Clinical Study Protocol

Anti-VEGF therapy for Acute Thyroid Eye Disease (AcTED study)

Compound:	Sub-tenon aflibercept injection
Study Name:	Anti-VEGF therapy for Acute Thyroid Eye Disease (AcTED study)
Date of Issue:	June 7, 2023
Protocol Version:	9.0
Primary Investigator:	Nahyoung Grace Lee, MD

CLINICAL STUDY PROTOCOL SYNOPSIS

TITLE	Anti-VEGF therapy for Acute Thyroid Eye Disease (AcTED study)
SITE LOCATION(S)	Massachusetts Eye and Ear Infirmary 243 Charles St., 10th floor Boston, MA 02114
Principal Investigator	Nahyoung Grace Lee, MD Grace_Lee@MEEI.HARVARD.EDU

OBJECTIVE(S)	The primary objective is to assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from the Baseline Visit (Day 1) to day 45. The secondary objectives are: From the Baseline Visit (Day 1) to Days 45 and 90: • assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from baseline to day 90 • change in clinical activity as measured by a ≥ 2 point decrease on the clinical activity score (CAS) • change in extraocular muscle diameter as measured by computed tomography (CT) scan • change in proptosis as measured by Hertel exophthalmometry and CT scan. • change in degree of ocular misalignment during physical exam (and documented with external photography) and limitation in ocular motility as measured with physical examination • change in vision as measured by ETDRS BCVA • change in vision as measured by Ishihara color test • change in visual function as determined by automated Humphrey visual field • change in subjective score of pain/discomfort based upon patient Graves' Ophthalmopathy Quality of Life (GO-QOL) questionnaire • change in eyelid retraction/position as measured by the mm of inferior and superior scleral show on physical examination

STUDY DESIGN	The study sequentially enrolls one patient at a time until a total of 24 patients with acute thyroid eye disease have completed the study. We estimate that it will be necessary to enroll up to 30 subjects in order to have 24 complete the study. Subjects will be randomized to receive one of 3 treatment arms (8 patients in each group): • Group 1: Sub-tenon's injection of saline followed by injection of 4 mg sub-tenon aflibercept (total volume 1.1 ml) in the worse eye • Group 2: Sub-tenon's injection of hyalronidase (HA) followed by sub-tenon injection of 4 mg aflibercept (total volume 1.1 ml) in the worse eye • Group 3: Sub-tenon injection of HA injection alone (total volume 1.1 ml) in the worse eye Both the subject and the investigator will be blinded as to which treatment arm the subject has been assigned. At the time of enrollment, patients will undergo a full ophthalmologic examination including external photography, BCVA, Ishihara color test, a 30-2 automated Humphrey visual field, intraocular pressure, ocular motility and alignment, exophthalmometry, calculation of the CAS score, medical history and smoking status, Go QOL, measurement of blood pressure, and pregnancy test if applicable. At baseline, patients will undergo a pre-treatment CT scan if they have not had one performed within 3 months prior to enrollment and pre-treatment laboratory work-up (including TSI testing and baseline measurement of systemic VEGF levels and cytokine levels). Patients in each treatment assignment will receive bi-weekly injections on day 1, day 14 and day 28. Primary and secondary end-points will be measured at each clinical visit by a masked observer. The final endpoint will be assessed at Day 90, Visit 8.
STUDY DURATION	90 days
ESTIMATED STUDY COMPLETION DATE	December 2023
POPULATION	
Sample Size:	24 patients
Target Population:	Adult patients over 18 with Acute Thyroid Eye Disease (AcTED)
TREATMENT(S)	
Study Drug Dose/Route:	Sub-tenon's injection of saline or hyalronidase followed by sub-tenon's aflibercept 4 mg aflibercept injection bi-weekly
ENDPOINT(S)	
Primary:	To assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from the Baseline Visit (Day 1) to day 45.

Secondary:	The secondary endpoints are: From the Baseline Visit (Day 1) to Days 45 and 90 • assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from baseline to day 90 • change in clinical activity as measured by a ≥ 2 point decrease on the clinical activity score (CAS) • change in extraocular muscle diameter as measured by computed tomography (CT) scan • change in proptosis as measured by Hertel exophthalmometry and CT scan. • change in degree of ocular misalignment during physical exam (and documented with external photography) and limitation in ocular motility as measured with physical examination • change in vision as measured by ETDRS BCVA • change in vision as measured by Ishihara color test • change in visual function as determined by automated Humphrey visual fields • change in subjective score of pain/discomfort based upon patient Graves' Ophthalmopathy Quality of Life (GO-QOL) questionnaire • change in eyelid retraction/position as measured by the mm of inferior and superior scleral show on physical examination
PROCEDURES AND ASSESSMENTS	Ophthalmologic examination including external photography, BCVA, Ishihara color test, a 30-2 automated Humphrey visual field, intraocular pressure, ocular motility and alignment, calculation of the clinical activity score (CAS), exophthalmometry, medical history and smoking status, Go QOL, measurement of blood pressure, CT scan, and laboratory work-up (including pregnancy test, Thyroid Stimulating Immunoglobulin (TSI) testing, anti-thyroglobulin antibody testing, anti thyroid peroxidase testing, cytokine testing, and baseline measurement of systemic VEGF levels prior to injection)
STATISTICAL PLAN	Descriptive statistics will be used to summarize study results. Summary statistics including mean, median, standard deviation and range will be provided for continuous variables. Because of the small number of patients and the short-term nature of the study, no interim data analysis is scheduled.

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1. INTRODUCTION

1.1 Background of Thyroid Eye Disease

Thyroid eye disease occurs in patients with autoimmune thyroid disease affecting about 60,000 US patients per year. TED causes swelling of the orbital contents, which disfigures one's physical appearance and can lead to disabling double vision or even irreversible blindness. The treatment options for these patients all have significant limitations including debilitating side effects and the need for repeat surgeries. In many cases even with intensive treatment, patients are left disfigured and with vision loss.

Clearly, a better treatment is needed. Here we propose the investigation of a novel, local therapy intended to treat TED while avoiding the systemic side effects and need for surgery. The MEEI oculoplastics service is well-positioned to develop a better treatment given the large number of TED patients seen routinely as referrals from the MGH thyroid center. The current gold standard therapy in abating the inflammatory and acute phase of this disease is to treat with systemic steroids. Unfortunately, steroids often have deleterious side effects and are poorly tolerated in patients with certain conditions including diabetes. Urgent surgery may also be necessary to decompress the bony orbit and to allow more "breathing room" for the swollen orbital tissues. A local therapy would be preferable over systemic medications as the rest of the body (including the thyroid gland) usually remain unaffected. Unfortunately, steroid injections into the orbit have demonstrated disappointing results.

1.2 Our Prior Research on TED

In order to develop novel and specific therapies for TED we first wanted to better understand the disease pathogenesis. Toward this end, we instituted a human experiment where orbital fat biopsies were taken from patients with acute TED. Our analysis revealed that there was a robust orbital angiogenic response in patients with active TED, which is not present in control or chronic TED orbital tissues. In addition, we found increased expression of multiple VEGF ligands and receptors in the adipose tissue of human orbit in patients with active TED. These findings suggest that the VEGF signaling pathway may be a potential therapeutic target for TED. These results were recently

published by our group in the journal *Ophthalmology*.

2. RATIONAL FOR THIS STUDY

2.1 Rationale for Study Design

Based on these data, we hypothesize that upregulation of the VEGF signaling pathway in the TED orbit leads to orbital angiogenesis and increased vascular permeability. These vascular changes likely lead to the edema, inflammatory infiltration and expansion of orbital tissues seen in acute TED. Inhibition of VEGF signaling pathway (including inactivation of VEGFA, VEGFC and VEGFD) could reverse or reduce the compressive forces in the orbit and provide a novel, local therapy for active TED. Importantly, local delivery of anti-VEGF medication is already commonplace in the eye for many ocular conditions. Local, orbital delivery (as was attempted with steroids) could obviate the need for systemic steroids and prove to be an ideal therapy for a disease currently lacking an effective treatment.

