Statistical Analysis Plan (SAP)

RRR

Title: A Randomized, Active-Controlled, Phase II Study of the Efficacy and Tolerability of Intravitreal Injections of Ranibizumab Compared to Intravitreal Injections of Ranibizumab Combined with Targeted Retinal Photocoagulation in Subjects with Radiation Retinopathy (RRR Study)

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Abbreviations

AE	Adverse Events
ATE	Arterial Thromboembolic Events
CMT	Central Macular Thickness
CNV	Choroidal Neovascularization
COMS	Collaborative Ocular Melanoma Study
EGF	Epithelial Growth Factor
ETDRS BCVA	Early Treatment Diabetic Retinopathy Study Best Corrected Visual Acuity
FGF	Fibroblast Growth Factor
IOP	Intraocular Pressure
IVT	Intravitreal
NV	Neovascularization
SD-OCT	Spectral-Domain Optical Coherence Tomography
PDGF	Platelet-Derived Growth Factor
PRN	*Pro Re Nata*, As Needed
SAE	Serious Adverse Events
TRP	Targeted Retinal Photocoagulation
USA	United States of America
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor
VH	Vitreous Hemorrhage

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1. DESCRIPTION OF STUDY

RRR is a phase II, randomized, multicenter, clinical study to assess the tolerability and efficacy of ranibizumab treatment administered in subjects with radiation retinopathy. Subjects will be randomized into one of 3 arms; ranibizumab treatment administered IVT monthly vs. ranibizumab treatment administered IVT monthly combined with peripheral targeted photocoagulation vs. ranibizumab treatment administered IVT for three months followed by as needed treatment of ranibizumab combined with peripheral targeted photocoagulation over 48 weeks. From week 52 to week 1010, all 3 treatment arms will employ a treat and extend protocol for IVT ranibizumab treatment.

2. OVERVIEW OF STUDY DESIGN

This trial will compare the results of 3 different combinations of therapy to assess the efficacy of peripheral targeted retinal photocoagulation (TRP) applied to areas of retinal nonperfusion and poor perfusion combined with ranibizumab injections for the treatment of macular edema secondary to radiation retinopathy. Specifically, this trial will evaluate the ability to reduce the monthly treatment burden by eliminating areas of peripheral ischemia as detected by wide field fluorescein angiography. This peripheral ischemic retina is likely a major VEGF production site. By selectively eliminating these ischemic areas with angiography-guided laser ablation while preserving more perfused areas of peripheral retinal, pathological levels of VEGF may be significantly reduced, translating into fewer necessary monthly intravitreal injections while preserving useful peripheral visual field. The study will include 3 cohorts followed for a total of 104 weeks.

Subjects will be randomized into one of three treatment cohorts in a 1:2:2 ratio.

Day 1 through Week 48:

- Cohort A (n=8) will receive monthly treatment of 0.5 mg ranibizumab for 48 weeks. Monthly treatment is defined as every 28 days (+/- 7 days).
- Cohort B (n=16) will receive monthly treatment of 0.5mg ranibizumab for 48 weeks. 1 week (+/- 3 days) after the initial dose of ranibizumab is delivered, the subject will have peripheral targeted-retinal photocoagulation (TRP) to areas of peripheral retinal ischemia based on wide field angiography that was performed at the screen visit. After the first session of TRP, subjects will have a repeat wide field angiogram at week 24 and week 36 and will receive additional TRP as needed (PRN) to areas of peripheral retinal ischemia.
- Cohort C (n=16) will receive a loading dose (3 consecutive monthly doses) of 0.5mg ranibizumab followed by PRN treatment with 0.5mg ranibizumab. 1 week (+/- 3 days) after the initial dose of ranibizumab is delivered, the subject will have peripheral TRP to areas of peripheral retinal ischemia based on wide field angiography that was performed at the screen visit. After the first session of TRP, subjects will have a repeat wide field angiogram at week 24 and week 36 and will receive additional TRP as needed to areas of peripheral retinal ischemia.

See RRR protocol for TRP and PRN treatment criteria.

