Diabetic Retinopathy Clinical Research Network

Intravitreous Anti-VEGF Treatment for Prevention of Vision Threatening Diabetic Retinopathy in Eyes at High Risk

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CHAPTER 1. BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.1 Background Information

1.1.1 Diabetic Retinopathy Complications and Public Health Impact

The age-adjusted incidence of diabetes mellitus in the United States has reportedly doubled in recent history.[1] Estimates suggest that by the year 2035, approximately 592 million individuals worldwide will be affected by this chronic disease.[2] The increasing global epidemic of diabetes implies an increase in rates of associated vascular complications from diabetes. At present at least 5 million people over the age of 40 in the United States are estimated to have diabetic retinopathy (DR) in the absence of diabetic macular edema (DME), and an additional 800,000 have DME, according to data from the Centers for Disease Control.[3] Despite advances in diagnosis and management of ocular disease in patients with diabetes, eye complications from diabetes mellitus continue to be a leading cause of vision loss and new onset blindness in working-age individuals throughout the United States.[4, 5]

1.1.2 PDR and Its Treatment

Worsening DR is characterized by the development of increasing areas of retinal vascular non-perfusion causing ischemia or infarction of retina tissue. The anatomic sequel of retinal vascular ischemia is retinal neovascularization (NV) or proliferative diabetic retinopathy (PDR), a major cause of preventable and potentially irreversible vision loss in patients with diabetes. Data from the Wisconsin Epidemiologic Study of Diabetic Retinopathy published in 1985 suggest that given long enough duration of diabetes, approximately 60% of patients with diabetes mellitus will develop PDR.[6] Although current rates may be lower, they are still substantial. More recently, the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS) and Diabetic Retinopathy Study (DRS)-2 study groups reported that eyes with moderate to severe non-proliferative DR enrolled in 2 separate phase 3 trials of the protein kinase C inhibitor, ruboxistaurin, demonstrated approximate rates of 60% and 40%, respectively, of worsening of 3 steps on the Early Treatment for Diabetic Retinopathy Study (ETDRS) person scale across both eyes, 2 steps on the ETDRS individual eye scale, or application of PRP over 3 years.[7, 8]

It is also well-documented that worsening to PDR is associated with worse visual outcomes in many eyes. According to the DRS without intervention, nearly half of eyes with high-risk PDR will experience profound vision loss from associated complications including vitreous hemorrhage or traction retinal detachment, but rates are reduced dramatically with panretinal photocoagulation (PRP).[9] The ETDRS demonstrated PRP reduces the risk of severe vision loss to 4% for eyes with or approaching high risk PDR.[10] Although remarkably effective at reducing visual loss if applied in a timely and appropriate manner, PRP treatment destroys viable retinal tissue and is associated with well-documented potential side effects that may lead to transient or permanent loss of visual function, including exacerbation of existing macular edema,[11] peripheral visual field defects, night vision loss, loss of contrast sensitivity, potential complications from misdirected or excessive burns. In addition, subsequent need for vitrectomy for vitreous hemorrhage or traction retinal detachment has been reported in at least 5% of individuals despite appropriate laser treatment.[12, 13]

Intravitreous anti-vascular endothelial growth factor (anti-VEGF) in eyes with PDR led to decreased risk of DR worsening (need for PRP, vitreous hemorrhage, or vitrectomy for

complications of PDR) compared with no anti-VEGF therapy in a secondary outcome reported by the Diabetic Retinopathy Clinical Research Network (DRCR.net) in a trial evaluating ranibizumab for DME.[14, 15] However, some eyes still worsen despite anti-VEGF therapy and DR severity can worsen when anti-VEGF therapy is discontinued.

The efficacy and safety of intravitreous anti-VEGF for treatment of PDR have been evaluated over a 2 year period in the ongoing DRCR.net trial, Prompt PRP versus Intravitreous Ranibizumab with Deferred PRP for PDR (Protocol S, NCT01489189). This study randomized eyes with PDR either with or without DME to either standard care PRP delivered at baseline or to treatment with ranibizumab as per a predefined treatment algorithm with deferred PRP given only if these eyes met failure or futility criteria. The study demonstrated that anti-VEGF treatment led to visual acuity at 2 years that was non-inferior to that obtained with PRP. The mean VA letter change was +2.8±15.2, ranibizumab group, versus +0.2±13.7, PRP group (difference +2.2, 95% confidence interval [CI]: -0.5 to +5.0). Other, secondary outcomes appeared to favor the ranibizumab-treated group, including mean change in visual acuity letter area under the curve over 2 years (difference +4.2, 95% CI: +3.0 to +5.4, $P < 0.001$), visual field sensitivity loss (mean difference 368 dB; 95% CI: 213 to 531, $P < 0.001$) and rates of vitrectomy (difference in surgical rates 11% ($P < 0.001$). Ranibizumab was well-tolerated with few ocular events (1 case of endophthalmitis) and no substantial differences identified in rates of systemic adverse events between the treatment groups.[16]

1.1.3 DME and Its Treatment

DME is another manifestation of DR that produces loss of central vision. DME is currently a leading cause of moderate vision loss in patients with diabetes.[17] Without intervention, 33% of 221 eyes included in the ETDRS with center-involved DME (CI-DME) experienced "moderate visual loss" (defined as a 15 or more letter score decrease in visual acuity) over a 3 year period.[18] The DRCR.net study "Intravitreal Ranibizumab or Triamcinolone Acetonide in Combination with Laser Photocoagulation for Diabetic Macular Edema" (Protocol I) indicated that treatment for DME with intravitreous anti-VEGF therapy (0.5 mg ranibizumab) with prompt or deferred focal/grid laser provides visual acuity outcomes at 1 year and 2 years that are superior to focal/grid laser alone or focal/grid laser combined with intravitreous corticosteroids,[14] providing definitive confirmation of the important role of VEGF in DME and the superiority of anti-VEGF agents in the treatment of DME. Additional phase 3 studies have since confirmed the superiority of anti-VEGF agents to manage DME.[19-21]

1.1.4 Rationale for Prevention of PDR and DME in Eyes at High Risk

Worse baseline NPDR severity is strongly associated with increased risk of worsening to PDR. Data from the ETDRS suggest that eyes with severe non-proliferative diabetic retinopathy (NPDR) have a 52% risk of progressing to PDR within 1 year and a 60% risk of worsening to PDR with high risk characteristics within 5 years.[10] Although PRP is performed in some select cases of severe NPDR, there is no clear treatment mandate generalizable to most eyes with severe NPDR that are at high risk of worsening to PDR. However, the high risk of vision loss from untreated PDR and potential complications from PDR treatment with PRP support a rationale to explore possible therapeutic modalities for prevention of PDR.

A higher risk of incident DME in eyes with more severe levels of baseline NPDR also has been reported.[22] Although there is similarly no clear current mandate to treat eyes with severe NPDR in the hopes of preventing DME, there is scientific rationale to support this approach. It is possible that the prevention of CI-DME onset in eyes at high risk might prevent vision loss associated with the development of CI-DME. Furthermore, it is possible that an initial, infrequently dosed anti-VEGF treatment regimen that prevents CI-DME onset might avoid adverse events associated with more frequent dosing required for treatment once CI-DME is present.

Multiple studies have implicated VEGF as a major causative factor in human eye diseases characterized by neovascularization including PDR and vascular permeability including DME.[23-33] Thus, inhibition of VEGF might be expected to reduce the risk of both PDR and DME onset in eyes with DR at high risk for worsening and over the long-term, reduce the risk of vision loss from these conditions. Indeed, as written above, substantial reductions in PDR-related outcomes such as worsening on fundus photographs or clinical examination from NPDR to PDR, having PRP, experiencing vitreous hemorrhage, or undergoing vitrectomy for PDR, have been reported from studies comparing eyes treated with ranibizumab to those given laser or no treatment to manage DME.[34, 35,19,20] Furthermore, anti-VEGF treatment appears not only to prevent worsening to PDR, but also to result in some improvement in the DR severity level as demonstrated by DRCR.net Protocol I, RIDE/RISE trials with ranibizumab, and VIVID/VISTA trials with aflibercept [14, 19, 20]

While there is strong evidence that PDR outcomes are markedly reduced in eyes that are treated with monthly anti-VEGF therapy (RIDE/RISE) and moderately reduced in eyes that received fairly frequent dosing during the 1st year of treatment (DRCR protocol I), it is yet unknown whether or not an earlier but less frequent dosing regimen would result in similar, favorable anatomic outcomes, and whether favorable anatomic outcomes subsequently would result in favorable visual acuity outcomes. Indeed, recently available data reveal that in the open label extension phase that followed the RIDE/RISE core studies, 28% of eyes that did not receive further ranibizumab treatment experienced 2 or more step worsening over the subsequent year, suggesting that the beneficial effects on DR severity of anti-VEGF therapy may not be sustained in all eyes once that therapy is withheld or given at decreasing frequency.[36]

The ability of anti-VEGF therapy to prevent DME onset has not been addressed by data from large scale clinical studies, since these studies largely have enrolled eyes with CI-DME at baseline. However, given the efficacy of anti-VEGF therapy in ameliorating retinal thickening in the RIDE/RISE, VIVID/VISTA, and Protocol I trials, as well as the very low rates of DME worsening in patients treated with anti-VEGF in these studies, it is plausible that anti-VEGF injections also might be effective in reducing the onset of and worsening to CI-DME in eyes at risk for CI-DME development and subsequently result in improved vision outcomes. In addition, ranibizumab reduced the rates development of CI-DME with decreased visual acuity in eyes with PDR in DRCR.net Protocol S (10% with ranibizumab vs. 27% with PRP, $P < 0.001$).