2.2 Rationale for Dose Selection

Intravitreal aflibercept injection (2 mg) has been approved by the FDA in the treatment of neovascular AMD, DME, and macular edema following RVO.

The recommended dose in this study is 4 mg due to the sub-tenon delivery route and amount of drug needed within the orbital space, which is approximately 2-3 times the volume of the eye. When delivering a sub-tenon block (local anesthesia), approximately 3-4 mL of lidocaine and hyaluronidase (HA) is given to help disperse the anesthesia. The orbit is filled with muscle and fat, but the fat and muscle are separated into "compartments" with septae, or walls between the compartments. The very thin fibrous walls are elastic and will not allow fluid to disperse unless an agent like hyaluronidase or potentially saline can break through them. Therefore, the added volume of sub-tenon injected HA or saline followed by 4 mg aflibercept reduce an orbital depot effect and improve aflibercept dispersion. In addition, the congestion in the orbit is thought to be due in part to the hyaluronan deposition in the orbital tissues. Theoretically, it is possible that HA itself may have an effect on TED patients, although this has never been studied.

Therefore, we will assign 1/3 of the study patients to HA injection only to determine if there is a difference between HA alone versus HA + aflibercept.

3. STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from the Baseline Visit (Day 1) to day 45.

3.2 Secondary Objectives

From the Baseline Visit (Day 1) to Days 45 and 90:

- assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from the Baseline Visit (Day 1) to day 90
- change in clinical activity as measured by a ≥ 2 point decrease on the clinical activity score (CAS)
- change in extraocular muscle diameter as measured by computed tomography (CT) scan
- change in proptosis as measured by Hertel exophthalmometry and CT scan.
- change in degree of ocular misalignment during physical exam (and documented with external photography) and limitation in ocular motility as measured with physical examination
- change in vision as measured by ETDRS BCVA
- change in vision as measured by Ishihara color test
- change in visual function as determined by automated Humphrey visual fields
- change in subjective score of pain/discomfort based upon patient Graves' Ophthalmopathy Quality of Life (GO-QOL) questionnaire

- change in eyelid retraction/position as measured by the mm of inferior and superior scleral show on physical examination

4. STUDY DESIGN

4.1 Study Description and Duration

The study is an exploratory investigation to evaluate use of sub-tenon injected HA or saline followed by sub-tenon injection of 4 mg aflibercept for the treatment of acute thyroid disease. The study will prospectively and sequentially enroll a total of twenty-four patients who meet the study enrollment criteria and will be randomized to one of three treatment arms:

1. Group 1: Sub-tenon's injection of saline followed by injection of 4 mg aflibercept (total volume 1.1 ml) in the worse eye
2. Group 2: Sub-tenon's injection of HA followed by injection of 4mg aflibercept (total volume 1.1 ml) in the worse eye
3. Group 3: HA injection alone (total volume 1.1 ml) in the worse eye

Patients will receive bi-weekly sub-tenon injections on day 1, day 14 and day 28 for a total of 3 treatments.

Both the subject and the investigator will be blinded as to which treatment arm the subject has been assigned.

At the time of enrollment at Day 0, patients will be screened through a full ophthalmologic examination including external photography, best corrected visual acuity (BCVA), Ishihara color test, exophthalmometry, a 30-2 automated Humphrey visual field, intraocular pressure, ocular motility and alignment, calculation of the CAS, medical history and smoking status, measurement of blood pressure, Go QOL questionnaire, and pregnancy test. The CAS is a 7-point clinical activity score and includes 1 point for the presence of each of the following: Redness of eyelids, spontaneous pain, pain when moving the globe, redness of conjunctiva, swelling of plica and/or caruncle, swelling of

eyelid, and chemosis.

The first injection will occur at the Baseline Visit (Day 1) after it has been determined that the subject meets the eligibility requirements to be in the study, pre-treatment CT scan (if no CT scan had been performed within 3 months prior to enrollment), laboratory work-up (including plasma testing of TSI, thyroglobulin antibodies, thyroid peroxidase antibodies, cytokine levels [IL-2, IL-2 receptor (CD25), IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IFN-g, TNF-a, IL-1B, TGF-B)], baseline measurement of systemic VEGF levels), and randomization into one of the treatment groups. Careful clinical monitoring will follow that initial injection including a complete re-evaluation on day 4 (complete ophthalmic exam, BCVA, Ishihara color test, exophthalmometry, CAS, motility exam and alignment, photos, measurement of blood pressure). Patients will be re-evaluated in the same way on day 14, day 28, day 45, and day 60. Graves Ophthalmology Quality of Life questionnaire will also be performed on day 14 and day 45. Humphrey visual field, post-treatment CT scan, and repeat thyroid antibody and cytokine levels will be collected on day 45. A long-term follow-up visit will occur at day 90.

At each clinical visit following injection, If CAS worsens to greater than 5 and there is evidence of new optic neuropathy, or undergoes subjective severe worsening as assessed by investigator, rescue systemic steroid therapy will be initiated at the clinician's discretion. Systemic steroid rescue therapy consists of either an initial IV dose of 1gram of SoluMedrol given weekly for a total of 3 weeks and 500 mg of the same medication given weekly for a total of 3 doses OR oral prednisone given 1mg per kg with a taper of 10mg every 2 weeks. If the patient has a flare-up of symptoms and TED activity while on the taper, he/she will be tapered more slowly or the dose will initially be increased back to 1mg/kg.

Primary and secondary endpoints will be measured at each clinical visit as described in Table 1. The final visit will be at day 90. Sample collection will occur concurrently.

Final data analyses and manuscript preparation will be completed after all the data has been analyzed.

4.2 Planned Interim Analysis

Because of the small number of patients and the short-term nature of the study, no interim data analysis is scheduled. Reports of adverse events from this study may be reviewed and summarized periodically while the study is ongoing to ensure the safety of patients.

4.3 Foreseeable Risks and Discomforts

Possible risks related to being in this study are those related to the study drug, risks related to the procedures to administer the study drug, and risks related to other study procedures. There are also possible risks to an embryo, fetus, or breastfeeding infant.

4.3.1 Risks Related to Study drug

The study agent has not been given to people with thyroid eye disease prior to this study, so the risks are not completely known. Potential risks include possible long-term problems that could occur years after the injection. The study drug has been approved for use by the FDA to treat other eye conditions such as age related macular degeneration (AMD), diabetic macular edema (DME), and macular edema following retinal vein occlusion (RVO), with no serious problems, though we cannot predict how people with thyroid eye disease will respond. Safety results from animal studies, if available, are summarized in the Investigator's Brochure.

As with any drug, an allergic reaction can occur. Allergic reactions can be mild or more serious, and can even result in death. Common symptoms of an allergic reaction are rash, itching, skin problems, swelling of the face and throat, or trouble breathing. There may be other risks of aflibercept that are currently unknown.

Subjects that are randomly assigned to study groups 2 or 3 will be injected with hyaluronidase which acts to disperse the aflibercept across the eye. It is possible that HA may have a negative effect on some subjects with thyroid eye disease although this has never been studied.

Subjects may experience an allergic reaction to HA. However, it is a rare event and especially in the orbit; there are only 5 patients reported in the literature. If treated within 24 hours with antihistamines and/or systemic steroids, the prognosis is good.

