DOCUMENT TYPE	STUDY PROTOCOL
PROTOCOL TITLE:	A PHASE 2, RANDOMISED, DOUBLE-MASKED, SHAM-CONTROLLED, MULTI-CENTRE STUDY TO EVALUATE THE EFFICACY AND SAFETY OF O**CRI**PLASMIN IN INDUCING TOTAL POSTERIOR VIT**R**EOUS DETA**C**HMENT (PVD) IN SUBJECTS WITH NON-PRO**L**IFERATIVE DIAB**E**TIC RETINOPATHY (NPDR) **(CIRCLE)**
PROTOCOL NUMBER:	TG-MV-015
CLINICALTRIALS.GOV IDENTIFIER:	NCT02681809
PROTOCOL DATE:	28-OCT-2016
PROTOCOL VERSION	AMENDMENT 2

CLARIFICATION NOTE

Recruitment in the study was discontinued early due to slow recruitment rate. As a consequence, a total of 48 subjects were randomised instead of the planned 115.

This led to the following changes to the analyses planned in the study protocol:

- A single analysis dataset was used instead of the 3 listed in the protocol.
- The first and third quartiles, and 5^{th} and 95^{th} percentiles were not included in the summary statistics of continuous variables.
- No statistical testing was performed.
- The last-observation-carried-forward method for data imputation was not used, and no sensitivity analysis was performed.
- The stratification variable, NPDR severity, was not taken into account in any summary statistics.
- The following exploratory endpoints were not summarised:
  - A ≥ 2-step progression on the ETDRS Severity Scale from baseline, at Month 15 and at Month 24, based on 7 standard field stereo colour fundus photography, as assessed by the masked CRC
  - Improvement of ≥ 2 steps on the ETDRS Severity Scale from baseline, at Month 15 and at Month 24, based on 7 standard field stereo colour fundus photography, as assessed by the masked CRC
  - Neovascularisation as compared to baseline, at Month 15 and at Month 24, based on 7 standard field stereo colour fundus photography, as assessed by the masked CRC
  - Vitreous / pre-retinal haemorrhage by Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
  - Development of iris neovascularisation as compared to baseline, at each study visit, based on slit lamp examination

PROTOCOL TITLE:	A PHASE 2, RANDOMISED, DOUBLE-MASKED, SHAM-CONTROLLED, MULTI-CENTRE STUDY TO EVALUATE THE EFFICACY AND SAFETY OF O**C**R**I**PLASMIN IN INDUCING TOTAL POSTERIOR VIT**R**EOUS DETA**C**HMENT (PVD) IN SUBJECTS WITH NON-PRO**L**IFERATIVE DIAB**E**TIC RETINOPATHY (NPDR) **(CIRCLE)**
PROTOCOL NUMBER:	TG-MV-015
STUDY PHASE:	PHASE 2
EudraCT NUMBER:	2015-002415-15
IND NUMBER:	126449
SPONSOR:	THROMBOGENICS NV GASTON GEENSLAAN 1 B-3001 LEUVEN BELGIUM
FINAL PROTOCOL DATE:	04-SEP-2015
AMENDMENT 1 DATE:	13-OCT-2015
AMENDMENT 2 DATE:	28-OCT-2016

STUDY CONTACTS

Table 1: Contact Information

Role in Study	Name	Address and Telephone Number
Sponsor Medical Expert	[redacted]	Gaston Geenslaan 1 B-3001 Leuven Belgium [redacted]

PERSONS AUTHORISED TO SIGN PROTOCOL AND AMENDMENTS

Table 2: Persons Authorised to Sign Protocol and Amendments

Name	**Name**
[redacted]	[redacted]
Title	**Title**
Sponsor Medical Expert	Global Head of Development

SPONSOR SIGNATURE PAGE

PROTOCOL TG-MV-015

AMENDMENT 2 DATE: 28-OCT-2016

A PHASE 2, RANDOMISED, DOUBLE-MASKED, SHAM-CONTROLLED, MULTI-CENTRE STUDY TO EVALUATE THE EFFICACY AND SAFETY OF O**C**R**I**PLASMIN IN INDUCING TOTAL POSTERIOR VIT**R**EOUS DETA**C**HMENT (PVD) IN SUBJECTS WITH NON-PRO**L**IFERATIVE DIAB**E**TIC RETINOPATHY (NPDR) **(CIRCLE)**

On behalf of ThromboGenics

Signed:_____ _____

[redacted]

Global Head of Development

Date

INVESTIGATOR'S AGREEMENT

PROTOCOL TG-MV-015

AMENDMENT 2 DATE:	28-OCT-2016

A PHASE 2, RANDOMISED, DOUBLE-MASKED, SHAM-CONTROLLED, MULTI-CENTRE STUDY TO EVALUATE THE EFFICACY AND SAFETY OF O**C**R**I**PLASMIN IN INDUCING TOTAL POSTERIOR VIT**R**EOUS DETA**C**HMENT (PVD) IN SUBJECTS WITH NON-PRO**L**IFERATIVE DIAB**E**TIC RETINOPATHY (NPDR) **(CIRCLE)**

I have read the protocol and by my signature below I agree that I will conduct this protocol as outlined therein and will make a reasonable effort to complete the study within the time designated. I agree to conduct this study in compliance with the Declaration of Helsinki, the ICH guideline for Good Clinical Practice, all applicable local and federal regulatory requirements and state / local customs or laws.

I will provide copies of the protocol and access to information furnished by ThromboGenics to study personnel under my supervision. I will discuss this material with them to ensure they are fully informed about the drug and the study.

I have read the Investigator's Brochure. I agree to ensure the confidentiality of my subjects; however, I agree to make available to the CRO, the Sponsor of this clinical study or relevant regulatory authorities those sections of my subjects' medical records which directly concern this study. I understand that the study may be terminated or enrolment suspended at any time by ThromboGenics, or by me if it becomes necessary to protect the best interests of the study subjects.

Signed:_____ _____

ADD INV. NAME AND TITLE Date

PROTOCOL SYNOPSIS

Name of Sponsor / Company:
ThromboGenics

Name of Test Products:
ocriplasmin 0.0625mg
ocriplasmin 0.125mg

Name of Active Ingredient:
ocriplasmin

Protocol Title:
A Phase 2, randomised, double masked, sham controlled, multi-centre study to evaluate the efficacy and safety of o**cri**plasmin in inducing total posterior vit**r**eous deta**c**hment (PVD) in subjects with non-pro**l**iferative diab**e**tic retinopathy (NPDR) **(CIRCLE)**

Protocol Number:
TG-MV-015

Study Period:	**Phase of Development:**
Estimated date first subject enrolled: December 2015 Estimated date last subject completed: July 2019	2

Objective:

- To assess the efficacy and safety of up to 3 intravitreal injections of ocriplasmin (0.0625mg or 0.125mg), in subjects with moderate to very severe NPDR, to induce total PVD in order to reduce the risk of disease progression to proliferative diabetic retinopathy (PDR)

Number of Subjects (Planned):
Approximately 115 subjects (approximately 46 in each ocriplasmin arm and 23 in the sham arm)

Study Population:
Diabetic subjects with moderate to very severe NPDR (International Clinical Diabetic Retinopathy Disease Severity Scale [ETDRS] Levels 43A -53E), who do not have total PVD

Study Design Overview:

- **Study design.** Multi-centre, randomised, sham-controlled, double-masked, Phase 2 clinical study with 3 parallel treatment arms
- **Study arms and treatments.**
    - **Ocriplasmin 0.0625mg arm.** Approximately 46 subjects will receive up to 3 intravitreal injections of ocriplasmin 0.0625mg, approximately 1 month apart. All subjects assigned to this arm will receive an intravitreal injection of ocriplasmin 0.0625mg at Day 0. These subjects will be re-treated with ocriplasmin 0.0625mg at Day 35 and at Day 70 if, by that time, they have not achieved total PVD on both B-scan ultrasound and spectral domain optical coherence tomography (SD-OCT), as assessed by the masked B-scan expert reader and the masked central reading centre (CRC), respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s)

- **Ocriplasmin 0.125mg arm.** Approximately 46 subjects will receive up to 3 intravitreal injections of ocriplasmin 0.125mg, approximately 1 month apart. All subjects assigned to this arm will receive an intravitreal injection of ocriplasmin 0.125mg at Day 0. These subjects will be re-treated with ocriplasmin 0.125mg at Day 35 and at Day 70 if, by that time, they have not achieved total PVD on both B-scan ultrasound and SD-OCT, as assessed by the masked B-scan expert reader and the masked CRC, respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s)
- **Sham arm.** Approximately 23 subjects will receive 3 sham injections, approximately 1 month apart. All subjects assigned to this arm will receive a sham injection at Day 0, Day 35 and Day 70

- **Test product, dose and mode of administration.** Up to 3 intravitreal injections of ocriplasmin 0.0625mg or ocriplasmin 0.125mg
- **Reference therapy (comparator), dose and mode of administration.** 3 sham injections
- **Treatment allocation**. Eligible subjects will be sequentially assigned according to a computer-generated randomisation list to the ocriplasmin 0.0625mg arm, the ocriplasmin 0.125mg arm or the sham arm in a 2:2:1 allocation ratio. Randomisation will be stratified by baseline NPDR severity (moderate and moderately severe, *i.e.* ETDRS Level 43A-47D *vs.* severe and very severe NPDR, *i.e.* ETDRS Level 53A-E). It is expected that more subjects with moderate and moderately severe than with severe and very severe NPDR will be enrolled, as a reflection of the natural population. Therefore, enrolment in the moderate and moderately severe NPDR stratum may become capped in the course of the study
- **Study duration**. For each subject enrolled, the study duration will be approximately 24 months from the first injection

Inclusion and Exclusion Criteria:

Subjects can have only 1 eye treated as part of this study. For eligible subjects where both eyes are eligible for injection, the eye with the more advanced NPDR stage (as determined by the CRC) will be selected as the study eye.

Inclusion Criteria

- Male or female aged 18 years or older
- Best corrected visual acuity (BCVA) of 65 letters read or greater (Snellen equivalent of 20/50 or better) in the study eye
- BCVA of 20 letters read or greater (Snellen equivalent of 20/400 or better) in the fellow eye
- Clear ocular media for adequate fundus imaging in the study eye
- HbA1c $\leq$ 12%, as assessed by the central laboratory
- Moderate to very severe NPDR as per ETDRS Severity Scale (Levels 43A-53E), based on 7-standard field stereo colour fundus photograph, as assessed by the CRC
- Central subfield thickness (CST) of $\leq$ 340µm on Spectralis SD-OCT or $\leq$ 320µm on non-Spectralis SD-OCT in the study eye, as assessed by the CRC, with or without mild centre-involved DME (CI-DME) (mild CI-DME is defined as cysts or intraretinal fluid in the central subfield on SD-OCT that in the Investigator's opinion will not require treatment with an anti-VEGF for at least up to 3 months after randomisation)
- No evidence of total PVD in the study eye, based on both B-scan ultrasound and SD-OCT, as assessed by the B-scan expert reader and the CRC, respectively

- Written informed consent obtained from the subject prior to screening procedures

Exclusion Criteria

- History of or current ocular condition in the study eye that may interfere with the assessment of the progression to PDR (*e.g.* vitreomacular traction [VMT], exudative age-related macular degeneration [AMD], retinal vein occlusion [branch or central vein], uveitis, angioid streaks, histoplasmosis, toxoplasmosis, rhegmatogenous retinal detachment, retinal tear, fibrovascular proliferation, lattice degeneration, macular hole, ocular tumours)
- Significant ocular trauma in the study eye within 6 months prior to screening (including corneo-scleral laceration, lens subluxation, cryo-retinopexy)
- Corneal, lenticular, or ocular media abnormalities in the study eye that preclude observation with the slit lamp or accurate readings with a tonometer
- Presence of epiretinal membrane in the study eye, based on SD-OCT, as assessed by the CRC
- Presence of foveal ischemia in the study eye, based on fluorescein angiograph, as assessed by the CRC
- Presence of pre-retinal or vitreous haemorrhage in the study eye
- Presence of iris or angle neovascularisation in the study eye
- Any active ocular / intraocular infection or inflammation in either eye (*e.g.* blepharitis, infectious conjunctivitis, keratitis, scleritis, endophthalmitis, uveitis)
- Uncontrolled glaucoma in the study eye (uncontrolled glaucoma is defined as intraocular pressure [IOP] ≥ 26mmHg despite treatment with anti-glaucoma medication)
- More than 8D high myopia in the study eye
- Aphakic study eye
- Previous treatments / procedures as follows:

Treatment / Procedure In the Study Eye	**Excluded Period prior to Randomisation**
Vitrectomy	Any time
Pan-retinal photocoagulation (PRP)	Any time
Intraocular surgery	4 months
Focal / grid laser photocoagulation	3 months
Intravitreal anti vascular endothelial growth factor (anti-VEGF)	1 month
Topical ocular steroids	1 month
Intravitreal and peri-ocular steroids	4 months
Steroid implants	Any time

- Uncontrolled hypertension in the opinion of the Investigator (*e.g.* systolic blood pressure > 160mmHg or diastolic blood pressure > 100mmHg for at least 30 days prior to screening despite antihypertensive treatment, or any finding in the Investigator's opinion suggesting hypertensive retinopathy)
- Pseudoexfoliation, Marfan's syndrome, phacodonesis or any other finding in the Investigator's opinion suggesting lens / zonular instability

- Current use of or possible need for systemic medications known to be toxic to the lens, retina or optic nerve, including Deferoxamine, Chloroquine / hydroxychloroquine (Plaquenil), Tamoxifen, Phenothiazines and Ethambutol
- Known hypersensitivity to ocriplasmin, its excipients or any of the medications that will be used for study procedures (*e.g.* fluorescein, antibiotics, anaesthetic eye drops, eye drops for pupil dilation)
- Pregnant or lactating female, or female of child-bearing potential not utilising an adequate form of contraception, or male of reproductive potential not utilising contraception (where 1 method is barrier at the minimum)
- Previous ocriplasmin injection in the study eye
- History and / or current evidence of a systemic medical condition or any other reason that may, in the Investigator's opinion, preclude adherence to the scheduled study visits / assessments and safe participation in the study
- Concurrent participation in another clinical study, at any time during the entire study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device)
- Use of any investigational, non-registered or off-label product within 30 days prior to screening, or planned use during the entire study period

Diabetic Retinopathy-related Treatment:

Escape Criteria

- Subjects may be treated with pan-retinal photocoagulation (PRP) following the confirmation of progression from NPDR to PDR, as assessed by the masked CRC. No minimum time interval between study treatment and PRP needs to be considered
- Subjects may be treated with an anti-VEGF treatment following the confirmation of development of CI-DME with CST of ≥ 340µm on Spectralis SD-OCT or ≥ 320µm on non-Spectralis SD-OCT, or an increase of 50µm from baseline in CST, based on SD-OCT, as assessed by the masked CRC. There are no clinical data on concomitant use of ocriplasmin with anti-VEGF treatment, however, as ocriplasmin is a proteolytic enzyme with serine protease activity which could be present in the eye for several days after intravitreal injection, administration in close temporal association with other medicinal products in the same eye may affect the activity of both medicinal products and is therefore not recommended. A minimum time of 7 days between study treatment and anti-VEGF injection is therefore required

Study Masking:

The study will be conducted in a double-masked manner up to the Month 15 analysis. As of the Month 15 analysis, the Sponsor will be unmasked, while the masking will be kept for the B-scan expert reader, the CRCs, the masked study staff and the subjects up to the end of the study.