1.1.5 Aflibercept

The anti-VEGF drug to be used in this trial is intravitreous aflibercept injection, also known as VEGF Trap-Eye or Aflibercept (Eylea®), which is a soluble decoy receptor fusion protein that

has a high binding affinity to all isoforms of VEGF as well as to placental growth factor. Aflibercept received approval by the United States Food and Drug Administration (FDA) for the treatment of neovascular age-related macular degeneration in 2011[37], for treatment of macular edema due to central retinal vein occlusion in 2012[38-40], and for treatment of macular edema due to branch retinal vein occlusion in 2014. In 2014, the FDA approved aflibercept for treatment of DME based on data from two phase III studies, VISTA and VIVID, which included 872 eyes with DME with central involvement that received either intravitreous aflibercept every 4 weeks, intravitreous aflibercept every 8 weeks after 5 initial monthly doses, or macular laser photocoagulation. The mean change in visual acuity letter score at 1 year compared to baseline was 12.5 and 10.7 letters in the aflibercept groups compared with 0.2 letters in the laser group in VISTA ($P < 0.0001$) and 10.5 and 10.7 compared with 1.2 letters in VIVID ($P < 0.0001$). The visual gains in the aflibercept arms as compared with the macular laser arm were sustained through 100 weeks. The FDA further approved aflibercept for treatment of diabetic retinopathy in patients with diabetic macular edema in March 2015 based on VIVD and VISTA data that showed that eyes treated with q4 or q8 week aflibercept had a significantly higher chance of at least a 2 step improvement in Diabetic Retinopathy Severity Scale score as compared to eyes treated with laser control (VIVID: 29.3% and 32.6% vs. 8.2%, respectively; $P < 0.0004$ for q4wk and $P < 0.0001$ for q8wk; VISTA: 37.0% and 37.1% vs. 15.6%, $P < 0.0001$ for both aflibercept vs control comparisons).[41] With regard to safety, the incidences of ocular and non-ocular adverse events were similar across treatment groups. The incidence of APTC-defined thromboembolic events was similar across treatment groups. There were no reported cases of endophthalmitis, and intraocular inflammation occurred in less than 1% of injections.[42]

Although there is no currently available head-to-head data on the available anti-VEGF agents for treatment and prevention of PDR, a comparative effectiveness trial in DME reported that aflibercept was more effective than ranibizumab and bevacizumab in improving vision in eyes starting with CI-DME and worse levels of visual acuity (approximately 20/50 or worse).[43] No difference in efficacy was identified for eyes with CI-DME and mild visual acuity loss (approximately 20/40 or better).

1.1.6 Summary of Study Rationale

The prevention of PDR or DME in eyes that are high risk for PDR and DME onset might prevent vision loss secondary to retinal neovascularization or central retinal thickening and also might avoid potential complications and adverse effects on vision associated with more aggressive treatments for these diabetic ocular complications once established. Although anti-VEGF therapy given for DME improves PDR-related outcomes and results in regression of nonproliferative changes in some eyes with baseline NPDR, these data derive largely from trials of frequent, often monthly dosing of intravitreous anti-VEGF. No study to date has specifically evaluated the role of anti-VEGF in prevention of DME. This study will evaluate the safety and efficacy of an anti-VEGF regimen for prevention of PDR or CI-DME or both in eyes that are at high risk for worsening to PDR or CI-DME. Treatment will be deferred in the control (observation) arm since there is no clear treatment mandate for these eyes at this time. This protocol will evaluate both anatomic outcomes of development of either PDR within the 7-modified ETDRS fields or CI-DME on OCT associated with vision loss as well as whether favorable anatomic outcomes, if identified, result in longer-term beneficial visual outcomes.

If this study demonstrates that intravitreous aflibercept treatment is effective and safe for reducing the onset of PDR or CI-DME in eyes that are at high risk for these complications, a new strategy to prevent vision threatening complications of diabetes will be available for patients. The application of intravitreous aflibercept earlier in the course of disease (i.e., at the time when an eye has baseline severe NPDR) could help to reduce future potential treatment burden in patients, at the same time resulting in similar or better long-term visual outcomes, if PDR and DME are prevented.

1.2 Study Objective

The objectives of this study are to 1) determine the efficacy and safety of intravitreous aflibercept injections versus sham injections (observation) for prevention of PDR or CI-DME in eyes at high risk for development of these complications and 2) compare long-term visual outcomes in eyes that receive anti-VEGF therapy early in the course of disease with those that are observed initially, and treated only if high-risk PDR or CI-DME with vision loss develops.

1.3 Study Design and Synopsis of Protocol

A. Study Design

- Phase III, multi-center randomized clinical trial

B. Major Eligibility Criteria

- Age >=18 years
- Type 1 or type 2 diabetes
- Study eye with
    - Best corrected Electronic-ETDRS (E-ETDRS) visual acuity letter score in the study eye ≥79 (approximate Snellen equivalent 20/25 or better)
    - Severe NPDR (based on the 4:2:1 rule) on clinical examination and on digital imaging as judged by the investigator
        - Reading Center grading of less than ETDRS level 43 or greater than 53 is an exclusion
    - No evidence of neovascularization on fluorescein angiography within the 7-modified ETDRS fields, confirmed by Reading Center grading.
    - No clinical exam evidence of neovascularization including active neovascularization of the iris (small iris tufts are not an exclusion) or angle neovascularization (if the angle is assessed).
    - No prior PRP (defined as ≥ 100 burns placed previously outside of the posterior pole)
    - No CI-DME on clinical exam and OCT central subfield thickness below the following gender and OCT-machine specific thresholds:
        - Zeiss Cirrus: 290µm in women and 305µm in men
        - Heidelberg Spectralis: 305µm in women and 320µm in men
    - No history of DME or DR treatment with laser or intraocular injections of medication within the prior 12 months and no more than 4 prior intraocular injections at any time in the past.

C. Treatment Groups

Study eyes will be assigned randomly (1:1) to one of the following two groups:

- Sham injections
- Intravitreous 2 mg aflibercept injections

Study participants may have one or two study eyes. Study participants with two study eyes will receive intravitreous aflibercept in one eye and sham injection in the other eye. Further details on randomization are located in section 2.4.

Injections (intravitreous or sham) will be given at baseline, 1 and 2 months in all participants. Thereafter, injections will be given at each 4-month visit until 2 years. At and after the 2-year visit, retreatment with injections (intravitreous or sham) will be based on DR level, as assessed by the investigator.

Treatment for DME or PDR, if developed, may only be given once protocol-specified criteria are met and will follow a protocol-specified regimen (see Section 4.4 and 4.5).

D. Sample Size

- A minimum of 386 eyes (approximately 322 study participants assuming 20% have two study eyes)

E. Duration of Follow-up

- Primary outcome: 2 years
- Total follow-up: 4 years

F. Follow-up Schedule

- All participants will have visits at 1 month, 2 months, and 4 months, followed by visits every 4 months thereafter through 4 years.
- Eyes may be seen more frequently depending on disease progression and treatment administered. Further details on the follow-up visit schedule are described in Section 3.1.

G. Main Efficacy Outcomes

Primary outcome:

Development of PDR or DME, defined as the first occurrence of any of the following (composite time-to-event outcome):

- NV within the 7-modified ETDRS fields on fundus photography or FA, confirmed by a masked grader at the central reading center
  - At non-annual visits, fundus photography and FA will only be submitted to the reading center to assess for this component of the primary outcome if the investigator thinks treatment is necessary.
- NV of the iris (at least 2 cumulative clock hours), definitive NV of the angle, or neovascular glaucoma on clinical exam (photographic documentation not required)

- Other outcomes presumed to be from PDR and documented: traction retinal detachment, vitreous hemorrhage, or pre-retinal hemorrhage greater than ½ disc area
- Procedures undertaken for the treatment of PDR (when present or presumed to be present): PRP, anti-VEGF, or vitrectomy
- CI-DME on clinical exam with at least 10% increase in central subfield thickness from baseline and either (1) at least a 10-letter decrease in visual acuity from baseline at a single visit or (2) 5-to-9-letter decrease in visual acuity from baseline at 2 consecutive visits at least 21 days apart, with vision loss presumed to be from DME
- Non-topical treatment for DME performed without meeting the above criteria, including focal/grid laser or intravitreous injections for DME

The primary outcome analysis will be performed when the last randomized participant reaches 2 years of follow up, using all available follow up data. The treatment groups will be compared using the hazard ratio.

Other Key Outcomes:

- Development of PDR or DME outcome through 4 years
- Mean visual acuity change from baseline at 2 years
- Mean visual acuity change from baseline at 4 years

See section 7.3 for methods of handling multiplicity.

Additional secondary outcomes at 2 and 4 years:

- Development of PDR or PDR-related outcome (as defined above within the composite time-to-event outcome)
- Development of CI-DME with visual acuity impairment (as defined above within the composite time-to-event outcome)
- Development of PDR or DME based only on the objective components defined in the composite outcome, including OCT, visual acuity, and reading center assessment of photos and FA (i.e. not including investigator-only assessments)
- Development of each component of the composite outcome assessed individually
- Proportion of eyes with at least 10 or at least 15 letter loss from baseline, or gain or loss of at least 5 letters at consecutive study visits, consisting of the visits just before and the 2- or 4-year visit
- Visual acuity area under the curve (AUC) between randomization and the 2- and 4-year visits
- Mean change in OCT central subfield thickness from baseline
- Mean change in OCT volume from baseline
- Development of CI-DME on clinical exam with at least 10% increase in central subfield thickness and at least a 25-micron increase from baseline, regardless of visual acuity change
- Proportion of eyes with at least 2-step worsening of DR severity level (scale for individual eyes) by central reading center from baseline
- Proportion of eyes with at least 2-step improvement of DR severity level (scale for individual eyes) by central reading center from baseline

- Proportion of eyes with at least 3-step worsening of DR severity level (scale for individual eyes) by central reading center from baseline
- Proportion of eyes with at least 3-step improvement of DR severity level (scale for individual eyes) by central reading center from baseline
- Level of retinopathy on color photos
- Number of aflibercept injections performed
- Follow-up costs and patient-centered outcomes from the Workplace Productivity and Activity Impairment Questionnaire

H. Main Safety Outcomes

Ocular: endophthalmitis, inflammation, retinal detachment, traumatic cataract from injection, vitreous hemorrhage

Systemic: Antiplatelet Trialists' Collaboration (APTC) events and hypertension

I. Schedule of Assessment Visits and Examination Procedures

	Screening	Randomization	Follow-Up Visits*	Annual Visits
Visit Window		within 35 days of screening	(±1to8w)	(±8w)
Usual care visual acuity[a]	X			
E-ETDRS best corrected visual acuity[b]		X	X	X
Questionnaires[c]		X		X
OCT[d]	X	X	X	X
Eye Exam[e]	X	X	X	X
Fundus Photography[f]	X		X[g]	X
Fluorescein angiography[f]	X		X[g]	X
Blood pressure		X		X
HbA1c[h]		X		X
OCT angiography[i]	X		X[g]	X

*= Assessment Visits at 1 month (±1w), 2 months (±1w), 4 months (±8w) and every 4 months (±8w) thereafter; additional study visits may occur for treatment of DME/PDR as needed

a=study eye only; refraction and/or electronic ETDRS testing may be performed at the discretion of the site for usual care visual acuity.

b=both eyes including protocol refraction in the study eye at each study visit. Protocol refraction in non-study eye is only required at baseline and annual visits. E-ETDRS refers to electronic ETDRS testing using the Electronic Visual Acuity Tester that has been validated against 4-meter chart ETDRS testing.

c= only in participants with one study eye

d= study eye only at randomization and annual visits and at other study visits only if evaluating for DME treatment (see section 3.2 for more details) or prior to initiating more frequent anti-VEGF treatment for PDR, if the DME outcome was not confirmed previously.

e=both eyes at randomization; study eye only at each additional study visit including slit lamp exam, lens assessment, measurement of intraocular pressure, and dilated ophthalmoscopy

f= study eye only. Fundus photography is 7MF or 4WF and FA is using the widest approach available at the site.

g= fundus photography, FA, and OCTA (if available at the site) is also required in the study eye at 4 months AND 1) the first time traction retinal detachment, vitreous hemorrhage, or preretinal hemorrhage is identified to confirm the primary outcome has been met, or 2) prior to initiating PRP or vitrectomy, if the primary outcome was not confirmed previously or 3) prior to initiating more frequent anti-VEGF treatment for either DME or PDR, if the primary outcome was not confirmed previously. Fundus photography is 7MF or 4WF and FA is using the widest approach available at the site.

h= does not need to be repeated if HbA1c is available from within the prior 3 months. If not available, can be performed within 3 weeks after randomization.

i=study eye only; only at sites with OCT angiography capabilities.