4.3.2 Risks Related to Procedures to Administer the Study Drug

Risks associated with any periocular injection include possible pain, bleeding, or swelling in the eye, bruising around the eye, infection, inflammation, retinal holes, retinal tears, retinal detachments, corneal abrasion, corneal edema, decreased vision, dry eye, macular

hole, or cataract development that could require additional eye surgery. Infection that may occur after an injection can usually be treated with antibiotics. All of these complications could result in worsening of vision. There may also be an increase in the pressure in subjects' eyes after treatment that would require treatment with medications to lower the pressure.

There is a low risk of complications from the proparacaine drops which are used to anesthetize the subject's worse eye prior to the sub-tenon injection. Subjects may experience irritation, stinging, burning, conjunctival redness, tearing or increased winking after the drops are given. Some local irritation and stinging may occur several hours after the proparacaine is applied. More rarely, subjects will experience pupil dilatation, and/or an inability to focus their eye also have been observed. While the drug appears to be safe for use in patients sensitive to other local anesthetics, local or systemic sensitivity occasionally occurs. Rarely, a severe corneal reaction may occur which includes acute, intense and diffuse foreign-body sensation, light sensitivity, redness, and blurred vision

Common risks of topical lidocaine gel include irritation, stinging or burning immediately after use, conjunctival redness, tearing or increased winking, and allergic reaction. Uncommon risks include local irritation and stinging occurring several hours after the lidocaine gel is applied. In rare cases, systemic sensitivity to the drug and severe corneal reaction (including acute, intense and diffuse foreign-body sensation, light sensitivity, redness, and blurred vision) may occur.

The most common risks associated with applying steroid medication for subtenon injections include increased pressure within the eye, infection, and cataract formation. Steroids applied in or around the eye may also cause blurred vision, temporary burning or stinging, itching, or eye pain. All risks described in the intravitreal injection of the study drug apply for intravitreal and subtenon applications of the steroids.

The most common risks associated with taking steroid pills include high blood pressure, increased glucose level or worsening of glycemic control in patients with existing diabetes, laboratory abnormalities (including increased white blood cell count), increased appetite, weight gain, acne, and formation of cataracts or glaucoma. Other risks include infection (for example, herpetic infection) gastrointestinal upset/bleeding, aggression, anxiety, blurred vision, dizziness, headache, hormonal imbalance, mood changes, facial and body hair growth, bone loss, numbness or tingling in the arms or legs, shortness of breath, swelling of the fingers, hands, feet, or lower leg, flushed, dry skin, skin thinning, fracture, heartburn or indigestion (severe and continuous), nausea, and insomnia. These side effects are generally associated with long-term steroid use.

4.3.3 Risks Related to other study procedures

Eye exams utilizing drops for pupil dilation: Rarely, people may have redness, discomfort or allergic reactions to the drops used to dilate the pupil during eye tests. This is usually not serious and can be treated if necessary. Bright lights may be uncomfortable while the

pupil is dilated, but this can be relieved by wearing sunglasses. The drops may also cause high blood pressure, abnormal heart beats, or worsening of some types of glaucoma, but these can be treated. In rare cases, the drops can cause high pressure in the eye that needs immediate attention. If this side effect occurs it can be treated. Subjects' eyes will be dilated for the eye exam. Their eyes will remain dilated for 4-6 hours or maybe the rest of the day. This may cause vision to be blurry and sensitive to bright lights. The PI will recommend that subjects wear sunglasses with UV protection.

CT Scans: Standard x-ray procedures, such as routine chest x-rays and mammography, use relatively low levels of ionizing radiation. The radiation exposure from CT is higher than that from standard x-ray procedures, but the increase in cancer risk from one CT scan is still small. Only the head is placed inside the CT scanner. Subjects must lie still during the scan that takes about 30 seconds. The entire process takes about 15 minutes. Subjects will be exposed to a maximum of approximately six milli sieverts (mSv) for this research.

Visual Fields: There are no known risks associated with these procedures.

Blood draw: There may be a bruise (a black and blue mark) or pain where the blood samples are taken. There is also a small risk of infection, lightheadedness, and/or fainting.

4.3.4 Risks to an Embryo or Fetus, or to a Breastfeeding Infant

The effect of aflibercept on an embryo or fetus (developing baby still in the womb), or on a breastfeeding infant, is unknown and may be harmful. Because of these unknown risks, women cannot take part in this study if they are pregnant, trying to become pregnant, or breastfeeding.

Pregnancy tests will be required for all female subjects except post-menopausal women who have not had a menstrual period for the past 12 months or more, or those with a well-documented method of surgical sterilization.

Female subjects that are sexually active and able to become pregnant, must agree to use birth control for the entire study and for at least 30 days after their last dose of study drug/agent.

Male subjects that are sexually active and able to father a child, must agree to use birth control for the entire study and for at least 30 days after their last dose of study drug/agent.

If the female partner of a male subject becomes pregnant, the study doctor will follow the outcome of the pregnancy. Male subjects will be asked to notify the PI if their female partner becomes pregnant. We will work with the subject and his female partner to provide contact information to the sponsor. The sponsor may ask the female partner to

sign a release of medical information form that gives her doctors permission to provide information to the sponsor.

5. SELECTION, WITHDRAWAL, AND REPLACEMENT OF SUBJECTS

5.1 Number of Patients Planned

We are planning to have twenty-four subjects complete the study. To achieve that number of participants, we anticipate enrolling and screening thirty patients.

Potential study participants (those with a diagnosis of acute Thyroid Eye Disease (TED)) will be from the clinical practice of the principal investigator at the Department of Ophthalmology at Massachusetts Eye and Ear Infirmary and are also anticipated to come from referrals to the practice. The medical records of these patients will be reviewed by the PI in light of study eligibility criteria. In addition, if a potentially eligible patient is seen in the clinic of an investigator during a routine consultation or follow-up visit, the study may be discussed. In this scenario, if the patient were interested, then a separate screening visit would be arranged on a different day to provide ample time for the individual to consider participation, generate additional questions, and discuss the study with other health care providers. We would recommend that the patient discuss participation with other health care providers. It will be confirmed to the individual that his or her decision to participate is completely voluntary and will have no effect on the care that he or she receives from MEE. A healthcare provider (preferably a physician) other than the investigator, who is known to the potential subject and has first-hand knowledge of the patient's medical history, will (1) give approval for his/her patient to be contacted for research purposes, (2) initially introduce the study to the patient **and** (3) obtain the patient's permission to be contacted by study staff. The health care provider can introduce the study and obtain the patient's permission to be contacted by study staff verbally during the course of providing medical care.

Patients who volunteer to participate in the study will meet with the PI to go over the study design and risks in detail at the first visit. Patients will be given time to ask

questions about any part of the study. When it is clear that the patient understands the study, including its risks, and agrees to participate, they will be asked to sign a written consent document.

5.2 Inclusion Criteria

A patient must meet the following criteria to be eligible for inclusion in the study:

1. Age $\geq$ 18 years
2. Active Thyroid Eye Disease
3. Clinical Activity Score (CAS) ≥ 3 and ≤ 5
4. Phakic and pseudophakic eyes are allowed in the study.
5. Willing and able to provide written informed consent after the nature of the study has been explained, and prior to any research-related procedures
6. Willing and able to comply with clinic visits and study-related procedures
7. Only one eye will be enrolled. The worse eye (demonstrating any of these features: worse HVF results, worse restricted motility, worse visual acuity, worse edema, worse proptosis, worse eyelid retraction) will be enrolled. If both eyes are equivalent, we will enroll the non-dominant eye.

