the study, such as adjudication of AEs or possible safety signals. The SAC Charter will describe the committee's structure, roles, and responsibilities.

10.2 Quality Control and Assurance

10.2.1 Monitoring and Audits

The Investigator will permit regular study-related monitoring by the Sponsor or designee, audits, IRB/IEC review, and regulatory inspections by providing direct access to source data and documents.

10.2.2 Protocol Deviations

Protocol violations/deviations will be documented in accordance with good documentation practice and reported to the IRB/IEC in accordance with IRB/IEC Policy. In case of a deviation necessary to eliminate an immediate hazard to a research participant, the deviation will be reported to the IRB/IEC as soon as possible. Investigational sites should make every effort to adhere to the processes and procedures described in this protocol.

10.2.3 Records

10.2.3.1 Data Capture and Management

An EDC system will be designed and managed on behalf of the Sponsor by the Sponsor's designated contract research organization. Clinical data will be entered by study site personnel within 5 business days of the patient visit or activity conduct. Monitoring of the study will be conducted on site by a designee of the Sponsor (clinical research associate [CRA]), who will conduct document and source data review, as well as remote data monitoring in the intervals between monitoring visits. Data will be reviewed remotely by the Medical Monitor for safety oversight.

Investigator

All study-related information will be recorded on source documents. All required study data will be recorded in the eCRFs. All eCRF data must be submitted to the Sponsor throughout and at the end of study in a timely and accurate manner.

If an Investigator retires, relocates, or otherwise withdraws from conducting the study, the Investigator must notify the Sponsor to agree upon an acceptable storage solution.

Regulatory agencies will be notified with the appropriate documentation.

Sponsor

The data will be checked for completeness and correctness in real-time online.

Data are checked as they are entered into the EDC system. Offline checks will also be run to assess the need for additional data review. Discrepancy reports will be generated and transferred to the study center for resolution by the Investigator or its designee.

10.2.3.2 Records Retention

The Investigator shall retain and preserve one copy of all data generated in the course of the study, specifically including, but not limited to, those defined by GCP as essential, for the duration of (i) 2 years after the last marketing authorization for the Study Drug has been approved or the Sponsor has discontinued its research with respect to such Study Drug or (ii) such longer period as required by applicable global regulatory requirements. At the end of such period, the Investigator shall notify the Sponsor, in writing, of the intent to destroy all such material. The Sponsor shall have 30 days to respond to the Investigator's notice, and the Sponsor shall have a further opportunity to retain such materials at the Sponsor's expense.

11 PUBLICATION POLICY

Any formal presentation or publication of data collected from this study will be considered as a joint publication by the Investigator(s) and the appropriate personnel at UNITY Biotechnology,Inc.

Authorship of any publications resulting from this study will be mutually agreed and determined on the basis of the Uniform Requirement for Manuscripts submitted to Biomedical Journals (International Committee of Medical Journal Editors), which states the following:

- Authorship credit should be based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or critically revising it for important intellectual content; (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.
- When a large, multicenter group has conducted the work, the group should identify the individuals who accept direct responsibility for the manuscript. These individuals should fully meet the criteria for authorship defined above.
- Acquisition of funding, collection of data, or general supervision of the research group, alone, does not justify authorship.
- All persons designated as authors should qualify for authorship, and all those who qualify should be listed.
- Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content. (Additional information on the current guidelines for publications can be found at the following location: http://www.icmje.org/).
- All publications (e.g., manuscripts, abstracts, oral/slide presentations, or book chapters) based on this study must be submitted to the Sponsor, for review. The Clinical Trial Agreement among the institution, Investigator, and the Sponsor will detail the procedures for, and timing of, the Sponsor’s review of publications.

12 FINANCING AND INSURANCE

Financial disclosure, site budget, and any insurance policies relevant to this clinical study are described in each Clinical Trial Agreement.

13 REFERENCES

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