In order to maintain the masking, the following measures will be taken:

- **Study staff.** In order to maintain the masking, 2 teams of study staff will be identified. One (1) team will be masked while the other will be unmasked. The unmasked team will include an unmasked treating investigator who will be performing the (sham) injections and will ensure immediate follow-up of the subject after injection (1-hour post-injection assessment)
- **Drug kits.** Each carton will contain a single vial (ocriplasmin or sham). The carton and the vial label will be masked. However, as ocriplasmin vials will have a different appearance

than sham vials, it is important that drug kits are opened by unmasked study personnel only. The vials will have a peel-off label, which will be put in the accountability log

- **Administration kits.** The carton label will be masked. As there will be 2 different types of administration kits, it is important that the kits are opened by unmasked study personnel only. All administration kits will contain 2 syringes, 2 needles 19-gauge (19G) and 1 needle 30-gauge (30G). Some administration kits will also contain an ampoule of sodium chloride (NaCl) 9mg/mL (0.9%), whereas others will not. The administration kits will have a peel-off label, which will be put in the accountability log
- **Injection procedures.** In order to maintain the subject masked, injection procedures will, as much as possible, be the same for the ocriplasmin and sham injections. Pre-injection procedures will be identical for ocriplasmin and sham injections, including antisepsis and use of topical anaesthesia. Study drug preparation will not be identical for ocriplasmin and sham injections and should be done outside of the subject's view. A sterile drape may be used to cover the subject's fellow eye and the subject will be asked to direct his / her gaze away at all times. Subjects in the sham arm will not receive an actual injection (no penetration of the globe will occur). For the sham injection, no needle will be attached to the syringe. The unmasked treating investigator will press the hub of the syringe firmly against the sclera / conjunctiva and slowly depress the plunger to mimic the test product injection procedure
- **B-scan expert reader and CRCs.** The B-scan expert reader, the CRC readers and, where applicable, the adjudicators at the CRCs will be masked to the treatment assignment
- **Immunogenicity assessment.** The laboratory in charge of the immunogenicity assay will be masked to the treatment assignment

Safety:

Withdrawal from Repeat Injection

The following events should be assessed at Visit 4a (Day 28) and Visit 6a (Day 63), before repeat injection. If any of these events apply, no further injection(s) should be given. For events that are deemed to be temporary, the injection can be postponed, if this is feasible within the protocol-specified study visit interval

- Severe intraocular inflammation associated with previous injection
- Active intraocular inflammation or infection in either eye at the time of repeat injection
- Any signs or symptoms of lens subluxation associated with previous injection
- Any condition associated with previous injection requiring surgical treatment (*e.g.* new macular hole, worsening of existing macular hole, retinal tear, retinal detachment, IOP increase)
- Persistent presence of subretinal fluid or inner segment / outer segment (IS / OS) junction changes with functional impairment, associated with previous injection, at the time of repeat injection
- Persistent complaint of dim vision, dark adaptation problems or impaired night vision, or afferent pupillary defect, associated with previous injection, at the time of repeat injection
- BCVA decrease $\geq$ 15 letters from baseline or BCVA worse than 20/400, in the study eye, at the time of repeat injection
- Significant ocular trauma in the study eye at the time of repeat injection
- Any other intraocular injection since previous study injection
- Pregnancy

- Subject's individual treatment assignment has been unmasked (emergency unmasking)

In addition, subjects may be withdrawn from repeat injection at any time at the discretion of the Investigator (*e.g.* based on significant abnormalities on ophthalmic examination or functional test associated with previous injection not listed above)

Safety Oversight

In order to maintain a general safety oversight and to monitor the risk / benefit balance, a data monitoring committee (DMC) will be established for this study

From the time the first subject is injected until the last subject completed the Month 3 visit (Visit 8), the following treatment-emergent adverse events (TEAEs) will be escalated to the DMC on an ongoing basis:

- Lens subluxation
- Iso-electric full-field electroretinograms (ffERGs)
- Clinically significant increased intraocular inflammation after repeat (2^{nd} or 3^{rd}) injection
- BCVA decrease ≥ 15 letters from baseline, without evidence of resolution or stabilisation within 24 hours
- Foveal detachment or worsening of a foveal detachment
- Any other event at the discretion of the Sponsor

The DMC will conduct periodic reviews at the following pre-defined timepoints:

- After approximately 20% of the subjects completed the Month 2 visit (Visit 6b)
- After approximately 50% of the subjects completed the Month 3 visit (Visit 8)
- After all subjects completed the Month 3 visit (Visit 8)
- After all subjects completed the Month 15 visit (Visit 10)

In addition, *ad hoc* DMC meetings may be organised

The DMC will be empowered to make recommendations to the Sponsor on further study conduct. Such recommendations include continuing or terminating the study or modifications to the study. Another recommendation may be temporary suspension of enrolment and / or study treatment until some uncertainty is resolved

Criteria for Evaluation:

Efficacy

Primary Efficacy Endpoint:

- Total PVD by the Month 3 visit, confirmed on both B-scan ultrasound and SD-OCT (6mm), as assessed by the masked B-scan expert reader and the masked CRC, respectively

Exploratory Efficacy Endpoints:

- A ≥ 2-step progression on the ETDRS Severity Scale from baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Progression from NPDR at baseline to PDR (at least ETDRS level 61) at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Improvement of ≥ 2 steps on the ETDRS Severity Scale from baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC

- Neovascularisation as compared to baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Development or progression of macular oedema as compared to baseline, at each study visit, based on SD-OCT, as assessed by the masked CRC
- BCVA change from baseline at each study visit
- Total PVD at each study visit, confirmed on both B-scan ultrasound and SD-OCT (6mm), as assessed by the masked B-scan expert reader and the masked CRC, respectively
- Total PVD by the Month 3 visit, based on widefield SD-OCT, as assessed by the masked CRC, in a subset of sites where this is available
- Vitreous / pre-retinal haemorrhage by Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Development of iris neovascularisation as compared to baseline, at each study visit, based on slit lamp examination
- PRP treatment by the Month 15 visit
- Anti-VEGF treatment by the Month 15 visit
- Vitrectomy by the Month 15 visit

Safety

Principal Safety Endpoint:

- Ocular TEAEs in the study eye

Safety will be further assessed through reported TEAEs, full ophthalmic examination, BCVA assessment, assessment of colour vision (in a subset of sites where the Roth 28 Colour Test is available), SD-OCT, ffERG (at sites where this is available, in approximately 25% of the subjects) and assessment of immunogenicity

Statistical Methods:

Determination of Sample Size

One hundred and fifteen (115) subjects (46 subjects in each of the ocriplasmin arms and 23 subjects in the sham arm) will provide 80% power to detect a significant difference in total PVD rate by the Month 3 visit, for the comparison of sham to each of the ocriplasmin arms at a 2-sided alpha level of 0.05, assuming total PVD is achieved in 5% and 40% of the subjects in the sham and ocriplasmin arms, respectively. The sample size takes into account a drop-out rate of 10% by the Month 3 visit

In order to protect the alpha level to 0.05, a hierarchical testing procedure will be used. Ocriplasmin 0.125mg will be first compared to sham. If the p-value of this first comparison is found < 0.05, then ocriplasmin 0.0625mg will be compared to sham

Statistical Analyses

Primary Efficacy Analysis:

- The proportion of subjects with total PVD by the Month 3 visit will be calculated in each treatment arm with corresponding 95% confidence interval (CI), stratified for NPDR severity at baseline. The adjusted differences in proportions between sham and each of the ocriplasmin arms will be calculated with its corresponding 95% CI and tested using the Cochran-Mantel-Haenszel test. In addition, the effect of treatment on the proportion of subjects with total PVD will be computed using a logistic regression with effects for treatment and NPDR severity at baseline

Principal Safety Analyses:

- The proportion of subjects with ocular TEAEs in the study eye at any time during the study will be calculated in each treatment arm with corresponding 95% CI
- The proportion of subjects with a loss of ≥ 15 ETDRS letters at any time during the study and between Day 0 and Day 7 after each injection will be calculated in each treatment arm with corresponding 95% CI

Sequence of Analyses

- While the Sponsor is masked to the individual treatment assignments, the data analyses for the DMC meetings will be performed by an unmasked, independent external statistician. The independent external statistician will maintain secure custody of unmasked or partially unmasked data
- The analysis of the primary endpoint will be performed by an unmasked, independent, external statistician when all subjects have completed the Month 3 visit (Visit 8). The independent external statistician will maintain secure custody of unmasked or partially unmasked data
- The analysis of the exploratory efficacy and the safety endpoints up to the Month 15 visit will be performed when all subjects have completed the Month 15 visit (Visit 10)
- An additional analysis will be performed when all data is available, after the database lock

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LIST OF ABBREVIATIONS

The following abbreviations are used in this study protocol.

Table 3: List of Abbreviations

AE	Adverse Event
AMD	Age-related Macular Degeneration
Anti-VEGF	Vascular Endothelial Growth Factor
APE	Autologous Plasmin Enzyme
BCVA	Best Corrected Visual Acuity
°C	Degrees Celsius
CI	Confidence Interval
CI-DME	Centre-Involved Diabetic Macular Oedema
CRC	Central Reading Centre
CRO	Contract Research Organisation
CST	Central Subfield Thickness
CSR	Clinical Study Report
DMC	Data Monitoring Committee
DME	Diabetic Macular Oedema
DNA	Deoxyribonucleic Acid
DR	Diabetic Retinopathy
eCRF	Electronic Case Report Form
ETDRS	International Clinical Diabetic Retinopathy Disease Severity Scale
°F	Degrees Fahrenheit
ffERG	Full-field electroretinogram
G	Gauge
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HbA1c	Glycated Haemoglobin
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IOP	Intraocular Pressure
IRB	Institutional Review Board

IS / OS	Inner Segment / Outer Segment
IXRS	Interactive Voice / Web Response System
kDa	Kilodalton
LOCF	Last-Observation-Carried-Forward
MedDRA	Medical Dictionary for Regulatory Activities
µg	Microgram
Mg	Milligram
mL	Millilitre
µm	Micrometer
Mw	Molecular Weight
NaCl	Sodium Chloride
NPDR	Non-Proliferative Diabetic Retinopathy
OC	Observed Case
OCT	Optical Coherence Tomography
PDR	Proliferative Diabetic Retinopathy
PRP	Pan-Retinal Photocoagulation
PT	Preferred Term
PVD	Posterior Vitreous Detachment
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD-OCT	Spectral Domain Optical Coherence Tomography
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reactions
TEAE	Treatment-Emergent Adverse Event
US	United States
USPI	United States Package Insert
VMA	Vitreomacular Adhesion
VMT	Vitreomacular Traction
WHO	World Health Organization

GLOSSARY OF TERMS

The following specialist terms are used in this study protocol.

Table 4: Glossary of Terms

Adequate contraception	Adequate methods of birth control include intrauterine device, oral, implanted or injected contraceptives, and barrier methods with spermicide
Eligible	Qualified for enrolment into the study based upon strict adherence to inclusion / exclusion criteria
Foveal ischemia	Foveal ischemia is defined as evidence of disrupted foveal avascular zone
Study drug	Test product, placebo or comparator being tested or used as a reference in the clinical study
Masking	A procedure in which one or more parties in the study are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. In a double-masked study, the subject, the Investigator and Sponsor staff who are involved in clinical evaluation of the subjects and the review or analysis of data are all unaware of the treatment assignment
Principal Investigator	The Investigator who leads the study conduct at an individual site. Every site has a Principal Investigator
Study Treatment	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo / sham intended to be administered to a subject, identified by a unique number, according to the study randomisation or treatment allocation
Test product	The ThromboGenics compound being used in the study
Subject	Term used throughout the protocol to denote an individual who participates in the clinical study, either in one of the ocriplasmin arms or in the sham arm

1. BACKGROUND INFORMATION

1.1. Introduction

1.1.1. Diabetic Retinopathy

According to the World Health Organization (WHO), in 2014, 9% of adults 18 years and older had diabetes (WHO, 2015). In the United States (US), an estimated 3 out of 5 people with diabetes have one or more of the complications associated with the disease (American Association of Clinical Endocrinologists, 2007). Diabetic retinopathy (DR) is one of those complications and it is a leading cause of visual disability and blindness among professionally active adults (Cunha-Vaz, 1998; Fong *et al.*, 1999). Worldwide, the prevalence rate of vision-threatening DR (*i.e.* proliferative diabetic retinopathy [PDR] and / or diabetic macular oedema [DME] – see below) was estimated at 11.72% of the people with diabetes in 2010 (Yau *et al.*, 2012). Patients with severe stages of DR are reported to have poorer quality of life and they utilise more health care resources.

DR progresses from mild, non-proliferative to more severe, proliferative stages when there is no appropriate treatment. The initial, non-proliferative stages of DR are characterised by retinal vascular related abnormalities, such as micro-aneurysms, intraretinal haemorrhages, venous dilation and cotton-wool spots. As DR progresses, there is a gradual closure of retinal vessels that results in impaired perfusion and retinal ischemia. When this progresses beyond certain thresholds, severe non-proliferative diabetic retinopathy (NPDR) is diagnosed. The more advanced stage, PDR, is characterised by the onset of neovascularisation at the inner surface of the retina induced by retinal ischemia. These new vessels are prone to bleed, resulting in vitreous haemorrhage. They may also undergo fibrosis and contraction, which may lead to epiretinal membrane formation, vitreoretinal traction bands, retinal tears and traction or rhegmatogenous retinal detachments. When new vessels are accompanied by vitreous haemorrhage, or when new vessels at the optic disc occupy greater than or equal to about ¼ to ⅓ disc area, even in the absence of vitreous haemorrhage, PDR is considered high risk. Patients with high risk PDR are at high risk of severe vision loss.

DME is macular oedema arising in patients with DR due to increased retinal vascular permeability and is characterised by retinal thickening and / or adjacent hard exudates that either involve the centre of the macula or threaten to involve it. DME is an important complication of DR and is assessed independently from the DR stage, as it can occur at any stage and follow an independent course.

1.1.2. Current Treatment Options

Good control of blood glucose, blood pressure and blood lipids is important, as it can delay the onset of DR and slow its progression.

Once DR is established and the PDR stage is reached, pan-retinal photocoagulation (PRP) is effective in reducing the development of blindness (Cunha-Vaz, 1998). However, some patients treated with PRP will still progress to severe vision loss or even complete vision loss resulting from persistent or recurrent disease. In addition, PRP treatment may lead to clinically relevant complications such as visual field loss or worsening macular oedema (Fong *et al.*, 1999; Bailey *et al.*, 1998; Bailey *et al.*, 1999).

Multiple studies have demonstrated the benefit of intravitreal anti-vascular endothelial growth factor (anti-VEGF) for the treatment of centre-involved DME (CI-DME). At the present time, anti-VEGF is the initial treatment choice for CI-DME, with possible subsequent or deferred focal / grid laser photocoagulation (American Academy of Ophthalmology Retina/Vitreous Panel, 2014). In addition, the corticosteroids Ozurdex® (dexamethasone) and Iluvien® (fluocinolone acetonide) have recently been approved for the treatment of DME and the role of other corticosteroids in the treatment strategy for patients with DME is currently under investigation (American Academy of Ophthalmology Retina/Vitreous Panel, 2014).

It has been suggested that total posterior vitreous detachment (PVD) can prevent the progression of NPDR to PDR. A meta-analysis including 2188 analysable eyes showed that diabetic patients with total PVD had a statistically significant lower prevalence of PDR (odds ratio 0.1, 95% confidence interval [CI] 0.05-0.18) than patients with no PVD. In the same meta-analysis, progression of PDR was significantly associated with no PVD or partial PVD (odd ratio 0.3, 95% CI 0.09-0.91) (De Smet *et al*, 2013). This could be explained by total PVD leading to:

1. Elimination of the scaffold for retinal neovascular outgrowth
2. Improvement of the oxygen supply to the retina, thereby reducing retinal ischemia, production of VEGF, vascular outgrowth and neovascularisation

Currently, the only method to achieve total PVD is by vitrectomy. However, due to the risk of complications, such as recurrent vitreous haemorrhage, retinal tear or detachment, vision loss, infectious endophthalmitis and cataract, vitrectomy is only indicated in DR patients who are at the high risk PDR stage and who are not amenable to PRP, or in patients with macula-threatening vitreomacular traction (VMT) or epiretinal membrane on optical coherence tomography (OCT) (American Academy of Ophthalmology Retina/Vitreous Panel, 2014). As vitrectomy is only performed in patients who are already at the PDR stage, therapy targeted at earlier stages of DR, when the disease process may still be reversible or halted, is urgently needed.

1.2. Test Product

Ocriplasmin, a recombinant human DNA derived protein (*Mw* 27.2kDa), is a truncated form of the human protein, plasmin, with retained protease activity. Ocriplasmin acts on several components of the vitreoretinal interface, including laminin, fibronectin and collagen IV and is able to induce PVD in a dose- and time-dependent fashion, leaving a clean retinal surface devoid of protein debris on electron microscopy (De Smet *et al.,* 2009; Gad Elkareem *et al.*, 2010).

A single intravitreal injection of JETREA® (ocriplasmin 0.125mg) is approved in the US for the treatment of symptomatic vitreomacular adhesion (VMA) and in Europe for the treatment of VMT including when associated with macular hole of diameter less than or equal to 400µm.

In this study, the potential of multiple injections of ocriplasmin 0.0625mg or 0.125mg to safely induce total PVD in subjects with moderate to very severe NPDR will be assessed, ultimately aiming to reduce the risk of progression to PDR.

1.3. Preclinical / Clinical Data

1.3.1. Preclinical Data

Please refer to the current Investigator's Brochure (IB) for a description of the preclinical data.

1.3.2. Clinical Data

As part of the clinical development programme for ocriplasmin's initial indication 'treatment of VMT (symptomatic VMA)', a single intravitreal injection of ocriplasmin has been assessed. Subsequent to, or in conjunction with multiple Phase 2 studies, 2 Phase 3 studies (TG-MV-006 and TG-MV-007) were conducted to support applications for marketing authorisations. As of 16-Apr-2016, it was estimated that 1638 eyes had been treated with an intravitreal injection of ocriplasmin (any dose) in clinical studies, whereas exposure from marketing experience was estimated at 23220 eyes.

The current study is the first of the clinical development programme to support ocriplasmin's new indication 'to reduce the risk of progression to PDR in patients with NPDR', assessing the effect of up to 3 intravitreal ocriplasmin injections in subjects with NPDR. During the clinical development programme supporting the initial indication, there were a limited number of subjects who received multiple injections of ocriplasmin 0.125mg as well as a limited number of subjects with DR who were treated with ocriplasmin (0.025mg, 0.075mg, 0.125mg or 0.175mg):

- In the Phase 2 study TG-MV-004, 9 subjects received multiple injections of ocriplasmin 0.125mg. In 1 of the cohorts in this study, 15 subjects with VMT were assigned to ocriplasmin 0.125mg (n=12) or sham injection (n=3). Five (5) out of 12 (41.7%) subjects who were assigned to ocriplasmin and 1 out of 3 (33.3%) subjects who were assigned to sham injection achieved total PVD by the Day 28 visit. The 9 subjects who did not achieve resolution of VMT by the Day 28 visit received 2 additional open-label injections of ocriplasmin 0.125mg. This led to 7 subjects who were assigned to ocriplasmin receiving a total of 3 ocriplasmin injections each, and to 2 subjects who were assigned to sham injection receiving a total of 2 ocriplasmin injections each. At 28 days after the second open-label ocriplasmin injection, 1 out of 2 subjects who were assigned to sham injection achieved total PVD and none of the 7 subjects who were assigned to ocriplasmin injection achieved total PVD. All except 1 of the subjects who received multiple injections of ocriplasmin experienced adverse events (AEs), with events occurring both before and after the open label injections. With the exception of 1 moderate ocular AE (macular hole) reported after 3 injections of ocriplasmin, all ocular events reported after 2 or 3 ocriplasmin injections were mild in intensity. The type of the treatment-related AEs reported after 2 or 3 ocriplasmin injections were not different from those observed in the total population included in the Phase 2 and 3 studies.
- In order to group data on subjects with DR who were treated with ocriplasmin, a pooled analysis has been performed on the treatment-emergent adverse events (TEAE) reported for the subjects with DR enrolled in the studies TG-MV-002, TG-MV-004, TG-MV-006, TG-MV-007, TG-MV-014 and J-12-075. A total of 125 subjects with DR were enrolled in these studies, of which 90 received ocriplasmin (any dose; 0.025mg, 0.075mg, 0.125mg or 0.175mg) and 62 received

ocriplasmin 0.125mg. In the subjects who received ocriplasmin 0.125mg, ocular treatment-emergent adverse events (ocular TEAEs) in the study eye were reported for 37 (59.7%) subjects. The most frequently reported TEAEs were eye pain, conjunctival haemorrhage, vitreous floaters and retinal haemorrhage. This safety profile is not different from that observed in the population included in the Phase 2 and 3 studies.