1.4 General Considerations

The study is being conducted in compliance with the policies described in the DRCR.net Policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice.

The DRCR.net Procedures Manuals (Visual Acuity-Refraction Testing Procedures Manual, OCT procedures manuals, photography and FA procedures manuals, and Study Procedures Manual) provide details of the examination procedures and intravitreous injection procedure.

Photographers, OCT technicians, and visual acuity testers, including refractionists, will be masked to treatment group at the annual visits. Study participants will be masked to their treatment group assignment and will continue to be masked to their original treatment assignment even once they initiate treatment for PDR or CI-DME. Investigators and study coordinators are not masked to treatment group.

Data will be directly collected in electronic case report forms, which will be considered the source data.

There is no restriction on the number of study participants to be enrolled by a site.

A risk-based monitoring approach will be followed, consistent with the FDA “Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring” (August 2013).

The risk level is considered to be research involving greater than minimal risk.

CHAPTER 2. STUDY PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Identifying Eligible Subjects and Obtaining Informed Consent

A minimum of 386 eyes (322 participants assuming 20% have two study eyes) are expected to be enrolled into the randomized trial. As the enrollment goal approaches, sites will be notified of the end date for recruitment. Study participants who have signed an informed consent form can be randomized up until the end date, which means the recruitment goal might be exceeded.

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

Consent may be given in two stages (if approved by the IRB). The initial stage will provide consent to complete any of the screening procedures needed to assess eligibility that have not already been performed as part of a usual-care exam. The second stage will be obtained prior to randomization and will be for participation in the study. A single consent form will have two signature/date lines for the study participant: one for a study participant to give consent for the completion of the screening procedures and one for the study participant to document consent for the randomized trial. Study participants will be provided with a copy of the signed Informed Consent Form.

Once a study participant is randomized, that participant will be counted regardless of whether the assigned treatment is received. Thus, the investigator must not proceed to randomize an individual until he/she is convinced that the individual is eligible and will accept assignment to either of the two treatment groups.

2.2 Subject Eligibility Criteria

2.2.1 Individual-level Criteria

Inclusion

To be eligible, the following inclusion criteria must be met:

1. Age >= 18 years
   - *Individuals <18 years old are not being included because DR is so rare in this age group that the diagnosis of NPDR may be questionable.*
2. Diagnosis of diabetes mellitus (type 1 or type 2)
   - Any one of the following will be considered to be sufficient evidence that diabetes is present:
     - *Current regular use of insulin for the treatment of diabetes*
     - *Current regular use of oral anti-hyperglycemia agents for the treatment of diabetes*
     - *Documented diabetes by ADA and/or WHO criteria (see Procedures Manual for definitions)*

3. At least one eye meets the study eye criteria listed in section 2.2.2.
4. Able and willing to provide informed consent.

Exclusion

An individual is not eligible if any of the following exclusion criteria are present:

5. History of chronic renal failure requiring dialysis or kidney transplant.
6. A condition that, in the opinion of the investigator, would preclude participation in the study (e.g., unstable medical status including blood pressure, cardiovascular disease, and glycemic control).
7. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to randomization or plans to do so in the next 4 months.
8. Participation in an investigational trial that involved treatment within 30 days of randomization with any drug that has not received regulatory approval for the indication being studied.
    - *Note: study participants cannot participant in another investigational trial that involves treatment with an investigational drug while participating in the study.*
9. Known allergy to any component of the study drug or any drug used in the injection prep (including povidone iodine prep).
10. Known allergy to fluorescein dye.
11. Blood pressure > 180/110 (systolic above 180 or diastolic above 110).
    - *If blood pressure is brought below 180/110 by anti-hypertensive treatment, individual can become eligible.*
12. Systemic anti-VEGF or pro-VEGF treatment within 4 months prior to randomization.
    - *These drugs should not be used during the study.*
13. For women of child-bearing potential: pregnant or lactating or intending to become pregnant within the next 2 years.
    - *Women who are potential study participants should be questioned about the potential for pregnancy. Investigator judgment is used to determine when a pregnancy test is needed.*
14. Individual is expecting to move out of the area of the clinical center to an area not covered by another DRCR.net certified clinical center during the next 2 years.

2.2.2 Study Eye Criteria

The potential study participant must have at least one eye meeting all of the inclusion criteria and none of the exclusion criteria listed below.

A study participant can have two study eyes only if both are eligible at the time of randomization. For study participants with two eligible eyes, the logistical complexities of the protocol must be considered for each individual prior to randomizing both eyes.

The eligibility criteria for a study eye are as follows:

Inclusion

a. Best corrected E-ETDRS visual acuity letter score ≥79 (approximate Snellen equivalent 20/25 or better)

b. Severe NPDR (based on the 4:2:1 rule) evident on clinical examination and/or on digital imaging as judged by the investigator. Severe NPDR is defined as:

    1. 4 fields show severe hemorrhages or microaneurysms (at least as great as Standard photograph 2A), or
    2. At least 2 fields of definite venous beading or at least 1 field at least as severe as Standard photograph 6A, or
    3. At least 1 field of moderate intraretinal microvascular abnormalities (IRMA), at least as severe as Standard photograph 8A

c. No evidence of neovascularization on clinical exam including active neovascularization of the iris (small iris tufts are not an exclusion) or angle neovascularization (if the angle is assessed).

d. No evidence of neovascularization on fluorescein angiography within the 7-modified ETDRS fields, confirmed by the central Reading Center prior to randomization.

- *The widest method of imaging available at the site must be used to document whether there is NV present in the periphery; however, presence of NV outside of the 7-modified ETDRS fields on ultra-widefield imaging will not be an exclusion provided treatment is not planned.*

e. No CI-DME on clinical exam and OCT central subfield thickness must be below the following gender and OCT-machine specific thresholds:

    - Zeiss Cirrus: 290µ in women and 305µ in men
    - Heidelberg Spectralis: 305µ in women and 320µ in men

    AND investigator and potential participant are comfortable withholding treatment for DME until there is at least a 10% increase in OCT central subfield thickness with confirmed visual acuity loss (10 letter loss at a single visit or 5 to 9 at two consecutive visits).

f. Prompt PRP or anti-VEGF treatment not required AND investigator and potential participant are willing to wait for development of high-risk characteristics (defined in Section 4.5.2) to treat PDR.

g. Media clarity, pupillary dilation, and study participant cooperation sufficient to obtain adequate fundus photographs, FA, and OCT.

- *Investigator must verify accuracy of OCT scan by ensuring it is centered and of adequate quality (including segmentation line placement)*

Exclusion

The following exclusions apply to the study eye only (i.e., they may be present for the non-study eye):

h. Central Reading Center grading of DR severity level on fundus photographs less severe than ETDRS level 43 or more severe than level 53.

- *Enrollment will be limited to a maximum of 50% of the planned sample size with DR severity level 47A or 43 by RC grading (with a maximum of 25% of the planned sample size with DR severity level 43 by RC grading). Once the number of eyes has been enrolled for each severity level, RC grading of that level will be an exclusion criterion.*

i. Exam or photographic evidence of vitreous hemorrhage or preretinal hemorrhage presumed to be from PDR.

j. History of prior vitreous hemorrhage or preretinal hemorrhage presumed to be from PDR.

k. History of prior PRP (defined as ≥100 burns outside of the posterior pole).

l. An ocular condition is present (other than DR) that, in the opinion of the investigator, might alter visual acuity during the course of the study (e.g., retinal vein or artery occlusion, uveitis or other ocular inflammatory disease, vitreomacular traction, etc.).

m. History of DME or DR treatment with laser or intraocular injections of medication within the prior 12 months and no more than 4 prior intraocular injections at any time in the past.

- *Enrollment will be limited to a maximum of 25% of the planned sample size with any history of treatment for DME/DR. Once this number of eyes has been enrolled, any history of treatment for DME/DR will be an exclusion criterion.*

n. History of major ocular surgery (including cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 4 months or anticipated within the next 6 months following randomization.

o. Any history of vitrectomy.

p. History of YAG capsulotomy performed within 2 months prior to randomization.

q. Aphakia.

r. Exam evidence of severe external ocular infection, including conjunctivitis, chalazion, or substantial blepharitis.

s. Evidence of uncontrolled glaucoma.

- *Intraocular pressure must be <30, with no more than one topical glaucoma medication, and no documented glaucomatous field loss for the eye to be eligible.*

2.2.3 Non-Study Eye Criteria

If anti-VEGF treatment is indicated for any condition in the non-study eye at any time during the study, the investigator must be willing to use the study anti-VEGF drug (2 mg aflibercept) for the non-study eye. If the non-study eye is currently being treated with a different anti-VEGF drug for any condition, then the investigator and patient must be willing to switch to aflibercept. If the investigator or patient is unwilling to change anti-VEGF treatment in the non-study eye, the patient should not be enrolled.

2.3 Screening Evaluation and Baseline Testing

2.3.1 Historical Information

A history will be elicited from the potential study participant and extracted from available medical records. Data to be collected will include: age, gender, ethnicity and race, diabetes history and current management, other medical conditions, medications being used, as well as ocular diseases, surgeries, and treatment.

2.3.2 Baseline Testing Procedures

2.3.2.1 Screening Visit

The following procedures are needed to assess eligibility at Screening.

