STUDY PROTOCOL

PROTOCOL TITLE:
Identifying ocular and systemic biomarkers for response to aflibercept in Asian patients with centre involving diabetic macular edema: A prospective clinical trial

PROTOCOL NUMBER: R1436/19/2017

PROTOCOL VERSION: 4.0
PROTOCOL DATE: 26 Feb 2019
STUDY PERIOD: 16 months from the initiation date of the contract with Santen

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RESPONSIBILITY:

Singapore Eye Research Institute, Singapore National Eye Centre	Research planning and management Implementation of clinical treatment and sample/information acquisition Sample and data analysis Sample and data storage for future study
Santen Pharmaceutical Co., Ltd	Research planning and management Sample and data analysis for proteomic vitreous biomarkers

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PROTOCOL SIGNATURE PAGE

Protocol Title**: Identifying ocular and systemic biomarkers for response to aflibercept in Asian patients with center involving diabetic macular edema: A prospective interventional trial**

Protocol Number: R1436/19/2017

Protocol Version/ Date: Version 4.0/ 26 Feb 2019

Sponsor Name: IAF_ICP (Singapore) and Santen Pharmaceutical Co., Ltd.

Declaration of Investigator

I confirm that I have read the above-mentioned protocol and its attachments. I agree to conduct the described study in compliance with all stipulations of the protocol, regulations and ICH E6 Guideline for Good Clinical Practice (GCP).

SERI Principal Investigator Name: Dr Gavin Tan Siew Wei

Principal Investigator Signature: 

Date: 05/03/2019

Santen Principal Investigator Name: Takahiro Imanaka

Santen Principal Investigator Signature: 

Date: 05/03/2019

1. BACKGROUND AND RATIONALE

Diabetic Macular Edema is a major Public Health Problem in Singapore and Worldwide.
Diabetes is chronic disease with significant morbidity from multi systemic complications. It is estimated that by 2030, more than 400 million persons worldwide will be affected by this disease. Diabetic retinopathy (DR) is a specific microvascular complication of diabetes and a major public health problem in Singapore and globally.1-4 We have led a global effort to show that there are now nearly 100 million people with DR, of whom nearly 30 million have vision-threatening DR, including diabetic macular edema (DME), now considered the major cause of vision loss in diabetes.4,5 Our group has made substantial discovery of novel risk factors for DR from population-based and clinic-based studies in Singapore and around the world.1,3,4,6-20

DME is a major cause of visual impairment in persons with diabetes. DME is an advanced, vision-limiting manifestation of DR in which swelling of the central retina causes loss of central vision. The Wisconsin Epidemiologic Study found that the prevalence of DME increases from 0–3% in recently diagnosed individuals to 28–29% in those living with type 1 or 2 diabetes for at least 20 years.21-25 Without treatment, 33% of eyes in the Early Treatment Diabetic Retinopathy study (ETDRS) with center involved DME experience moderate visual loss (15 or more letter decrease in visual acuity) over a three year period. Historically, laser photocoagulation was the gold standard treatment for DME based on the results of the ETDRS. However, less than 3% of treated eyes in the ETDRS demonstrated an improvement in visual acuity of 15 or more letters.26-32

The current therapeutic approach is limited to Anti-Vascular Endothelial Growth Factor (VEGF) agents.
Anti- VEGF agents such as ranibizumab, aflibercept and bevacizumab have emerged as the treatment of choice for center involving diabetic macular edema. The RISE and RIDE trial, randomized trials assessing ranibizumab for diabetic macular edema found that a significantly greater proportion of patients receiving ranibizumab (33.6 to 45.7%) gained 15 letter or more in best corrected visual acuity compared with sham injection (12.3 to 18.1%) at 2 years.33 The Diabetic Retinopathy Clinical Research Network (DRCRnet) demonstrated that patients treated with ranibizumab plus laser had significantly better visual outcomes (9±12 letter gain) compared with those treated with sham injections plus prompt laser (3±13 letter gain).34,35 A Prospective Randomized Trial of Intravitreal Bevacizumab or Laser Therapy in the Management of Diabetic Macular Edema (BOLT study) randomized 80 eyes from 80 study participants to intravitreal bevacizumab (given every six weeks with a minimum of three injections in the first 12 months) or macular laser treatment and found that whereas the bevacizumab group gained a median of eight letters in visual acuity over 12 months, the laser group lost a median of 0.5 letters over the same time period (P = 0.0002).36 In the Da Vinci study, subjects with DME gained up to 13.1 letters at one year in the 2mg aflibercept every 4 weekly group.37

