Diabetic Retinopathy Clinical Research Network

A Comparative Effectiveness Study of Intravitreal Aflibercept, Bevacizumab and Ranibizumab for Diabetic Macular Edema

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CHAPTER 1.
BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.1 Background Information

1.1.1 Public Health Impact of DME

The age-adjusted incidence of diabetes mellitus in the United States has reportedly doubled in recent history,[1] and estimates suggest that by the year 2030, approximately 439 million individuals worldwide will be affected by this chronic disease.[2] The increasing global epidemic of diabetes implies an associated increase in rates of vascular complications from this chronic disease, including diabetic retinopathy. Despite advances in diagnosis and management of ocular disease in diabetic patients, eye complications from diabetes mellitus continue to be the leading cause of vision loss and new onset blindness in working-age individuals throughout the United States.[3]

Diabetic macular edema (DME) is a manifestation of diabetic retinopathy that produces loss of central vision.[4] In a review of three early studies concerning the natural history of diabetic macular edema, Ferris and Patz found that 53% of 135 eyes with DME, presumably all involving the center of the macula, lost two or more lines of visual acuity over a two year period.[5] Without intervention, 33% of 221 eyes included in the Early Treatment Diabetic Retinopathy Study (ETDRS) with center-involved DME experienced "moderate visual loss" (defined as a 15 or more letter score decrease in visual acuity) over a three year period.[6]

1.1.2 Rationale for Anti-VEGF Treatment for DME

Diabetic macular edema results from abnormal leakage of fluid and macromolecules, such as lipoproteins, from retinal capillaries into the extravascular space. This is followed by an influx of water into the extravascular space due to increased oncotic pressure.[7] The retinal pigment epithelium normally acts as a barrier to fluid flow from the choriocapillaris to the retina and also actively pumps fluid out of the retina. Thus, abnormalities in the retinal pigment epithelium may contribute to diabetic macular edema by allowing increased fluid access from the choriocapillaries or decreasing the normal efflux of fluid from the retina.[7] The mechanism of breakdown of the blood retina barrier at the level of the retinal capillaries and the retinal pigment epithelium may be mediated by changes in tight junction proteins such as occludin.[8]

Vascular endothelial growth factor (VEGF), a 45 kD homodimeric glycoprotein, potently increases retinal capillary permeability and subsequent retinal edema in part by inducing breakdown of the blood retina barrier.[9]

1.1.3 Evolution of Standard Therapy for DME

For the past 25 years, focal/grid laser photocoagulation had been the mainstay of treatment for DME. In the ETDRS, focal/grid photocoagulation of eyes with DME reduced the risk of moderate visual loss by approximately 50% (from 24% to 12%) three years after initiation of treatment.[10] A modified ETDRS focal/grid photocoagulation protocol (M-ETDRS) adapted from the original ETDRS approach has been adopted as the standard laser technique for DME used in DRCR.net studies. A study conducted by DRCR.net, A Randomized Trial Comparing Intravitreal Triamcinolone Acetonide and Focal/grid Photocoagulation for DME (DRCR.net Protocol B), showed that efficacy over 2 years of use with the M-ETDRS focal/grid laser technique was comparable to results in similar eyes in the ETDRS, and that intravitreal

triamcinolone as monotherapy was not superior to use with the M-ETDRS focal/grid laser technique for central-involved DME in eyes with some visual acuity loss.[11,12]