For a more detailed description of the clinical data, please refer to the current IB.

1.4. Study Rationale

1.4.1. Rationale for the Study

As described above, it has been suggested that total PVD can prevent the progression of NPDR to PDR. This Phase 2 study in diabetic subjects with moderate to very severe NPDR is conducted to assess the efficacy and the safety of up to 3 injections of ocriplasmin (0.0625mg or 0.125mg) to induce total PVD, in order to reduce the risk of disease progression to PDR.

1.4.2. Rationale for the Dose and the Injection Regimen

Up to 3 intravitreal injections of ocriplasmin 0.0625mg or 0.125mg will be administered. All subjects assigned to an ocriplasmin arm will receive an intravitreal injection of ocriplasmin 0.0625mg or 0.125mg at Day 0. These subjects will be re-treated with the same dose of ocriplasmin at Day 35 and at Day 70 if, by that time, they do not have total PVD on both B-scan ultrasound and spectral domain optical coherence tomography (SD-OCT), as assessed by the masked B-scan expert reader and the masked central reading centre (CRC), respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s).

1.4.2.1. Rationale for the Dose

The evaluation of 2 different ocriplasmin doses (0.0625mg and 0.125mg) will help to inform the dose selection for investigation in the Phase 3 studies.

The dose of 0.125mg will be assessed as this is the dose marketed for treatment of VMT (symptomatic VMA). This dose was selected as part of the previous clinical development programme supporting ocriplasmin's initial indication. A single dose of ocriplasmin 0.125mg was able to induce VMT release without undue safety findings in 2 separate, dose finding clinical studies. Positive results were obtained with this dose in 2 adequate and well-controlled Phase 3 studies (TG-MV-006 and TG-MV-007).

The dose of 0.0625mg will be assessed as preclinical data showed that PVD can be induced with ocriplasmin doses of 0.0125 to 0.250mg, with higher doses inducing progressively more pronounced PVD. In the dose-ranging studies performed as part of the previous clinical development programme, VMT resolution was also observed with a single dose of ocriplasmin 0.075mg, albeit at a lower rate than what was observed with ocriplasmin 0.125mg. In the current study however, up to 3 injections with ocriplasmin will be given, and a lower dose may be sufficient to induce total PVD while reducing the number of AEs.

For a more detailed description of the preclinical and the clinical data, please refer to the current IB.

1.4.2.2. Rationale for the Injection Regimen

In the pivotal Phase 3 studies (TG-MV-006 and TG-MV-007), a single intravitreal injection of ocriplasmin 0.125mg led to total PVD in 13.4% of the subjects at Day 28 (62 out of 464 subjects). In most subjects, a single intravitreal ocriplasmin injection may hence not be sufficient to induce total PVD. Repeat administration is expected to increase the total PVD rate. This has been observed in a study with the autologous plasmin enzyme (APE) in patients with PDR or DME, where a single APE injection led to total PVD in 38% (24 out of 63) of the eyes and repeat injection in subjects with no evidence of total PVD at least 1 month after the initial injection led to total PVD in 51% (32 out of 63) of the eyes (Diaz-Llopis *et al*., 2013). In the current study, subjects who do not achieve total PVD will therefore receive (an) additional ocriplasmin injection(s), with a maximum of 3 injections per subject.

The time interval between injections has been selected based on the observation that the onset of total PVD induced by a single intravitreal injection of ocriplasmin is rapid. In the pivotal Phase 3 studies (TG-MV-006 and TG-MV-007), 75% of subjects who responded to the treatment, responded within 1 week and almost all subjects who responded did so within 1 month. Therefore, if a subject has not responded by 1 month after the injection, it is not likely that release will occur, justifying repeat injection.

1.4.3. Rationale for the Use of a Sham Control

As there is currently no non-surgical treatment available to induce total PVD in subjects with NPDR, an inactive control will be used. For ethical reasons, sham was chosen over placebo given the inherent risks associated with the intravitreal injection procedure, particularly when administered repeatedly, including pain, intraocular haemorrhage, infection, transient increase in IOP and retinal detachment. Moreover, it has been shown that the majority of subjects receiving a sham injection believe that they are receiving a real injection (Glassman *et al*., 2012). The use of a sham control will hence minimise the risks for the subjects in the control arm while permitting double masking.

1.5. Risks / Benefits

This is the first study in which the effect of up to 3 intravitreal injections of ocriplasmin in diabetic subjects with moderate to very severe NPDR will be assessed. Based on available preclinical and clinical data, it is believed that multiple injections of ocriplasmin will have an acceptable safety profile and will have the potential to induce total PVD. Given the measures taken to ensure safety of the subjects in this study, the study Sponsor believes that the potential benefits of the study outweigh the potential risks, as outlined below.

1.5.1. Potential Risks

1.5.1.1. Potential Risks Related to the Test Product

Risks of a Single Intravitreal Injection of Ocriplasmin 0.125mg in Subjects with VMT (Symptomatic VMA)

As of 16-Apr-2016, it was estimated that 1638 eyes received an intravitreal injection of ocriplasmin (any dose) and 416 eyes received a placebo / sham injection in clinical studies.

The AEs observed after a single intravitreal injection of ocriplasmin 0.125mg in subjects with VMT (symptomatic VMA) mostly occurred within the first week after the injection. The majority of these AEs were non-serious, mild to moderate in intensity and resolved within 2 to 3 weeks without treatment. The most commonly reported AEs were vitreous floaters, eye pain, photopsia and chromatopsia, as well as conjunctival haemorrhage resulting from the injection procedure. Visual symptoms perceived in the contralateral eye or bilaterally have also been reported (such as floaters, flashes).

With data from the paediatric study TG-MV-009 excluded (16 eyes treated with ocriplasmin and 8 eyes treated with placebo), 435 serious adverse events (SAEs) have been reported in total in clinical trials; 336 of which were reported for subjects treated with ocriplasmin and 99 of which were reported for subjects treated with sham or placebo. The most frequently reported SAEs were macular hole (108 occurrences; 24.8%), retinal detachment (26 occurrences; 6.0%), visual acuity reduced (16 occurrences; 3.7%) and vitreous adhesions (14 occurrences; 3.2%). Cumulatively, 15 SAEs with a fatal outcome were reported, all of which were assessed to be unrelated to ocriplasmin.

Please refer to the current IB / the US Package Insert (USPI) / the European Summary of Product Characteristics (SmPC) for more detailed information on the risks associated with a single intravitreal injection of ocriplasmin in subjects with VMT (symptomatic VMA).

Risks of Multiple Injections in Subjects with Diabetic Retinopathy

During the clinical development programme supporting the indication of VMT (symptomatic VMA), there were a limited number of subjects who received 2 or 3 injections of ocriplasmin 0.125mg as well as a limited number of subjects with DR who were treated with ocriplasmin (0.025mg, 0.075mg, 0.125mg or 0.175mg). Overall, the risks observed in subjects with DR or after repeat injections did not seem to differ from those observed in the population included in the Phase 2 and Phase 3 studies (refer also to Section 1.3.2).

However, there may be undesirable effects that are unknown at this time.

In order to ensure safety of the subjects in this study, the following measures will be taken:

- For each subject, AEs will be recorded from the time of providing consent until the end of the study (approximately 24 months follow-up from the first injection). At each study visit, the study personnel will inquire about AEs, and a full ophthalmic examination (including pupillary examination, slit lamp examination, IOP assessment and dilated fundus examination), best corrected visual acuity (BCVA) assessment and SD-OCT will be performed. In addition, 24-72 hours after each injection visit, there will be a scripted post-injection phone call to inquire about AEs and concomitant

medications / treatments. Based on this phone call, the subject may be invited back for an unscheduled study visit.

- A list of withdrawal criteria from repeat injection has been compiled. If for a subject, any of these criteria apply at the time of repeat injection, and it is not possible to postpone the repeat injection, within the protocol-specified study visit interval, until the event has resolved, he / she should not receive further injection(s). In addition, the Investigator may decide not to administer repeat injection(s) at his / her discretion. Subjects who are not given repeat injection(s) will continue all other study procedures to ensure complete follow-up.
- In addition to safety follow-up on an individual subject level, a data monitoring committee (DMC) will be established to maintain the general safety oversight. The DMC will conduct periodic review of safety and efficacy data. In addition, certain TEAEs will be escalated to the DMC on an ongoing basis and *ad hoc* DMC meetings may be convened whenever deemed necessary. At any time during the study, the DMC will be empowered to make recommendations to the Sponsor on further study conduct.

1.5.1.2. Potential Risks Related to Other Study Procedures

Intravitreal Injection

The risk of complications from intravitreal injections itself is low. The most common complications associated with intravitreal injections are pain, intraocular haemorrhage, infection, transient increase in IOP and retinal detachment (Aiello *et al,* 2004; Avery *et al*, 2014; Jager *et al,* 2004). The prevalence of endophthalmitis, intraocular haemorrhage and retinal detachment after intravitreal injections was estimated at 0.2%, 1.3% and 0.9% per injection, respectively in a systematic search of the literature (Jager *et al,* 2004). Nevertheless, careful attention to injection technique and appropriate post-injection monitoring are essential because uncommon injection-related complications may be associated with permanent vision loss.

Antibiotic Eye Drops

The Investigator may decide at his / her discretion to administer prophylactic topical antibiotics. The risks associated with the ocular antibiotic drops are the same as those for oral antibiotics and include mild to moderate local allergic reactions characterised by eye redness and itching and rare systemic drug reactions including Stevens-Johnson syndrome and / or anaphylaxis.

Anaesthetic Eye Drops

Before the intravitreal injection and for some of the ophthalmic assessments, the eye will be anaesthetised with anaesthetic drops. Allergic reactions to anaesthetic eye drops are rare, but include itching, lid swelling and eye redness. Another risk associated with these drops is scratching of the cornea due to rubbing of the numbed eye. Subjects should therefore be instructed not to rub their eyes until the anaesthetic wears off (about 15 minutes).

Pupil Dilation

For some of the ophthalmic examinations, the pupils will be dilated. The pupils will remain dilated for at least 4-6 hours. This may result in light sensitivity and blurred vision (especially for near tasks). Subjects should be told to bring sunglasses to the study visits and to not drive while their pupils are dilated. Allergic reactions are rare but include lid swelling and eye redness. The procedures themselves do not pose any risks to the subjects.

Tonometry

Tonometry (IOP measurement) carries a small risk for corneal scratching (abrasion), which usually heals without treatment within a few days.

Fluorescein Angiography

Fluorescein will be injected in the systemic circulation, which may cause pain at the injection site and which carries a small risk of bleeding, bruising and / or infection at the injection site. Other side effects may include nausea, vomiting, gastrointestinal distress, headache, syncope and hypotension. In addition, the fluorescein may lead to temporary, limited darkening of the skin and urine, subsiding within a few hours.

In very rare cases, a severe allergic reaction can occur, which can cause breathing problems and exceptionally lead to death. For this reason, people with a history of drug reaction or allergy will be carefully monitored.

Full-Field ERG

The full-field ERG (ffERG) test requires an electrode to be placed onto the surface of the eye along the lower eyelid, in contact with the tear film and cornea, which may cause irritation and a slight foreign-body sensation while it is in place.

Blood Draw

Blood draw carries a small risk of pain, excessive bleeding, fainting or light-headedness, haematoma under the skin at the site of the needle insertion, and a rare risk of infection.

1.5.2. Potential Benefits

This is the first study in which the efficacy and safety of up to 3 intravitreal injections of ocriplasmin in diabetic subjects with moderate to very severe NPDR will be assessed, and there may not be any direct benefit for the study participants.

An indirect benefit of study participation is that this study may contribute to the development of a drug that reduces the risk of disease progression to PDR. In addition, this study may realise overall advancement of medical and scientific knowledge that may benefit future patients. In addition, subjects will be closely followed up for DR progression while being in the study.

2. STUDY OBJECTIVE

- To assess the efficacy and safety of up to 3 intravitreal injections of ocriplasmin (0.0625mg or 0.125mg), in subjects with moderate to very severe NPDR, to induce total PVD in order to reduce the risk of disease progression to PDR

3. STUDY DESIGN AND METHODOLOGY

3.1. Study Endpoints

3.1.1. Efficacy Endpoints

Primary Efficacy Endpoint

- Total PVD by the Month 3 visit, confirmed on both B-scan ultrasound and SD-OCT (6mm), as assessed by the masked B-scan expert reader and the masked CRC, respectively

Exploratory Efficacy Endpoints

- A ≥ 2-step progression on the International Clinical Diabetic Retinopathy Disease Severity Scale (ETDRS Severity Scale) from baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Progression from NPDR at baseline to PDR (at least ETDRS level 61) at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Improvement of ≥ 2 steps on the ETDRS Severity Scale from baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Neovascularisation as compared to baseline, at Month 15 and at Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Development or progression of macular oedema as compared to baseline, at each study visit, based on SD-OCT, as assessed by the masked CRC
- BCVA change from baseline at each study visit
- Total PVD at each study visit, confirmed on both B-scan ultrasound and SD-OCT (6mm), as assessed by the masked B-scan expert reader and the masked CRC, respectively
- Total PVD by the Month 3 visit, based on widefield SD-OCT, as assessed by the masked CRC, in a subset of sites where this is available
- Vitreous / pre-retinal haemorrhage by Month 24, based on 7-standard field stereo colour fundus photography, as assessed by the masked CRC
- Development of iris neovascularisation as compared to baseline, at each study visit, based on slit lamp examination
- PRP treatment by the Month 15 visit
- Anti-VEGF treatment by the Month 15 visit
- Vitrectomy by the Month 15 visit

3.1.2. Safety Endpoints

Principal Safety Endpoint

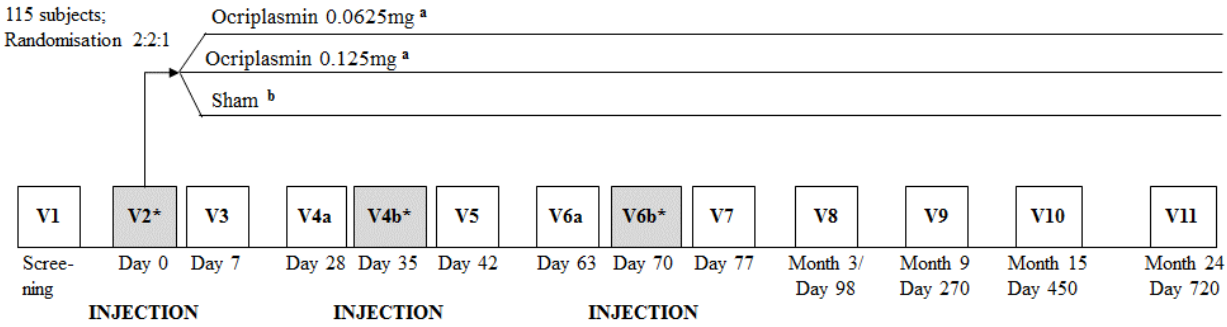
- Ocular TEAEs in the study eye

Other Safety Endpoints

Safety will be further assessed through reported TEAEs, full ophthalmic examination, BCVA assessment, assessment of colour vision (in a subset of sites where the Roth 28 Colour Test is available), SD-OCT, ffERG (at sites where this is available, in approximately 25% of the subjects) and assessment of immunogenicity.

3.2. Study Design Overview

Figure 1: Study Design Overview



Light grey highlighted visits indicate visits with ocriplasmin/ sham injection.
[a] All subjects assigned to the ocriplasmin arms will receive an intravitreal injection of ocriplasmin 0.0625mg or 0.125mg at Day 0. These subjects will be re-treated with the same dose of ocriplasmin at Day 35 and at Day 70 if, by that time, they have not achieved total PVD on both B-scan ultrasound and SD-OCT, as assessed by the masked B-scan expert reader and the masked CRC, respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s).
[b] Subjects assigned to the sham arm will receive 3 sham injections at Day 0, Day 35 and Day 70.
* The masked Investigator / designee will make a **scripted phone call** to each subject 24-72 hours after the injection visit. Based on this phone call, the subject may be invited back for an unscheduled study visit, as per the masked Investigator's clinical judgement.

