Statistical Analysis Plan (SAP)

RECOVERY

Title: Intravitreal Aflibercept for Retinal Non-Perfusion in Proliferative Diabetic Retinopathy (The RECOVERY Study)

Principal Investigator: Charles C. Wykoff, MD PhD

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Abbreviations

ATE	Arterial Thromboembolic Events
CI-DME	Center-Involving DME
CST	Central Subfield Thickness
DME	Diabetic Macular Edema
DR	Diabetic Retinopathy
DRSS	Diabetic Retinopathy Severity Scale
ETDRS BCVA	Early Treatment Diabetic Retinopathy Study Best Corrected Visual Acuity
IAI	Intravitreal Aflibercept Injection
IOP	Intraocular Pressure
IUD	Intrauterine Device
NVD	Neovascularization of the Disc
NVE	Neovascularization Elsewhere
OCT	Optical Coherence Tomography
PDR	Proliferative Diabetic Retinopathy
PRP	Panretinal Photocoagulation
RNP	Retinal Non-Perfusion
USA	United States of America
VA	Visual Acuity
VEGF	Vascular Endothelial Growth Factor
VH	Vitreous Hemorrhage

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1. INTRODUCTION

Diabetic retinopathy (DR) is a leading cause of visual loss around the world, and remains the most common cause of blindness among working age people in the United States (USA) and many developed countries[1]. Through pathologic retinal ischemia DR leads to visual loss primarily through diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR).

PDR is defined by the development of neovascularization (NV) that originates from the retinal vasculature and aberrantly grows through the internal limiting membrane into the vitreous. Retinal NV can either involve the optic disc (NVD) or more peripheral retina (NVE). PDR typically leads to visual loss through either rupture of the unstable, pathologic vessels causing vitreous hemorrhage (VH) or retinal distortion and traction-detachments due to concurrent proliferation of a fibrous scaffold along with the abnormal vessels.

Epidemiologic data suggests that given a long enough duration of diabetes, approximately 60% of diabetics will develop PDR[2]. Such transition to PDR significantly increases the risk of progressive visual loss and without intervention, approximately half of eyes will ultimately experience severe visual loss[3]. Currently, PDR results in 12,000 to 24,000 new cases of blindness each year in the USA[4].

Validated through the Diabetic Retinopathy Study, panretinal photocoagulation (PRP) has been the standard treatment for PDR since the 1970s[5]. Application of appropriate PRP reduces the risk of severe visual loss to approximately 4%[6]. However, despite its effectiveness for PDR treatment, PRP can have substantial untoward effects including peripheral visual field defects, night vision loss, loss of contrast sensitivity, and loss of visual acuity (VA); furthermore, PRP itself can be incompletely effective in some eyes, with subsequent need for additional PRP in nearly half of patients[4] and need for traditional vitrectomy surgical intervention in at least 5% of eyes despite appropriate laser treatment[7].

Therefore, supplemental or alternative therapies for PDR could be of substantial clinical value. Pathologic over-expression of vascular endothelial growth factor-A (VEGF)[8] is a key driver of DR, DME and PDR. Pharmaceutical agents that specifically inhibit VEGF including aflibercept (Eylea, Regeneron), ranibizumab (Lucentis, Genentech), and bevacizumab (Avastin, Genentech) have revolutionized the management of DME. These medications are remarkably well-tolerated by patients and administered in the clinic using a 30-gauge or smaller needle inserted through the pars plana directly into the vitreous cavity.

Multiple prospective, randomized trials focusing on the management of DME have demonstrated that anti-VEGF therapy can significantly blunt the progression of DR to PDR. For example, PDR events were reduced in the RIDE/RISE phase 3 trials at 2 years from approximately 34% with sham treatment to 11% with monthly ranibizumab treatment (either 0.3mg or 0.5mg)[9, 10]. Anti-VEGF treatments have the additional benefit of not only slowing progression to PDR but also improving diabetic retinopathy severity scales (DRSS) in a substantial proportion[10, 11], a clinical finding not observed with PRP alone. For example, in the RISE/RIDE trials at 2-years, 35.9-37.2% of ranibizumab treated eyes experienced ≥2 step DRSS improvements compared to 5.4% of sham treated eyes[9]. Similarly, at the 2-year point of VISTA/VIVID study program, 29.3-37.1% of aflibercept treated eyes experienced ≥2 step DRSS improvements compared to 8.2-15.6% of sham-treated eye[11].

The Diabetic Retinopathy Clinical Research Network (DRCR.net) protocol S, *Prompt PRP versus Intravitreous Ranibizumab with Deferred PRP for PDR,* stands as the first large, prospective trial to directly compare PRP to anti-VEGF treatment specifically for the management of PDR[4].