Recent results from a DRCR.net study ("Intravitreal Ranibizumab or Triamcinolone Acetonide in Combination with Laser Photocoagulation for Diabetic Macular Edema"[DRCR.net Protocol I]) indicate that treatment for DME with intravitreal anti-vascular endothelial growth factor (anti-VEGF) therapy (0.5 mg ranibizumab) plus deferred (≥24 weeks) or prompt focal/grid laser provides visual acuity outcomes at one year and two years that are superior to prompt focal/grid laser alone or intravitreal triamcinolone with prompt focal/grid laser,[13] providing definitive confirmation of the important role of VEGF in DME and the role of anti-VEGF drugs in the treatment of DME. The study enrolled 854 study eyes of 691 study participants with DME involving the fovea and with visual acuity (approximate Snellen equivalent) of 20/32 to 20/320. Eyes were randomized to sham injection+prompt focal/grid laser (N = 293), 0.5-mg ranibizumab+prompt laser (within 3-10 days, N = 187), and 0.5-mg ranibizumab+deferred laser (deferred for at least 24 weeks, N = 188). Treatment with ranibizumab was generally continued on a monthly basis unless the participant's vision stabilized or reached 20/20, or the retinal swelling resolved. Treatment could be stopped if failure criteria were met (persistent swelling with poor vision), but this occurred in very few participants (less than 5% in any group). The mean change (± standard deviation) in visual acuity letter score at one year from baseline was significantly greater in the ranibizumab+prompt laser group (+9 ± 11) and the ranibizumab+deferred laser group (+9 ± 12) as compared with the control laser group (+3 ± 13, $P < 0.001$ for both comparisons) or triamcinolone+prompt laser group (+4 ± 13, $P < 0.001$ for both comparisons). The one-year optical coherence tomography (OCT) results paralleled the visual acuity results in the ranibizumab and control laser groups. No apparent increases in treatment-related systemic events were observed.

These results provided definitive confirmation of the promising role of ranibizumab therapy suggested by phase 2 trials,[14, 15].and have been further supported by findings from additional phase 3 trials, including the RISE, RIDE[16] and RESTORE[17]studies. Participants in RISE and RIDE were randomized to 0.5 or 0.3 mg ranibizumab versus sham injections as treatment for DME with macular laser available to all treatment arms. The percentage of individuals gaining ≥ 15 letters from baseline at 24 months was significantly higher in the ranibizumab groups in both studies (RISE: sham- 18.1%, 0.3mg ranibizumab- 44.8%, 0.5mg ranibizumab 39.2%; RIDE sham- 12.3%, 0.3mg ranibizumab- 33.6%, 0.5mg ranibizumab 45.7%). Neither the 0.3 mg or 0.5 mg was consistently shown to have a greater benefit compared with the other in terms of visual outcomes across the two studies. In RESTORE, both ranibizumab (0.5mg) monotherapy and combination ranibizumab+laser treatment resulted in better visual acuity outcomes than laser alone in patients with DME. The percentage of participants gaining ≥ 15 letters from baseline at month 12 were 22.6%, 22.9% and 8.2% in the ranibizumab alone, ranibizumab+laser and laser alone groups, respectively. In general, ranibizumab therapy was well-tolerated in these studies although the overall rate of Antiplatelet Trialists' Collaboration events was slightly higher in the 0.3 mg (5.6%) and 0.5 mg (7.2%) groups as compared with the sham group (5.2%) in the pooled data from the RISE and RIDE studies. Deaths were also more frequent in the ranibizumab groups (0.8% and 1.6% of sham and 2.4-4.8% of ranibizumab treated patients) in these trials. The rate of non-fatal cerebrovascular events in this pooled analysis was numerically higher in the 0.5mg group (2%) than in the sham (1.2%) or 0.3mg group (0.8%) but the rate of non-fatal myocardial infarctions was similar across treatment groups (2.8%, 2.8% and 2.4% in the sham,

0.3mg and 0.5mg groups, respectively). In August 2012, the U.S. Food and Drug Administration approved 0.3 mg ranibizumab (Lucentis) for treatment of DME.