- **Study design.** Multi-centre, randomised, sham-controlled, double-masked, Phase 2 clinical study with 3 parallel treatment arms
- **Study arms and treatments**.
  - **Ocriplasmin 0.0625mg arm.** Approximately 46 subjects will receive up to 3 intravitreal injections of ocriplasmin 0.0625mg, approximately 1 month apart. All subjects assigned to this arm will receive an intravitreal injection of ocriplasmin 0.0625mg at Day 0. These subjects will be re-treated with ocriplasmin 0.0625mg at Day 35 and at Day 70 if, by that time, they have not achieved total PVD on both B-scan ultrasound and SD-OCT, as assessed by the masked B-scan expert reader and the masked CRC, respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s).

  - **Ocriplasmin 0.125mg arm.** Approximately 46 subjects will receive up to 3 intravitreal injections of ocriplasmin 0.125mg, approximately 1 month apart. All subjects assigned to this arm will receive an intravitreal injection of ocriplasmin 0.125mg at Day 0. These subjects will be re-treated with ocriplasmin 0.125mg at Day 35 and at Day 70 if, by that time, have not achieved total PVD on both B-scan ultrasound and SD-OCT, as assessed by the masked B-scan expert reader and the masked CRC, respectively. Subjects who have total PVD on both B-scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s)
  - **Sham arm.** Approximately 23 subjects will receive 3 sham injections, approximately 1 month apart. All subjects assigned to this arm will receive a sham injection at Day 0, Day 35 and Day 70

- **Test product, dose and mode of administration.** Up to 3 intravitreal injections of ocriplasmin 0.0625mg or ocriplasmin 0.125mg
- **Reference therapy (comparator), dose and mode of administration.** 3 sham injections
- **Treatment allocation.** Eligible subjects will be sequentially assigned according to a computer-generated randomisation list to the ocriplasmin 0.0625mg arm, the ocriplasmin 0.125mg arm or the sham arm in a 2:2:1 allocation ratio. Randomisation will be stratified by baseline NPDR severity (moderate and moderately severe, *i.e.* ETDRS Level 43A-47D *vs.* severe and very severe NPDR, *i.e.* ETDRS Level 53A-E). It is expected that more subjects with moderate and moderately severe than with severe and very severe NPDR will be enrolled, as a reflection of the natural population. Therefore, enrolment in the moderate and moderately severe NPDR stratum may become capped in the course of the study
- **Study duration.** For each subject enrolled, the study duration will be approximately 24 months from the first injection

3.3. Number of Subjects

Approximately 115 subjects are planned to be enrolled (approximately 46 in each ocriplasmin arm and 23 in the sham arm). For a detailed description of the sample size determination, refer to Section 9.1.

3.4. Data Monitoring Committee

In order to maintain a general safety oversight and to monitor the risk / benefit balance, a DMC will be established for this study.

From the time the first subject is injected until the last subject completed the Month 3 visit (Visit 8), the following TEAEs will be escalated to the DMC on an ongoing basis:

- Lens subluxation
- Iso-electric ffERG

- Clinically significant increased intraocular inflammation after repeat (2nd or 3rd) injection
- BCVA decrease ≥ 15 letters from baseline, without evidence of resolution or stabilisation within 24 hours
- Foveal detachment or worsening of a foveal detachment
- Any other event at the discretion of the Sponsor

The DMC will conduct periodic reviews at the following pre-defined timepoints:

- After approximately 20% of the subjects completed the Month 2 visit (Visit 6b)
- After approximately 50% of the subjects completed the Month 3 visit (Visit 8)
- After all subjects completed the Month 3 visit (Visit 8)
- After all subjects completed the Month 15 visit (Visit 10)

In addition, *ad hoc* DMC meetings may be organised.

The DMC will be empowered to make recommendations to the Sponsor on further study conduct. Such recommendations include continuing or terminating the study or modifications to the study. Another recommendation may be temporary suspension of enrolment and / or study treatment until some uncertainty is resolved.

The DMC responsibilities and procedures will be documented in the DMC Charter.

3.5. Treatment Allocation

3.5.1. Treatment Allocation at First Injection

The target is to enrol approximately 115 eligible subjects who will be sequentially assigned, according to a computer-generated randomisation list, to the ocriplasmin 0.0625mg arm, the ocriplasmin 0.125mg arm or the sham arm in a 2:2:1 allocation ratio (approximately 46 subjects in each ocriplasmin arm and approximately 23 subjects in the sham arm).

Randomisation will be stratified by baseline NPDR severity (moderate and moderately severe NPDR, *i.e.* ETDRS Level 43A-47D *vs.* severe and very severe NPDR, *i.e.* ETDRS Level 53A-E). It is expected that more subjects with moderate and moderately severe than with severe and very severe NPDR will be enrolled, as a reflection of the natural population. Therefore, enrolment in the moderate and moderately severe NPDR stratum may become capped in the course of the study.

Allocation of eligible subjects to a treatment arm will be performed through an interactive voice / web response system (IXRS). The unmasked treating investigator / designee will access the system to obtain the subject's treatment allocation and the treatment number for the first injection. The assigned treatment number will be loaded automatically by IXRS into the subject's electronic case report form (eCRF).

3.5.2. Treatment Allocation at Repeat Injection

For subjects assigned to the ocriplasmin arms, the treatment allocation at repeat injection will depend on their total PVD status, as assessed on B-scan ultrasound and SD-OCT by the masked B-scan expert reader and the masked CRC, respectively. Once the total PVD status has been assessed, treatment allocation for repeat injection can occur.

For each injection subsequent to the first one, the unmasked treating investigator / designee will access IXRS to obtain the treatment number for the subsequent injection. The assigned treatment number will automatically be loaded by IXRS into the subject's eCRF.

3.6. Masking

3.6.1. Masking Procedures

The study will be conducted in a double-masked manner up to the Month 15 analysis. As of the Month 15 analysis, the Sponsor will be unmasked, while the masking will be kept for the B-scan expert reader, the CRCs, the masked study staff and the subjects up to the end of the study.

In order to maintain the masking, the following measures will be taken:

- **Study staff.** In order to maintain the masking, 2 teams of study staff will be identified. One (1) team will be masked while the other will be unmasked. The unmasked team will include an unmasked treating investigator who will be performing the (sham) injections and will ensure immediate follow-up of the subject after injection (1-hour post-injection assessment). For an overview of the responsibilities of the masked and the unmasked study staff, refer to Table 8
- **Drug kits.** Each carton will contain a single vial (ocriplasmin or sham). The carton and the vial label will be masked. However, as ocriplasmin vials will have a different appearance than sham vials, it is important that drug kits are opened by unmasked study personnel only. The vials will have a peel-off label, which will be put in the accountability log
- **Administration kits.** The carton label will be masked. As there will be 2 different types of administration kits, it is important that the kits are opened by unmasked study personnel only. All administration kits will contain 2 syringes, 2 needles 19-gauge (19G) and 1 needle 30-gauge (30G). Some administration kits will also contain an ampoule of sodium chloride (NaCl) 9mg/mL (0.9%), whereas others will not. The administration kits will have a peel-off label, which will be put in the accountability log
- **Injection procedures.** In order to maintain the subject masked, injection procedures will, as much as possible, be the same for the ocriplasmin and sham injections. Pre-injection procedures will be identical for ocriplasmin and sham injections, including antisepsis and use of topical anaesthesia. Study drug preparation will not be identical for ocriplasmin and sham injections and should be done outside of the subject's view. A sterile drape may be used to cover the subject's fellow eye and the subject will be asked to direct his / her gaze away at all times. Subjects in the sham arm will not receive an actual injection (no penetration of the globe will occur). For

the sham injection, no needle will be attached to the syringe. The unmasked treating investigator will press the hub of the syringe firmly against the sclera / conjunctiva and slowly depress the plunger to mimic the test product injection procedure

- **B-scan expert reader and CRCs.** The B-scan expert reader, the CRC readers and, where applicable, the adjudicators at the CRCs will be masked to the treatment assignment
- **Immunogenicity assessment.** The laboratory in charge of the immunogenicity assay will be masked to the treatment assignment

3.6.2. Emergency Unmasking

Unmasking of a subject's individual treatment assignment should occur only in the case of a medical emergency, or in the event of a serious medical condition, when knowledge of the treatment is essential for the clinical management or welfare of the subject, as judged by the Investigator.

In order to unmask a subject's individual treatment assignment, the masked Investigator will need to access IXRS using the Investigator-specific access code to obtain the subject's treatment assignment. When IXRS is not available, please refer to the IXRS user guide for instructions.

The Investigator has the option of contacting the Sponsor Medical Expert if he / she needs medical advice or needs support to perform the unmasking.

For practical details about emergency unmasking, please refer to the study-specific manuals.

3.6.3. Responsibilities Masked / Unmasked Study Staff

Refer to Table 8 for an overview of the responsibilities of the masked and the unmasked study staff.

3.7. Study Duration

For each subject enrolled, the study duration will be approximately 24 months from the first injection.

The end of the study is defined as the last visit of the last subject.

3.8. Data Capturing

Data will be collected via eCRFs.

At each site, subject numbers will be assigned sequentially to subjects who have consented to participate in the study. Data obtained for each subject will be identified with the subject number. Subject names will not be collected.

For subjects who do not meet the eligibility criteria, the minimum information that will be captured is the following: date of screening, subject number, procedures completed and reason for screening failure.

3.9. Regulatory and Ethical Considerations

The study will be conducted in accordance with all applicable regulatory requirements.

This study will be conducted in compliance with the protocol approved by the relevant Independent Ethics Committee (IEC) / Institutional Review Board (IRB) and according to the Principles of the International Conference of Harmonisation (ICH) guidelines for Good Clinical Practice (GCP) E6(R1). In addition, it will be conducted in accordance with all local, federal and / or regional regulatory requirements and the principles which have their origin in the Declaration of Helsinki.

The Sponsor will obtain favourable opinion / approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Protocol waivers or exemptions are not allowed unless necessary to eliminate apparent immediate hazards to the subject. This should not normally arise in the context of inclusion / exclusion criteria, since the subject is not yet fully included in the study at that point in time. Adherence to the study design requirements, including those specified in the study plan / flowchart, the study visit intervals and the responsibilities of masked and unmasked study staff and need for certification (refer to Table 6, Table 7 and Table 8), are essential and required for study conduct.

3.10. Criteria for Discontinuation of the Study

The study may be terminated at all sites or at selected sites, or modified at any time by the Sponsor or on the acceptance of DMC recommendation. Reasons for termination or modification may include, but are not restricted to, the following:

1. The incidence or severity of AEs indicates a potential health hazard to subjects
2. The risks are found to outweigh the potential benefits
3. Data indicate that an inappropriate dose regimen is being used in the study
4. The Sponsor decides that the indication under examination will no longer be pursued
5. The Regulatory Authority demands that the study be terminated
6. Termination of the study by the IEC / IRB
7. Investigator fails to comply with the protocol or GCP guidelines
8. Unsatisfactory enrolment of subjects
9. Issues related to quality or quantity of data

4. SELECTION AND WITHDRAWAL OF SUBJECTS

4.1. Study Population

Diabetic subjects with moderate to very severe NPDR (ETDRS Levels 43A-53E), who do not have total PVD.

4.1.1. Inclusion and Exclusion Criteria

Subjects can have only 1 eye treated as part of this study. For eligible subjects where both eyes are eligible for injection, the eye with the more advanced NPDR stage (as determined by the CRC) will be selected as the study eye.

4.1.1.1. Subject Inclusion Criteria

A subject must meet all of the following inclusion criteria in order to be enrolled in the study:

- Male or female aged 18 years or older
- BCVA of 65 letters read or greater (Snellen equivalent of 20/50 or better) in the study eye
- BCVA of 20 letters read or greater (Snellen equivalent of 20/400 or better) in the fellow eye
- Clear ocular media for adequate fundus imaging in the study eye
- HbA1c ≤ 12%, as assessed by the central laboratory
- Moderate to very severe NPDR as per ETDRS Severity Scale (Levels 43A-53E), based on 7-standard field stereo colour fundus photograph, as assessed by the CRC
- Central subfield thickness (CST) of ≤ 340µm on Spectralis SD-OCT or ≤3 20µm on non-Spectralis SD-OCT in the study eye, as assessed by the CRC, with or without mild CI-DME (mild CI-DME is defined as cysts or intraretinal fluid in the central subfield on SD-OCT that in the Investigator's opinion will not require treatment with an anti-VEGF for at least up to 3 months after randomisation)
- No evidence of total PVD in the study eye, based on both B-scan ultrasound and SD-OCT, as assessed by the B-scan expert reader and the CRC, respectively
- Written informed consent obtained from the subject prior to screening procedures

4.1.1.2. Subject Exclusion Criteria

If a subject has any of the following criteria, the subject cannot be enrolled in the study:

- History of or current ocular condition in the study eye that may interfere with the assessment of the progression to PDR (*e.g.* VMT, exudative age-related macular degeneration [AMD], retinal vein occlusion [branch or central vein], uveitis, angioid streaks, histoplasmosis, toxoplasmosis, rhegmatogenous retinal detachment, retinal tear, fibrovascular proliferation, lattice degeneration, macular hole, ocular tumours)

- Significant ocular trauma in the study eye within 6 months prior to screening (including corneo-scleral laceration, lens subluxation, cryo-retinopexy)
- Corneal, lenticular, or ocular media abnormalities in the study eye that preclude observation with the slit lamp or accurate readings with a tonometer
- Presence of epiretinal membrane in the study eye, based on SD-OCT, as assessed by the CRC
- Presence of foveal ischemia in the study eye, based on fluorescein angiograph, as assessed by the CRC
  Refer to the Glossary of Terms for the definition of foveal ischemia
- Presence of pre-retinal or vitreous haemorrhage in the study eye
- Presence of iris or angle neovascularisation in the study eye
- Any active ocular / intraocular infection or inflammation in either eye (*e.g.* blepharitis, infectious conjunctivitis, keratitis, scleritis, endophthalmitis, uveitis)
- Uncontrolled glaucoma in the study eye (uncontrolled glaucoma is defined as IOP ≥ 26mmHg in spite of treatment with anti-glaucoma medication)
- More than 8D high myopia in the study eye
- Aphakic study eye
- Previous treatments / procedures as follows:

Treatment / Procedure In the Study Eye	**Excluded Period prior to Randomisation**
Vitrectomy	Any time
PRP	Any time
Intraocular surgery	4 months
Focal / grid laser photocoagulation	3 months
Intravitreal anti-VEGF	1 month
Topical ocular steroids	1 month
Intravitreal and peri-ocular steroids	4 months
Steroid implants	Any time

- Uncontrolled hypertension in the opinion of the Investigator (*e.g.* systolic blood pressure > 160mmHg or diastolic blood pressure > 100mmHg for at least 30 days prior to screening despite antihypertensive treatment, or any finding in the Investigator's opinion suggesting hypertensive retinopathy)
- Pseudoexfoliation, Marfan's syndrome, phacodonesis or any other finding in the Investigator's opinion suggesting lens / zonular instability
- Current use of or possible need for systemic medications known to be toxic to the lens, retina or optic nerve, including Deferoxamine, Chloroquine / hydroxychloroquine (Plaquenil), Tamoxifen, Phenothiazines and Ethambutol

- Known hypersensitivity to ocriplasmin, its excipients or any of the medications that will be used for study procedures (*e.g.* fluorescein, antibiotics, anaesthetic eye drops, eye drops for pupil dilation)
- Pregnant or lactating female, or female of child-bearing potential not utilising an adequate form of contraception, or male of reproductive potential not utilising contraception (where 1 method is barrier at the minimum)
  Refer to the Glossary of Terms for the definition of adequate contraception
- Previous ocriplasmin injection in the study eye
- History and / or current evidence of a systemic medical condition or any other reason that may, in the Investigator's opinion, preclude adherence to the scheduled study visits / assessments and safe participation in the study
- Concurrent participation in another clinical study, at any time during the entire study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device)
- Use of any investigational, non-registered or off-label product within 30 days prior to screening, or planned use during the entire study period

4.2. Subject Withdrawal Criteria

Withdrawals will not be replaced.

4.2.1. Withdrawal from Repeat Injection

A withdrawal from repeat injection refers to any subject who does not receive repeat injection(s) (ocriplasmin or sham). Subjects withdrawn from repeat injection(s) should not be withdrawn from the study and, apart from repeat injection-related procedures, should continue all scheduled study visits and procedures until the end of the study to ensure complete follow-up.

The following events should be assessed at Visit 4a (Day 28) and Visit 6a (Day 63), before repeat injection. If any of these events apply, no further injection(s) should be given. For events that are deemed to be temporary, the injection can be postponed, if this is feasible within the protocol-specified study visit interval (refer to Table 7).

- Severe intraocular inflammation associated with previous injection
- Active intraocular inflammation or infection in either eye at the time of repeat injection
- Any signs or symptoms of lens subluxation associated with previous injection
- Any condition associated with previous injection requiring surgical treatment (*e.g.* new macular hole, worsening of existing macular hole, retinal tear, retinal detachment, IOP increase)
- Persistent presence of subretinal fluid or inner segment / outer segment (IS / OS) junction changes with functional impairment, associated with previous injection, at the time of repeat injection

- Persistent complaint of dim vision, dark adaptation problems or impaired night vision, or afferent pupillary defect, associated with previous injection, at the time of repeat injection
- BCVA decrease ≥ 15 letters from baseline or BCVA worse than 20/400, in the study eye, at the time of repeat injection
- Significant ocular trauma in the study eye at the time of repeat injection
- Any other intraocular injection since previous study injection
- Pregnancy
- Subject's individual treatment assignment has been unmasked (emergency unmasking, refer to Section 3.6.2)

In addition, subjects may be withdrawn from repeat injection at any time at the discretion of the Investigator (*e.g.* based on significant abnormalities on ophthalmic examination or functional test associated with previous injection not listed above).