5.3 Exclusion Criteria

A patient who meets any of the following criteria will be excluded from the study:

1. History of orbital, strabismus, or eyelid surgery or orbital radiation
2. Optic neuropathy or other vision-threatening signs
3. Patients currently on systemic or topical steroids. If they received steroids in the past, they would require a 6 week washout period and the cumulative dose of steroids must be less than 1 gram of methylprednisolone (or equivalent). During the washout period we will contact patients by phone weekly to monitor their safety and address any concerns they may have related to no longer taking steroids. We will encourage patients to contact us during the washout period to discuss any concerns they may have regarding their condition.
4. Patients who have taken teprotumumab (Tepezza).
5. Patients who have received intraocular anti-VEGF medications within 1 year of screening

6. Patients who have a history of receiving systemic anti-VEGF
7. Presence of other retinal vascular diseases (diabetic retinopathy, vein occlusion) that could affect the VEGF levels within the eye
8. History of stroke or prior myocardial infarction
9. Known hypersensitivity to aflibercept
10. Infectious conjunctivitis, keratitis, or endophthalmitis of either eye
11. Presence of a glaucoma shunting or filtration device that is subconjunctival
12. Previous participation in any studies of investigational drugs within 1 month preceding Day 0
13. Any clinically significant acute or chronic medical condition that would preclude participation in a clinical study
14. Pregnant or breast-feeding women
15. Sexually active men* or women of childbearing potential** who are unwilling to practice adequate contraception during the study (adequate contraceptive measures include stable use of oral contraceptives or other prescription pharmaceutical contraceptives for 2 or more menstrual cycles prior to screening; intrauterine device [IUD]; bilateral tubal ligation; vasectomy; condom plus contraceptive sponge, foam, or jelly, or diaphragm plus contraceptive sponge, foam, or jelly)
16. History of hyalronidase (HA) injection*** after the diagnosis of thyroid eye disease.
17. Taken part in other research studies in the past 12 months that have involved radiation exposure
18. Participated in other research studies in the past 12 months that required radiologic imaging (particularly CT scan and not MRI)

* Contraception is not required for men with documented vasectomy.

Postmenopausal women must be amenorrheic for at least 12 months in order **not to be considered of child bearing potential. Pregnancy testing and contraception are not required for women with documented hysterectomy or tubal ligation.

*** The past use of HA in general would not exclude a patient from participating in this study. However, we are concerned about the placebo effect: if a potential subject had used HA to treat thyroid eye disease (TED), he or she may be biased in terms of HA's positive effect. We are therefore excluding potential subjects who have prior use of HA to treat TED.

5.4 Premature Withdrawal from the Study

A patient has the right to withdraw from the study at any time, for any reason, and without repercussion.

Should a patient (or a patient's legally authorized guardian or representative) decide to withdraw, all efforts will be made to complete and report observations as thoroughly as possible. Early termination procedures will be followed.

The investigator may withdraw a patient from the study in the event of any of the following:

- an intercurrent illness
- the worsening of a subject's visual field, indicating that there is additional optic neuropathy
- the need to prescribe rescue systemic steroid therapy
- a serious adverse event
- a protocol violation
- signs of an allergy to the study drugs
- a recommendation by the DSMB that a patient should be withdrawn
- a subject's failure to appear for the treatment regimen within the required time interval
- a female subject who becomes pregnant, or
- administrative or other reasons.

An excessive rate of withdrawals would render the study uninterpretable; therefore, unnecessary withdrawal of patients will be avoided.

5.5 Replacement of Patients

Patients prematurely withdrawn from the study will continue to receive standard of care treatment based on recommendations from their ophthalmologist. Withdrawn patients will be replaced.

6. STUDY TREATMENTS

6.1 Treatment Arms

In the first arm, subjects will receive a sub-tenon injection of saline (total volume 1.1

mL), followed by a sub-tenon injection of 4 mg of aflibercept. In the second arm, subjects will receive a sub-tenon injection of HA (total volume 1.1 mL), followed by a sub-tenon injection of 4 mg of aflibercept. The third group will receive 1.1 mL of HA only. For all three arms, injection will be administered as bi-weekly sub-tenon injections on day 1, day 14 and day 28 for a total of 3 treatments.

The study will utilize a MediMop device that can withdraw aflibercept in an aseptic manner into one syringe from two, separate vials. A 1 mL syringe will be used to withdraw 0.05 mL for the 2 mg dose from the first vial via the MediMop device and then the same syringe will withdraw from a second vial an additional 0.05 mL via the MediMop device for a total of 4 mg per 1 mL. Bacteriostatic saline will be utilized in conjunction with aflibercept in the first arm and HA will be used in conjunction with aflibercept in the second arm. The saline syringe and the aflibercept syringe will be two separate syringes connected by a Luer-lock apparatus; similarly aflibercept + HA will also be contained in two separate syringes connected by a Luer-lock apparatus. The Luer-lock apparatus will obviate the need to perform two, separate injections as the needle will be kept in place and the syringe will rotate from one to the other. Saline or HA will be administered first, followed by intravitreal aflibrecept.

In the third arm, HA will be administered in an aseptic manner using two 1 cc syringes with a Luer-lock apparatus with 1mL in one and 0.1mL in the second syringe as to keep the PI blinded. The Luer-lock apparatus in all 3 arms will be attached to a 30 gauge ½ inch needle which will be injected into the sub-Tenon space.

A 30 gauge ½ inch needle will be used for delivery into the sub-tenon space.

6.2 Dose Modification

Dose modification for an individual patient is not allowed.

6.3 Stopping Rules

After considering the information provided to them, the DSMB will determine whether the study should continue as planned, proceed with modifications, or be terminated. The justification to terminate the study may be due to the DSMB's analysis that there is overwhelming effectiveness, futility, or safety issues. If the DSMB votes to terminate the study, a member of the DSMB will be chosen to prepare a report and a final DSMB meeting will be held. The DSMB's recommendations at the final DSMB meeting may include continuing action items to the sponsor-investigator based upon the final review.

6.4 Treatment Logistics and Accountability

6.4.1 Packaging, Labeling, and Storage

Intravitreal aflibercept injection (2.0 mg) is formulated as a sterile liquid to a final concentration of 40 mg/mL aflibercept in 5% sucrose, 10 mM sodium phosphate pH 6.3, 0.03% polysorbate 20, and 40 mM NaCl. Aflibercept 2.0 mg study drug will be supplied by Regeneron Pharmaceuticals Inc. in sealed, sterile 3 mL vials with a "withdrawable" volume of approximately 0.5 mL. Vials must be used only once (defined as entered with a MediMop device). For study drug in vials, the study drug will be withdrawn using aseptic technique with a MediMop device.

Each HA vial contains 1mL of solution, therefore one vial will be used per patient.

Each saline vial contains 20mL of solution. One vial will be used per patient to prevent possible contamination.

Intravitreal aflibercept will be shipped to the site via overnight shipping using cold packs to maintain a temperature of 2° to 8° C. The Investigator, or an approved representative (e.g. pharmacist), will ensure that all study drugs are stored in a secured area, under recommended storage conditions and in accordance with applicable regulatory requirements. The shipping box is to be opened and stored immediately at the site in a refrigerator intended for investigational products at a temperature of 2° to 8°C.

Immediately prior to use, the vials will be removed from the refrigerator. Prior to preparing the injection, the solution must be visually inspected and it should have no evidence of turbidity. If particulates, cloudiness, or discoloration are observed, the vial must not be used. Exposure of the material to temperatures outside these limits, except for warming prior to administration, is not recommended and may result in loss of activity. Records of actual storage conditions (i.e. temperature log) at the study site must be maintained; and must include a record of the dates, when the refrigerator was checked, the initials of person checking, and the temperature.

Saline vials will be ordered and shipped to the site at the beginning of the trial. They will be kept in a locked cabinet at room temperature for the duration of the study. Prior to injection, the solution must be visually inspected and it should have no evidence of turbidity. If particulates, cloudiness, or discoloration are observed, the vial must not be used.