However, there is a significant group of DME patients who are non-responders to Anti-VEGF agents, and do not improve vision. In the Rise and Ride trial, 20-25% of subjects had no change (<4 letter change) or worsening of visual acuity.33 In the DRCRnet DME study, 28% had no significant improvement in vision (<4 letter improvement) at 3 years. In the DRCRnet protocol T comparing the efficacy of intravitreal aflibercept, bevacizumab, and ranibizumab in the treatment of diabetic macular edema, only 50-60% of subject benefited from a significant visual improvement of at least 10 ETDRS letters.38

There is a need to identify poor and non-responders to antiVEGF and discover novel biochemical/genetic biomarkers for DME.
Accumulating evidence suggests that several overlapping pathophysiological mechanisms play an important role in the development of DR, including inflammation, microvascular dysfunction, increased oxidative stress, endothelial dysfunction and genetic factors.1 Clinical, systemic and

imaging features in DME patients may be able to predict the response to anti-VEGF agents. Mediators other than VEGF may be involved in the pathogenesis of DME, which may account for the poor response to anti-VEGF in these patients. Ocular and blood biomarkers of poor response may be potential therapeutic targets.

Significance
This study will contribute to the unmet needs of the 40 million persons worldwide with diabetic macular edema. Clinicians treating patients with DR are looking accessible biomarkers that will help them predict disease outcomes and response to therapy. They also need alternatives to anti-VEGF treatment for these diseases. This is the case not only for the roughly 30-40% of patients who have poor or suboptimal response to current therapy, but also for the patients that must undergo repeated intravitreal injections for control of DME. In addition to alternatives to anti-VEGF treatment, add-on therapeutics that can potentially enhance the effects of current front line therapies and/or prolong their effectiveness (thus reducing the numbers of injections) are also of considerable potential value to clinicians, their patients, as well as the pharmaceutical industry.

2. HYPOTHESIS AND OBJECTIVES

Aim 1: To investigate whether ocular and systemic biomarkers predict treatment response to intravitreal aflibercept in a cohort of patients with DME.

Hypothesis: That novel ocular and systemic biochemical markers can predict response to intravitreal aflibercept in subjects with DME.

3. EXPECTED RISKS AND BENEFITS

The benefits to this patient will be that by studying the blood and biomarker study we will be able to better predict prognosis and treatment response in future patients. The recruited patient will also have a direct benefit in receiving free intravitreal aflibercept treatment during the course of the study. Risks to the patient would be the adverse events from the intravitreal injections like endophthalmitis, cerebral or cardiac complications, which are very rare; the risk of infection from blood which is rare; and the risk of minor trauma from tear collection which is uncommon.

4. STUDY POPULATION

4.1. List the number and nature of subjects to be enrolled.

Up to 36 study participants with DME in at least 1 eye are expected to be enrolled. Study participants can only have one study eye. If both eyes are eligible for the study, the eye without previous intravitreal anti-VEGF treatment will be selected. If both eyes are treatment naïve, the eye with worse VA should be selected as the study eye, or the eye with worse central retinal thickness will be chosen as the study eye. If both the VA and CRT are equal then the right eye will be chosen. The non-study eye will continue to receive treatment as per current clinical standard of care. They can receive treatment at the same time as the study eye to ensure there is no delay of treatment in the non-study eye.

4.2. Criteria for Recruitment and Recruitment Process

Potential eligibility will be assessed as part of a routine-care examination. Prior to completing any procedures or collecting any data that are not part of usual care, written informed consent will be obtained. For potential study participants who are considered potentially eligible for the study based on a routine-care exam, the study protocol will be discussed with the potential study participant by a study investigator and clinic coordinator. The potential study participant will be given the Informed Consent Form to read. Potential study participants will be encouraged to discuss the study with family members and their personal physician(s) before deciding whether to participate in the study.