- If a procedure has been performed (using the study technique and by study certified personnel) as part of usual care, it does not need to be repeated specifically for the study if it was performed within the defined time windows specified below.
- The testing procedures are detailed in the DRCR.net Procedures Manuals. Visual acuity testing, ocular exam, fundus photography, fluorescein angiography and OCT will be performed by DRCR.net certified personnel.
- The fundus photographs and fluorescein angiograms will be promptly sent to the central reading center for grading and a participant cannot be randomized until reading center confirmation of eligibility has been received.
- OCTs meeting DRCR.net criteria for manual grading may be sent to a reading center, but study participant eligibility regarding DME status is determined by the site (i.e., individuals deemed eligible by the investigator will be randomized without pre-randomization reading center confirmation of the OCT central subfield thickness).

1. Visual acuity using clinic's usual care method or Electronic-ETDRS visual acuity to confirm vision is 20/25 or better in the study eye *(within prior 8 days).*
2. Spectral Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on the study eye *(within prior 8 days).*
3. Ocular examination on the study eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy *(on day of Screening)*.
4. Digital fundus photography in the study eye. *(within prior 8 days)*
5. Digital fluorescein angiogram (FA) in the study eye, using the widest approach available at the clinical site (e.g. ultra-widefield imaging device, if available). *(within prior 8 days)*
6. OCT angiography on the study eye*. (within prior 8 days)*
    - Only obtained by a subset of sites with OCT angiography capabilities. If a site has OCT angiography systems from more than one manufacturer, the images should be obtained on each system available.
    - *See procedure manual for more details on acquisition, including which fields to collect on a given OCT angiography system.*

2.3.2.2 Randomization Visit

The randomization visit must be completed within 35 days of Screening. The visit should not be completed until Reading Center confirmation of eligibility based on the Screening fundus

photographs and FA has been received. The following procedures are needed to confirm eligibility and to serve as baseline measures for the study:

1. Electronic-ETDRS visual acuity testing at 3 meters using the Electronic Visual Acuity Tester (including protocol refraction) in each eye. *(on day of randomization)*
2. Spectral Domain OCT using Zeiss Cirrus or Heidelberg Spectralis on study eye *(on day of randomization)*
    - *The same OCT machine type as Screening should be used.*
3. Ocular examination on each eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy. *(on day of randomization)*
4. Workplace Productivity and Activity Impairment (WPAI) Questionnaire (only in participants with one study eye, *on day of randomization*).
5. Measurement of blood pressure.
6. Laboratory testing of Hemoglobin A1c.
    - *HbA1c does not need to be repeated if available in the prior 3 months. If not available at the time of randomization, the potential study participant may be enrolled but the test must be obtained within 3 weeks after randomization.*

2.4 Randomization of Eligible Study Participants

1. Prior to randomization, the study participant's understanding of the trial, willingness to accept the assigned treatment group, and commitment to the follow-up schedule should be reconfirmed.
2. The initial injection must be given on the day of randomization. A study participant should not be enrolled until this is possible
3. Randomization is completed on the DRCR.net website.

    - <u>Study participants with one study eye</u> will be randomly assigned (stratified by Reading Center grading of DR severity level [43, 47A, 47B-D, 53 with no NV in the periphery, or 53 with NV in the periphery]) with equal probability to one of the treatment groups:
        - Group A: Sham injections
        - Group B: Intravitreous 2 mg aflibercept injections

    - <u>For study participants with two study eyes</u> (both eyes eligible at the time of randomization), the study participant will be randomly assigned with equal probability to receive either:
        - Group A in the eye with greater DR severity and Group B in the eye with lower DR severity
        - Group B in the eye with greater DR severity and Group A in the eye with lower DR severity

Note: if both eyes have the same DR severity, the right eye will be considered the eye with the greater DR severity.

CHAPTER 3. FOLLOW-UP VISITS AND TESTING

3.1 Visit Schedule

3.1.1 Assessment Visits

The schedule of protocol-specified Assessment Visits for all participants is as follows:

- Visits at 1 and 2 months (± 1 week)
  - *Study injections for prevention of PDR and DME must be at least 21 days apart; therefore, follow-up visits should be scheduled accordingly so that the eye is eligible for retreatment.*
- Visits at 4 months and then every 4 months at 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 months (±8 weeks)
  - *The every 4-month study visits/injections for prevention of PDR and DME must be no closer than 8 weeks apart.*

3.1.2 Additional Study Visits

1. DME Outcome Visits
   - If the eye has not already met the primary outcome and visual acuity has decreased 5 to 9 letters from baseline and the loss is attributable to DME, the participant will return in 4 weeks (± 1 week) to assess whether the eye has met the primary outcome for DME progression and is eligible for treatment.

2. DME Treatment Visits
   - Once DME treatment has been initiated, follow-up visits for DME treatment occur every 4 weeks for the first 6 months from initial aflibercept treatment for DME. After 6 months, if the injection is deferred at the current and previous 2 visits (see section 4.4 for retreatment criteria), the next study follow-up visit is in twice the time since the last visit up to a maximum of 16 weeks between visits. Otherwise, the next study follow-up visit is in 4 weeks.

3. PDR Treatment Visits
   - Once NV is present, the participant may return sooner than the next scheduled Assessment Visit to evaluate for progression to high-risk, at the discretion of the investigator.
   - Once PDR treatment has been initiated, follow-up visits for PDR treatment occur every 4 weeks for the first 6 months from initial aflibercept treatment for PDR. After 6 months, if the injection is deferred at the current and previous 2 visits (see section 4.5.4 for retreatment criteria), the next study follow-up visit is in twice the time since the last visit up to a maximum of 16 weeks between visits. Otherwise, the next study follow-up visit is in 4 weeks.

Note: Regardless of the timing of additional visits for DME/PDR treatment, the participant will return for the protocol-specified Assessment Visits listed above in Section 3.1.1.

3.2 Testing Procedures

The following procedures will be performed at each study visit (listed in 3.1.1 and 3.1.2) on the study eye only unless otherwise specified. A grid in section 1.3 summarizes the testing performed at each visit. Photographers, OCT technicians, and visual acuity testers, including refractionists, will be masked to treatment group at annual visits.

1. E-ETDRS visual acuity testing in each eye (best corrected).
    - A protocol refraction in the study eye is required at all study visits. Refraction in the non-study eye is only required at annual visits. When a refraction is not performed, the most-recently performed refraction is used for the testing.
2. Workplace Productivity and Activity Impairment (WPAI) Questionnaire (only in participants with one study eye) at annual visits.
3. OCT on the study eye at annual visits and if any of the following are met:
    - If visual acuity has decreased by at least 5 letters (equivalent to approximately 1 or more line) since baseline in an eye that has not previously met the DME outcome and there is no other apparent cause (e.g. cataract), an OCT must be performed to determine if DME is the cause of vision loss.
    - If DME treatment is being considered, an OCT must be done to confirm the eye has met the primary outcome before proceeding with treatment.
    - Once DME treatment has been initiated, an OCT must be done at each subsequent DME Treatment Visit.
    - Prior to initiating more frequent anti-VEGF treatment for PDR, if DME outcome was not met previously.
    - ➢ *The same OCT machine type as Randomization should be used.*
4. Ocular exam on the study eye, including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy
    - Undilated exam of the iris and examination of the angle is at investigator discretion.
5. Digital fundus photographs on the study eye at the 4-month visit and annual visits.
    - Digital fundus photography must also be performed 1) the first time traction retinal detachment, vitreous hemorrhage, or preretinal hemorrhage is identified to confirm the primary outcome has been met, or 2) prior to initiating PRP or vitrectomy, if the primary outcome was not confirmed previously or 3) prior to initiating more frequent anti-VEGF treatment for either DME or PDR, if the primary outcome was not confirmed previously.
6. Digital FA using the widest approach available (e.g. ultra-widefield imaging device, if available) on the study eye at the 4-month visit and annual visits.
    - Digital FA must also be performed 1) the first time traction retinal detachment, vitreous hemorrhage, or preretinal hemorrhage is identified to confirm the primary outcome has been met, or 2) prior to initiating PRP or vitrectomy, if the primary outcome was not confirmed previously or 3) prior to initiating more frequent anti-

VEGF treatment for either DME or PDR, if the primary outcome was not confirmed previously.

- *If a site obtains a new ultra-widefield imaging device during the course of the study, the widest approach available should be used for all study visits going forward.*
- *For participants with two study eyes, the transit eye at follow-up should be consistent with the transit eye selected at baseline.*

7. OCT angiography on the study eye at the 4-month visit and annual visits, as well as the time points above when fundus photographs and FA are obtained for primary outcome documentation.

   - Only obtained by a subset of sites with OCT angiography capabilities. If a site has OCT angiography systems from more than one manufacturer, the images should be obtained on each system available.
   - *See procedure manual for more details on acquisition, including which fields to collect on a given OCT angiography system.*

8. Measurement of blood pressure at annual visits only.
9. Laboratory testing of Hemoglobin A1c at annual visits only.
   - *HbA1c does not need to be repeated at annual visits if available in the prior 3 months.*

All of the testing procedures do not need to be performed on the same day, provided that they are completed within the time window of a visit and prior to initiating any treatment.

Testing procedures at unspecified visits are at investigator discretion. However, it is recommended that procedures that are performed should follow the standard DRCR.net protocol for each procedure. If a primary outcome criterion is identified at an unspecified visit, the imaging requirements above apply and best-corrected visual acuity testing should be performed whenever possible.

CHAPTER 4. TREATMENT REGIMEN

4.1 Treatment Groups

The treatment groups are as follows:

A: Sham injections

B: Intravitreous 2 mg aflibercept injections

For both groups, the baseline injection (sham or intravitreous) must be given on the day of randomization.

4.2 Injection Procedure

4.2.1 Intravitreous Aflibercept Injection (Eylea®)

Eylea® (intravitreal aflibercept injection) is made by Regeneron Pharmaceuticals, Inc. and is approved by the FDA for the treatment of neovascular age-related macular degeneration, macular edema due to central retinal vein occlusion, macular edema due to branch retinal vein occlusion, diabetic macular edema, and diabetic retinopathy in eyes with diabetic macular edema.

Study eyes that receive anti-VEGF will receive a dose of 2 mg aflibercept in 0.05 cc each time a study injection is performed. The physical, chemical and pharmaceutical properties and formulation are provided in the Clinical Investigator Brochure. Aflibercept for the study and non-study eye will be distributed by the Network.

4.2.2 Intravitreous Injection Technique

The injection is preceded by a povidone iodine prep of the conjunctiva. In general, topical antibiotics in the pre-, peri-, or post-injection period should not be used.

The injection will be performed using sterile technique. The full injection procedure is described in the protocol-specific study procedures manual.

4.2.3 Sham Injection Technique

The prep will be performed as for an intravitreous injection. A syringe without a needle will be used, with the hub pressed against the conjunctival surface to simulate the force of an actual injection.