HA vials will be ordered and shipped overnight with cold packs. The Investigator, or an approved representative (e.g. pharmacist), will ensure that all study drugs are stored in a secured area, under recommended storage conditions and in accordance with applicable regulatory requirements. The shipping box is to be opened and stored immediately at the site in a refrigerator at a temperature of 2° to 8°C.

6.4.2 Supply and Disposition of Treatments

Intravitreal aflibercept will be shipped at a temperature of 2 to 8ºC to the investigator or designee at regular intervals or as needed during the study. At the end of the study, and following drug reconciliation and documentation, all opened and unopened vials of study drug will be destroyed or returned to Regeneron Pharmaceuticals, Inc. or designee.
HA vials will be shipped at a temperature of 2 to 8ºC to the investigator or designee at the beginning of the study. The HA has a shelf life of 36 months. At the end of the study, and following drug reconciliation and documentation, all opened and unopened vials of study drug will be destroyed.

Bacteriostatic saline vials will be shipped at room temperature to the investigator or designee at the beginning of the trial and may be stored at room temperature throughout the length of the trial. Bacteriostatic saline has a shelf life of 5 years.

6.4.3 Treatment Accountability

All drug accountability records will be kept current.

The investigator will account for all opened and unopened vials of study drug. These records will contain the dates, quantity, and study medication

- dispensed to each patient,
- returned from each patient (if applicable), and
- disposed of at the site or returned to Regeneron Pharmaceuticals, Inc. or designee.

All accountability records will be made available for inspection by regulatory agency inspectors.

6.4.4 Treatment Compliance

All drug compliance records will be kept current and will be made available for inspection by regulatory agency inspector.

6.4.5 Concomitant Medications and Procedures

After the patient receives the injection of drug in sequence with the vehicle, antibiotic drops (without steroid) will be placed on the ocular surface three times per day for 5 days to ensure proper healing of the conjunctiva and prevent infection.

Patients may continue to receive all medications and standard treatments administered for their conditions at the discretion of their treating physician. If the patient has a diagnosis of glaucoma or any other ocular condition that requires local therapy, they will be permitted to use their regular eye drops with the exception of topical steroid drops. Patients with Graves' disease may continue to take their prescribed medications.

Any medications with steroids (topical, local injection, or systemic) will be prohibited in the study unless the benefits outweigh the risks for the patient at which point the patient will be removed from the study.

6.4.6 Prohibited Medications and Procedures

Intravitreal vascular endothelial growth factor inhibitors other than intravitreal aflibercept injection in both study and non-study eyes are prohibited. Investigational drugs are also not allowed.

6.4.7 Post-Study Treatment

Treatment for Thyroid Eye Disease will continue in the usual manner following completion of the study. Depending on the response to the study medication, the patient will be carefully monitored and may undergo surgeries including orbital decompression, eyelid surgery, or strabismus surgery or they may undergo treatment with systemic steroids.

7. STUDY SCHEDULE OF EVENTS AND VISIT DESCRIPTIONS

7.1 Schedule of Events

Study assessments and procedures are presented by study period and visit in Table 1.

Table 1 Schedule of Events

	Screening Visit 1	**Baseline Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8/ Early Termination**
Day	0	1	4 (+/- 2 days)	14 (+/- 3 days)	28 (+/- 3 days)	45 (+/- 3 days)	60 (+/-3 days)	90 (+/- 5 days)
Inclusion/Exclusion	X							
Informed Consent	X							
Pregnancy test (Urine)	X							
Medical History/Smoking Status	X							
Demographics	X							
Complete Ophthalmic Exam[1]	X	X	X	X	X	X	X	X
Motility exam	X		X	X	X	X	X	X
Clinical Activity Score (CAS) Assessment/Calculation	X	X	X	X	X	X	X	X
Administer Study Drug[2]		**X**		**X**	**X**			
External Photos[3]	X		X	X	X	X	X	X
Exophthalmometry	X		X	X	X	X	X	X
Humphrey Visual Field	X					X		X
Concomitant Meds and Treatments	X	X	X	X	X	X	X	X
Adverse Events		X	X	X	X	X	X	X
Best Corrected Visual Acuity (BCVA)	X	X	X	X	X	X	X	X
Ishihara Color Test	X	X	X	X	X	X	X	X
Blood draw sample (VEGF levels)		X						
Blood draw sample (TSI, anti-thyroglobulin, anti thyroid peroxidase, cytokine panel)		X				X		X
Blood Pressure (taken by technician or nurse)	X	X	X	X	X	X	X	X
CT Scan		X[4]				X		X
Go QOL questionnaire	X			X		X		X
Randomization to treatment arm		X						

[1] Including slit lamp biomicroscopy and dilated fundus examination
[2] saline or HA followed by injection of 4 mg sub-tenon aflibercept or injection of HA (total volume 1.1 ml)
[3] External photos taken from a high definition DSLR clinic camera of patient in primary gaze, upgaze, downgaze, leftgaze and rightgaze
[4] Pre-treatment CT scan will be done at Baseline/ Visit 2 if patient has not had a CT scan within 3 months prior to study enrollment.

7.2 Study Visits

7.2.1 Screening/Day 0

After the patient has provided informed consent, the following information will be collected:

- Inclusion/exclusion
- Demographics
- Medical history, smoking status, and concurrent illnesses
- Concomitant medications
- Go QOL questionnaire

The following procedures and assessments will be conducted:

- Urine pregnancy test (female subjects of childbearing potential)
- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam
- Humphrey Visual Field
- Motility exam
- Clinical Activity Score
- External Photos
- Exophthalmometry

Stopping Current Medications ("The Washout Period")

Subjects who have taken a cumulative dose of steroids of less than 1 gram (of methylprednisolone or equivalent) within the 30 days prior to screening will be asked to stop taking systemic or topical steroids for a 6-week washout period. We will encourage patients to contact us during the washout period to discuss any concerns they may have regarding their condition. We will also initiate contact with patients during the third week of the washout period to determine how they are doing and if additional follow-up is required.

7.2.2 Baseline/Day 1 (Within 60 days of the Screening Visit to allow for washout period)

- Concomitant medications

- Clinical Activity Score
- Blood draw sample (TSI, anti-thyroglobulin, anti thyroid peroxidase, cytokine panel and VEGF levels—to be collected before treatment injection)
- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam
- CT Scan (to be done before treatment injection if patient has not had a CT scan within 3 months prior to study enrollment)
- Adverse events
- Randomization to treatment group
- Sub-tenon injection according to what arm of study subject has been randomly assigned

7.2.3 Day 4, Visit 3

The following information will be collected:

- Adverse Events
- Concomitant medications

The following procedures and assessments will be conducted:

- Blood pressure measurement
- Motility exam
- Clinical Activity Score
- External Photos
- Exophthalmometry
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam

If CAS worsened to greater than 5, new optic neuropathy, perform rescue systemic steroid therapy.

7.2.4 Day 14, Day 28 (Visit 4 and Visit 5)

The following information will be collected:

- Go QOL questionnaire on Day 14
- Adverse Events
- Concomitant medications

The following procedures and assessments will be conducted:

- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam
- Motility exam
- Clinical Activity Score
- External Photos
- Exophthalmometry
- Sub-tenon injection according to what arm of study subject has been randomly assigned will be given at day 14 and day 28.

If CAS worsened to greater than 5, new optic neuropathy, perform rescue systemic steroid therapy.