4.3. Inclusion Criteria

Participant

1. Age >=21 years
2. Diagnosis of Diabetes Mellitus (Type 1 or type 2)
    a. Current regular use of insulin or oral hypoglycemic agents for treatment of diabetes
    b. Documented diabetes by ADA and/or WHO criteria.
3. Able and willing to provide informed consent.

Study Eye

1. Best corrected ETDRS visual acuity score <= 78 (i.e. 20/32 or worse)
2. On clinical examination, definite retinal thickening due to diabetic macular edema involving the center of the macula.
3. Diabetic macular edema present on optical coherence tomography (OCT) (central subfield thickness on OCT >=340um with Spectralis (Heidelberg)
4. Media clarity, pupillary dilation and individual cooperation sufficient for study procedure including fundus photography.

4.4. Exclusion Criteria

Participant

1. End stage renal failure requiring hemodialysis or peritoneal dialysis.
2. Medical condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status including blood pressure, cardiovascular disease, and glycemic control).
3. Participation in an investigational trial within 30 days of enrolment which involves treatment with unapproved investigational drug
4. Known allergy to any component of aflibercept or any other medication to be used in the study.
5. Blood pressure > 180/110 (systolic above 180 OR diastolic above 110 on repeated measurements). If blood pressure is brought below 180/110 by anti-hypertensive treatment, individual can become eligible.
6. Myocardial infarction, other acute cardiac event requiring hospitalization, stroke, transient ischemic attack, or treatment for acute congestive heart failure within 6 months prior to participation to the study.
7. Systemic anti-VEGF or pro-VEGF treatment within 6 months prior to randomization or anticipated use during the study.
8. For women of child-bearing potential: pregnant or lactating or intending to become pregnant within the next 24 months. Women who are potential study participants should be questioned about the potential for pregnancy.

9. Patient with non-study eye VA: counting finger or worse (i.e. only one seeing eye) will be excluded.

Study Eye

1. Macular edema is considered to be due to a cause other than diabetic macular edema. An eye should not be considered eligible if: (1) the macular edema is considered to be related to ocular surgery such as cataract extraction or (2) clinical exam and/or OCT suggest that vitreoretinal interface abnormalities (e.g., a taut posterior hyaloid or epiretinal membrane) are the primary cause of the macular edema.
2. An ocular condition is present such that, in the opinion of the investigator, visual acuity loss would not improve from resolution of macular edema (e.g., foveal atrophy, pigment abnormalities, dense subfoveal hard exudates, nonretinal condition).
3. An ocular condition is present (other than diabetes) that, in the opinion of the investigator, might affect macular edema or alter visual acuity during the course of the study (e.g., vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, etc.)
4. Substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by more than three lines (i.e., cataract would be reducing acuity to worse than 20/40 if eye was otherwise normal).
5. History of an anti-VEGF treatment for DME in the past 6 months or history of any other treatment for DME at any time in the past 6 months (such as focal/grid macular photocoagulation, intravitreal or peribulbar corticosteroids).
6. History of pan-retinal photocoagulation in the past 6 months or anticipated need for immediate pan-retinal photocoagulation. (e.g. Proliferative diabetic retinopathy. Cases with severe non-proliferative diabetic retinopathy will still be eligible)
7. History of ocular anti-VEGF treatment for a disease other than DME in the past 6 months
8. History of major ocular surgery (including vitrectomy, cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 6 months or anticipated during the study.
9. History of YAG capsulotomy performed within two months prior to recruitment.
10. Aphakia.
11. Exam evidence of external ocular infection, including conjunctivitis, chalazion, or significant blepharitis.
12. History of intravitreal steroids within the last 6 months.

5. STUDY DESIGN AND PROCEDURES/METHODOLOGY

This is a prospective interventional non-comparative clinical trial. Treatment received will be based on study design. All study eyes will receive intravitreal aflibercept with the initial injection given on within 2 weeks of the screening visit and x 4 monthly injections including at month 3.