If a subject withdraws / is withdrawn from repeat injection, information relative to the withdrawal will be documented in the subject's medical record and in the eCRF. The Investigator will document whether the decision to withdraw from repeat injection was made by the subject himself / herself or by the Investigator, as well as the reason for withdrawal.

4.2.2. Withdrawal from the Study

Subjects may withdraw from the study at any time for any reason without jeopardy or prejudice or compromising their clinical care. However, every effort should be made to adhere to as many study assessments as possible. If a subject indicates that he / she wishes to withdraw from the study, whenever possible, the subject should be seen by the Investigator and the procedures for the Exit Visit should be performed. The Investigator also has the right to withdraw subjects from the study in the event of intercurrent illness, AEs, protocol violations and administrative or other reasons. Data for all visits to the point of withdrawal must be recorded in the subject's medical record and in the eCRF.

The data to be collected for subjects who withdraw from the study should include:

- Date of withdrawal
- Reason for withdrawal
- Data on assessments conducted (if possible) as part of the Exit Visit as described in Section 5.17
- Data on any AEs present at withdrawal

If withdrawal is a result of an SAE / death or pregnancy, an SAE Form or Pregnancy / Prenatal Exposure Form will also have to be completed, respectively. The Investigator will follow subjects who are withdrawn from the study as a result of an (S)AE until the event has resolved, subsided, stabilised, or until the event is otherwise explained, or the subject is lost to follow-up (refer to Section 8.4.1.4). The Investigator will follow-up and document the outcome of the pregnancy for pregnant women who are withdrawn from the study (refer to Section 8.4.2).

5. STUDY OBSERVATIONS AND PROCEDURES

There will be a total of 13 scheduled study visits in this study, including a screening visit, 3 injection visits and a follow-up period of 24 months from the first injection visit. 24-72 hours after each injection visit, there will be a scripted post-injection phone call to inquire about AEs and concomitant medications / treatments. Based on this phone call, the subject may be invited back for an unscheduled study visit. Additional unscheduled study visits can be conducted at any time during the study, if deemed necessary.

Refer to Table 6 for an overview of the study procedures per scheduled study contact and to Table 7 for an overview of the timing and the intervals between those study contacts. Refer to Table 8 for an overview of which study procedures should be done by masked and the unmasked study staff and for which study procedures certification is needed. A more detailed description of the study procedures and the names and addresses of the CRCs and the clinical laboratories are provided in the study-specific manuals.

5.1. Informed Consent

Subjects are required to provide signed and dated informed consent prior to participation in the study.

Before signing the Informed Consent Form, the Investigator or designee will explain the nature, purpose, risks and benefits associated with the study and ensure that the subject is given full and adequate written information. Subjects must be informed that they are free to discontinue their participation in the study at any time. Subjects should be given the opportunity to ask questions and be allowed time to consider the information provided. If a subject agrees to participate in the study, he / she should sign the Informed Consent Form to indicate that he / she understands all aspects of the study. The Investigator will provide the subject with a copy of the Subject Information Sheet / Informed Consent Form.

5.2. Visit 1 - Screening Visit

At the screening visit, the subject's eligibility for study participation will be determined by checking all in- and exclusion criteria as specified in Section 4.1.1. It is advised to do eligibility assessment in the order presented below. If a subject fails 1 of the in- / exclusion criteria, the subject will be a screening failure and no further assessments will be done (note that certain inclusion / exclusion criteria will be confirmed by the B-scan expert reader / the CRC, or the central laboratory). The eligibility assessment will entail the following evaluations:

1. Demography (year of birth, gender, race [except in countries where Regulatory Authorities / IEC / IRB do not permit to collection of race])
2. Medical history, including concomitant medications / treatments / interventions (refer to Section 6.4)
3. Ocular history
4. Blood pressure
5. BCVA (ETDRS) - **both eyes**
6. B-scan ultrasound – **potential study eye(s)** *

7. Full ophthalmic examination – **both eyes**
8. 7-standard field stereo colour fundus photography – **potential study eye(s)** *
9. SD-OCT (6mm) – **potential study eye(s)** *
10. Fluorescein angiography – **potential study eye(s)** *
11. Blood sampling for serum pregnancy test (for women of childbearing potential only)
    Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
12. Blood sampling for HbA1c
    Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
13. (S)AE monitoring (refer to Section 8.4)

* These examinations need to be performed in potential study eye(s) only. If 1 of the subject's eyes is excluded based on an eligibility criterion that does not need confirmation by the B-scan expert reader or the CRC, these examinations do not need to be performed for that eye. If both eyes potentially qualify as the study eye, these examinations need to be performed for both eyes.

For screening failures due to reasons that are expected to be temporary, and for subjects for whom eligibility cannot be timely determined due to a problem with one of the eligibility assessments (*e.g.* ungradable B-scan ultrasound), 1 re-screening visit can be organised. The re-screening visit should be scheduled at least 28 days after the 1st screening visit. Subjects who are re-screened will be given a new subject number and need to have all screening procedures repeated (including signing of a new Informed Consent Form).

For eligible subjects, all information must be recorded in the subject's eCRF. For subjects who do not meet the eligibility criteria, the minimum information to be recorded in the eCRF will be the following: date of screening, subject number, procedures completed and reason for screening failure.

5.3. Visit 2 –Day 0

The following procedures will be performed:

- Urine pregnancy test (for women of childbearing potential only)
  Pregnant women will not be eligible for study participation
- Blood sampling for immunogenicity assessment
  Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
- BCVA (ETDRS) –**both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- Full ophthalmic examination – **study eye only**

- SD-OCT (widefield) – **study eye only** (only at sites where this is available)
- ffERG – **both eyes** (in approximately 25% of the subjects, at sites where this is available)
- Treatment number allocation (refer to Section 3.5.1)
  Randomisation can only occur after confirmation of eligibility by the B-scan expert reader and the CRC
- Study treatment
- Post-injection assessment (IOP measurement and indirect ophthalmic examination)
  The post-injection assessment should be performed by the unmasked treating investigator as many times as needed at his / her discretion but at least once within 60 minutes after the injection to exclude central retinal artery non-perfusion or other complications
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.4. Post-injection Phone Call

The masked Investigator / designee will make a scripted phone call to each subject 24-72 hours after the injection visit. Based on this phone call, the subject may be invited back for an unscheduled study visit as per the masked Investigator's clinical judgement.

For the phone script, please refer to Appendix 2.

5.5. Visit 3 - Day 7

The following procedures will be performed:

- BCVA (ETDRS) –**both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- Full ophthalmic examination – **study eye only**
- SD-OCT (6mm) – **study eye only**
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.6. Visit 4a – Day 28

The following procedures will be performed:

- Urine pregnancy test (for women of childbearing potential only)
- BCVA (ETDRS) –**both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- B-scan ultrasound – **study eye only**
- Full ophthalmic examination – **study eye only**
- SD-OCT (6mm) – **study eye only**
- SD-OCT (widefield) – **study eye only** (only at sites where this is available).
- Assess list of events that lead to withdrawal from repeat injection (refer to Section 4.2.1)
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.7. Visit 4b – Day 35

The following procedures will be performed:

- Treatment number allocation for repeat injection (refer to Section 3.5.2)
- Study treatment
- Post-injection assessment (IOP measurement and indirect ophthalmic examination)
  The post-injection assessment should be performed by the unmasked treating investigator as many times as needed at his / her discretion but at least once within 60 minutes after the injection to exclude central retinal artery non-perfusion or other complications
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.8. Post-injection Phone Call

Refer to Section 5.4.

5.9. Visit 5 –Day 42

The procedures to be performed at Visit 5 (Day 42) are the same as those to be performed at Visit 3 (Day 7). Refer to Section 5.5 for the description of the study procedures at this visit.

5.10. Visit 6a – Day 63

The procedures to be performed at Visit 6a (Day 63) are the same as those to be performed at Visit 4a (Day 28). Refer to Section 5.6 for the description of the study procedures at this visit.

5.11. Visit 6b – Day 70

The procedures to be performed at Visit 6b (Day 70) are the same as those to be performed at Visit 4b (Day 35). Refer to Section 5.7 for the description of the study procedures at this visit.

5.12. Post-injection Phone Call

Refer to Section 5.4.

5.13. Visit 7 – Day 77

The following procedures will be performed:

- BCVA (ETDRS) –**both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- B-scan ultrasound – **study eye only**
- Full ophthalmic examination – **study eye only**
- SD-OCT (6mm) – **study eye only**
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.14. Visit 8 – Month 3 (Day 98)

The following procedures will be performed:

- Blood pressure
- Blood sampling for HbA1c
  Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
- Blood sampling for immunogenicity assessment
  Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples

- BCVA (ETDRS) – **both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- B-scan ultrasound – **study eye only**
- Full ophthalmic examination – **both eyes**
- 7-standard field stereo colour fundus photography – **study eye only** (only if the Investigator suspects that the subject has progressed to PDR)
- SD-OCT (6mm) – **study eye only**
- SD-OCT (widefield) – **study eye only** (only at sites where this is available).
- ffERG – **both eyes** (in approximately 25% of the subjects, at sites where this is available)
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.15. Visit 9 – Month 9 (Day 270)

The following procedures will be performed:

- Blood pressure
- Blood sampling for HbA1c
  Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
- BCVA (ETDRS) – **both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- B-scan ultrasound – **study eye only**
- Full ophthalmic examination – **both eyes**
- 7-standard field stereo colour fundus photography – **study eye only** (only if the Investigator suspects that the subject has progressed to PDR)
- SD-OCT (6mm) – **study eye only**
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.16. Visit 10 – Month 15 (Day 450)

The following procedures will be performed:

- Blood pressure
- Blood sampling for HbA1c
  Refer to the study-specific manuals for instructions on the collection, processing, storage and shipping of blood samples
- BCVA (ETDRS) – **both eyes**
- Colour vision (Roth 28-hue) – **study eye only** (only at sites where the Roth 28 Colour Test is available)
  Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline
- B-scan ultrasound – **study eye only**
- Full ophthalmic examination – **both eyes**
- 7-standard field stereo colour fundus photography – **study eye only**
- SD-OCT (6mm) – **study eye only**
- ffERG – **both eyes** (only for subjects with expert-defined ffERG abnormalities at the previous assessment)
- Record concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AE monitoring (refer to Section 8.4)

All information must be recorded in the subject's eCRF.

5.17. Visit 11 – Month 24 (Day 720) / Exit Visit

The procedures to be performed at Visit 11 (Month 24) are the same as those to be performed at Visit 10 (Month 15). Refer to Section 5.16 for the description of the study procedures at this visit.

5.18. Unscheduled Visits

Unscheduled visits can be conducted at any time during the study (*e.g.* based on scripted post-injection phone call).

For each unscheduled visit, the following information must be recorded in the subject's eCRF:

- Reason for visit
- Procedures performed and results obtained
- Concomitant medications / treatments / interventions (refer to Section 6.4)
- (S)AEs (refer to Section 8.4)

6. TREATMENT OF SUBJECTS

6.1. Description of Study Drugs

Table 5: Study Drugs

Study Drug	**Ocriplasmin 0.0625mg**		**Ocriplasmin 0.125mg**		**Sham**
Study Drug Components	ocriplasmin 0.5mg/0.2mL	Sodium chloride (NaCl) 9mg/mL (0.9%)	ocriplasmin 0.5mg/0.2mL	Sodium chloride (NaCl) 9mg/mL (0.9%)	-
Formulation	**Active Substance** ocriplasmin 0.5mg/0.2mL **Excipients** Mannitol Citric acid Sodium hydroxide (NaOH) for pH adjustment Water for injection	NaCl 9mg/mL (0.9%)	**Active Substance** ocriplasmin 0.5mg/0.2mL **Excipients** Mannitol Citric acid Sodium hydroxide (NaOH) for pH adjustment Water for injection	NaCl 9mg/mL (0.9%)	-
Presentation	Clear and colourless solution	Clear and colourless solution	Clear and colourless solution	Clear and colourless solution	-
Volume for dilution	0.2mL	0.6mL	0.2mL	0.2mL	-
Volume to be administered	0.1mL		0.1mL		-

Table 5: Study Drugs (Continued)

Study Drug	Ocriplasmin 0.0625mg	Ocriplasmin 0.125mg	Sham
Route of Administration	Intravitreal injection	Intravitreal injection	Sham injection
Dosing Schedule	• Up to 3 injections for subjects in the ocriplasmin 0.0625mg arm, administered at Day 0, Day 35 and Day 70	• Up to 3 injections for subjects in the ocriplasmin 0.125mg arm, administered at Day 0, Day 35 and Day 70[a]	• 3 sham injections for subjects in the sham arm, administered at Day 0, Day 35 and Day 70 • Up to 2 sham injections for subjects in the ocriplasmin arms, administered at Day 35 and / or Day 70[a]

[a] All subjects assigned to the ocriplasmin arms will receive an intravitreal injection of ocriplasmin 0.0625mg or 0.125mg at Day 0. These subjects will be re-treated with the same dose of ocriplasmin at Day 35 and at Day 70 if, at that time, they do not have total PVD on both B-scan ultrasound and SD-OCT, as assessed by the masked B-scan expert reader and the masked CRC, respectively. Subjects who have total PVD on both B scan ultrasound and SD-OCT will receive subsequent sham injection(s) in place of ocriplasmin injection(s)

Ocriplasmin is supplied in a single use, glass (type I glass) vial containing 0.5mg ocriplasmin in 0.2mL solution (2.5mg/mL). Ocriplasmin vials are packed in carton boxes, each of which contains a single vial. Vials are closed with a chlorobutyl rubber stopper and a polypropylene flip-off cap.

NaCl (0.9%) is supplied in a single use ampoule containing 5mL NaCl 9mg/mL (0.9%).

Sham is supplied as an empty glass (type I glass) vial. Sham vials are packed in carton boxes, each of which contains a single vial. Vials are closed with a chlorobutyl rubber stopper and a polypropylene flip-off cap.

All vials and cartons are labelled and packed in accordance with current ICH guidelines on Good Manufacturing Practice (GMP) and GCP E6(R1).

6.2. Storage and Handling of Study Drug

6.2.1. Study Drug Kit Storage

Study drug kits should be stored protected from light at -20°C±5°C (-4°F±9°F) in a temperature controlled, secure facility with limited access.

If temperature excursions are noted, this must be reported immediately to the CRO, who works on behalf of the Sponsor. Study drug subject to a temperature excursion should be immediately quarantined under the indicated storage conditions, until further instructions from the CRO.

After Thawing

Once thawed, the study drug should be diluted and used immediately. Do not refreeze a vial once it has been thawed.

After Opening / Dilution

From a microbiological point of view, the study drug must be used immediately after opening / dilution.

6.2.2. Administration Kit Storage

Administration kits should be stored at ambient temperature.

6.2.3. Study Drug Preparation and Administration

The unmasked treating investigator / designee will prepare the injections. The unmasked treating investigator will administer the injections (ocriplasmin 0.0625mg, ocriplasmin 0.125mg and sham).

In addition to the procedures described below, added safety measures in adherence to specific institutional policies associated with intravitreal injections will be used. Also refer to (Avery *et al*., 2014) for a guideline on intravitreal injection technique and monitoring.

6.2.3.1. Pre-injection Procedures

Pre-injection procedures will be implemented to minimise the risk of potential AEs associated with intravitreal injections.

The pre-injection procedures, including injection tray assembly and anaesthetic preparation, will be performed under controlled aseptic conditions. This will include surgical hand disinfection, sterile gloves and disinfection of the periocular skin, eyelid and ocular surface (*i.e.* cleansed with povidone-iodine).

Adequate anaesthesia should be administered prior to the injection as per local clinical practice and in line with the JETREA USPI / SmPC. A prophylactic topical antibiotic may be administered at the discretion of the unmasked treating investigator.

6.2.3.2. Ocriplasmin 0.0625mg Injection

Study drug preparation will be done outside of the subject's view. In addition, a sterile drape may be used to cover the fellow eye and the subject will be instructed to direct his / her gaze away at all times. It is recommended to follow the guidelines published by Avery *et al* on intravitreal injections (Avery *et al*., 2014). The injection should be carried out under aseptic conditions, which includes the use of surgical hand disinfection, sterile gloves, a surgical mask, a sterile drape and a sterile eyelid speculum (or equivalent).

1. Remove the drug kit from the freezer and allow to thaw at room temperature (takes about 2 minutes)
2. Once completely thawed, remove the protective polypropylene flip-off cap from the ocriplasmin vial and discard the flip-off cap immediately
3. Disinfect the top of the ocriplasmin vial with an alcohol wipe
4. Disinfect the top of the NaCl (0.9%) ampoule with an alcohol wipe. Twist off the head of the ampoule to decapsulate. Insert the syringe cone into the ampoule and withdraw NaCl (0.9%). Detach the syringe from the ampoule and attach 1 of the 19G needles. Carefully expel the air and excess NaCl (0.9%) from the syringe and adjust the volume to the 0.6mL mark
5. Add 0.6mL NaCl (0.9%) into the ocriplasmin vial. Discard the needle and the syringe
6. Swirl the vial
7. Visually inspect the vial for particulate matter. Only a clear, colourless solution without visible particles should be used
8. Using aseptic technique, withdraw all of the solution using the other 19G needle (slightly incline the vial to ease withdrawal) and discard the needle after withdrawal of the vial contents. Do not use this needle for the intravitreal injection
9. Replace the needle with the sterile 30G injection needle, carefully expel the air and excess drug from the syringe and adjust the volume to the 0.1mL mark on the syringe (corresponding to 0.0625mg ocriplasmin)
10. Inject 0.1mL of the solution into the mid-vitreous:
    a. The injection should be made through the pars plana in the inferotemporal quadrant 3.5-4mm posterior to the limbus
    b. The needle should be inserted at least 6mm towards the centre of the eye avoiding the horizontal meridian

c. Use a moderately slow injection technique in order to allow the test product to enter the vitreous

11. Slowly remove the needle and use a sterile cotton-tip applicator to prevent reflux of both the test product and the vitreous
12. Discard the syringe and the needle immediately after the injection
13. Put the NaCl (0.9%) ampoule back in its administration kit carton and the ocriplasmin vial in its study drug kit carton. Seal both cartons and store securely for drug accountability

6.2.3.3. Ocriplasmin 0.125mg Injection

Study drug preparation will be done outside of the subject's view. In addition, a sterile drape may be used to cover the fellow eye and the subject will be instructed to direct his / her gaze away at all times. It is recommended to follow the guidelines published by Avery *et al* on intravitreal injections (Avery *et al*., 2014). The injection should be carried out under aseptic conditions, which includes the use of surgical hand disinfection, sterile gloves, a surgical mask, a sterile drape and a sterile eyelid speculum (or equivalent).