It is expected that retina physician practice patterns with regard to treatment of center-involved DME will change in response to the results from Protocol I and these other trials with a corresponding rise in the nationwide use of anti-VEGF therapy for DME. This is especially true given the widespread influence of previous DRCR.net studies on U.S. practice patterns for treatment of DME (e.g., the marked drop in nationwide use of intravitreal steroid for DME after the publication of the DRCR.net Protocol B primary outcome results[11]). Although ranibizumab plus prompt or deferred laser has clearly demonstrated efficacy over focal/grid laser treatment alone for center-involved DME, its clinical use may divert limited resources of physicians and payors by its high cost and the need for multiple injections at frequent (monthly) dosing intervals when bevacizumab is available and when bevacizumab has been shown potentially to be efficacious in the treatment of DME.[18] Furthermore, prioritizing resources from a public health policy perspective could be easier if more precise estimates regarding the risks and benefits of other anti-VEGF therapies were available. Thus, there is a clear rationale at this time to explore potential anti-VEGF alternatives to ranibizumab that might prove to be as efficacious or more efficacious, might prove to deliver equally lasting or longer-lasting treatment effects, and cost substantially less.

1.1.4 Alternative (Non-Ranibizumab) Anti-VEGF Drugs

Several anti-VEGF agents exist that might serve as an alternative to ranibizumab, including bevacizumab (Avastin, Genentech, Inc.), pegaptanib (Macugen, Eyetech Pharmaceuticals) and aflibercept (Eylea, Regeneron, Inc.). Bevacizumab is a full-length recombinant humanized monoclonal antibody that, in contrast to pegaptanib's isoform-specific actions, blocks all isoforms of VEGF-A. It shares a similar molecular structure with ranibizumab, which was designed as a monoclonal antibody fragment from the same parent murine antibody. It was originally approved by the Food and Drug Administration (FDA) as a systemic therapy for the treatment of metastatic colorectal cancer and has subsequently been approved for the treatment of non-squamous non-small cell lung cancer, glioblastoma, and metastatic renal cell carcinoma.[19] The FDA also initially granted approval of bevacizumab for the treatment of metastatic breast cancer, but the agency subsequently recommended removal of the breast cancer indication from the drug's label after an independent advisory committee determined that the drug has not been shown to be safe and effective for that use.[20] Bevacizumab has been used widely in clinical practice for DME but has not been extensively studied in large scale, randomized controlled trials for this indication. Pegaptanib is an aptamer consisting of a pegylated modified oligonucleotide which binds to extracellular VEGF isoform 165 (the predominant isoform) and is approved for the treatment of neovascular age-related macular degeneration. Pegaptanib has been studied in phase 2 trials for DME, and results have demonstrated some ability to decrease edema compared with no treatment, although the magnitude of the effect did not appear to be similar to that reported with ranibizumab.[21, 22]

Aflibercept is a fully human, soluble VEGF receptor fusion protein that binds all isoforms of VEGF-A in addition to Placental Growth Factor and is approved by the FDA for the treatment of neovascular age related macular degeneration. Aflibercept has been evaluated in Phase 2 clinical trials of DME and is currently being investigated in phase 3 clinical trials for DME.

1.1.5 Efficacy and Safety of Alternative Anti-VEGF Agents for DME Treatment

1.1.5.1 Bevacizumab

In 2007, the DRCR.net reported results from a phase two randomized clinical trial that suggested intravitreal bevacizumab treatment had an effect on the reduction of DME in some eyes (Protocol H).[15] Study eyes were randomized to one of five treatment groups: macular laser alone, 1.25 mg bevacizumab at baseline and six weeks, 2.5 mg bevacizumab at baseline and 6 weeks, 1.25 mg bevacizumab at baseline only, or 1.25 mg bevacizumab at baseline and 6 weeks and macular laser at 3 weeks. At three weeks, there was a reduction of OCT central subfield thickness > 11% (reliability limit) in 36 of 84 (43%) eyes treated with any bevacizumab. Compared with the eyes in the laser control group, both the 1.25 and 2.5 mg bevacizumab-treated eyes had a greater reduction in central retinal thickness at 3 weeks, although there was no statistically significant difference between the groups after the 3 week time point. The Pan-American Collaborative Retina Group (PACORES) also reported an apparent benefit of bevacizumab treatment for DME in a retrospective review of data from 101 eyes of 82 patients, with statistically significant improvements from baseline in best corrected visual acuity and central macular thickness that were sustained over 12 months.[23] A Prospective Randomized Trial of Intravitreal Bevacizumab or Laser Therapy in the Management of Diabetic Macular Edema (BOLT study) randomized 80 eyes from 80 study participants to intravitreal bevacizumab (given every six weeks with a minimum of three injections in the first 12 months) or macular laser treatment and found that whereas the bevacizumab group gained a median of eight letters in visual acuity over 12 months, the laser group lost a median of 0.5 letters over the same time period ($P = 0.0002$).[18] Central macular thickness also decreased to a greater extent in the bevacizumab group as compared with the laser group (mean change $\pm$ SD: -130 $\pm$ 122 versus -68 $\pm$ 171 µm).