This trial randomized 394 eyes with PDR at 55 sites across the USA to either baseline PRP or intravitreal ranibizumab (0.5mg) injections[4]. At the 2-year primary endpoint, non-inferiority of ranibizumab compared to PRP was achieved with a mean VA improvement of +2.8 vs +0.2 letter in the ranibizumab and PRP arms respectively. While VA outcomes were similar between the arms, secondary efficacy outcomes strongly favored the anti-VEGF treatment arm. Mean Humphrey visual field sensitivity loss was worse, vitrectomy was more frequent, and DME development was more common in the PRP vs anti-VEGF groups, findings that were all highly statistically significant (P<0.001).

The role of anti-VEGF pharmaceuticals in the management of advanced forms of DR may be much more important than blunting progression to PDR and obviating the negative consequences of PRP. Indeed the mechanism by which VEGF blockade leads to DRSS improvements may be fundamental to the underlying disease process of DR itself: retinal vascular perfusion. Specifically, VEGF blockade in the context of DR appears to have a significant impact on the underlying retinal vasculature. In RISE/RIDE, development of angiographically-identified RNP was significantly reduced at 2 years from approximately 30% to <10% with monthly VEGF blockade[12]. Ongoing prospective angiographic analyses of patients with moderately severe and severe NPDR, corresponding to DRSS levels 47 and 53, in the randomized DRCR-W and PANORAMA studies may provide further evidence of anti-VEGF-medicated modification of RNP using aflibercept.

Preliminary data from a small series involving eyes with PDR presented by Jeff Heier, MD in October, 2015 indicate that some eyes may experience substantial and impressive RNP improvements with aflibercept treatment[13]. More data is needed to further understand the effect of aflibercept treatment on RNP in PDR.

2. STUDY DESIGN

The RECOVERY trial will assess the safety and tolerability of 2 mg intravitreal aflibercept injections (IAI) given monthly (Q4WK) or every 12 weeks (Q12WK) for the treatment of retinal non-perfusion (RNP) associated with proliferative diabetic retinopathy (PDR) primarily assessed through retinal capillary non-perfusion.

Study eyes will be assigned randomly (1:1 ratio) to one of the following 2 treatment arms:

- **Group 1** – aflibercept 2mg every 4 weeks (defined as every 28 days (±7 days) and at least 21 days between injections) through week 48. Subjects will have a mandatory year 1 visit at week 48. Subjects have a mandatory visit at week 52 and will not receive treatment. During the second year of follow-up, subjects will be monitored and treated every 12 weeks (Week 60, 72, 84 and 96) with an end of study visit at week 100. If NV or PDR are worse per the pre-specified criteria at week 60, or at any study visit thereafter, the subject will be treated monthly through the end of the study.
- **Group 2** – aflibercept 2mg every 12 weeks for 48 weeks. Subjects will be followed every 4 weeks through week 12, and can be treated if the pre-specified criteria are met. Starting at week 12 if NV or PDR are stable or improved (as assessed by investigator) the subject will be monitored and treated at a 12-week interval through week 48. If NV or PDR are worse per the pre-specified criteria at week 12, or at any study visit thereafter, the subject will be treated monthly through week 48. At week 52-
    - For subjects without any retinal non-perfusion, monitoring and treatment will continue at every 12 weeks (Week 60, 72, 84 and 96) with an end of study visit at week 100.

    - For subjects with visible retinal non-perfusion, monitoring and treatment will be at a 4-week interval (defined as every 28 days (±7 days) and at least 21 days between injections). If retinal non-perfusion has completely resolved at week 72, the subject will be switched back to monitoring and treatment every 12 weeks (Week 72, 84, 96).
- *Pre-specified criteria* (subjects must meet at least one criterion which must be documented with imaging):
    - Increased neovascularization
    - Decrease in BCVA by 5 or more letters due to progressive DME or PDR
    - Worsening central subfield diabetic macular edema causing vision loss, with principal investigator or other delegated investigator confirmation
    - Total area of retinal ischemia increases by 10% as determined by the central reading center

2.1 Sample Size Calculations

Power calculations were conducted based on an assessment of retinal perfusion in twelve eyes of six patients with diabetic retinopathy treated with intravitreal bevacizumab; this retrospective analysis by Schwartz et al presented at AAO 2015 demonstrated a significant increase in the average perfused area by 57.3 % with treatment (P=0.015)[14]. A priori power calculation revealed that a sample size of n=36 is needed to achieve 90% power to detect non-inferiority compared to the reported data using a one-sided t-test when the margin of non-inferiority is set at 10% with a significance level (alpha) of 0.05. The standard deviation was estimated at 20% due to the wide range of percent change in retinal perfusion between patients[15,16]. Assumption is equivalent efficacy at improving retinal perfusion between the two aflibercept arms in the current study.