1. Remove the drug kit from the freezer and allow to thaw at room temperature (takes about 2 minutes)
2. Once completely thawed, remove the protective polypropylene flip-off cap from the ocriplasmin vial and discard the flip-off cap immediately
3. Disinfect the top of the ocriplasmin vial with an alcohol wipe
4. Disinfect the top of the NaCl (0.9%) ampoule with an alcohol wipe. Twist off the head of the ampoule to decapsulate. Insert the syringe cone into the ampoule and withdraw NaCl (0.9%). Detach the syringe from the ampoule and attach 1 of the 19G needles. Carefully expel the air and excess NaCl (0.9%) from the syringe and adjust the volume to the 0.2mL mark
5. Add 0.2mL NaCl (0.9%) into the ocriplasmin vial. Discard the needle and the syringe
6. Swirl the vial
7. Visually inspect the vial for particulate matter. Only a clear, colourless solution without visible particles should be used
8. Using aseptic technique, withdraw all of the solution using 1 of the 19G needles (slightly incline the vial to ease withdrawal) and discard the needle after withdrawal of the vial contents. Do not use this needle for the intravitreal injection
9. Replace the needle with the sterile 30G injection needle, carefully expel the air and excess drug from the syringe and adjust the volume to the 0.1mL mark on the syringe (corresponding to 0.125mg ocriplasmin)
10. Inject 0.1mL of the solution into the mid-vitreous:
    a. The injection should be made through the pars plana in the inferotemporal quadrant 3.5-4mm posterior to the limbus

b. The needle should be inserted at least 6mm towards the centre of the eye avoiding the horizontal meridian
c. Use a moderately slow injection technique in order to allow the test product to enter the vitreous

11. Slowly remove the needle and use a sterile cotton-tip applicator to prevent reflux of both the test product and the vitreous
12. Discard the syringe and the needle immediately after the injection
13. Put the NaCl (0.9%) ampoule back in its administration kit carton and the ocriplasmin vial back in its study drug kit carton. Seal both cartons and store securely for drug accountability

6.2.3.4. Sham Injection

Study drug preparation will be done outside of the subject's view. In addition, a sterile drape may be used to cover the fellow eye and the subject will be instructed to direct his / her gaze away at all times. It is recommended to follow the guidelines published by Avery *et al* on intravitreal injections (Avery *et al*., 2014). The injection should be carried out under aseptic conditions, which includes the use of surgical hand disinfection, sterile gloves, a surgical mask, a sterile drape and a sterile eyelid speculum (or equivalent).

1. Remove the drug kit from the freezer and allow to attain room temperature (leave at room temperature for at least 2 minutes for masking reasons)
2. Remove the protective polypropylene flip-off cap from the sham vial and discard the flip-off cap immediately
3. Disinfect the top of the sham vial with an alcohol wipe
4. Using aseptic technique, mimic solution withdrawal using 1 of the 19G needles (slightly incline the vial to ease withdrawal) and discard the needle after
5. Adjust the dose volume to the 0.1mL mark on the syringe
6. Press the hub of the syringe firmly against the sclera / conjunctiva and slowly depress the plunger to mimic an actual injection procedure
7. Discard the syringe immediately after the sham injection. Also discard the unused syringe, 30G and 19G needles
8. Put the sham vial back in the study drug kit carton. Seal the study drug kit carton and the empty drug administration kit carton and store both cartons securely for drug accountability

6.2.4. Post-Injection Assessment

During the 1-hour period after injection (ocriplasmin or sham), the subject should be monitored for elevated IOP. Elevated IOP may occur when the central retinal artery remains closed for approximately 2 minutes, if the subject reports no light perception. The following will be done:

1. Visualise the optic nerve head to verify reperfusion of the central retinal artery in the immediate post-injection period

2. Monitor IOP with tonometry
3. Increased IOP may warrant treatment (*e.g.* anterior chamber paracentesis). However, transient graying or obscuration of vision following injection is expected and does not need to be treated

Verify that the retina is attached and that there is no haemorrhage.

Instruct subjects that they should avoid rubbing their eye. Also instruct subjects to report any symptoms suggestive of infection, bleeding, retinal break or tear, increased IOP or lens subluxation without delay (*e.g.* eye pain, worsening eye redness, severely blurred or decreased vision, increased sensitivity to light, floaters, fluctuation of vision, double vision, headache, halos around light, nausea and vomiting).

6.2.5. Study Drug Accountability

A full accountability record must be maintained. The unmasked treating investigator / designee (*e.g.* study nurse, study coordinator or pharmacist) will be responsible for recording the receipt, administration and return of all supplies using the supplied forms.

Unused study drug and administration kits must be stored securely. Used study drug kits (containing used ocriplasmin or sham vials) and used administration kits (containing the used NaCl [0.9%] ampoule where applicable) must be sealed and stored securely. An unmasked study monitor will visit the site periodically to check inventories and ensure that IXRS assigned treatments have correctly been administered and that all unused supplies remain intact or quarantined.

After study treatment is completed for all subjects, and upon request from the Sponsor, all used and unused study drug and administration kits must be reconciled by the unmasked study monitor and returned to the Sponsor or its representative, together with the drug return form and a copy of the accountability form.

Under no circumstances will the Investigator allow the study drug to be used in any manner other than directed by this protocol.

6.3. Treatment Compliance

As the treatment is not dispensed to the subject, but administered by the unmasked treating investigator, no procedures for monitoring subject treatment compliance are required.

6.4. Concomitant Medications / Treatments / Interventions

At each study visit, the Investigator should question the subject about any medications / treatments / interventions taken / received by the subject. All concomitant medications / treatments / interventions should be recorded in the subject's eCRF.

6.4.1. Diabetic Retinopathy-related Treatment

6.4.1.1. Escape Criteria

- Subjects may be treated with PRP following the confirmation of progression from NPDR to PDR, as assessed by the masked CRC.
  No minimum time interval between study treatment and PRP needs to be considered.
- Subjects may be treated with an anti-VEGF treatment following the confirmation of development of CI-DME with CST of ≥ 340µm on Spectralis SD-OCT or ≥ 320µm on non-Spectralis SD-OCT, or an increase of 50µm from baseline in CST, based on SD-OCT, as assessed by the masked CRC.
  There are no clinical data on concomitant use of ocriplasmin with anti-VEGF treatment, however, as ocriplasmin is a proteolytic enzyme with serine protease activity which could be present in the eye for several days after intravitreal injection, administration in close temporal association with other medicinal products in the same eye may affect the activity of both medicinal products and is therefore not recommended. A minimum time of 7 days between study treatment and anti-VEGF injection is therefore required.

7. ASSESSMENT OF EFFICACY

7.1. Efficacy Parameters

Refer to Section 3.1.1 for the efficacy endpoints.

7.2. Methods and Timing for Assessing Efficacy Parameters

Refer to Table 8 for an overview of which study procedures should be done by masked and the unmasked study staff and for which study procedures certification is needed.

7.2.1. B-scan Ultrasound

B-scan ultrasounds will be used to assess total PVD. The images will be submitted to and evaluated by the B-scan expert reader.

B-scan ultrasounds will be taken for all subjects:

- At Visit 1 (screening visit) for **potential study eye(s)**
  If 1 of the subject's eyes is excluded based on an eligibility criterion that does not need confirmation by the B-scan expert reader or the CRC, no B-scan ultrasound needs to be performed for that eye. If both eyes potentially qualify as the study eye, B-scan ultrasounds need to be taken for both eyes
- At Visit 4a (Day 28), Visit 6a (Day 63), Visit 7 (Day 77), Visit 8 (Month 3), Visit 9 (Month 9), Visit 10 (Month 15) and Visit 11 (Month 24) / the Exit Visit for the **study eye**

7.2.2. SD-OCT (6mm)

6mm SD-OCTs will be used for assessment of efficacy (total PVD, macular oedema) and safety. The images will be submitted to and evaluated by the masked CRC.

6mm SD-OCT imaging will be performed for all subjects:

- At Visit 1 (screening visit) for **potential study eye(s)**
  If 1 of the subject's eyes is excluded based on an eligibility criterion that does not need confirmation by the B-scan expert reader or the CRC, no SD-OCT needs to be taken for that eye. If both eyes potentially qualify as the study eye, SD-OCT imaging needs to be performed for both eyes
- At each scheduled study visit, with the exception of Visit 2 (Day 0) and the repeat injection visits (Visit 4b [Day 35] and Visit 6b [Day 70]), for the **study eye**

7.2.3. 7-Standard Field Stereo Colour Fundus Photography

7-standard field stereo colour fundus photography will be used to assess DR stage, neovascularisation and vitreous / pre-retinal haemorrhage. The images will be submitted to and evaluated by the masked CRC.

7-standard field stereo colour fundus photographs will be taken for all subjects:

- At Visit 1 (screening visit) for **potential study eye(s)**
  If 1 of the subject's eyes is excluded based on an eligibility criterion that does not need confirmation by the B-scan expert reader or the CRC, no 7-standard field stereo colour fundus photograph need to be taken for that eye. If both eyes potentially qualify as the study eye, 7-standard field stereo colour photographs need to be taken for both eyes
- At Visit 10 (Month 15) and at Visit 11 (Month 24) / the Exit Visit for the **study eye**

In addition, 7-standard field stereo colour fundus photographs will be taken for subjects that the Investigator suspects progressed to PDR:

- At Visit 8 (Month 3) and at Visit 9 (Month 9)

7.2.4. Slit Lamp Examination

Slit lamp examination will be performed for all subjects:

- At Visit 1 (screening visit), Visit 8 (Month 3), Visit 9 (Month 9), Visit 10 (Month 15) and at Visit 11 (Month 24) / the Exit Visit for **both eyes**
- At Visit 2 (Day 0), Visit 3 (Day 7), Visit 4a (Day 28), Visit 5 (Day 42), Visit 6a (Day 63) and at Visit 7 (Day 77) for the **study eye**

7.2.5. Best Corrected Visual Acuity

BCVA will be reported as the number of letters read correctly by the subject on the ETDRS chart.

BCVA will be assessed for all subjects:

- At each scheduled study visit, with the exception of the repeat injection visits (Visit 4b [Day 35] and Visit 6b [Day 70]), for **both eyes**

7.2.6. Widefield SD-OCT

Widefield SD-OCT will be used for exploratory assessment of total PVD. The images will be submitted to and evaluated by the masked CRC.

Widefield SD-OCT imaging will be performed in a subset of subjects, enrolled at sites where this is available:

- At Visit 2 (Day 0), Visit 4a (Day 28), Visit 6a (Day 63) and Visit 8 (Month 3) for the **study eye**

8. ASSESSMENT OF SAFETY

8.1. Safety Parameters

Refer to Section 3.1.2 for the safety endpoints.

8.2. Methods and Timing for Assessing Safety Parameters

Refer to Table 8 for an overview of which study procedures should be done by masked and the unmasked study staff and for which study procedures certification is needed.

8.2.1. Adverse Events

All AEs must be recorded from the time of providing consent until the end of the study for all subjects.

8.2.2. Full Ophthalmic Examination

A full ophthalmic examination (pupillary examination, slit lamp examination, IOP assessment and dilated fundus examination) will be performed for all subjects:

- At Visit 1 (screening visit), Visit 8 (Month 3), Visit 9 (Month 9), Visit 10 (Month 15) and at Visit 11 (Month 24) / the Exit Visit for **both eyes**
- At Visit 2 (Day 0), Visit 3 (Day 7), Visit 4a (Day 28), Visit 5 (Day 42), Visit 6a (Day 63) and at Visit 7 (Day 77) for the **study eye**

8.2.3. Best Corrected Visual Acuity

BCVA will be both a measure of efficacy and safety. Refer to Section 7.2.5 for more information on the method and timing of BCVA measurement.

8.2.4. Colour Vision (Roth 28-hue)

Hue discrimination ability will be assessed by the Roth 28 Colour Test.

The Roth 28-hue colour vision test will be performed in a subset of subjects, enrolled at sites where the Roth 28 Colour Test is available, and who are ≤ 80 years of age and do not have a diagnosis of dyschromatopsia at baseline:

- At each scheduled study visit, with the exception of Visit 1 (screening) and the repeat injection visits (Visit 4b [Day 35] and Visit 6b [Day 70]), for the **study eye**

8.2.5. SD-OCT

SD-OCTs will be used both as a measure of efficacy and safety. Refer to Section 7.2.2 and Section 7.2.6 for more information on the timing of SD-OCT imaging.

8.2.6. Full-field ERG

ffERG readings will be submitted to and evaluated by the masked CRC.

ffERG will be performed for approximately 25% of the subjects, enrolled at sites where this is available:

- At Visit 2 (Day 0) and Visit 8 (Month 3) for **both eyes**

In addition, ffERG will be performed for subjects with expert-defined ffERG abnormalities:

- At Visit 10 (Month 15) for **both eyes** - for subjects with expert-defined ffERG abnormalities at Visit 8 (Month 3)
- At Visit 11 (Month 24) / the Exit Visit for **both eyes -** for subjects with expert-defined ffERG abnormalities at Visit 10 (Month 15)

8.2.7. Immunogenicity

An immunogenicity assay will be performed to assess whether multiple injections of ocriplasmin 0.0625mg or 0.125mg induce an immune response. Blood samples for assessment of immunogenicity will be sent to the central laboratory for immunogenicity testing.

Blood samples for immunogenicity assessment will be taken from all subjects:

- At baseline (Visit 2 [Day 0]) and at Visit 8 (Month 3)

8.3. Safety Definitions

8.3.1. Definition of an Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

8.3.2. Definition of a Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening
  NOTE: The term "life-threatening" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires in-patient hospitalisation or prolongation of existing hospitalisation
- Results in persistent or significant disability / incapacity
- Is a congenital abnormality / birth defect

Medical and scientific judgment should be exercised in deciding whether other situations should be considered SAEs, such as important medical events that might not be immediately

life-threatening or result in death or hospitalisation but might jeopardise the patient or might require intervention to prevent one of the other outcomes listed above.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, neoplasms or convulsions that do not result in hospitalisation.

8.3.3. Clarifications

Hospitalisation

Hospitalisation for elective treatment of a pre-existing condition (*i.e.* a condition present prior to the subject's signature of the informed consent) that did not worsen during the study is **not** considered an (S)AE. Complications that occur during hospitalisation are AEs. If a complication prolongs hospitalisation, or meets any of the other SAE criteria, the complication is an SAE.

Pre-existing Conditions, Including DR

Pre-existing conditions (*i.e.* conditions present or detected at the start of the study) which worsen during the study, exacerbation of a pre-existing illness or an increase in frequency or intensity of a pre-existing episodic event or condition are (S)AEs. Anticipated day-to-day fluctuations of pre-existing condition(s) that do not worsen with respect to baseline are **not** (S)AEs.

Worsening or progression of DR is considered to be a "lack of efficacy" or "failure of expected pharmacological action" per se and is already recorded in the efficacy data and therefore does **not** need to be reported as an (S)AE. However, the signs and symptoms and / or clinical sequelae resulting from the lack of efficacy may be reported as an (S)AE if considered by the Investigator to fulfil the definition of an (S)AE.

Medical or Surgical Procedures

Medical or surgical procedures (*e.g.* endoscopy, colonoscopy) are **not** (S)AEs; the condition that leads to the procedure is an (S)AE.

In case of elective medical or surgical procedures, or pre-study planned medical or surgical procedures for pre-existing conditions (*i.e.* a condition present prior to the subject's signature of the informed consent) that did not worsen during the study, the condition that leads to the procedure does **not** need to be reported as an (S)AE.

Death

Death is **not** an SAE; the condition that leads to death is an SAE.

Abnormal Laboratory Values

In the absence of diagnosis, abnormal laboratory values that are judged by the Investigator to be clinically significant must be recorded as an (S)AE. Clinically significant abnormal laboratory findings that are present at baseline and significantly worsen following the start of the study will also be reported as an (S)AE. However, clinically significant abnormal laboratory findings that are associated with DR, unless judged by the Investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will **not** be reported as (S)AEs.

8.4. Recording of Adverse Events

8.4.1. Adverse Events

Adverse Events (Non-serious and Serious)

(S)AEs occurring from the time of providing consent until the end of the study (Visit 11 – Month 24 / Exit Visit) must be recorded in the subject's eCRF.

(S)AEs observed during the 1-hour post-injection assessment should be recorded by the unmasked treating investigator / designee in the unmasked AE pages of the eCRF. If such an (S)AE is ongoing after the post-injection assessment, it will be followed up in a masked manner by the masked Investigator. All other (S)AEs should be recorded by the masked Investigator / designee in the masked AE pages of the eCRF.