Data from comparative efficacy studies directly comparing bevacizumab to ranibizumab for treatment of neovascular macular degeneration suggest that the two drugs may have similar efficacy as therapy for this non-diabetic disease process. Both 1 and 2 year results from the Comparison of Age-Related Macular Degeneration Treatments Trial (CATT)[24]demonstrated that mean gain in visual acuity was similar for bevacizumab versus ranibizumab treated eyes with neovascular age-related macular degeneration, although anatomic measures such as proportion of eyes without fluid at 2 years and mean decrease in central retinal thickness at 1 year appeared more favorable in the ranibizumab-treated groups. One year results from another head to head comparison of ranibizumab to bevacizumab for neovascular age-related macular degeneration, the IVAN trial, were inconclusive, demonstrating neither inferiority nor equivalence of bevacizumab to ranibizumab using a 3.5 letter limit (Mean acuity of bevacizumab minus ranibizumab group = -1.99 letters, (95% CI, -4.04 to 0.06).[25] Two year safety data from the CATT study did not reveal significant differences in rates of arterial thromboembolic events or death between bevacizumab and ranibizumab treated participants. Overall rates of serious adverse events, however, were higher among bevacizumab-treated patients (39.9%) than ranibizumab-treated patients (31.7%), with the greatest imbalance in gastrointestinal disorders not previously linked to anti-VEGF therapy. In contrast, at 1 year in the IVAN study, fewer arteriothrombotic events or heart failure cases were seen in the bevacizumab treated group and there was no difference in the percentage of patients experiencing serious adverse events between the treatment groups. A large retrospective cohort study of 146,942 Medicare beneficiaries being treated for age-related macular degeneration found no significant difference in rates of all cause mortality, incident myocardial infarction, bleeding, and incident stroke in

patients treated with bevacizumab versus ranibizumab in a subgroup analysis that included only practices that exclusively used one or the other of these two drugs.[26]

In diabetic patients intravitreal bevacizumab appears to have a reasonably good safety profile overall with regard to ocular and systemic adverse events. No increased rates of thromboembolic events or death in bevacizumab versus control groups have been reported in smaller, prospective randomized studies including the DRCR.net Protocol H or the BOLT study.[18] Retrospective, observational data from larger patient groups also does not appear to indicate an increased risk of ocular or systemic events with intravitreal bevacizumab treatment. In 2006, an internet-based survey of 70 international sites from 12 countries was reported that described outcomes after 7,113 injections given to 5,228 patients. Rates were 0.21% or less for each category of doctor-reported adverse events, including blood pressure elevation, transient ischemic attack, cerebrovascular accident, death, endophthalmitis, retinal detachment, uveitis, or acute vision loss.[27] The PACORES group reported 12 month safety of intravitreal injections of 1.25 and 2.5 mg doses of bevacizumab given for a variety of conditions in a large group of study participants including 548 patients with diabetes.[28] A total of 1,174 patients were followed for at least 1 year. Systemic adverse events were reported in 1.5% (N = 18); including elevated blood pressure in 0.6% (7), cerebrovascular accidents in 0.5% (6), myocardial infarctions in 0.4% (5), iliac artery aneurysms in 0.2% (2), toe amputations in 0.2% (2), and deaths in 0.4% (5) of patients. The overall mortality rate of diabetic patients in this study was low at 0.55% (3/548). Ocular complications were reported as bacterial endophthalmitis in 0.2% (7), traction retinal detachments in 0.2% (7), uveitis in 0.1% (4), and a single case each of rhegmatogenous retinal detachment and vitreous hemorrhage.