Screening Evaluation and Baseline Testing

Historical Information & Study Questionnaires & The Brief Impact of Vision Impairment Profile (B_IVI)

A history will be elicited from the potential study participant and extracted from available medical records. Socio-demographic and medical history details via a face-to-face interview in a study questionnaire. Data to be collected will include: age, gender, ethnicity and race, diabetes history and current management, other medical conditions, medications being used, as well as ocular

diseases, surgeries, and treatment. Visual impact quality of life will also be assessed via the brief impact of vision impairment profile (B IVI) questionnaire.

Baseline Testing Procedures

The following procedures are needed to assess eligibility and/or to serve as baseline measures for the study.
• If a procedure has been performed (using the study technique and by study certified personnel) as part of usual care, it does not need to be repeated specifically for the study if it was performed within the defined time windows specified below or within 8 days of the baseline injection otherwise they need to be repeated.

1. Best-corrected Visual Acuity: 'best corrected visual acuity（BCVA) will be measured using the ETDRS VA protocol following manifest refraction.
2. Optical Coherence Tomography: OCT and OCT-angiography (OCTA) on both eyes will be performed. Both standard and enhanced depth imaging scans will be performed.
3. Baseline ocular examination on each eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy.
4. Fundus Photography & ultra-wide field photography.
5. Anthropometric and blood pressure measurements
6. Blood Sample: 30mls of blood sample will be obtained for HbA1c measurement as well as assessment of biomarkers and genetics.
7. Vitreous sample: 0.1-0.2 ml of vitreous humour will be obtained (detailed procedure outlined below) together with the first intravitreal injection at baseline.
8. Tears sample

Follow-up visits and re-treatment

Visit schedule
Follow-up visits occur every 1 month (within +/- 7 days; and a minimum of 21 days between visits) during the first 4 months.

Testing procedures
The following procedure will be performed at each protocol visit as per the protocol schedule in table
1. Best corrected visual acuity / ETDRS visual acuity
2. OCT and OCT A on both eyes
3. Ocular examination on both eyes including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy.
4. Fundus photography & ultra-wide field photography
6. Anthropometric and blood pressure measurements
7. Vitreous sample: 0.1-0.2 ml of vitreous humour will be obtained (detailed procedure outlined below) at the month 3 visit together with the 4^{th} intravitreal injection.
8. Tears sample

Treatment during follow- up
All subjects will receive an intravitreal anti-VEGF injection monthly for a total of 4 mandatory intravitreal treatments.

Response to aflibercept will be assess based on CRT and VA at 4 months

Considerations in follow-up

Endophthalmitis
Diagnosis of endophthalmitis is based on investigator's judgment. In the case of suspected endophthalmitis, the patient will be immediately referred to the retina and uveitis team for management. This will include obtaining cultures of vitreous and/or aqueous fluid, expedient intravitreal antibiotic treatment for presumed endophthalmitis, and vitrectomy where clinically indicated. The cost of treatment for endophthalmitis will be covered by the study institution.

Surgery for Vitreous Hemorrhage and Other Complications of Diabetic Retinopathy
A study eye could develop a vitreous hemorrhage and/or other complications of diabetic retinopathy that may cause visual impairment. The timing of vitrectomy for the complications of proliferative diabetic retinopathy such as vitreous hemorrhage is left to investigator discretion.

Panretinal Photocoagulation
Panretinal photocoagulation (PRP) can be given if it is indicated in the judgment of the investigator. In general, PRP should not be given if the study participant has less than severe non-proliferative diabetic retinopathy. In general, PRP should be given promptly for previously untreated eyes exhibiting proliferative diabetic retinopathy with high-risk characteristics and can be considered for persons with non high-risk proliferative diabetic.

Study Participant Withdrawal and Losses to Follow-up
A study participant has the right to withdraw from the study at any time. If a study participant is considering withdrawal from the study, the principal investigator should personally speak to the individual about the reasons, and every effort should be made to accommodate him or her.

Study participants who withdraw will be asked to have a final closeout visit at which the testing described for the protocol visits will be performed. Study participants who have an adverse effect attributable to a study treatment or procedure will be asked to continue in follow-up until the adverse event has resolved or stabilized.