4.2.4 Deferral of Injections Due to Pregnancy

Female study participants of child-bearing age must be questioned regarding the possibility of pregnancy prior to each injection. In the event of pregnancy, study injections must be discontinued during the pregnancy and any post-partum period of breastfeeding.

4.2.5 Non-Study Eye Injections

If the non-study eye is going to be treated for any condition which requires treatment with an anti-VEGF agent, study provided aflibercept must be used. However, if intravitreous treatment is planned on the same day as an intravitreous injection in the study eye, the study eye will be injected first, followed by the non-study eye (see Procedures Manual for additional details). If a non-study anti-VEGF medication is desired to be administered by intravitreous injection in the non-study eye, a discussion with the Protocol Chair is required first.

4.3 Follow-Up Treatment Protocol for Prevention of PDR/DME

Injections for prevention of PDR/DME will be given according to the criteria below at each Assessment Visit (listed in 3.1.1). Additional injections must not be given in-between the Assessment Visits, unless criteria are met for PDR or DME treatment (see Sections 4.4 and 4.5).

If an eye experienced adverse effects from a prior intravitreous injection, retreatment is at the discretion of the investigator.

4.3.1 Injections at 4 and 8 Weeks and Each 4-Month Interval Visit until 2 Years

During Years 1 and 2, study eyes receive an injection (sham or intravitreous) at each Assessment Visit (listed in 3.1.1). Group A receives a sham injection and group B receives a 2 mg aflibercept injection.

4.3.2 Injections at and After the 2-Year Visit

At and after the 2-year visit, the study eye is evaluated for intravitreous (sham) injection retreatment at each Assessment Visit. Group A receives a sham injection and group B receives a 2 mg aflibercept injection.

- If the DR level is mild NPDR or better (≤ Level 35) based on the investigator's assessment, the injection should be deferred.
    - Level 35 can be clinically defined as microaneurysms plus venous loops, hard exudates, cotton wool spots and/or mild retinal hemorrhages (less than present in ETDRS Standard photograph 2a).
- If the DR level is worse than mild NPDR (> Level 35, defined above), the injection (or sham) is given.

4.4 Treatment for CI-DME

Treatment for CI-DME must not be given until the following criteria have been met:

- CI-DME on clinical exam with ≥10% increase in central subfield thickness from baseline and either:
    - 1) at least 10 letter decrease in visual acuity presumed to be from DME at a single visit or
    - 2) 5 to 9 letter decrease in visual acuity presumed to be from DME at two-consecutive visits at least 21 days apart.

Once the above criteria have been met, an injection of 2 mg aflibercept will be given. Thereafter, the eye will be evaluated at each visit for retreatment. In general, an eye will continue to receive an injection if the eye is improving or worsening on OCT or visual acuity. The first time an eye has not improved or worsened, the eye will receive an injection. If the eye has not improved or worsened for at least 2 consecutive 4-week injections and the OCT CSF thickness is less than the gender specific spectral domain OCT threshold (see below) and visual acuity is 20/20 or better, then injection will be deferred. If the eye has not improved or worsened for at least 2 consecutive 4-week visits and the OCT CSF thickness is ≥ the gender specific spectral domain OCT threshold or visual acuity is worse than 20/20, the following will be done:

- If less than 24 weeks from the initial injection for DME, an injection will be given.
- At and after 24 weeks, the injection will be deferred.

The protocol chair or designee must be contacted prior to deviation from the injection protocol. See the DRCR.net Procedure Manual for additional details.

Spectral domain OCT central subfield gender-specific threshold:

- Zeiss Cirrus: 290 microns in women, and 305 microns in men
- Heidelberg Spectralis: 305 microns in women, and 320 microns in men

4.4.1 Initiation of Focal/Grid Photocoagulation While Receiving Anti-VEGF Injections

In general, focal/grid laser will be initiated at or after the 24 week visit if 1) the OCT central subfield thickness is greater than the OCT central subfield gender-specific threshold (above) or there is edema that is threatening the fovea and 2) the eye has not improved on OCT or visual acuity from the last two consecutive injections. Once focal/grid laser has been initiated, retreatment with focal/grid laser will be given unless one of the following is present: 1) focal/grid laser has been given in the previous 13 weeks, 2) complete focal/grid laser has already been given in the investigator's judgment, 3) the OCT central subfield thickness is less than the OCT central subfield gender-specific threshold (above) and there is no edema threatening the fovea, 4) the eye has improved since the last laser treatment. The protocol chair or designee must be contacted prior to deviating from the focal/grid laser protocol. See the DRCR.net Procedure Manual for additional details.

4.4.2 Continuation of Prevention Treatment Protocol

Eyes for which the above anti-VEGF treatment regimen is initiated for DME will continue injections as part of the prevention protocol. At each Assessment Visit, if an injection has not been given within the prior 21 days, the eye will be treated per protocol (years 1 and 2) or evaluated for a prevention injection (sham or intravitreous) following section 4.3.2 in years 3 and 4, regardless of DME status.

4.5 Treatment for PDR

4.5.1 Primary Outcome for PDR

An eye will be considered to have met the primary outcome for PDR if any of the following are met:

- Development of NV within the 7-modified ETDRS fields on fundus photography or FA, confirmed by a masked grader at the central reading center
  - At non-annual visits, fundus photography and FA will only be submitted to the reading center to assess for this component of the primary outcome if the investigator thinks treatment is necessary.
- NV of the iris (at least 2 cumulative clock hours), definitive NV of the angle, or neovascular glaucoma development on clinical exam (photographic documentation not required)
- Other outcomes presumed to be from PDR and documented: traction retinal detachment, vitreous hemorrhage, pre-retinal hemorrhage greater than ½ disc area
- Procedures undertaken for the treatment of PDR (when present or presumed to be present): PRP, anti-VEGF, or vitrectomy

Once NV develops, the participant may return sooner than the next scheduled Assessment Visit to evaluate for initiation of treatment (see below), at the discretion of the investigator.

4.5.2 Initiating Treatment for PDR

If at any point NV of the angle develops, treatment with anti-VEGF and/or PRP is at investigator discretion; otherwise, treatment for PDR must not be given until one of the following criteria has been met:

1. The eye has PDR with high-risk characteristics, defined as:
   - NVD greater than Standard photograph 10A (1/4 to 1/3 disc area), or
   - Any NVD with pre-retinal or vitreous hemorrhage, or
   - NVE greater than ½ disc area with pre-retinal or vitreous hemorrhage
2. The eye has vitreous hemorrhage requiring treatment that is presumed to be from PDR (either NV identified on FA or unable to assess NV due to density of the hemorrhage but there is no other attributable cause)
3. The reading center has confirmed NV is present within the 7-modified fields and protocol chair approval has been received to initiate treatment prior to high-risk characteristics being present.
   - Treatment for NVE outside of the 7-modified fields without the presence of pre-retinal or vitreous hemorrhage is discouraged. If the investigator believes treatment for peripheral NV is necessary, protocol chair approval is required.

If at least 4 study injections have been given in the prior 4 months (for DME) and the eye has developed high-risk PDR as defined above, PRP may be performed at the discretion of the investigator.

Otherwise, once one of the above criteria for treatment has been met, an injection of 2 mg aflibercept will be given. Thereafter, the eye will be evaluated at each visit for retreatment using the criteria below (Sections 4.5.3 to 4.5.4). If an anti-VEGF injection was already given in the prior 5 weeks for prevention, it will be considered the baseline injection, and retreatment will begin with section 4.5.3.

4.5.3 Intravitreous Injection for PDR at 4 weeks, 8 weeks and 12 weeks

All eyes that initiate treatment for PDR will receive injections at 4, 8, and 12 weeks following the initial injection. If an eye experienced adverse effects from a prior intravitreous injection, retreatment with intravitreous aflibercept is at the discretion of the investigator.

4.5.4 Intravitreous Injection for PDR at and after 16 weeks

Starting at 16 weeks, the eye will be evaluated for retreatment with intravitreous injection for PDR based on appearance of neovascularization.

If an eye has experienced adverse effects from prior intravitreous injection treatment, retreatment with intravitreous aflibercept is at the discretion of the investigator. In addition, if any future treatment with aflibercept is contraindicated based on a previous adverse reaction, treatment with PRP for PDR is at investigator discretion after discussion with and approval from the Protocol Chair or Coordinating Center designee. Each eye with no contraindication to additional injections will be categorized into one of the following 5 categories based on neovascularization (NV) status:

** Note: examination of the angle is at investigator discretion; however, if the angle is examined, then the results from this examination should be factored into the subsequent treatment decision.*

- **Resolved**
  - NV (of the retina, disc, AND iris/angle*) is absent and visualization of the entire retina is adequate to completely assess for NV. Decision to re-inject is at investigator discretion. In general, if NV is completely regressed the injection should be deferred. PRP should not be given.

- **Improved**

  NV (of the retina, disc OR iris/angle*) still persists, but there is evidence of improvement (improvement defined as a decrease in the size of NV or diminished density of NV) since the last visit and visualization of the entire retina is adequate to completely assess for NV. An injection is given. PRP should not be given.

- **Stable**
  - NV (of the retina, disc AND iris/angle*) is clinically unchanged since the last visit and visualization of the entire retina is adequate to completely assess for NV. Once the eye meets criteria for stability, at least 2 more injections must be given, each one month apart (one at the visit at which stability criteria are met and the second at the following study visit one month later if still stable). Further reinjection is then at investigator discretion as long as the eye remains stable. PRP should not be given.

- **Not fully treated**
  - Failure/futility criteria not met and recurrent or worsening NV (of the retina, disc *OR* iris) is present since the last visit in an eye that has had fewer than 4 injections over the previous 4 months or there is vitreous or preretinal hemorrhage preventing adequate visualization of the fundus to assess NV status. An injection is given. PRP should not be given.

- **Failed/futile**
  - Failure/futility criteria met. Decision to re-inject is at investigator discretion. PRP may be given at this time (see below for cases that first require discussion with the Protocol Chair or Coordinating Center designee).

    - *Failure criteria are defined as*
      1. *growth of NV or new NV of the retina, disc OR iris since the last visit such that the NV, including fibrosis, is greater in extent than when treatment for NV was initiated and at least 4 study injections have been given over the previous 4 months. The investigator may perform PRP.*

    OR

      2. *New or worsened NV of the angle* has developed since the last visit. The investigator may perform PRP.*

OR

3. *definite worsening of NV or fibrous proliferation of the retina, disc OR iris at least 1 day after the last injection that the investigator believes is likely to lead to substantial vision loss if PRP is not performed within 1 week. PRP may only be performed after discussion with and approval from the Protocol Chair or Coordinating Center designee.*

- *Futility criteria are defined as continued persistence or recurrence of NV at least 1.5 years from initial study aflibercept injection that is equal to or greater than the extent of the NV when treatment for NV was initiated and at least 5 study injections performed over the preceding 6 months. PRP may only be performed after discussion with and approval from the Protocol Chair or Coordinating Center designee.*

4.5.5 Continuation of Prevention Treatment Protocol

Eyes for which the above anti-VEGF treatment regimen is initiated for PDR will continue injections as part of the prevention protocol. At each Assessment Visit, if an injection has not been given within the prior 21 days, the eye will treated per protocol (years 1 and 2) or be evaluated for a prevention injection (sham or intravitreous) following section 4.3.2 in years 3 and 4, regardless of whether the injection can be deferred according to the PDR treatment criteria.