In order to escalate relevant TEAEs in a timely manner to the DMC (refer to Section 3.4), all (S)AEs need to be recorded in the eCRF **within 24 hours** after receipt or awareness of the information.

The (S)AE term should be reported in standard medical terminology when possible. For each (S)AE, the Investigator will assess and record the date of onset, date of resolution, intensity, causality, action taken, seriousness, outcome (if applicable), and whether or not it led to withdrawal from the study.

When possible, signs and symptoms indicating a common underlying pathology should be documented as one comprehensive event with an overall diagnosis. If a diagnosis cannot be made initially, the main signs and symptoms should be documented and updated with a diagnosis when available.

Serious Adverse Events

The unmasked treating investigator / masked Investigator shall report SAEs **immediately**, and no later than 24 hours after receipt or awareness of the information, **by completing the SAE Form in the subject's eCRF**. The SAE Form should be completed as thoroughly as possible and verified with the corresponding source documents. It is very important that the Investigator provides an assessment of the relationship to the study drug or the injection procedure at the time of the initial SAE report. The eCRF system will automatically send the information to the Sponsor for processing.

If the eCRF system cannot be used for any reason, the Investigator shall submit the completed SAE Form by email or fax to the Sponsor (email: pharmacovigilance@thrombogenics.com; fax: + 1 8775274503), within the same timeframe. The data should be entered in the eCRF as soon as the system becomes available again.

8.4.1.1. Relationship to Study Drug or the Injection Procedure

For each (S)AE, the unmasked treating investigator / masked Investigator must determine whether the event is related to the study drug or the injection procedure. To do so, the Investigator shall determine whether, in his / her medical judgment, there is a reasonable possibility that the event may have been caused by the study drug or the injection procedure, as defined below.

Related

- There is reasonable probability that the (S)AE may have been caused by the study drug or the injection procedure; the (S)AE follows a reasonable temporal sequence from study treatment and the (S)AE cannot be reasonably explained by the subject's clinical status or other factors (*e.g.* disease under study, concurrent disease[s], and concomitant medications)

Unrelated

- There is no reasonable probability that the (S)AE has been caused by the study drug or the injection procedure; the (S)AE does not follow a reasonable sequence from study treatment, or can be reasonably explained by the subject's clinical status or other factors (*e.g.* disease under study, concurrent disease[s], and concomitant medications)

8.4.1.2. Adverse Event Intensity

The unmasked treating investigator / masked Investigator shall assess and record the intensity of the (S)AE according to the following scale:

- Mild (awareness of sign or symptom, but easily tolerated)
- Moderate (discomfort sufficient to cause interference with normal activities)
- Severe (incapacitating, with inability to perform normal activities)

An AE that is assessed as 'Severe' should not be confused with an SAE. 'Severe' is part of a scale used for rating the intensity of an event. An event is defined as 'Serious' when it meets 1 of the pre-defined outcomes as described in Section 8.3.2. A severe AE can be of relatively minor medical significance / non-serious (*e.g.* severe itching). Similarly, an SAE can be of mild intensity (*e.g.* mild chest pain).

For AEs that change in intensity, the start and stop date of each intensity should be recorded.

8.4.1.3. Adverse Event Outcome

At the time of reporting the outcome of an AE, it must be recorded as ongoing, resolved, or resolved with sequelae / death.

8.4.1.4. Adverse Event Follow-Up

Non-Serious Adverse Events

The masked Investigator will follow subjects from the study who are withdrawn from the study as a result of an AE until the event has resolved, subsided, stabilised, or until the event is otherwise explained, or the subject is lost to follow-up.

Other AEs will be followed until the subject has recovered or the subject's participation in the study is complete.

Serious Adverse Events

The Sponsor or delegated Pharmacovigilance Service Provider will follow-up SAE reports to completion. Investigators are expected to provide the additional information required for complete assessment and documentation of the SAE reports in a timely manner.

When more information becomes available, the masked Investigator must update the SAE Form in the subject's eCRF. The eCRF system will automatically send the follow-up report to the Sponsor for processing.

If the eCRF system cannot be used for any reason, the Investigator shall submit the follow-up report by email or fax to the Sponsor (email: pharmacovigilance@thrombogenics.com; fax: + 1 8775274503), within the same timeframe. The data should be entered in the eCRF as soon as the system becomes available again.

Copies of hospital reports, autopsy reports and other documents should be provided if they are relevant to the evaluation of the SAE.

SAEs should be followed-up until the event has resolved, subsided, stabilised, or until the event is otherwise explained, or the subject is lost to follow-up.

If the Investigator learns of any SAE at any time after a subject has completed the study, and he / she considers the event to be possibly related to the study drug or the injection procedure, he / she will notify the Sponsor by emailing or by faxing (email: pharmacovigilance@thrombogenics.com; fax: + 1 8775274503).

Screening Failures

For screening failures, only (S)AEs related to participation at the screening visit will be followed-up as described in the sections above.

8.4.2. Pregnancy / Prenatal Exposure

Pregnancies will be recorded from the time of providing consent until the end of the study (Visit 11 – Month 24 / Exit Visit).

Subjects with reproductive potential, whether male or female, must agree to use contraception for the duration of the study. The Investigator should counsel subjects with regard to the possible untoward effects on the foetus.

Female subjects of childbearing potential will need to have a negative pregnancy test at Visit 1 (Screening) and at Visit 2 (Day 0). In addition, a urine pregnancy test will be taken at each repeat injection visit and for any woman of childbearing potential who missed 1 period at any time during the study. Female subjects who are pregnant at the time of repeat injection must not receive further injection(s). Pregnant subjects should not be withdrawn from the study, but should be asked to continue with all scheduled study visits and procedures until the end of the study to ensure complete follow-up.

Pregnancy occurring in a female subject or in the partner of a male subject should be reported immediately to the Investigator. The Investigator should report pregnancies immediately to the Sponsor by using the Pregnancy / Prenatal Exposure Form in the eCRF (for study subjects) or via email or fax (email: pharmacovigilance@thrombogenics.com; fax: + 1 8775274503) (for

pregnant partners). The eCRF system will automatically send the form to the Sponsor for processing. If the eCRF system cannot be used for any reason, the Investigator shall submit the Pregnancy / Prenatal Exposure report by email or fax to the Sponsor (email: pharmacovigilance@thrombogenics.com; fax: + 1 8775274503). The data should be entered in the eCRF as soon as the system becomes available again.

In case of a pregnancy in the partner of a male subject, the Investigator should obtain written informed consent from the pregnant partner prior to obtaining information about the pregnancy. The Investigator will provide the pregnant partner with a copy of the Pregnant Partner Information Sheet / Informed Consent Form.

Pregnancies should be monitored until conclusion. The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented via the Pregnancy Follow-up Form, even for subjects withdrawn from the study or who completed the study.

Pregnancy in itself is not regarded as an AE unless there is a suspicion that the study drug may have interfered with the effectiveness of a contraceptive medication. If there is an adverse condition associated with the pregnancy, the adverse condition is recorded on the AE eCRF page, but not the pregnancy itself. If an adverse condition as a result of pregnancy is considered serious, an SAE Form must be completed. All reports of congenital abnormalities / birth defects in the offspring of study participants are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs.

Screening Failures

Subjects who are pregnant before study drug administration (and who will hence be screening failures) will not be followed up.

8.5. Treatment of Adverse Events

Treatment of any AE is at the sole discretion of the Investigator and according to current good medical practice. The treatment / procedure should be recorded in the eCRF.

8.6. Reporting Requirements for Serious Adverse Events

In accordance with ICH GCP, the Sponsor is responsible for the ongoing safety evaluation of an investigational medicinal product under clinical investigation. As such, the Sponsor is responsible for prompt notification to all concerned Investigators, IECs / IRBs and Regulatory Authorities of findings that could adversely affect the health of the subjects, impact the conduct of the study or alter the authorisation to continue the study.

The Sponsor or delegated Pharmacovigilance Service Provider will submit Suspected Unexpected Serious Adverse Reactions (SUSARs) to the Regulatory Authorities in an expedited manner as follows:

- Fatal or life-threatening SUSARs – as soon as possible but no later than 7 calendar days after the Sponsor’s first knowledge of the minimum criteria for expedited reporting

- Non-fatal and non-life-threatening SUSARs – as soon as possible but no later than 15 calendar days after the Sponsor's first knowledge of the minimum criteria for expedited reporting
- Relevant follow-up information for fatal or life-threatening SUSARs – within an additional 8 calendar days

Where required by IEC / IRB procedure, it is the Principal Investigator's responsibility to notify the IEC / IRB of all SUSARs / SAEs that occur at his / her site.

SUSARs that have arisen in other clinical studies of the same sponsor and with the same study drug and that could have consequences for the safety of the subjects involved in the clinical study that was assessed by the concerned IEC / IRB will be reported in accordance with the requirements of the concerned IEC / IRB.

9. STATISTICS

9.1. Determination of Sample Size

One hundred and fifteen (115) subjects (46 subjects in each of the ocriplasmin arms and 23 subjects in the sham arm) will provide 80% power to detect a significant difference in total PVD rate by the Month 3 visit, for the comparison of sham to each of the ocriplasmin arms at a 2-sided alpha level of 0.05, assuming total PVD is achieved in 5% and 40% of the subjects in the sham and ocriplasmin arms, respectively. The sample size takes into account a drop-out rate of 10% by the Month 3 visit.

In order to protect the alpha level to 0.05, a hierarchical testing procedure will be used. Ocriplasmin 0.125mg will be first compared to sham. If the p-value of this first comparison is found < 0.05, then ocriplasmin 0.0625mg will be compared to sham.

9.2. Subject Analysis Sets

9.2.1. Safety Analysis Set

The Safety Analysis Set consists of all subjects who received at least 1 dose of study drug. Analyses on the Safety Analysis Set will be performed based on treatment actually administered at Visit 2 (Day 0).

9.2.2. Full Analysis Set

The Full Analysis Set consists of all subjects who received at least 1 dose of study drug and for whom data of at least 1 post-baseline efficacy assessment is present. Analysis on the Full Analysis Set will be based on the assigned randomised treatment.

9.2.3. Per Protocol Set

The Per Protocol Set consists of a subset of subjects of the Full Analysis Set, excluding those subjects with major deviations from the protocol that may substantially affect the results of the efficacy analysis. Full details of the criteria leading to exclusion of data from the Per Protocol Set will be specified in the statistical analysis plan (SAP). Analysis on the Per Protocol Set will be based on the assigned randomised treatment.

9.3. Statistical Methods

Statistical methodology that will be applied to the study data will be described in detail in the SAP to be finalised prior to the Month 3 analysis. Deviations from the SAP will be described in the final CSR.

Descriptive statistics for continuous variables will include the number of subjects with available data, mean with its 95% CI (where indicated), standard deviation, median, Q1 and Q3, P05 and P95, minimum and maximum.

Categorical data will be summarised by presenting count and percentage with its 95% CI, where indicated.

9.3.1. Significance

All statistical tests will be performed at the two-sided 5% (α=5%) level of significance unless otherwise specified. All p-values, with the exception of the 2 p-values resulting from the comparisons of the primary efficacy endpoint, will be considered as supportive and explorative. CIs will be two-sided 95%, unless otherwise specified.

9.3.2. Procedure for Accounting for Missing, Unused and Spurious Data

All reasonable efforts will be made to obtain complete data for all subjects. However, missing observations may occur due to subjects lost to follow-up or to noncompliance with required assessments. Management of dropouts and missing data will depend on their frequency and the nature of the outcome parameter.

The primary analysis of the efficacy parameters will be performed on the Full Analysis Set using the last-observation-carried-forward (LOCF), unless otherwise specified. Sensitivity analysis to evaluate the robustness of the data will be performed using alternative imputation method on the Full Analysis Set and the Per Protocol Set.

In the safety analysis, missing data will not be replaced and data analysis will be performed based on the Observed Case (OC) imputation method.

9.4. Analysis of Subject Disposition, Demographics and Baseline Characteristics

Subject disposition (including withdrawals), demographics and baseline characteristics and withdrawal status will be summarised by treatment arm and overall using descriptive statistics.

9.5. Analysis of Efficacy

Efficacy analyses will be performed on the Full Analysis Set.

9.5.1. Primary Efficacy Analysis

The proportion of subjects with total PVD by the Month 3 visit will be calculated in each treatment arm with corresponding 95% CI, stratified for NPDR severity at baseline. The adjusted differences in proportions between sham and each of the ocriplasmin arms will be calculated with its corresponding 95% CI and tested using the Cochran-Mantel-Haenszel test. In addition, the effect of treatment on the proportion of subjects with total PVD will be computed using a logistic regression with effects for treatment and NPDR severity at baseline.

9.5.2. Exploratory Efficacy Analyses

The analysis of exploratory dichotomous endpoints will be similar to the methodology used for the primary endpoint. For each exploratory endpoint, the proportion of subjects who achieve the endpoint at a certain study visit will be calculated in each treatment arm with corresponding 95% CI, stratified for NPDR severity at baseline. The adjusted differences in proportions between sham and each of the ocriplasmin arms will be calculated with their corresponding 95% CIs and tested using the Cochran-Mantel-Haenszel test. In addition, the effect of treatment on the proportion of subjects who achieve the endpoint will be computed using a logistic regression with effects for treatment and NPDR severity at baseline. Mean change from baseline in ETDRS

letters will be calculated by treatment arms at follow-up visits, stratified for NPDR severity at baseline. The adjusted differences in means between treatment arms will be summarised and analysed using a PROC GLM model including factors for treatment, BCVA at baseline and NPDR severity at baseline.

9.6. Analysis of Safety

Safety analyses will be performed on the Safety Analysis Set. No formal statistical comparison will be performed and no p-value will be calculated. Analyses will be performed irrespective of stratum.

9.6.1. Principal Safety Analyses

The proportion of subjects with ocular TEAEs in the study eye at any time during the study will be calculated in each treatment arm with corresponding 95% CI.

The proportion of subjects with a loss of ≥ 15 ETDRS letters at any time during the study and between Day 0 and Day 7 after each injection will be calculated in each treatment arm with corresponding 95% CI.

9.6.2. Other Safety Analyses

Adverse Events

AEs (ocular, in the study and the non-study eye, and non-ocular) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Summaries of AEs will use the system organ class (SOC) and preferred term (PT) codes, as appropriate.

AEs will be summarised by treatment arm, SOC and PT. Both the number and percentage of subjects who experience the event and the number of events will be summarised.

TEAEs, study drug-related AEs, AEs leading to withdrawal from the study and SAEs will be summarised in a similar manner. AEs will also be tabulated by maximum severity, relationship to study treatment, and onset from study treatment.

Other Safety Assessments

Descriptive statistics will be calculated by study visit and treatment arm for slit lamp examination parameters, IOP, loss of vision in BCVA, Roth 28-hue colour vision status, SD-OCT parameters and ffERG assessments.

Concomitant Medications

Concomitant medications will be listed by subject and medication name.

9.7. Sequence of Analyses

- While the Sponsor is masked to the individual treatment assignments, the data analyses for the DMC meetings will be performed by an unmasked, independent statistician. The independent external statistician will maintain secure custody of unmasked data

- The analysis of the primary endpoint will be performed by an unmasked, independent, external statistician when all subjects have completed the Month 3 visit (Visit 8). The independent external statistician will maintain secure custody of unmasked data
- The analysis of the exploratory efficacy and the safety endpoints up to the Month 15 visit will be performed when all subjects have completed the Month 15 visit (Visit 10)
- An additional analysis will be performed when all data is available, after the database lock

10. DATA VERIFICATION AND DIRECT ACCESS TO SOURCE DOCUMENTATION

During the conduct of the study and thereafter until the final clinical study report (CSR) has been completed, unmasked / masked study monitors from the CRO, working on behalf of the Sponsor, will visit the study site at regular intervals by prior arrangement. During these visits, data recorded in the eCRFs and other data related to the study will be reviewed, and any discrepancies or omissions will be resolved. The monitors require direct access to appropriate parts of medical records relating to the subject's participation in this study, for the purpose of verifying the data provided to the company. The Investigator agrees to allow the study monitors to inspect the drug storage area, study drug stocks, drug accountability records, subject charts and study source documents, and other records relative to study conduct.

Other people, working for the CRO, Sponsor, IEC / IRB or Regulatory Authority may also wish, by prior arrangement, to have direct access to these records. Direct access, by prior arrangement, must also be permitted if an audit is requested by a regulatory authority or the Sponsor. An inspection may occur during the study or at any time subsequently until the time that the Investigator is informed by the Sponsor that the documentation no longer needs to be stored.

Monitoring will be performed according to applicable provisions of the CRO's clinical monitoring procedures and in compliance with applicable regulatory requirements.

11. QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor and people working on behalf of the Sponsor (including the CRO) will implement and maintain quality assurance and quality control systems with written SOPs (Sponsor's and / or CRO's) to ensure that data are generated and recorded in accordance with the protocol. The Informed Consent Form will be verified for all subjects enrolled in the study. The monitor will verify that all Informed Consent Forms are signed and dated prior to enrolment of the subject into the study. 100% of all SAEs, inclusion / exclusion criteria, demographic data and efficacy measures will be source verified. In addition, the complete eCRF for a subset of subjects will be 100% source verified. The subject source documents will be cross-checked with the eCRF and the SAE Forms. Drug accountability will be performed for all subjects in the study.

To ensure compliance with GCP and applicable regulatory requirements, the Sponsor may conduct a quality assurance audit. Additionally, authorised representatives of the Sponsor, a Regulatory Authority, an IEC / IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a Sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded, analysed, and accurately reported according to the protocol, ICH GCP, and any applicable regulatory requirements. The Investigator should contact the Sponsor or the CRO immediately if contacted by a Regulatory Authority about an inspection.