Treatment details

Intravitreal Injections

Anti-VEGF agent

Study eyes will receive a dose of 2.0mg in 0.05 ml of Aflibercept (Eylea)

Intravitreal Injection Technique
Antibiotics in the pre-, peri-, or post-injection period are not necessary and will not be prescribed in this study.

Prior to the injection, the study eye will be anaesthetized with topical anesthetic, followed by a povidone iodine prep of the conjunctiva. (Instill 5% povidone iodide on to the ocular surface and allow adequate time prior to injection)

The injection will be performed using sterile technique. Investigator will use a surgical hand disinfection technique and wear sterile gloves. Periocular skin and eyelid margins and eye lashes will be cleaned with 5-10% povidone iodine.
Skin will be dried and drape applied. Investigator will insert eyelid speculum, ensuring that it is well positioned underneath the eyelids to direct the eyelashes away from the field. Callipers should be used to mark the injection site. The entry site of the needle should be 3.0-3.5 mm from the limbus in pseudophakic patients, and 3.5-4.0 mm in phakic patients.

The conjunctiva may be displaced anteriorly using either forceps or cotton tipped applicator so that no direct route between vitreous and ocular surface remains. The needle is inserted perpendicular through sclera with the tip aimed towards the centre of the globe (to avoid any contact with the posterior lens). A vitreous tap will be performed at baseline and before the month 3 injection where 0.1-0.2 ml of the vitreous will be drawn out before injection of the intravitreal medication.

IOP measurement post-injection is not mandatory. While small volume injections (0.05ml) are unlikely to cause IOP rise, it should be considered inpatients with ocular hypertension or glaucoma, and in all cases where patients are symptomatic for pain or reduced vision immediately following injection. Should a high intraocular pressure resulting in non-perfusion of the central retinal artery occur, indicated by no perception of light (NPL) in the treated eye, an anterior chamber paracentesis is indicated. Such decompression needs to be achieved within 3-5 minutes. Patients should be instructed to report any symptoms regarding eye pain or discomfort, increased redness of the eye, or additional blurring of vision (which may indicate endophthalmitis) to the treating ophthalmologist without delay.

Vitreous and tears collection procedures

Vitreous Tap

Done at baseline injection and repeated with month 3 injection. Subconjunctival lignocaine anaesthesia. Sterile caliper was used to mark 4.0 mm and 3.5 mm posterior to the corneoscleral limbus for phakic and pseudophakic patients, respectively. A 27 or 25-gauge, 5/8-inch needle will be introduced through the marked site into the mid vitreous cavity. 0.1-0.2 mL of vitreous fluid will be gently aspirated into a 1-mL or 3-mL syringe. Procedure will be abandoned if it is a dry tap (<0.05ml of vitreous aspirated).

Vitreous Humour samples should be collected into Protein Lo bind Eppendorf tubes. The sample will be placed on ice and centrifuge at 5000 rpm, 4°C for 10 minutes (Only for samples with debris/blood). The supernatant will be transferred into a new Lo bind Eppendorf tube labelled correctly* (Sample volume will be measured). The sample in the tube will be snap-freeze sample in dry ice / liquid nitrogen, after which it will be stored at -80°C promptly.

Tears Collection

Done at baseline injection and repeated with month 4. Tears will be collected using a 5x35mm strip of filter paper will be placed in the lower lid with your eyes closed, and kept in place for 5 minutes. Special care will be taken not to touch the lid margin or ocular surface.

Storage and Disposal

All samples of blood, vitreous fluid and tear will be collected into sterile tubes, placed on ice, centrifuged to remove cells and debris, and stored at –80°C for up to 15 years until analysis at SERI.

Unused samples will be stored indefinitely for future research as permitted by the IRB. Waste samples will undergo proper disposal as per SERI biohazard waste requirements.

Laboratory analysis

Samples will undergo proteomic, metabolomic and cytokine discovery assessment using, mass spectrometry, gas and liquid chromatography as well as ELISA based platforms.