4.6 Panretinal Photocoagulation Technique

An eye may receive PRP only if failure/futility criteria for intravitreous injection for PDR above are met. Study eyes that receive panretinal photocoagulation should have 1200 to1600 burns with a spot size on the retina of approximately 500 microns (or the equivalent area treated with a PASCAL) given over 1 to 3 sittings and completed within 8 weeks (56) days of initiation.

The burn characteristics for non-automated photocoagulation will be as follows:

Size (on retina)	500 microns [e.g. argon laser using 200 micron spot size with Rodenstock lens (or equivalent) or 500 micron spot size with 3 mirror contact lens]
Exposure	0.1 seconds recommended, 0.05 to 0.2 allowed
Intensity	mild white (i.e. 2+ to 3+ burns)
Distribution	edges 1 burn width apart
No. of Sessions/Sittings	1 to 3
Nasal proximity to disk	No closer than 500 microns
Temp. proximity to center	No closer than 3000 microns
Superior/inferior limit	No further posterior than 1 burn within the temporal arcades

Extent	Arcades (~3000 microns from the macular center) to at least the equator
Total # of burns	1200 to 1600: *There may be instances where 1200 burns are not possible such as development of vitreous hemorrhage or study participant inability to complete a sitting precluding completion of the PRP session. Similarly, there may be clinical situations in which more than 1600 burns are needed such as initial difficulty with laser uptake due to media opacity.*
Wavelength	Green or yellow (red can be used if vitreous hemorrhage is present precluding use of green or yellow)

An anesthetic injection (retrobulbar, peribulbar or sub-Tenon's) can be used at investigator discretion.

An indirect laser approach can be used at investigator discretion.

If a laser is used that has the capability of producing an automated pattern (e.g. the PASCAL), the automated pattern producing mode is permissible. Guidelines for use of the automated pattern are included in the study procedure manual.

4.7 Surgery for Vitreous Hemorrhage, Traction Detachment, and Other Complications of DR

A study eye could develop a vitreous hemorrhage or traction detachment that may cause visual impairment. In these cases, vitrectomy may be performed at the discretion of the investigator; however, vitrectomy for hemorrhage alone should not be performed without first confirming presence of neovascularization on color photographs and/or FA. If NV has not been confirmed by the investigator in the setting of vitreous hemorrhage alone, review with the Protocol Chair or Coordinating Center designee must occur prior to proceeding with vitrectomy.

CHAPTER 5.
MISCELLANEOUS CONSIDERATIONS IN FOLLOW-UP

5.1 Endophthalmitis

Diagnosis and treatment of endophthalmitis is based on investigator's judgment. Obtaining cultures of vitreous and aqueous fluid is highly recommended prior to initiating antibiotic treatment for presumed endophthalmitis.

5.2 Treatment in Non-study Eye

Treatment of PDR or DME in the non-study eye is at investigator discretion. However, if anti-VEGF treatment will be given in the non-study eye, study aflibercept must be used.

5.3 Diabetes Management

Diabetes management is left to the study participant's medical care provider.

5.4 Study Participant Withdrawal and Losses to Follow-up

A study participant has the right to withdraw from the study at any time. If s/he is considering withdrawal from the study, the principal investigator should personally speak to the individual about the reasons, and every effort should be made to accommodate the study participant to allow continued participation if possible.

The goal for the study is to have as few losses to follow-up as possible. The Coordinating Center will assist in the tracking of study participants who cannot be contacted by the site. The Coordinating Center will be responsible for classifying a study participant as lost to follow-up.

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

Study participants who withdraw or are determined to have been ineligible post-randomization will not be replaced.

5.5 Discontinuation of Study

The study may be discontinued by the Executive Committee (with approval of the Data and Safety Monitoring Committee) prior to the preplanned completion of follow-up for all study participants.

5.6 Contact Information Provided to the Coordinating Center

The Coordinating Center will be provided with contact information for each study participant. Permission to obtain such information will be included in the Informed Consent Form. The contact information may be maintained in a secure database and will be maintained separately from the study data.

Phone contact from the Coordinating Center will be made with each study participant in the first month after enrollment, and approximately every six months thereafter. Additional phone contacts from the Coordinating Center will be made if necessary to facilitate the scheduling of

the study participant for follow-up visits. A study participant-oriented newsletter will be sent at least twice a year. A study logo item may be sent once a year.

Study participants will be provided with a summary of the study results in a newsletter format after completion of the study by all study participants.

5.7 Study Participant Reimbursement

The study will be providing the study participant with a $25 merchandise or money card per completed non-annual study visit and $100 in merchandise or money cards per annual visit. Additional travel expenses will be paid in select cases for study participants with higher expenses.

CHAPTER 6.
ADVERSE EVENTS

6.1 Definition

An adverse event is any untoward medical occurrence in a study participant, irrespective of whether or not the event is considered treatment-related. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal lab finding), symptom or disease temporally associated with the use of the treatment, whether or not related to the treatment. This includes preexisting medical conditions (other than the condition being studied) judged by the investigator to have worsened in severity or frequency or changed in character.

6.2 Recording of Adverse Events

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

All adverse events whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, laboratory test, or other means will be reported on an adverse event form online. Each adverse event form is reviewed by the Medical Monitor to verify the coding and the reporting that is required.

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

To ensure consistency of adverse event causality assessments, investigators should apply the following general guideline when determining whether an adverse event is related:

Yes

There is a plausible temporal relationship between the onset of the adverse event and administration of the study treatment, and the adverse event cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study treatment; and/or the adverse event abates or resolves upon discontinuation of the study treatment or dose reduction and, if applicable, reappears upon re-challenge.

No

Evidence exists that the adverse event has an etiology other than the study treatment (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to study treatment administration (e.g., cancer diagnosed 2 days after first dose of study drug).

The intensity of adverse events will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe. It is emphasized that the term severe is a measure of intensity: thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

Adverse events will be coded using the MedDRA dictionary.

Definitions of relationship and intensity are listed on the DRCRnet website data entry form.

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

6.3 Reporting Serious or Unexpected Adverse Events

A serious adverse event is any untoward occurrence that:

- Results in death
- Is life-threatening; (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (sight threatening)
- Is a congenital anomaly/birth defect
- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above)

Unexpected adverse events are those that are not identified in nature, severity, or frequency in the current Eylea® Clinical Investigator's Brochure, protocol, or informed consent form.

Serious or unexpected adverse events must be reported to the Coordinating Center immediately via completion of the online serious adverse event form.

The Coordinating Center will notify all participating investigators of any adverse event that is both serious and unexpected. Notification will be made within 10 days after the Coordinating Center becomes aware of the event.

Each principal investigator is responsible for informing his/her IRB of serious study-related adverse events and abiding by any other reporting requirements specific to their IRB.

6.4 Data and Safety Monitoring Committee Review of Adverse Events

A Data and Safety Monitoring Committee (DSMC) will advise the Coordinating Center regarding the protocol, template informed consent form, and substantive amendments and will provide independent monitoring of adverse events. Cumulative adverse event data are semi-annually tabulated for review by the DSMC. Following each DSMC data review, a summary will be provided to institutional review boards. A list of specific adverse events to be reported to the DSMC expeditiously, if applicable, will be compiled and included as part of the DSMC Standard Operating Procedures document.

6.5 Risks

6.5.1 Potential Adverse Effects of Aflibercept

The most common adverse reactions (≥ 5%) reported in patients receiving aflibercept were conjunctival hemorrhage, eye pain, cataract, vitreous floaters, intraocular pressure increased, and vitreous detachment.

Serious adverse reactions related to the injection procedure have occurred in <0.1% of intravitreal injections with aflibercept including endophthalmitis and retinal detachment.

The DA VINCI study, a phase II study evaluating aflibercept for treatment of DME, reported common adverse events that were consistent with those previously seen with intravitreal injections. Over one year of follow-up, two cases of endophthalmitis and one case of uveitis occurred (all in aflibercept treatment groups). Seven deaths (4.0%) occurred in the groups randomized to aflibercept treatment as compared with 1 (2.3%) in the group treated with laser. Myocardial infarction or cerebrovascular accident occurred in 6 (3.4%) participants treated with aflibercept as compared with 1 (2.3%) participant treated with laser alone.[44] Percentages of study participants that experienced events meeting APTC criteria were 5.1% (N = 9) in the combined aflibercept groups and 4.5% (N = 2) in the laser group.[45]

The DRCR.net Protocol T study assessed ocular and systemic adverse events in eyes with central-involved DME treated with aflibercept over 1 year.[43] In the aflibercept-treated study eyes, there were no cases of endophthalmitis and 2 cases of ocular inflammation. Non-study eyes treated with aflibercept had 1 case of endophthalmitis and 3 cases of ocular inflammation. Systemic adverse events were infrequent with only 6 APTC events (4 nonfatal myocardial infarctions, 2 deaths from a potential vascular cause or unknown cause, 6% of participants) over the 1 year period in the aflibercept group.

Additional safety data were published from phase III studies VISTA and VIVID, which included 872 eyes with DME with central involvement that received either intravitreal aflibercept every 4 weeks, intravitreal aflibercept every 8 weeks after 5 initial monthly doses, or macular laser photocoagulation. Overall, the incidences of ocular and non-ocular adverse events were similar across treatment groups at 52 weeks.[42] The incidence of APTC-defined thromboembolic events was similar across treatment groups. There were no reported cases of endophthalmitis, and intraocular inflammation occurred in less than 1% of injections. Through 100 weeks, an integrated safety analysis found that the most frequent serious ocular adverse event was cataract (2.4% and 1.0% in the aflibercept groups compared with 0.3% in the laser group).[41]

There may be side effects and discomforts that are not yet known.

6.5.2 Potential Adverse Effects of Intravitreous Injection

Rarely, the drugs used to anesthetize the eye before the study drug injections (proparacaine, tetracaine, or xylocaine) can cause an allergic reaction, seizures, and an irregular heartbeat.