Week 52 through Week 101:

All cohorts will enter a treat and extend protocol for IVT ranibizumab retreatment beginning at week 52. At any visit where a dry macula is achieved din the study eye, the visit interval between IVT ranibizumab treatments will be lengthened by 1 week. A dry macula is defined as the resolution of recurrent or persistent fluid based on SD-OCT, slit lamp examination, and/or indirect ophthalmoscopy.

After a subject has been extended beyond a monthly visit interval between IVT ranibizumab treatments, the visit interval will be reduced by 1 week, if the study eye develops recurrent disease activity. Recurrent disease activity is defined as recurrent or persistent fluid based on SD-OCT, slit lamp examination, and/or indirect ophthalmoscopy.

IVT ranibizumab treatment will be administered in the study eye at every visit, no earlier than 7 days before the target date and no longer than 7 days after the target date. The visit interval between IVT ranibizumab treatments is individualized based on each patient's response to treatment. The visit interval between IVT ranibizumab treatments will not exceed 12 weeks.

2.1 Sample Size Calculations

A priori power calculation was performed and revealed that a sample size of n=41 was needed to achieve 80% power using single factor ANOVA with a significance level (alpha) of 0.05. Based on this result, the final study size was 40. Patients randomized in a 1:2:2 ratio. Sample size estimation was performed using RStudio (Version 1.1.463, RStudio Inc., Boston, Massachusetts). Statistical analyses were performed using Prism (GraftPad Software, San Diego, CA) and the data analysis function in Microsoft Excel (Microsoft Inc., Redmond, WA).

The reason for the 1:2:2 randomization ratio was that the monthly group would have less variability in terms of treatment course because each patient would receive an injection each month without alterations or additional factors to their treatment regimen, allowing for detection of the group's trend with a smaller cohort size. On the other hand, the other two cohorts could have different treatment schedules and therefore would require more patients to capture the trend. The exception to this randomization was that patients whose central vision of 20/50 or better at screening exam were randomized into the three cohorts in a 1:1:1 ratio in order to minimize a disproportionate population of subjects with good vision in any one group and to minimize the chance of significant difference in baseline vision between groups. Randomization occurred on Day 0 in which a randomly selected envelope containing a cohort assignment was chosen for each patient.

3. AIMS AND OBJECTIVES

3.1 Primary Objective

To evaluate the mean change in ETDRS visual acuity at 48 weeks and 104 weeks from Day 0.

3.2 Secondary Objectives

To evaluate the total number of intravitreal injections required during a 104 week study period (laser versus non laser). To evaluate the percentage of patients with persistent macular edema based on SD-OCT at 48 weeks and 104 weeks.

4. OUTCOMES

4.1 Primary Outcomes

- Mean change in ETDRS visual acuity at week 48 and week 104 from day 0.

4.2 Secondary Outcomes

- The mean number of intravitreal injections required per subject per cohort.

- Percentage of subjects with persistent macular edema, based on SD-OCT, at week 48 and week 104 compared to day 0.
- Percentage of subjects avoiding the development of neovascular glaucoma and/or vitreous hemorrhage.

4.3 Safety Outcomes

The safety and tolerability of intravitreal ranibizumab have been investigated in previous Phase I, I/II and III studies in AMD, RVO, and DME trials. Potential safety issues associated with the route of administration or the pharmacology of aflibercept in the study population include decreased BCVA, intraocular inflammation, intraocular infection, transient and/or sustained elevation of intraocular pressure (IOP), cataract development or progression, retinal or intraretinal hemorrhage, macular edema, retinal break or detachment, and arterial thromboembolic events (ATEs). Safety will be assessed by visual acuity, ophthalmic examinations, fluorescein angiograms, SD-OCT, intraocular pressure, vital signs, and adverse event documentation.

To minimize the risks of intraocular infections, all injections will be performed employing sterile techniques. Study drug administration will be held for subjects who experience certain ocular events or infections. In the event any subject develops an adverse event in the study eye that is considered by the evaluating physician to be severe in intensity, serious consideration should be given to withdrawing the subject from the study.

The PI or designated Sub-Investigators will review all adverse events on an ongoing basis to determine causality and relationship to study drug and/or study procedures.