In the analyses, proteomic analysis of vitreous will be conducted as collaborative research between SERI and Santen for the purpose of biomarker investigation. The protein analysis will be conducted by service provider outside of Singapore under the contract research agreement with SERI and Santen. Preliminary test will be conducted to evaluate detectability of proteins by technology of the service provider. Vitreous fluid which was obtained and stored in SERI with the protocol of R1256/62/2015 (title: Ocular Biomarker Study for Retinal Angiogenic Diseases) will be used for the preliminary test.

Delay in Giving Injections
If a scheduled injection is not given by the end of the visit window, it can still be given up to one week prior to the next visit window opening. If it is not given by that time, it will be considered missed. If an injection is given late, the next scheduled injection should occur no sooner than three weeks after the previous injection.

Non-Study Eye Injections
The non-study eye will continue to receive treatment as per current clinical standard of care. They can receive treatment at the same time as the study eye to ensure there is no delay of treatment in the non-study eye. If the non-study eye is going to be treated for any condition which requires treatment with an anti-VEGF agent, where possible, the non-study eye should be treated with the same antiVEGF as the study eye.

Focal/Grid Photocoagulation
If focal/grid photocoagulation will be deferred until after completion of the month 4 visit.

Discontinuation of Study
The study may be discontinued by the Data and Safety Monitoring Committee [DSMC] prior to the preplanned completion of follow-up for all study participants.

Study Participant Reimbursement
The study will be providing the study participant with $50 per completed protocol visit to cover travel and other visit-related expenses.

6. SAFETY MEASUREMENTS

6.1. Definitions

An adverse event (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.

Recording of Adverse Events

Throughout the course of the study, all efforts will be made to remain alert to possible adverse events or untoward findings. The first concern will be the safety of the study participant, and appropriate medical intervention will be made.

All adverse events whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported to the study committee.

The study investigator will assess the relationship of any adverse event to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by the treatment (including treatment of the non-study eye with study treatment).

Adverse events that continue after the study participant's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

Reporting Serious or Unexpected Adverse Events

A serious adverse event (SAE) or reaction is any untoward medical occurrence that :

- results in death
- is life-threatening; (a non life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity or
- is a congenital anomaly/birth defect
- is a medical event that may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

Unexpected adverse events are those that are not identified in nature, severity, or frequency in the current study protocol

6.2. Collecting, Recording and Reporting of Adverse Events and Serious Adverse Events to CIRB

Reporting of adverse events involves the PI submitting to the approving CIRB the completed SAE Reporting Form within the stipulated timeframe. PI is responsible for informing the institution representative (local SAE resulting in death), sponsor or regulatory bodies as required and appropriate.

Reporting timeline to CIRB:

- SAE that result in death, regardless of causality, should be reported immediately - within 24 hours of the PI becoming aware of the event.
- Local life-threatening (unexpected/ expected) SAE should be reported no later than 7 calendar days after the Investigator is aware of the event, followed by a complete report within 8 additional calendar days.
- Local unexpected SAE that are related events, but not life-threatening, should be reported no later than 15 calendar days after the investigator is aware of the event.
- An increase in the rate of occurrence of local expected SAE, which is judged to be clinically important, should be reported within 15 calendar days after the PI is aware of the event.
- Local expected SAE should be reported annually (together with Study Status Report for annual review).
- Local unexpected and unlikely related SAE that are not life-threatening should also be reported annually (together with Study Status Report for annual review).
- Local unexpected AE that are related events should be reported at least annually (together with Study Status Report for annual review).
- Non-local unexpected SAE that are fatal or life threatening and definitely/probably/possibly related should be reported not later than 30 calendar days after the PI is aware of the event.

6.3. Safety Monitoring Plan

A Data and Safety Monitoring Committee (DSMC) will approve the protocol, template informed consent form, and substantive amendments and provide independent monitoring of adverse events. Cumulative adverse event data are semi-annually tabulated for review by the DSMC. Following each DSMC data review, a summary will be made available for submission to Institutional Review Board. A list of specific adverse events to be reported to the DSMC expeditiously will be compiled and included as part of the DSMC Standard Operating Procedures.