1.1.5.2 Pegaptanib

In a phase 2 trial of pegaptanib for the treatment of diabetic macular edema, intravitreal pegaptanib (0.3mg, 1mg, or 3mg) or sham injection was administered every 6 weeks for 12 weeks with the option of subsequent doses and/or focal laser photocoagulation thereafter (N = 172). At week 36, 73% of those treated with pegaptanib gained $\geq$ 0 lines of vision compared with 51% of the sham group (P = 0.02); 18% of treated patients gained $\geq$ 3 lines of vision compared with 7% of the sham group (P =0.12). Central retinal thickness decreased 68μm in the 0.3mg group compared with 3.7μm in the sham group (P = 0.021); a decrease in central retinal thickness of $\geq$100 μm was demonstrated in 42% of patients in the 0.3mg group compared with 16% in the sham group (P = 0.02). Twenty-five percent of patients in the 0.3mg group underwent laser photocoagulation compared with 48% in the sham group. One case of endophthalmitis (not associated with severe vision loss) was observed.[29]

A subsequent phase three study enrolled 260 study participants from 56 sites worldwide who were randomized to 0.3 mg intravitreal pegaptanib injections versus sham every six weeks for one year, followed by as needed dosing for a second year.[30] Up to three macular laser treatments were allowed per year beginning at week 18. Study participants who received pegaptanib treatment were significantly more likely to gain two or more lines of vision at two years than study participants who only received sham (37% vs. 20%, P = 0.005). The mean visual acuity gain at two years was 6.1 letters in the pegaptanib group versus 1.2 letters in the sham group (P = 0.01). Cardiac disorders were present at a slightly greater rate in the pegaptanib versus sham group (6.9% versus 5.6%). However, no deaths were related to use of the study drug.

1.1.5.3 VEGF Trap

Intravitreal aflibercept injection, also known as VEGF Trap Eye or Aflibercept (Eylea) 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. This drug was first reported as possible treatment for DME in 2009 in phase one study that enrolled five study participants with center involved DME.[31] After a single injection of 4.0 mg VEGF Trap-Eye, five out of five eyes demonstrated reduction in retinal thickening at four weeks which was maintained in 4/5 eyes at six weeks. There was a median improvement in visual acuity of nine and three letters at four and six weeks, respectively. No ocular toxicity was seen over the six week observation period. Results from a larger, phase two trial have been subsequently published.[32] In this study, 221 participants with center-involved DME were randomized to one of five groups: macular laser therapy, 0.5 mg aflibercept every four weeks, 2 mg aflibercept every four weeks, 2 mg aflibercept every four weeks times 3 doses followed by every 8-week dosing or 2 mg aflibercept every four weeks times three doses followed by as needed dosing. Eyes that received aflibercept had greater mean improvement in visual acuity from baseline at week 24 as compared with eyes that received macular laser (8.5-11.4 letter score increase versus a 2.5 letter score increase). Ocular adverse events were similar to those reported in other trials involving intravitreal injections. Two cases of endophthalmitis and one case of uveitis occurred (all in aflibercept treatment groups). Three participants out of 175 in the VEGF Trap-Eye groups experienced arterial thromboembolic events as compared with 0/44 participants treated with laser. In addition, three VEGF Trap-Eye treated individuals died (of renal failure, myocardial infarction and "sudden death") as compared with no study participants treated with laser.