7.2.5 Day 45 (Visit 6)

The following information will be collected:

- Adverse Events
- Go QOL questionnaire
- Concomitant medications

The following procedures and assessments will be conducted:

- Blood draw sample (TSI, anti-thyroglobulin, anti thyroid peroxidase, cytokine panel)
- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam
- Motility exam

- Clinical Activity Score
- External Photos
- Exophthalmometry
- CT scan
- Humphrey visual field

7.2.6 Day 60 (Visit 7)

The following information will be collected:

- Adverse Events
- Concomitant medications

The following procedures and assessments will be conducted:

- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam
- Motility exam
- Clinical Activity Score
- External Photos
- Exophthalmometry

7.2.7 Day 90 (Visit 8/Early Termination):

The following information will be collected:

- Go QOL questionnaire
- Adverse Events
- Concomitant medications

The following procedures and assessments will be conducted:

- Blood draw sample (TSI, anti-thyroglobulin, anti thyroid peroxidase, cytokine panel)
- Blood pressure measurement
- ETDRS BCVA
- Ishihara Color Test
- Complete ophthalmic exam

- Motility exam
- Clinical Activity Score
- External Photos
- Exophthalmometry
- Humphrey Visual Field
- CT Scan

7.2.8 Early Termination Visit

Patients who are withdrawn from the study should be instructed to return to the clinic for end of study assessments, as described in section 7.2.7.

7.2.9 Unscheduled Visits

All attempts should be made to keep patients on the study schedule. Unscheduled visits may be necessary to repeat testing following abnormal laboratory results, for follow-up of AEs, or for any other reason, as warranted.

7.3 Study Procedures

7.3.1 Procedures Performed Prior to Treatment

The following procedures will be performed for the purpose of determining study eligibility or characterizing the baseline population at the Screening visit or Baseline visit prior to treatment.

Patients will undergo full ophthalmic examination including calculation of Clinical Activity Score. A thorough review regarding their medical health and smoking history will be conducted. They will also undergo examination of their ocular alignment and motility, Humphrey visual field to detect any occult optic neuropathy. Patients will receive a baseline CT scan to determine the amount of extraocular muscle enlargement and crowding in the apex of the orbit if they have not had a CT scan performed within 3 months before study enrollment. Complete ophthalmic exam including ETDRS BCVA, Ishihara color test, pupils, ocular motility, intraocular pressure

measurement, exophthalmometry, resistance to retropulsion, slit lamp biomicroscopy, and dilated fundus examination will also be conducted.

7.3.2 Procedures to Determine Efficacy

For documentation of diagnosis and initial response to sub-tenon aflibercept injection, Clinical Activity Scale, extraocular muscle diameter as measured by computed tomography (CT), Humphrey Visual Field, BCVA, and Ishihara color test will be performed at each study visit. Patients will also undergo TSI blood testing and serum VEGF level testing, which can sometimes be associated with activity in TED.

7.3.3 Safety Procedures

Adverse events (AEs) will be monitored and reported at each visit starting with the Baseline Visit. The investigator (or designee) will record all AEs that occur on the AE pages of the case report form (CRF). Information on follow-up for AEs is provided in section 8.2.2. The definition of an AE and SAE, and information on the determination of severity and relationship to treatment are provided in sections 8.2.3 and 8.2.4.

8. SAFETY DEFINITIONS, REPORTING, AND MONITORING

8.1 Definitions

8.1.1 Adverse Event

An AE is any untoward medical occurrence in a patient administered a study drug which may or may not have a causal relationship with the study drug. Therefore, an AE is any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease which is temporally associated with the use of a study drug, whether or not considered related to the study drug.

An AE also includes any worsening (i.e. any clinically significant change in frequency and/or intensity) of a pre-existing condition that is temporally associated with the use of the study drug.

8.1.2 Serious Adverse Event

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

1. Results in **death** – includes all deaths, even those that appear to be completely unrelated to study drug (e.g. a car accident in which a patient is a passenger).
2. Is **life-threatening** – in the view of the investigator, the patient is at immediate risk of death at the time of the event. This does not include an AE that had it occurred in a more severe form, might have caused death.
3. Requires in-patient **hospitalization** or prolongation of existing hospitalization. In-patient hospitalization is defined as admission to a hospital or an emergency room for longer than 24 hours. Prolongation of existing hospitalization is defined as a hospital stay that is longer than was originally anticipated for the event, or is prolonged due to the development of a new AE as determined by the investigator or treating physician.
4. Results in persistent or significant **disability/incapacity** (substantial disruption of one's ability to conduct normal life functions).
5. Is a **congenital anomaly/birth defect**
6. Is an **important medical event** – Important medical events may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the patient or may require intervention to prevent 1 of the other serious outcomes listed above (e.g., intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse). Any malignancy (other than basal cell skin cancers) would be considered a medically important event.

8.2 Recording and Reporting Adverse Events

8.2.1 The Criteria for Reporting Adverse Events

All AEs and SAEs will be recorded on the CRF and in the patient's source documents. Laboratory values, vital signs or ECG abnormalities will be recorded as AEs only if they are medically relevant.

The criteria for determining whether an abnormal objective test finding will be reported as an AE are as follows:

- the test result is associated with accompanying symptoms, and/or
- the test result requires additional diagnostic testing or medical/surgical intervention, and/or
- the test result leads to a change in dosing (outside of protocol-stipulated dose adjustments), discontinuation from the study, significant additional concomitant drug treatment, or other therapy.

Repeating an abnormal test, in the absence of any of the above conditions, does not constitute an adverse event. Any abnormal test result that is determined to be an error does not require reporting as an adverse event.

8.2.2 Adverse Event Follow-up

Adverse event information will be collected until the end of study visits, or the early termination visit, if the patient withdraws consent.

The investigator must make every effort to obtain follow-up information on the outcome of any SAE until the event is considered chronic and/or stable.

8.2.3 Evaluation of Severity

The severity of an AE will be graded by the investigator using a 3–point scale (mild, moderate, or severe) and reported in detail as indicated on the CRF and/or SAE form, as appropriate.

- **Mild:** Does not interfere in a significant manner with the patient's normal functioning level. It may be an annoyance. Prescription drugs are not ordinarily needed for relief of symptoms, but may be given because of personality of the patient.
- **Moderate:** Produces some impairment of functioning but is not hazardous to health. It is uncomfortable or an embarrassment. Treatment for symptom may be needed.
- **Severe:** Produces significant impairment of functioning or incapacitation and is a definite hazard to the patient's health. Treatment for symptom may be given and/or patient hospitalized.

8.2.4 Evaluation of Causality

The relationship to treatment will be determined by the investigator and reported on the CRF and/or SAE form, as appropriate. The following terms will be used:

Not Related: likely or clearly due to causes other than the study drug.

Related: possibly, probably, or definitely related to the study drug.

All SAEs, regardless of assessment of causal relationship to study drug will be reported to the study Data Safety Management Board, the IRB, the FDA, and Regeneron Pharmaceuticals, Inc.

8.2.5 Reporting to the Data Safety Management Board

The primary charge of this study's DSMB is to monitor participant safety. The safety and related data that the DSMB will review every 12 months or after every five participants receive their first dose of intervention, whichever is sooner:

- Participant recruitment, accrual, retention, and withdrawal information
- Adverse events (AEs) and serious adverse events (SAEs)
    - Tabulated by body system, relatedness, intensity, seriousness, duration, treatment given, and the relationship to the study device
    - Individual events of particular concern
    - Physician follow-up for each patient enrolled since last DSMB meeting, including review of Case Report Forms, summary data, and/or portions of the medical record to identify:
- Any side effects or post-operative complications as identified at follow-up
    - Any other safety-supporting data requested by the DSMB

Serious adverse events (SAEs) that are determined to be related or possibly related to the study drug will be monitored by the DSMB as they arise. SAEs that are related or possibly related to the study drug will be reported by the sponsor-investigator to the DSMB Chair via written communication (email or fax) within one working day of the coordinating center (MEE) learning of the event.

8.2.6 Reporting to the IRB

SAEs will be reported to the IRB within three days. All adverse events will be reported to the IRB in the annual continuing review application.

8.2.7 Reporting to the FDA

SAEs will be reported to the FDA within 10 days. All adverse events (AEs) will be reported to the FDA in the annual progress report.