Subconjunctival hemorrhage or floaters will commonly occur as a result of the intravitreous injection. Discomfort, redness, or itching lasting for a few days is also likely.

Immediately following the injection, there may be elevation of intraocular pressure. It usually returns to normal spontaneously, but may need to be treated with topical drugs or a

paracentesis to lower the pressure. The likelihood of permanent loss of vision from elevated intraocular pressure is less than 1%.

As a result of the injection, endophthalmitis (infection in the eye) could develop. If this occurs, it is treated by intravitreous injection of antibiotics, but there is a risk of permanent loss of vision including blindness. The risk of endophthalmitis is less than 1%.

As a result of the injection, a retinal detachment could occur. If this occurs, surgery may be needed to repair the retina. The surgery is usually successful at reattaching the retina. However, a retinal detachment can produce permanent loss of vision and even blindness. The risk of retinal detachment is less than 1%.

The injection could cause a vitreous hemorrhage. Usually the blood will resolve spontaneously, but if not, surgery may be needed to remove the blood. Although the surgery usually successfully removes the blood, there is a small risk of permanent loss of vision and even blindness. The risk of having a vitreous hemorrhage due to the injection is less than 1%.

6.5.3 Risks of Eye Examination and Tests

There is a rare risk of an allergic response to the topical medications used to anesthetize the eye or dilate the pupil. Dilating drops rarely could cause an acute angle closure glaucoma attack, but this is highly unlikely since the study participants in the study will have had their pupils dilated many times previously.

There are no known risks associated with OCT or fundus photographs. The bright flashes used to take the photographs may be annoying, but are not painful and cause no damage.

For fluorescein angiography, both the skin and urine are expected to turn yellow/orange for up to 24 hours after the injection of fluorescein dye. There is a small risk of discomfort or phlebitis at the site of the injection. Patients occasionally experience lightheadedness or nausea after dye injection which are usually transient and resolve after a few minutes without further intervention. An allergic reaction to the dye used to do the fluorescein angiography imaging is rare. A rash or pruritus (itching) can develop, but true anaphylactic reactions are very rare.

CHAPTER 7.
STATISTICAL METHODS

The approach to sample size and statistical analyses are summarized below. A detailed statistical analysis plan will be written and finalized prior to the first assessment of 2-year outcome data. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

7.1 Primary Objectives and Key Outcomes

This study has two objectives. First, to determine the efficacy and safety of intravitreous aflibercept injections versus sham injections (observation) for prevention of PDR and CI-DME in eyes at high risk for development of these complications. Second, to compare long-term vision outcomes in eyes that receive anti-VEGF therapy early in the course of disease with those that are initially observed and treated only if high-risk PDR or CI-DME with vision loss develops.

Primary outcome: development of PDR or DME defined as the first occurrence of any of the following (composite time-to-event outcome):

- NV within the 7-modified ETDRS fields on fundus photography or FA, confirmed by a masked grader at the central reading center
  - At non-annual visits, fundus photography and FA will only be submitted to the reading center to assess for this component of the primary outcome if the investigator thinks treatment is necessary.
- NV of the iris (at least 2 cumulative clock hours), definitive NV of the angle, or neovascular glaucoma on clinical exam (photographic documentation not required)
- Other outcomes presumed to be from PDR and documented: traction retinal detachment, vitreous hemorrhage, or pre-retinal hemorrhage greater than ½ disc area
- Procedures undertaken for the treatment of PDR (when present or presumed to be present): PRP, anti-VEGF, or vitrectomy
- CI-DME on clinical exam with at least 10% increase in central subfield thickness from baseline and either (1) at least a 10-letter decrease in visual acuity from baseline at a single visit or (2) a 5-to-9-letter decrease in visual acuity from baseline at 2 consecutive visits at least 21 days apart, with vision loss presumed to be from DME
- Non-topical treatment for DME performed without meeting the above criteria, including focal/grid laser or intravitreous injections for DME

The primary outcome analysis will be performed when the last enrolled participant reaches 2 years of follow up, using all available follow up data. The treatment groups will be compared using the hazard ratio.

Other Key Outcomes:

- Development of PDR or DME outcome through 4 years
- Mean visual acuity change from baseline at 2 years
- Mean visual acuity change from baseline at 4 years

The overall type 1 error for the primary outcome and all key outcomes will be controlled at 5%. To control the type 1 error for each time point, 2.5% type I error will be allocated to the 2-year

analysis, and 2.5% will be allocated to the 4-year analysis. To control the type 1 error for the multiple key outcomes, a hierarchical approach will be used. The visual acuity outcome will be formally compared (i.e., with a *P* value) only if there is a significant treatment group difference in the anatomic outcome at the same time point ($P \leq .025$). If not, only point estimates and confidence intervals for within and between group changes in visual acuity from baseline will be computed.

See Section 7.4 for secondary outcomes to be evaluated at 2 and 4 years.

7.2 Sample Size

The sample size has been computed for the primary outcome at 2 years. The primary analysis will consist of a treatment group comparison based on the hazard ratio for the composite time-to-event outcome, as defined in Section 7.1, estimated using the marginal Cox proportional hazards model (see Section 7.3).

7.2.1 Projected Control Group Proportion

Data from the ETDRS, the Protein Kinase C β Inhibitor Diabetic Retinopathy Study (PKC-DRS), Diabetic Retinopathy Study (DRS)-2, prior DRCR.net studies, and RIDE/RISE were used to estimate progression rates in the control group. Estimates for this study are based largely on PDR development, although approximately 4% of eyes in ETDRS developed CI-DME prior to PDR, which would increase the expected progression rate.

It should be noted that eligibility for this study is based primarily on investigator assessment of DR severity as severe NPDR (level 53). The data below are presented by DR severity level as assessed by central reading center grading of fundus photographs; however, it is unknown whether features of severe NPDR were evident on clinical exam or FA in these cohorts

Fifty-nine percent (N=249), 43% (N=461), and 23% (N=499) of eyes in the ETDRS assigned to observation with DR Severity levels graded on fundus photography of 53, 47, and 43, respectively, with no DME on fundus photography at baseline, progressed to PDR on fundus photography at 2 years (personal communication, Adam Glassman).

More recent data for longer-term progression rates are available from 2 separate phase 3 trials of the protein kinase C inhibitor, ruboxistaurin, which demonstrated rates of PDR progression of approximately 40% and 60% in the 2 trials, respectively, over 3 years with lower levels of DR (47A) being included in the first trial.[7, 8]

Data from DRCR.net Protocol A and Protocol B include eyes with baseline DME treated with laser alone and having DR severity levels 43 to 53 at baseline (Table 1, personal communication, Adam Glassman). Data from RIDE/RISE include eyes with DME and diabetic retinopathy less severe than active PDR on clinical exam that were treated with sham (Table 2).[34]

Table 1. Proportion of Eyes with PDR* at 2 years by Baseline Level of Retinopathy (Laser group only; DME at Baseline)

Protocol A – A Pilot Study of Laser Photocoagulation for Diabetic Macular Edema		
DR Severity	**N**	**Proportion of eyes with PDR at 2 years**
Level 43	27	7%
Level 47A	62	13%
Level 47B-47D	22	32%
Level 53	26	58%
Protocol B – A Randomized Trial Comparing Intravitreal Triamcinolone Acetonide and Laser Photocoagulation for Diabetic Macular Edema		
DR Severity	**N**	**Proportion of eyes with PDR at 2 years**
Level 43	41	10%
Level 47A	59	15%
Level 47B-47D	23	13%
Level 53	27	55%

* Defined as ETDRS DR Severity Score ≥ 60 (i.e. includes eyes with PRP prior to 2 years)

Table 2. RIDE/RISE Cumulative Proportion with PDR Events in Sham Eyes (includes eyes with DME)

RIDE/RISE	N	1 Year	N	2 Year
Sham	135	18%	116	30%

Based on these data and the expected proportion of eyes enrolled in each DR severity level, we estimate the overall outcome proportion in the control group to be 30%. This estimate includes approximately 5% that are expected to meet the outcome by development of CI-DME prior to PDR.

7.2.2 Projected Treatment Group Rate

Pooled data from the RIDE/RISE Open-Label Extension include only eyes with DME and diabetic retinopathy less severe than active PDR on clinical exam (Table 3).[34]

Table 3. RIDE/RISE Cumulative Proportion with PDR Events in the Anti-VEGF Treated Groups

RIDE/RISE	N	1 Year	N	2 Year
0.3 mg Ranibizumab	164	5%	152	12%
0.5 mg Ranibizumab	154	6%	147	10%

The cumulative proportions of eyes with progression from RIDE/RISE are supported by unpublished data of PDR progression in eyes with DR severity levels of 43 to 53 treated with anti-VEGF from DRCR.net Protocol T (personal communication, Adam Glassman). Based on these data, the projected cumulative outcome proportion for the treatment group is estimated to be no more than one-half the rate in the control group (10-15%).

7.2.3 Sample Size Estimates

Table 4 shows sample size estimates under varying assumptions for primary outcome proportions in the treatment and control groups at 2 years. These calculations assume a type I error rate of 5% with 90% power, and a null hypothesis of no difference between groups.

Table 4: Total Sample Size for Various Outcome Rates of PDR/DME Development

	Control Group Rate		
Treatment Group Rate	**20%**	**30%**	**40%**
10%	564	180	94
15%	2496	**346**	144
20%	--	822	236

For true outcome rates of 30% vs. 15%, a sample of N=346 (173 per group) gives 90% power to reject the null hypothesis of no difference for a 2-sided test with a type I error rate of 5%. Sample size is increased by 10% for possible dropouts giving **N=386 (193 per group)**. Sample size was selected based on the original study design, which included the parameters above. Considering that the study is sufficiently powered for the current design (see below), no change to sample size will be made.

Given the approach to control type 1 errors, the alpha allocation at 2 years will be 2.5%. Using the assumptions above, with a sample size of 386 and an alpha level of 2.5%, the study will have 89% power to reject the null hypothesis of no difference. As this power calculation does not include estimates for person-time beyond 2 years, which will be included in the primary analysis, and is not adjusted for the correlation between eyes of participants with two study eyes, power is expected to be greater than this projection.

7.2.4 Power for the Visual Acuity Outcome

Table 5 shows the expected statistical power to detect a difference in the mean change in visual acuity from baseline if the true difference between the groups is 3, 4, or 5 letters under varying assumptions for standard deviation, using the estimated sample size for 2 and 4 years.

Table 5. Expected Statistical Power for Mean Change in Visual Acuity Outcome Adjusted for Baseline Visual Acuity

Standard Deviation*	**N†**	**Difference in Letter Score**		
		3	**4**	**5**
6	346	>99%	>99%	>99%
	306	98%	>99%	>99%
8	346	89%	>99%	>99%
	306	85%	98%	>99%
10	346	71%	93%	>99%
	306	65%	89%	98%

Alpha = 0.025 for a 2-sided hypothesis test.