12. DATA HANDLING AND RECORD KEEPING

12.1. Source documents

All documents and data relating to the study will be kept in an orderly manner in secure study files provided by the CRO. While the study is ongoing, unmasked source documents should be stored separately from the masked source documents and all efforts should be made to ensure that they cannot be accessed by masked study personnel.

12.2. Electronic Case Report Forms

eCRFs will be used to collect subject data during the course of the study. eCRFs must be fully completed for each subject.

Each eCRF will consist of:

- an unmasked part, which will only be accessible and completed by the unmasked treating investigator / designee (with the exception of the unmasked AE pages for masked follow-up of AEs that start within the 1-hour post-injection period, refer to Section 8.4.1 for more information), and
- a masked part, which will be completed by the masked Investigator / designee

Access to the eCRFs will be by means of a unique username and password through a secure web browser. Changes to the original data entries will trigger a reason for change dialogue box while the time, date and name of the user making the change are automatically logged.

The unmasked treating investigator / masked Investigator is required to sign the unmasked part / the masked part of the eCRF, respectively, to verify that he / she has reviewed the recorded data.

12.3. Data Review

A data manager or monitor working on behalf of the sponsor will review eCRFs for completeness and clarity upon entry of data by the Investigator / designee. Missing, incorrect or unclear data will be identified by the data manager or the monitor and will need to be provided / clarified / corrected by investigational site personnel as necessary throughout the study. The CRO / Sponsor Representative may request additional documentation from the Investigator such as physician procedure notes or physician written summaries when AEs are observed and reported.

Development of the primary database for the study will be performed by the CRO / Sponsor Representative. The CRO / Sponsor Representative will also be responsible for the quality control of the database and confirming the overall integrity of the data.

12.4. Retention of Records

Following closure of the study, the Investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (*e.g.* audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff.

The Sponsor will inform the Investigator of the time period for retaining these records to comply with all applicable regulatory requirements. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations.

Study files may be discarded upon written notification by the Sponsor. To avoid error, the Investigator should contact the Sponsor before destroying any records or reports pertaining to the study to ensure they no longer need to be kept. Other source documents, such as subject's medical records, must be retained for the maximum period of time permitted by the hospital or institution and until such time when the Investigator is informed by the Sponsor that there is no further need to do so.

In addition, in accordance with the Investigator Agreement, the Sponsor should be contacted if the site's Principal Investigator plans to leave the site so that appropriate arrangements can be made.

13. FINANCE AND INSURANCE

13.1. Finance

Financial issues are addressed in a separate agreement.

There are no incentives for subjects for participation in the study. Subjects will be compensated for travel expenses only.

13.2. Insurance

In the event that a subject suffers injury or death directly attributable to participation in this study, appropriate treatment and / or compensation will be provided by and / or paid to the subject by the Sponsor in accordance with applicable national and international laws and / or guidelines.

14. PUBLICATION POLICY AND FINAL REPORT

The Publication Policy is addressed in a separate agreement.

During the course of and at the conclusion of the study, after the data are analysed, a CSR will be prepared. The Sponsor will provide Investigators with a summary of the results.

During the course of the study, the Sponsor will designate the CSR signatory Coordinating Investigator.

15. LIST OF REFERENCES

Aiello LP, Brucker AJ, Chang S, Cunningham ET Jr, D'Amico DJ, Flynn HW Jr, Grillone LR, Hutcherson S, Liebmann JM, O'Brien TP, Scott IU, Spaide RF, Ta C, Trese MT (2004). Evolving guidelines for intravitreous injections. *Retina* 24 (5 Suppl): S3-19.

American Academy of Ophthalmology Retina/Vitreous Panel (2014). Preferred Practice Pattern® Guideline. Diabetic Retinopathy. San Francisco, CA: American Academy of Ophthalmology.

American Association of Clinical Endocrinologists (2007). State of diabetes complications in America: a comprehensive report issues by the American Association of Clinical Endocrinologists. http://multivu.prnewswire.com/mnr/AACE/2007/docs/Diabetes_Complications_Report.pdf 04 August 2015

Avery RL, Bakri SJ, Blumenkranz MS, Brucker AJ, Cunningham ET Jr, D'Amico DJ, Dugel PU, Flynn HW Jr, Freund KB, Haller JA, Jumper JM, Liebmann JM, McCannel CA, Mieler WF, Ta CN, Williams GA (2014). Intravitreal injection technique and monitoring: updated guidelines of an expert panel. *Retina* 34 Suppl 12: S1-S18.

Bailey CC, Sparrow JM, Grey RH, Cheng H (1998). The National Diabetic Retinopathy Laser Treatment Audit. II. Proliferative retinopathy. *Eye (Lond)* 12 (Pt 1): 77-84.

Bailey CC, Sparrow JM, Grey RH, Cheng H (1999). The National Diabetic Retinopathy Laser Treatment Audit. III. Clinical outcomes. *Eye (Lond)* 13 (Pt 2): 151-159.

Cunha-Vaz J (1998). Lowering the risk of visual impairment and blindness. *Diabet Med.* 15 (Suppl 4): S47-50.

De Smet MD, Valmaggia C, Zarranz-Ventura J, Willekens B (2009). Microplasmin: ex vivo characterization of its activity in porcine vitreous. *Invest Ophthalmol Vis Sci.* 50 (2): 814-819.

De Smet MD, Gad Elkareem AM, Zwinderman AH (2013). The vitreous, the retinal interface in ocular health and disease. *Ophthalmologica* 230 (4): 165-178.

Diaz-Llopis M, Udaondo P, Millán JM, Arevalo JF (2013). Enzymatic vitrectomy for diabetic retinopathy and diabetic macular edema. *World J Diabetes* 4 (6): 319-323.

Fong DS, Ferris FL 3rd, Davis MD, Chew EY (1999). Causes of severe visual loss in the early treatment diabetic retinopathy study: ETDRS report no. 24. Early Treatment Diabetic Retinopathy Study Research Group. *Am J Ophthalmol.* 127 (2): 137-141.

Gad Elkareem AM, Willekens B, Vanhove M, Noppen B, Stassen JM, de Smet MD (2010). Characterization of a stabilized form of microplasmin for the induction of posterior vitreous detachment. *Curr Eye Res.* 35 (10): 909-915.

Glassman AR, Stockdale CR, Beck RW, Baker C, Bressler NM; Diabetic Retinopathy Clinical Research Network (2012). Evaluation of masking study participants to intravitreal injections in a randomized clinical trial. *Arch Ophthalmol.* 130 (2): 190-194.

Jager RD1, Aiello LP, Patel SC, Cunningham ET Jr (2004) Risks of intravitreous injection: a comprehensive review. *Retina* 24 (5): 676-698.

Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, Chen SJ, Dekker JM, Fletcher A, Grauslund J, Haffner S, Hamman RF, Ikram MK, Kayama T, Klein BE, Klein R, Krishnaiah S, Mayurasakorn K, O'Hare JP, Orchard TJ, Porta M, Rema M, Roy MS, Sharma T, Shaw J, Taylor H, Tielsch JM, Varma R, Wang JJ, Wang N, West S, Xu L, Yasuda M, Zhang X, Mitchell P, Wong TY; Meta-Analysis for Eye Disease (META-EYE) Study Group (2012). Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 35 (3): 556-564.

World Health Organization (WHO). (2015). Diabetes. Fact sheet N°312. http://www.who.int/mediacentre/factsheets/fs312/en/ 21 May 2015.

16. APPENDICES

APPENDIX 1. PROTOCOL TABLES

Table 6: Study Plan / Flowchart

Visit Type / Number	V1	V2	Phone Call	V3	V4a	V4b	Phone Call	V5	V6a	V6b	Phone Call	V7	V8	V9	V10	V11 / Exit Visit
Study Month as of First Injection / Study Day as of First Injection	**Scree-ning[a]**	**D0**	**D2[b]**	**D7**	**D28**	**M1 / D35**	**D37[b]**	**D42**	**D63**	**M2 / D70**	**D72[b]**	**D77**	**M3 / D98**	**M9 / D270**	**M15 / D450**	**M24 / D720**
Informed Consent	X															
Demography, medical and ocular history	X															
Blood pressure measurement	X												X	X	X	X
Pregnancy test[c]	X	X			X				X							
Blood sampling for HbA1c	X												X	X	X	X
Blood sampling for immunogenicity		X											X			

Table 6: Study Plan / Flowchart (Continued)

Visit Type / Number	V1	V2	Phone Call	V3	V4a	V4b	Phone Call	V5	V6a	V6b	Phone Call	V7	V8	V9	V10	V11 / Exit Visit
Study Month as of First Injection / Study Day as of First Injection	**Scree-ning[a]**	**D0**	**D2[b]**	**D7**	**D28**	**M1 / D35**	**D37[b]**	**D42**	**D63**	**M2 / D70**	**D72[b]**	**D77**	**M3 / D98**	**M9 / D270**	**M15 / D450**	**M24 / D720**
Ophthalmic assessments:																
• BCVA (ETDRS)	X[d]	X[d]		X[d]	X[d]			X[d]	X[d]			X[d]	X[d]	X[d]	X[d]	X[d]
• Colour vision (Roth 28-hue)[e f]		X		X	X			X	X			X	X	X	X	X
• B-scan ultrasound	X[g]				X				X			X	X	X	X	X
• Full ophthalmic examination[h]	X[d]	X		X	X			X	X			X	X[d]	X[d]	X[d]	X[d]
• 7-standard field stereo colour fundus photography	X[g]												O	O	X	X
• SD-OCT (6mm)	X[g]			X	X			X	X			X	X	X	X	X
• SD-OCT (widefield)[f]		X			X				X				X			
• Fluorescein angiography	X[g]															
• ffERG[i]		X[d]											X[d]		X[d j]	X[d j]

Table 6: Study Plan / Flowchart (Continued)

Visit Type / Number	V1	V2	Phone Call	V3	V4a	V4b	Phone Call	V5	V6a	V6b	Phone Call	V7	V8	V9	V10	V11 / Exit Visit
Study Month as of First Injection / Study Day as of First Injection	**Scree-ning**[a]	**D0**	**D2**[b]	**D7**	**D28**	**M1 / D35**	**D37**[b]	**D42**	**D63**	**M2 / D70**	**D72**[b]	**D77**	**M3 / D98**	**M9 / D270**	**M15 / D450**	**M24 / D720**
Assess list of events that lead to withdrawal from repeat injection[k]					X				X							
Treatment number allocation		T[l]				T				T						
Study treatment		T				T				T						
Post-injection assessment[m]		T				T				T						
Record concomitant medications / treatments / interventions	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
(S)AE monitoring	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

V = Visit; **D** = Day; **M** = Month

Procedures indicated by '**T**' should be performed by the unmasked treating investigator / designee. Procedures indicated by '**O**' are optional. At Visit 8 (Month 3) and Visit 9 (Month 9), 7-standard field stereo colour fundus photographs should only be taken if the Investigator suspects that the subject has progressed to PDR. Light grey coloured visits indicate injection visits.

[a] For screening failures due to reasons that are expected to be temporary, and for subjects for whom eligibility cannot be timely determined due to a problem with one of the eligibility assessments (*e.g.* in case of ungradable B-scan ultrasound), 1 re-screening visit can be organised. The re-screening visit should be scheduled at least 28 days after the 1st screening visit

[b] The masked Investigator / designee will make a scripted phone call to each subject 24-72 hours after the injection visit. Based on this phone call, the subject may be invited back for an unscheduled study visit as per the masked Investigator's clinical judgement

[c] Only for women of childbearing potential. Serum pregnancy test at screening (Visit 1), urine pregnancy test at Visit 4a and Visit 6a. Urine pregnancy testing should be repeated in any woman of childbearing potential who missed 1 period

[d] Performed in both eyes. Ophthalmic assessments without footnote are performed in the study eye only

[e] Not needed for subjects > 80 years of age or with a diagnosis of dyschromatopsia at baseline

[f] Only at sites where this is available

[g] Performed in potential study eye(s) only. If one of the subject's eyes is excluded based on an eligibility criterion that does not need confirmation by the B-scan expert reader or the CRC, these examinations do not need to be performed for that eye. If both eyes potentially qualify as the study eye, these examinations need to be performed for both eyes

[h] Full ophthalmic examination including pupillary examination, slit lamp examination, IOP assessment and dilated fundus examination. The same slit lamp machine should be used across study visits for a given subject

[i] ffERG will be performed at sites where this is available, in approximately 25% of the subjects

[j] Only for subjects with expert-defined ffERG abnormalities at the previous assessment

[k] Refer to Section 4.2.1 for the list of withdrawal criteria from repeat injection

[l] Randomisation can only occur after confirmation of eligibility by the B-scan expert reader, the CRC and the central laboratory

[m] Post-injection IOP measurement and indirect ophthalmic examination will be performed by the unmasked treating investigator as many times as needed at his / her discretion but at least once within 60 minutes after the injection to exclude central retinal artery non-perfusion or other complications

Table 7: Study Visit Intervals

Interval	Optimal Interval	Allowed Interval
Visit 1 (Screening) → Visit 2 (Day 0)	10-14 days	10-17 days
Visit 2 → Post-injection Phone Call	24–48 hours	24–72 hours[a]
Visit 2 (Day 0) → Visit 3 (Day 7)	7 days	7±3 days (4–10 days)
Visit 2 (Day 0) → Visit 4a (Day 28)	28 days	28±3 days (25-31 days)
Visit 4a (Day 28) → Visit 4b (Day 35)	7 days	6-10 days
Visit 4b (Day 35) → Post-injection Phone Call	24–48 hours	24–72 hours[a]
Visit 4b (Day 35) → Visit 5 (Day 42)	7 days	7±3 days (4–10 days)
Visit 4b (Day 35) → Visit 6a (Day 63)	28 days	28±3days (25-31 days)
Visit 6a (Day 63) → Visit 6b (Day 70)	7 days	6-10 days
Visit 6b (Day 70) → Post-injection Phone Call	24–48 hours	24–72 hours[a]
Visit 6b (Day 70) → Visit 7 (Day 77)	7 days	7±3 days (4–10 days)
Visit 6b (Day 70) → Visit 8 (Day 98)	28 days	28±3days (25-31 days)
Visit 2 (Day 0) → Visit 9 (Month 9)	270 days	270±14 days (256-284 days)
Visit 2 (Day 0) → Visit 10 (Month 15)	450 days	450±14 days (436-464 days)
Visit 2 (Day 0) → Visit 11 (Month 24)	720 days	720±14 days (706-734 days)

[a] If an unscheduled post-injection visit falls within the allowed interval for the 7-days post-injection visit (4-10 days after injection), the unscheduled post-injection visit and the Day 7 visit will take place on the same day

Table 8: Responsibilities Masked and Unmasked Study Staff and Need for Certification

Study Procedure	Masked Study Staff	Unmasked Study Staff	Certification needed
Study drug:			
Study drug accountability		X	
Injection-related procedures:			
IXRS access		X	
(Pre-)injection procedures		X	
1-hour post-injection assessment		X	
Scripted phone call 24-72 hours after the injection	X		
Conduct unscheduled study visit(s)	X		
Assess list of events that lead to withdrawal from repeat injection	X		
AE reporting:			
AEs during 1-hour post-injection assessment		X	
All other AEs	X		
Ophthalmic assessment:			
BCVA	X		
Colour vision (Roth 28-hue)	X		
B-scan ultrasound	X		X
Full ophthalmic examination	X		
7-standard field stereo colour fundus photography	X		X
SD-OCT (6mm and widefield)	X		X
Fluorescein angiography	X		X
ffERG	X		X
Other procedures:			
Informed consent	X		
Demography, medical and ocular history	X		
Blood sampling (serum pregnancy test, HbA1c, immunogenicity)	X	X	
Blood pressure measurement	X		
Urine pregnancy test	X	X	
Record concomitant medications / treatments / interventions	X		

APPENDIX 2. POST-INJECTION PHONE CALL SCRIPT

Within 24-72 hours after each injection visit, the **masked** Investigator (or designee) should call the subject to ask about post-injection complaints. During the telephone contact, the Investigator (or designee) will determine whether there is a need to bring the subject in for an unscheduled study visit. If the subject cannot be reached on the first attempt, the Investigator (or designee) must make at least 2 additional attempts to reach the subject. Each attempt should be documented in the eCRF with the date and time of the contact noted.

A successful telephone contact is one in which the Investigator (or designee) has spoken directly to the subject and has determined the need for an unscheduled post-injection visit based on the answers to the questions outlined below. A telephone contact with someone other than the subject does not qualify as a successful telephone contact.

The following questions should be asked in the order noted.

Adverse Events

1. How are you feeling in general?
2. Do you have any complaints since the injection?

The Investigator (or designee) should not in any way prompt or probe the subject about anything, in particular not about changes in vision. Any complaints (e.g. blurred vision) should be recorded in the eCRF as for any AE, with time of onset, severity etc. If the subject has taken a concomitant medication for treatment of a complaint (e.g. aspirin for a headache), then the medication should be recorded on the eCRF page with the appropriate indication.

Concomitant Medications

1. Did you take any NEW medications since the injection?
2. Did you change any medications you were taking since the injection (did you take more or less of the medications? Or stop any medications?)

The masked Investigator (or designee) should verify that there are no changes to the concomitant medications since the last study visit. If there has been a change in any medication or an additional medication that was taken, the masked Investigator (or designee) should complete all the information as required on the eCRF page for concomitant medications. If there is a new medication that has been taken the indication should also be noted on the eCRF page.

Once the Investigator (or designee) has asked about complaints and concomitant medications, a decision should be made as to whether or not the subject should return for an unscheduled post-injection visit.