6.4. Complaint Handling

Complaints will be handled by the research co-ordinators and if required the Quality Assurance team at the Singapore National Eye Centre

7. DATA ANALYSIS

7.1. Data Quality Assurance

Quality control and quality assurance will be conducted in this study. The Investigator(s)/ Singapore National Eye Centre will permit study-related monitoring audits, Medical Clinical Research Committee (MCRC) and or Ethics Committee (EC) review and regulatory inspection(s), providing direct access to source data/ document.

7.2. Data Entry and Storage

Research data should be stored in a password-protected database on the SERI shared drive, accessed via a password-protected delegated staff. As for the hard copies, it will be stored in academia, in a locked cabinet within SERI.

All investigators, coordinators as well as the Database Management team declared in this CIRB application will have access to the research data. The research coordinator will grant access to the research data, and the requestor's information will first be noted in hard copy and filed.

8. SAMPLE SIZE AND STATISTICAL METHODS

8.1. Determination of Sample Size

Based on an outcome of 33% non-responders (<20% reduction of CRT on OCT), a sample of 30 DME patients will 10 non responders which will enable as to identify biomarkers for further validation studies. Up to 36 patients will be enrolled based on expectation of 20% of patients will have a dry vitreous tap.

8.2. Main Statistical and Analytical Plans

The proportion of study patients achieving positive response in primary outcome will be estimated with 95% confidence interval. Summary statistics will be tabulated to describe patient demographics and characteristics. Univariate logistic regression will be performed to assess the effects of individual biomarkers, and multiple logistic regression analyses to assess associations between biomarkers and treatment outcome while adjusting for potential confounders. Some of exploratory analysis will be conducted to evaluate biomarkers. Incidence rates of adverse events will be computed as the number of events divided by the number of person-years at risk, and the events described in detail in the paper.

9. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The SERI's investigator(s)/institution(s) will permit study-related monitoring, audits and/or IRB review and regulatory inspection(s), providing direct access to source data/document.

10. QUALITY CONTROL AND QUALITY ASSURANCE

Quality control and quality assurance will be conducted in this study. The Investigator(s)/ Singapore National Eye Centre will permit study-related monitoring audits, MCRC and or EC review and regulatory inspection(s), providing direct access to source data/ document.

11. ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice, Japanese Ethical Guidelines for Medical and Health Research Involving Human Subjects and the applicable regulatory requirements.

This final study protocol, including the final version of the Patient Information and Informed Consent Form, must be approved in writing by the Centralised Institutional Review Board (CIRB), and Ethical Review Committee in Japan prior to enrolment of any patient into the study.

The principle investigator is responsible for informing the CIRB of any amendments to the protocol or other study-related documents, as per local requirement.

Conflicts of interest (COI)

This study is funded by IAF_ICP (Singapore) and Santen Pharmaceutical Co., Ltd.
COIs of SERI researchers are deliberated by IRB in Singapore.
Employees of Santen pharmaceutical Co., Ltd. are involved in this research.

11.1. Informed Consent

Potential eligibility will be assessed as part of a routine-care examination. Prior to completing any procedures or collecting any data that are not part of usual care, written informed consent will be obtained. For potential study participants who are considered potentially eligible for the study based on a routine-care exam, the study protocol will be discussed with the potential study participant by a study investigator and clinic coordinator. The potential study participant will be given the Informed Consent Form including the following items to read. Potential study participants will be encouraged to discuss the study with family members and their personal physician(s) before deciding whether to participate in the study.