* For reference, the adjusted standard deviation from the DRCR.net Protocol T aflibercept group

(baseline visual acuity 20/32 to 20/40) of change in visual acuity from baseline at 2 years, adjusted for baseline visual acuity, was 7.7 (personal communication, Adam Glassman)

† Based on 5% annual lost to follow-up

7.3 Primary Analysis Plan

7.3.1 Principles for Analysis

The primary analysis consists of a treatment group comparison based on the hazard ratio for the PDR/DME composite time-to-event outcome when the last participant reaches 2 years.

Other key analyses include a treatment group comparison of (1) the development of PDR/DME composite time-to-event outcome when the last participant reaches 4 years, (2) the difference in the mean change in visual acuity from baseline at 2 years, and (3) the difference in the mean change in visual acuity from baseline at 4 years. Note that (2) and (3), the comparisons of mean change in visual acuity, will only be conducted if there is a significant difference ($P \leq .025$) in the PDR/DME composite outcome at the corresponding time point. The 2-year analysis will be conducted when the last enrolled participant reaches 2 years of follow up and include all of the data collected through that point. The 4-year analysis will be conducted at the end of the study.

PDR/DME Outcome

The comparison of the PDR/DME composite time-to-event outcome will be based on the hazard ratio from a marginal Cox regression model that accounts for the correlation within study participants having two study eyes, and adjusts for randomization stratification factors.[46] The primary analysis is an intention-to-treat analysis. Data from participants not observed to meet outcome criteria who are lost to follow up will be censored at the time of the last completed visit. If there is evidence that assumptions are not reasonably satisfied, an alternative analysis method will be considered.

Visual Acuity Outcome

If there is a significant difference ($P \leq .025$) in the PDR/DME composite outcome, a treatment group comparison of the difference in the mean change in visual acuity from baseline to the outcome visit will be conducted. A linear mixed effects model will be used to estimate the treatment group difference. The analysis will adjust for baseline visual acuity and randomization stratification factors. This will also be an intention-to-treat analysis that includes all randomized eyes. Multiple imputation will be used to impute missing data. The correlation between eyes of participants having two study eyes will be modeled using random intercepts. If model assumptions are not reasonably satisfied, a transformation or non-parametric analysis will be considered.

Imbalances between groups in important covariates are not expected to be of sufficient magnitude to produce confounding. However, the presence of confounding will be evaluated in a sensitivity analysis by including factors potentially associated with the outcome for which there is an imbalance between groups as covariates in the mixed effects model.

Pre-planned subgroup analyses will be described in the detailed Statistical Analysis Plan. There are no data to suggest that the treatment effect will vary by gender or race/ethnicity. However, both of these factors will be evaluated in exploratory subgroup analyses.

7.3.2 Per-protocol Analysis

A per-protocol analysis for the 2- and 4-year outcomes will be performed including only eyes that received at least 80% of injections (sham or intravitreous) according to protocol and no other treatment for DR or DME. If the intention-to-treat and per-protocol analyses yield similar results, the per-protocol analyses will be used to provide supportive evidence of the magnitude of treatment effect among subjects who received the treatment. If the results of the methods differ, exploratory analyses will be performed to evaluate the factors that may have contributed to the differences.

7.3.3 Interim Analysis Plan

The DSMC will review tabulated safety and efficacy data at semi-annual meetings to assess the risk-benefit ratio of adverse events against benefits, if any, of anti-VEGF as compared with sham. No formal statistical analysis is planned during these reviews.

It is not expected that the trial will be stopped early for efficacy, based on the following reasons:

- Even if there is a significant difference in the primary outcome at 2 years, this may not translate to a long-term visual acuity difference.
- Even if there is a significant difference in mean visual acuity change from baseline at 2 years, it is important to know whether in the long term, treatment when progression occurs results in worse, equal, or better visual acuity outcome compared with treatment to prevent progression, and the relative differences in amount of treatment required with the two approaches to DR management.

7.4 Secondary Outcomes for Treatment Group Comparison

The treatment groups will be compared on the following outcomes of interest at the time of the 2- and 4-year analyses of the primary outcome:

- Development of PDR or PDR-related outcome (as defined above within the composite time-to-event outcome)
- Development of CI-DME with visual acuity impairment (as defined above within the composite time-to-event outcome)
- Development of PDR or DME based only on the objective components defined in the composite outcome, including OCT, visual acuity, and reading center assessment of photos and FA (i.e. not including investigator-only assessments)*
- Development of each component of the composite outcome assessed individually*
- Proportion of eyes with at least 10 or at least 15 letter loss from baseline, or gain or loss of at least 5 letters at consecutive study visits, consisting of the visits just before and the 2- or 4-year visit†
- Visual acuity area under the curve (AUC) between randomization and the 2- and 4-year visits†
- Mean change in OCT central subfield thickness from baseline
- Mean change in OCT volume from baseline
- Development of CI-DME on clinical exam with at least 10% increase in central subfield thickness and at least a 25-micron increase from baseline, regardless of visual acuity change*
- Proportion of eyes with at least 2-step worsening of DR severity level (scale for individual eyes) by central reading center from baseline

- Proportion of eyes with at least 2-step improvement of DR severity level (scale for individual eyes) by central reading center from baseline
- Proportion of eyes with at least 3-step worsening of DR severity level (scale for individual eyes) by central reading center from baseline*
- Proportion of eyes with at least 3-step improvement of DR severity level (scale for individual eyes) by central reading center from baseline*
- Level of retinopathy on color photos*
- Number of aflibercept injections performed*

* Outcomes will include descriptive statistics only with no statistical comparisons of treatment groups.

† If the statistical comparison of the mean change in visual acuity is not performed because the anatomic outcome comparison is not statistically significant, any analysis on visual acuity outcomes will be considered exploratory.

Descriptive statistics for the outcomes listed above will also be presented for the 1- and 3-year visits, with no statistical analyses conducted.

Binary outcomes will be analyzed using logistic regression with generalized estimating equations (GEE). Continuous outcomes will be analyzed using a linear mixed model. Time-to-event outcomes will be analyzed using the marginal Cox regression model. Analyses will be adjusted for baseline measure, correlation within study participants having two study eyes, and randomization stratification factors, where appropriate. If model assumptions are not reasonably satisfied, a transformation, nonparametric approach, or alternative method will be considered. Methods for handling missing secondary outcome data will be included in the detailed Statistical Analysis Plan.

7.5 Economic Analysis

The purpose of the economic analysis is to compare the treatment groups with respect to cost and workplace productivity loss. Data from the clinical trial on number of clinic visits completed, number of procedures performed (e.g., OCT, fundus photographs), and number of aflibercept injections will be used to estimate an average cost per patient for each treatment arm, using the Medicare Fee Schedule to estimate medical costs. The cost estimates, in combination with the percentage of productivity loss for each treatment arm, will be incorporated into the analysis. The following will be analyzed by treatment group:

- Mean change from baseline in the percentage of work time missed due to vision problems over the past week (Absenteeism score)
  - Tabulated without statistical comparison
- Mean change from baseline in the percentage of impairment while working due to vision problems over the past week (Presenteeism score)
  - Tabulated without statistical comparison
- Mean change from baseline in the percentage of overall work impairment due to vision problems over the past week (Work Productivity Loss score)
- Mean change from baseline in the percentage of activity impairment due to vision problems over the past week (Activity Impairment score)

For functional outcomes measured at the participant level, bilateral participants are non-informative with respect to the treatment comparison and will not be included in the analyses.

7.6 OCT Angiography Ancillary Study

At a subset of sites with OCT angiography capabilities, images will be taken at baseline and at least one annual visit. Features evident on OCT angiography alone will not be used for the primary outcome determination. Exploratory analyses of OCT angiography may include, but are not limited to, the following:

1. Comparison with current imaging modalities for detection of diabetic retinopathy pathology.
2. Identification of biomarkers at baseline that are associated with retinopathy progression.
3. Comparison of different OCT angiography systems at sites with more than one available.

7.7 Safety Analysis Plan

7.7.1 Ocular Adverse Events

The following ocular adverse events are of primary interest:

- Endophthalmitis
- Retinal detachment
- Traumatic cataract
- Vitreous hemorrhage
- Inflammation
- Neovascular glaucoma
- Iris neovascularization

The ocular adverse events of primary interest will be tabulated by treatment group. In addition, a tabulation will be made for non-study eyes receiving study aflibercept. The frequency of the event occurring at least once per eye will be calculated. Eye-level outcomes will be compared between treatment groups using logistic regression with GEE to account for the potential correlation within participants having two study eyes.

7.7.2 Systemic Adverse Events

Systemic adverse events will be reported in three groups: (1) unilateral participants randomized to sham, (2) unilateral participants randomized to aflibercept, and (3) bilateral study participants. The frequency of the event occurring at least once per participant will be calculated. However, statistical comparisons for systemic adverse events will only include unilateral participants randomized to sham and unilateral participants randomized to aflibercept. Analysis of participant-level adverse events will be conducted with Barnard's Unconditional Exact Test.

- Primary systemic adverse events of interest:
  - Death
  - Serious adverse event (proportion of participants with at least one)
  - Hospitalization (proportion of participants with at least one)
  - Cardiovascular and cerebrovascular events according to Antiplatelet Trialists' Collaboration (excerpted from BMJ Jan 8, 1994):

- Non-fatal myocardial infarction
- Non-fatal stroke (counted only if symptoms lasted at least 24 hours)
- Death of unknown cause
- Death attributed to cardiac, cerebral, hemorrhagic, embolic, or other vascular cause (does not need to be ischemic in origin)

Note that transient ischemic attack, angina, possible myocardial infarction, and possible stroke are not counted. Non-fatal myocardial infarction and non-fatal stroke require that the participant is alive at the end of the study. If not, then only the death is counted.

- Secondary systemic adverse events of interest to be tabulated without statistical comparison:
  - Hypertension
  - Frequency of at least one event per participant in each Medical Dictionary for Regulatory Activities (MedDRA) system organ class

Sensitivity analyses will replicate the analyses above within two groups: (1) participants who received study aflibercept in either eye and (2) participants who did not receive study aflibercept in either eye.

A tabulation of all study eye ocular, non-study eye ocular, and systemic adverse events by primary treatment groups will be created.

7.8 Additional Tabulations and Analyses

The following will be tabulated according to treatment group:

1) Baseline demographic and clinical characteristics
2) Visit completion rate
3) Treatment adherence

CHAPTER 8. REFERENCES

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