3. AIMS AND OBJECTIVES

To assess the safety and tolerability of 2mg intravitreal aflibercept injections given monthly or every 12 weeks for the treatment of RNP associated with PDR primarily assessed through retinal capillary non-perfusion.

4. OUTCOMES

4.1 Primary Outcomes

- Assess the safety and tolerability of IAI for the treatment of proliferative diabetic retinopathy by evaluating the incidence and severity of ocular and systemic adverse events through week 52
- Change in area of retinal capillary non-perfusion, as assessed by central reading center, from baseline to week 52

4.2 Secondary Outcomes

- Mean change in Early Treatment of Diabetic Retinopathy Severity Best Spectacle Corrected Visual Acuity (ETDRS-BCVA) from baseline to week 52
- Change in area of retinal capillary non-perfusion within the macula, as assessed by ultrawide-field fluorescein from baseline to week 52
- Change in area of retinal capillary non-perfusion outside of the macula from baseline to week 52

- Percentage of subjects with neovascularization regression from baseline to week 52
- Percentage of subjects with increased neovascularization from baseline to week 52
- Percentage of subjects who develop vitreous hemorrhage from baseline to week 52
- Percentage of subjects treated with PRP or vitrectomy for progression of PDR, from baseline to week 52
- Percentage of subjects at week 52, who develop center-involving diabetic macular edema who did not have center-involving diabetic macular edema at baseline
- Changes in visual function outcomes (Humphrey visual field and self reported visual function) from baseline to week 52
- Mean change in central subfield thickness (CST) from baseline to week 52

4.3 Safety Outcomes

The safety and tolerability of IAI 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

The targeted study population is men or women 18 years and older with retinal non-perfusion (RNP) associated with proliferative diabetic retinopathy (PDR).

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

1. Men or women $\geq$ 18 years of age with type 1 or type 2 diabetes mellitus
2. BCVA ETDRS $\geq$ 20/400 in the study eye
3. Willing and able to comply with clinic visits and study-related procedures
4. Provide signed informed consent
5. Substantial non perfusion (defined as greater than 20 disc areas), as assessed by the investigator
6. Early PDR, as assessed by the investigator, with no vitreous hemorrhage*
   * Early PDR is defined in which PRP can safely be deferred and vitreous hemorrhage that does not obscure the application of PRP

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

1. Any prior systemic anti-VEGF or IVT anti-VEGF treatment in the study eye,

2. SD-OCT central subfield thickness measurement of > 320 µm, in the study eye
3. Evidence of infectious ocular infection, in the study eye, at time of screening
4. History of vitreoretinal surgery in the study eye
5. Any prior Panretinal laser photocoagulation (PRP) in the study eye
6. Current vitreous hemorrhage obscuring retinal imaging in the study eye
7. Cataract surgery in the study eye within 4 weeks of Day 0
8. Uncontrolled blood pressure (defined as $\geq$ 180/110 mm Hg systolic/diastolic, while seated)
9. Significant renal disease defined as a history of chronic renal failure requiring dialysis or renal transplant
10. Tractional Retinal Detachment threatening the macula in the study eye
11. Corticosteroid treatment (intravitreal or peribulbar) in the study eye within 12 weeks of screening
12. Pregnant or breast-feeding women
13. 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.

    *Contraception is not required for men with documented vasectomy.

6. ANALYSES

All outcomes will be presented using descriptive statistics. Statistical comparisons will be performed with paired and unpaired 2-tailed Student's t-tests using SPSS commercial software (SPSS, inc, Chicago IL) where appropriate. A t-test distribution will be used to calculate 95% confidence intervals. The Welch-Satterthwaite method will be used to calculate the 95% CI for the differences between means.

6.1 Primary Outcome

The primary analysis will compare cohorts (Q4wks vs. Q12wks) on their mean change in area of retinal capillary non-perfusion, as assessed by central reading center, from baseline to week 52 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 (Q4 wks vs. Q12 wks) on their mean change in ETDRS BCVA, change in area of retinal capillary non-perfusion within the macula, change in area of retinal capillary non-perfusion outside of the macula, percentage of subjects with increased or regressed NV, percentage of subjects who develop VH, percentage of subjects treated with PRP or vitrectomy, percentage of subjects who develop CI-DME, changes in visual function outcomes, and mean change in CST using the same methods as for the primary outcome.

Agreement of RNP area measurements as assessed by two independent masked graders will be analyzed using intraclass correlation coefficients.

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