8.2.8 Reporting to Regeneron

Serious adverse events will be reported within 24 hours of learning of the event to Regeneron at the following:

Medical.safety@regeneron.com
Fax 914-345-7476
SAE hotline: 914-593-1504

Any AE that results in death is considered an SAE. Deaths that occur from the time the patient signs the informed consent form ("ICF") until 30 days after dosing will be reported to Regeneron Pharmacovigilance and Risk Management (or designee) within 24 hours of learning of the death. Any available autopsy reports and relevant medical reports will be sent to Regeneron Pharmaceuticals, Inc. as soon as possible.

The following events also will be reported to Regeneron Pharmaceuticals, Inc. within 24 hours of learning of the event:

- Overdose**:** Accidental or intentional overdose of the study drug or concomitant medication, whether or not it is considered an AE.

- Pregnancy: Although it is not considered an AE, the investigator will report to Regeneron Pharmaceuticals, Inc., any pregnancy occurring in a female patient or female

partner of a male patient, during the study or within 30 days following the last dose of study drug. The investigator will follow the pregnancy until delivery, or longer. If the pregnancy continues to term (delivery), the health of the infant will also be reported to Regeneron Pharmaceuticals, Inc.

- All AEs that lead to a patient's withdrawal from the study will be reported to Regeneron Pharmaceuticals Inc. within 30 days. All SAEs leading to a patient's withdrawal from the study will be reported.

9. STUDY VARIABLES

9.1 Demographic and Baseline Characteristics

Baseline characteristics will include standard demographics (e.g. age, date of birth, ethnicity, race, weight, height), disease characteristics including medical history, and medication history for each patient.

9.2 Primary and Secondary Endpoints

The primary objective is to assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from Baseline Visit (Day 1) to day 45.

The secondary endpoints are:

From Baseline (Day 1) to Days 45 and 90:

- assess the safety and tolerability of sub-tenon aflibercept in combination with either saline or hyaluronidase (HA) in patients with acute Thyroid Eye Disease (TED) as assessed by the incidence and severity of adverse events from Baseline Visit (Day 1) to day 90
- change in clinical activity as measured by a ≥ 2 point decrease on the clinical activity score (CAS)
- change in extraocular muscle diameter as measured by computed tomography (CT) scan
- change in proptosis as measured by Hertel exophthalmometry and CT scan.
- change in degree of ocular misalignment during physical exam (and documented with external photography) and limitation in ocular motility as measured with physical

examination

- change in vision as measured by ETDRS
- change in vision as measured by Ishihara color test
- change in visual function as determined by automated Humphrey visual fields
- change in subjective score of pain/discomfort based upon patient Graves' Ophthalmopathy Quality of Life (GO-QOL) questionnaire
- change in eyelid retraction/position as measured by the mm of inferior and superior scleral show on physical examination

10. STATISTICAL PLAN

10.1 Determination of Sample Size

The study is an exploratory investigation to evaluate use of sub-tenon aflibercept injection in patients with acute Thyroid Eye Disease. A sample size of twenty-four patients was chosen based on the Hypothesis that Group 1 will have a superior reduction in CAS score relative to Group 3 and Group 2 will have a superior reduction in CAS score relative to Group 3.

Group 1 (intervention - Eylea alone) mean: 3.0; SD 1.0
Group 2 (intervention - Eylea + HA) mean: 3.0, SD 1.0
Group 3 (control - HA alone) mean: 5.0; SD 1.5.

Assuming an average reduction in CAS in Group 1 of 2 points (with standard deviation=1 point), an average reduction in CAS in Group 2 of 2 points (with SD = 1 point), and an average reduction in CAS in Group 3 of 0 points (SD = 1 point), sample sizes of 8 patients per arm (total sample size of 24 patients) are needed in order to have 80% statistical power for detecting the difference in reduction of CAS between the three groups. This power calculation is based on Student's t-test and assumes a two-tailed Bonferroni-adjusted alpha level of 0.017 (0.05/3 comparisons) in order to control from the risk of false positive results due to multiple comparisons.
Assumptions:
alpha = 0.017 (two-sided)
power = 0.8000

m1 = 3

m2 = 3

m3 = 5

sd1 = 1

sd2 = 1

sd3 = 1

Using paired t-test to compare group 1 to group 3 and group 2 to group 3,

estimated required sample sizes:

n1 = 8

n2 = 8

n3 = 8

10.2 Analysis Sets

10.2.1 Efficacy Analysis Sets

Efficacy analysis sets include the full analysis set of all patients randomized who received any study drug; it is based on the treatment allocated (as randomized). Efficacy endpoints will be analyzed using the FAS.

10.2.2 Safety Analysis Set

The safety analysis set includes all patients who received any study drug. It is based on the treatment received (as treated). Treatment compliance/administration and all clinical safety variables will be analyzed using the SAF.

10.3 Statistical Methods

10.3.1 Demography and Baseline Characteristics

Demographic and baseline characteristics will be summarized descriptively by treatment group. Continuous variables will be summarized with mean, median, standard deviation, minimum, and maximum. Categorical variables will be summarized with frequency and percentage.

10.3.2 Safety Analysis

Treatment-emergent AEs (“TEAEs”) are defined as those that are not present at baseline or represent the exacerbation of a pre-existing condition during the on-treatment period.

Treatment-emergent AEs will be listed and summarized by treatment group.

10.3.3 Treatment Exposure

Treatment exposure will be defined as the duration of effect following the initial administration of sub-tenon aflibercept injection, lasting throughout the 90-day period of the study.

10.3.4 Treatment Compliance

As only three doses are administered and they are administered by a surgeon, compliance is not expected to apply to this study. The patients need only to show up for their injections and their follow-up visits and they are not required to comply with a treatment at home. If the patient fails to appear for the treatment regimen within the required time interval, they will be withdrawn from the study.

10.3.5 Interim Analysis

Because of the small number of patients and the short-term nature of the study, no interim data analysis is scheduled.

11. STUDY MONITORING

11.1 Source Document Requirements

Investigator will prepare and maintain adequate and accurate patient records (source documents). The investigator will keep all source documents on file with the CRF. Case report forms and source documents will be available at all times for inspection by authorized representatives of the regulatory authorities.

11.2 Case Report Form Requirements

A CRF for each patient enrolled in the study will be completed and signed by the study investigator or authorized designee. The CRF will be typed or filled out using indelible ink. The writing will be legible. Errors will be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the investigator or authorized designee. The investigator will ensure the accuracy, completeness, legibility, and timeliness of the data reported in the CRFs.

Case report forms will be available at all times for inspection by authorized representatives of the regulatory authorities.

12. AUDITS AND INSPECTIONS

This study may be patient to a quality assurance audit or inspection by the regulatory authorities. Should this occur, the investigator will be responsible for:

- Informing Regeneron of a planned inspection by the authorities as soon as notification is received
- Providing access to all necessary facilities, study data, and documents for the inspection or audit
- Communicating any information arising from inspection by the regulatory authorities to the Regeneron immediately
- Taking all appropriate measures requested by the regulatory authorities to resolve the problems found during the audit or inspection

Documents patient to audit or inspection include but are not limited to all source documents, CRFs, medical records, correspondence, ICFs, IRB files, documentation of certification and quality control of supporting laboratories, and records relevant to the study maintained in any supporting pharmacy facilities. Conditions of study material storage are also patient to inspection.

In all instances, the confidentiality of the data will be respected.

13. ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Good Clinical Practice Statement

It is the responsibility of the investigator(s) to ensure that this clinical 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 ICH guidelines for Good Clinical Practice (GCP) and applicable regulatory requirements.

13.2 Informed Consent

The principles of informed consent are described in ICH Guidelines for GCP.