(1) Title of the research and the fact that approval of the chief executive of the research implementing entity has been given concerning its implementation;
(2) Names of the research implementing entity and the principal investigator (including names of the collaborative research implementing entity(s) and principal investigators of such collaborative research implementing entity(s), when the research is conducted collaboratively with other research implementing entity(s));
(3) Purpose of the research study
(4) Study procedure and visit schedule: Method and time period of the research (including purpose of the utilization of specimens or information acquired from the research subject; administration of study drug and description of study procedures)
(5) Patient's responsibilities in this study
(6) What is not standard care or experimental in this study;
(7) Possible risks, discomforts and inconveniences
(8) Potential benefits: when the research involves any financial expenditure on or remuneration for the research subject, etc., a statement to that effect and details of such;
(9) Important information for women participant
(10) Alternatives: When the research involves any medical technique beyond usual medical practice, description of alternative procedure(s) or course(s) of treatment;
(11) Costs of participation
(12) Participant's rights: The fact that research subjects, etc. may withdraw their consent at any time even after they have given consent with regard that the research is commenced or continued (when it can be difficult to take measures that follow the withdrawal made by the research subject, etc., a statement to that effect and the reason for the difficulty);
(13) Withdrawal from study : The fact that the refusal or withdrawal of consent by a research subject, etc. with regard that the research is to be commenced or continued does not cause any disadvantage to such research subject, etc.
(14) Research related injury and compensation: When the research involves any invasiveness, whether or not compensation will be offered for research-related injury and details of such compensation;
(15) Confidentiality of study and medical records
(16) Who to contact if you have questions regarding the study
(17) Who has reviewed the study

11.2. Confidentiality of Data and Patient Records

All personal information and biological materials will be handled in a manner that is consistent with the Singapore Personal Data Protection Act (PDPA) and the Singapore Human Biomedical Research Act. Only personnel directly involved in the study will have access to study information. All Data collected will be anonymised by the SERI Data

Management group with the identify key kept separate on a password protected computer at SERI which cannot be accessed without the SERI's PI consent.
Anonymised clinical information and laboratory analysis results will be transferred to Santen only for the purpose of vitreous biomarker investigation. Santen and their affiliate subsidiary will only have access to the anonymised data. The following documents will be substituted for data transfer records.
Data collected from each site will be anonymized before sending to the SERI internal data management team for data entry into electronic system. All records will be accessible for inspection and copying by authorized authorities. The research data will be stored for at least 15 years and then deleted/destroyed.

- Documents stored at SERI
  - Study protocol and project agreement as records of items provided to Santen. List of the items will be prepared as additional document when the items are transferred, and then stored at SERI.
  - Informed consent form as records of patient's information and his/her consent
- Documents stored at Santen
  - Study protocol and project agreement as records of items provided from SERI and procedure of data acquisition. List of the items will be prepared as additional document when the items are transferred, and then stored at Santen.

The study related documents at Santen will be stored for five years after the final report of this study or three years of the final publication, whichever is later, and then destroyed or deleted.
Data collected from each site will be anonymized before sending to the SERI internal data management team for data entry into electronic system. All records will be accessible for inspection and copying by authorized authorities. The research data will be stored for at least 15 years and then deleted/destroyed.

12. PUBLICATIONS

This study will adhere to the Singapore Eye Research Institute and Santen Master Research Collaboration Agreement (DATED the 10th day of October 2014) publication policy.

Report
When research is finished (including the case of discontinuance), the principal investigator of the research shall report to the chief executive of the research institute.

Clinical Research Database
This study will be registered on JAPIC iyakuSearch database (http://database.japic.or.jp/is/top/index.jsp) in Japan, ClinicalTrial.gov (https://clinicaltrials.gov/), and Health Sciences Authority (http://www.hsa.gov.sg/content/hsa/en.html).

13. RETENTION OF STUDY DOCUMENTS

The research data will be stored for at least 15 years and then destroyed/deleted.

14. FUNDING and INSURANCE

The study will be funded by IAF_ICP (Singapore) and Santen Pharmaceutical Co., Ltd. This is an investigator initiated trial which will be covered by the institutional clinical trial insurance.

List of Attachments:

	Month 0	Month 1	Month 2	Month 3	Month 4
Clinic consultation (Follow-up visit)	X	X	X	X	X
Refraction	X	X	X	X	X
Study Questionnaires	X				
B_IVI	X				
OCT & OCTA	X	X	X	X	X
Fundus Photography (For DR Grading)	X				X
HbA1c	X				
Blood Pressure	X	X	X	X	X
Blood specimen	X				
Tears collection	X				X
Vitreous tap	X			X	
Intravitreal Aflibercept	X	X	X	X	

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