5. POPULATIONS

Subjects were eligible to participate if the following criteria were met:

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

1. Ability to provide written informed consent and comply with study assessments for the full duration of the study
2. Age ≥18 years
3. Active radiation retinopathy resulting from any form of radiation treatment. Radiation retinopathy is defined as any of the following: retinal hemorrhages, exudates, edema, and/or neovascularization, not attributable to other causes
4. BCVA of 20/25 to 20/400 in the study eye

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

1. Pregnancy (verified by positive pregnancy test) or lactation
2. Premenopausal women not using adequate methods of contraception
3. Any other condition that the investigator believes would pos a significant hazard to the subject if the investigational therapy were initiated
4. Participation in any other simultaneous medical investigation or trial
5. Previous participation in any studies involving investigational drugs within 30 days before Day 0 (excluding vitamins and minerals)
6. History of allergy fluorescein, not amenable to treatment
7. Previous intravitreal treatment with any anti-VEGF drug within 60 days of Day 0
8. Previous intravitreal or subconjunctival treatment with cortical steroids within 90 days of Day 0

9. History of vitrectomy
10. History of treatment with more than one form of radiation to the eye
11. Subjects who have more than 7DD of ischemia in the central macula that would hinder visual acuity improvement
12. History of panretinal photocoagulation treatment in the study eye
13. Inability to obtain fundus photographs or fluorescein angiograms of sufficient quality to be analyzed
14. Any concurrent intraocular condition in the study eye that, in the opinion of the investigator, could:
    a. Require medical or surgical intervention during the 104 week study period to prevent or treat visual loss that might result from that condition
    b. Contribute to loss of at least 2 Snellen equivalent lines of BCVA over the 104 week study period, if allowed to progress untreated
15. Active intraocular inflammation (grade 2+ or above) in the study eye
16. Current vitreous hemorrhage in the study eye
17. History of rhegmatogenous retinal detachment or macular hole (stage 3 or 4) in the study eye
18. Active infectious or conjunctivitis, keratitis, scleritis, or endophthalmitis in either eye
19. Aphakia or absence of the posterior capsule in the study eye
20. Intraocular surgery (including cataract surgery) in the study eye within 2 months preceding Day 0
21. Uncontrolled glaucoma in the study eye (defined as IOP $\geq$ 30 mmHg despite treatment with anti-glaucoma medication)
22. History of glaucoma-filtering surgery in the study eye
23. History of corneal transplant in the study eye
24. Uncontrolled blood pressure (defined as systolic and/or diastolic >180/110 mmHg while subject is seated). A second reading may be taken at least 30 minutes later. If the subject requires antihypertensive medication, the subject can become eligible if medication is taken continuously for at least 14 days prior to Day 0 and blood pressure is less than 180/110 mmHg
25. New diagnosis of atrial fibrillation not managed by subject's primary care physician or cardiologist within 3 months of Day 0
26. History of stroke within the last 3 months of Day 0
27. History of myocardial infarction within 3 months of Day 0
28. History of other disease, metabolic dysfunction, or physical examination finding giving reasonable suspicion of a disease or condition that contraindicates the use of an investigational drug or that might affect interpretation of the results of the study or renders the subject at high risk for treatment complications
29. Active malignancy other than uveal melanoma
30. Presence of metastases

6. ANALYSES

All outcomes will be presented using descriptive statistics. For comparison of groups for primary and secondary outcomes, variables of interest will be statistically analyzed using analysis of variance (ANOVA), Student's t-tests, Chi square and Fisher's exact test. ETDRS data will be obtained for all visits

for all 40 of the participants. Bonferroni correction was applied to all pairwise comparison analyses for this study.

6.1 Primary Outcome

The primary analysis will compare cohorts (A vs. B vs. C) on their mean change in ETDRS BCVA from baseline to week 48 and week 104 with the above described statistical tests. Adverse events will be reported using counts and percentages for each cohort.

6.2 Secondary Outcomes

The secondary analysis will compare cohorts (A vs. B vs. C) on their mean CMT measurements at baseline, week 48 and week 104. The secondary analysis will also compare percentage of eyes with intraretinal exudates, retinal hemorrhage, and neovascularization at baseline, week 48 and week 104. The frequency of injection will be compared between cohorts and the correlation between injection frequency and CMT and/or ETDRS BCVA will be assessed.