Regeneron will have the right to review and comment on the informed consent form.

It is the responsibility of the investigator or designee (if acceptable by local regulations) to obtain written informed consent from each patient prior to his/her participation in the study and after the aims, methods, objectives, and potential hazards of the study have been explained to the patient in language that he/she can understand. The ICF will be signed and dated by the patient and by the investigator or authorized designee who reviewed the ICF with the patient.

The original ICF will be retained by the investigator as part of the patient's study record, and a copy of the signed ICF will be given to the patient.

If new safety information results in significant changes in the risk/benefit assessment, the ICF will be reviewed and updated appropriately. All study patients will be informed of the new information and provide their written consent if they wish to continue in the study. The original signed revised ICF will be maintained in the patient's study record and a copy will be given to the patient.

13.3 Patient Confidentiality and Data Protection

The investigator will take all appropriate measures to ensure that the anonymity of each study patient will be maintained.

The patient's and investigator's personal data will be treated in compliance with all applicable laws and regulations.

13.4 Institutional Review Board

An appropriately constituted IRB, as described in ICH Guidelines for GCP, will review and approve:

- The protocol, ICF, and any other materials to be provided to the patients (e.g. advertising) before any patient may be enrolled in the study
- Any amendment or modification to the study protocol or ICF before implementation, unless the change is necessary to eliminate an immediate hazard to the patients, in which case the IRB will be informed as soon as possible

Ongoing studies will be reviewed by the IRB on an annual basis or at intervals appropriate to the degree of risk.

In addition, the IRB will be informed of any event likely to affect the safety of patients or the continued conduct of the clinical study.

A copy of the IRB approval letter will be sent to Regeneron prior to shipment of drug supplies to the investigator. The approval letter will include the study number and title, the documents reviewed, and the date of the review.

Records of the IRB review and approval of all study documents (including approval of ongoing studies) will be kept on file by the investigator.

14. PROTOCOL AMENDMENTS

The investigator will not implement a change in the design or operation of the protocol or ICF without an IRB-approved amendment.

15. PREMATURE TERMINATION OF STUDY

The investigator will notify Regeneron of a desire to close-out a site in writing, providing approximately 30 days' notice. The final decision will be made through mutual agreement with Regeneron. Both parties will arrange the close-out procedures after review and consultation.

In all cases, the appropriate IRB and Health Authorities will be informed according to applicable regulatory requirements, and adequate consideration will be given to the protection of the patients' interests.

16. DATA MANAGEMENT

16.1 Data Collected

As part of the responsibilities assumed by participating in the study, the investigator (Dr. Lee) agrees to maintain adequate case histories for patients treated as part of the research under this protocol. The investigator agrees to maintain accurate eCRFs and source documentation as part of the case histories. These source documents may include laboratory reports, etc. All eCRF

information is to be completed. If an item is not available or is not applicable, this fact must be indicated. Blank spaces must not be present unless otherwise directed.
Investigative site personnel will enter patient data into a database system. The analysis data sets will be a combination of these data and data from other sources (eg, laboratory data).

All study subjects will be identified by a unique code number.

After database lock, an electronic copy of all the study site's data will be created and sent to the sponsor. Information that may identify subjects will not be provided.

16.2 Certification of Accuracy of Data

Clinical data management will be performed in accordance with professional standards and data cleaning procedures to ensure the integrity of the data (eg, removing errors and inconsistencies in the data). Adverse events and medical history will be coded using the MedDRA terminology. Concomitant medications will be coded using the most current available WHO Drug Dictionary.

A declaration assuring the accuracy and content of the data recorded on the CRFs will be signed by the investigator. This certification form accompanies each set of CRFs.

16.3 Retention of Records

The investigator will retain all essential study documents, including ICFs, source documents, CRFs, and drug accountability records for at least 7 years following the completion or discontinuation of the study, or longer if a longer period is required by relevant regulatory authorities. Records will be destroyed in a manner that ensures confidentiality.

17. PUBLICATION PLAN

After completion of the study, the data may be considered for reporting at a scientific meeting or for publication in a scientific journal. The Principal Investigator will provide a draft copy of all presentations and manuscripts to the sponsor prior to presentation or submission.

18. REFERENCES

Orbital Angiogenesis and Lymphangiogenesis in Thyroid Eye Disease: An Analysis of Vascular Growth Factors with Clinical Correlation.Wong LL, Lee NG, Amarnani D, Choi CJ, Bielenberg DR, Freitag SK,D'Amore PA, Kim, LA. Ophthalmology. 2016 Sep;123(9):2028-36. doi:10.1016/j.ophtha.2016.05.052. Epub 2016 Jul 14.N Engl J Med. 2017 May 4;376(18):1748-1761. doi:10.1056/NEJMoa1614949.

Teprotumumab for Thyroid-Associated Ophthalmopathy. Smith TJ1, Kahaly GJ1, Ezra DG1, Fleming JC1, Dailey RA1, Tang RA1,Harris GJ1, Antonelli A1, Salvi M1, Goldberg RA1, Gigantelli JW1, CouchSM1, Shriver EM1, Hayek BR1, Hink EM1, Woodward RM1, Gabriel K1,Magni G1, Douglas RN Engl J Med. 2017 May 4;376(18):1748-1761. doi:10.1056/NEJMoa1614949

Thyroid autoimmunity and ophthalmopathy related to melanoma biological therapy.Min L1, Vaidya A, Becker C. Eur J Endocrinol. 2011 Feb;164(2):303-7. doi: 10.1530/EJE-10-0833. Epub 2010 Nov 18.

Transforming growth factor β1 (TGFβ1) and vascular endothelial growth factor (VEGF) in the blood of healthy people and patients with Graves'orbitopathy--a new mechanism of glucocorticoids action?26Kajdaniuk D1, Marek B, Niedziołka-Zielonka D, Foltyn W, Nowak M,Siemińska L, Borgiel-Marek H, Głogowska-Szeląg J, Ostrowska Z, Drożdż L, Kos-Kudła B. Endokrynol Pol. 2014;65(5):348-56. doi: 10.5603/EP.2014.0048

Bock F, Onderka J, Dietrich T, et al. Bevacizumab as a potent inhibitor of inflammatory corneal angiogenesis and lymphangiogenesis. *Invest Ophthalmol Vis Sci.* 2007;48:2545-2552

Mourits MP, Koornneef L, Wiersinga WM, Prummel MF, Berghout A, van der Gaag R. Clinical criteria for the assessment of disease activity in Graves' ophthalmopathy: a novel approach. *Br J Ophthalmol.* 1989;73:639-644

Nagy JA, Vasile E, Feng D, et al. Vascular permeability factor/vascular endothelial growth factor induces lymphangiogenesis as well as angiogenesis. *J Exp Med.* 2002;196:1497-1506.

Cursiefen C, Maruyama K, Jackson DG, Streilein JW, Kruse FE. Time course of angiogenesis and lymphangiogenesis after brief corneal inflammation. *Cornea.* 2006;25:443-447.

Sub-tenon Injection(s): A topical anesthetic (Proparacaine) will be placed into the eye followed by a wire lid speculum. A cotton swab soaked in 2% Lidocaine gel will be applied to the conjunctiva in the region of injection. The surface of the eye will be sterilized with 3 drops of 5% betadine solution. The patient will be asked to supraduct and abduct the injected eye. A 30-gauge needle that is connected to a Luer-lock, connected to two syringes will enter into the Sub-tenon's space while the tissues are elevated with toothed forceps. In patients receiving aflibercept and either saline or hyaluronidase, the first injection of hyaluronidase vs. saline will be placed followed by an aflibercept injection. Patients receiving only hyaluronidase will receive two syringes of the same solution so that neither the patient nor the doctor injecting can tell which group the patient received in the randomization process.