Aflibercept received approval in November 2011 by the United States Food and Drug Administration for the treatment of neovascular age-related macular degeneration (AMD) at a recommended dose of 2 mg every 4 weeks for the first 12 weeks, followed by 2 mg every8 weeks thereafter.[33] This approval was based on results from two Phase three clinical trials (VIEW 1 and VIEW 2) that assigned participants with neovascular AMD one of four dosing regimens: ranibizumab 0.5 mg every four weeks, aflibercept 2 mg every four weeks, aflibercept 0.5 mg every four weeks, and aflibercept 2 mg given every eight weeks following three initial monthly doses.[34] All three regimens of aflibercept demonstrated non-inferiority to monthly ranibizumab in terms of the proportion of subjects who lost fewer than a 15 letter score from baseline. All aflibercept treatment groups gained vision from baseline to one year, with mean gains ranging from 7.6 to 10.9 letter score across the two studies. Serious ocular adverse events, including endophthalmitis, occurred at rates <0.1% per injection in both studies and there did not appear to be a dose or drug-related increase in Anti-Platelet Trialists' Collaboration events in either study.

1.1.6 Scientific Rationale for a Comparative Effectiveness Study of Aflibercept, Bevacizumab and Ranibizumab for DME

Of all the currently available alternative anti-VEGF agents, bevacizumab has the closest molecular structure to ranibizumab, since they are derived from the same monoclonal antibody. Thus, there is scientific rationale to believe that the two drugs may have similar efficacy and safety when used as treatment for DME.

However, some preclinical comparisons between bevacizumab and ranibizumab have demonstrated potential differences between the two agents. Klettner and Roider showed that at clinically relevant concentrations (bevacizumab 0.25 mg/mL and ranibizumab 0.125 mg/mL),

both drugs were highly effective at neutralizing VEGF expression from porcine retina-retinal pigment epithelium choroid organ culture and retina-retinal pigment epithelium cell culture, although when the drugs were diluted, ranibizumab was more efficient at VEGF neutralization than bevacizumab at lower concentrations.[35] Another study examined the effect of bevacizumab and ranibizumab on human microvascular endothelial cells, and found that although there was a strong decrease in VEGF release with both agents, reduction of phosphorylated Erk, cellular migration, capillary formation and phosphorylated VEGFR2 expression were more significantly reduced with bevacizumab treatment.[36] In contrast, proliferation was more strongly affected by ranibizumab treatment.

Other, clinical studies however, suggest that the two agents may have similar efficacy in treating intraocular neovascularization in humans. A small, randomized, prospective study comparing the two agents for treatment of choroidal neovascularization for pathologic myopia did not find a statistically significant difference between the two, although a relatively small number (N = 32) of eyes were enrolled.[37] Another larger, retrospective case series of 452 participants with neovascular AMD found that 22.9% of bevacizumab-treated versus 25% of ranibizumab-treated patients achieved visual acuity of 20/40 or better at 12 months; while neither drug was shown to be superior to the other, the data also suggested that bevacizumab was non-inferior to ranibizumab.[38] The Comparison of the Age-related Macular Degeneration Treatment Trials: Lucentis-Avastin Trial (CATT) is a National Eye Institute-sponsored ongoing multi-center, prospective randomized clinical trial comparing bevacizumab to ranibizumab for treatment of neovascular AMD. One year results were published in May, 2011 and revealed that bevacizumab administered monthly was equivalent to ranibizumab administered monthly for the primary outcome of mean change in visual acuity at one year (an average of 8.0 and 8.5 letter scores were gained in the bevacizumab and ranibizumab groups respectively).[39] Equivalent visual outcomes were also seen between the groups treated with bevacizumab as needed and ranibizumab as needed and between the ranibizumab group treated monthly and the ranibizumab group treated as needed. In contrast, the comparison of monthly bevacizumab and bevacizumab as needed was inconclusive. Rates of thromboembolic events including death, myocardial infarction and stroke were similar in the bevacizumab and ranibizumab treated groups. Although a higher rate of serious systemic adverse events was present in the bevacizumab group, the excess events in this group were primarily hospitalizations due to events not previously attributed to anti-VEGF treatment. Even though this trial showed that bevacizumab is non-inferior to ranibizumab in neovascular AMD for vision outcomes and appears to have a similar safety profile, it does not mean that similar vision outcomes and safety would apply to bevacizumab in central DME; use of bevacizumab in DME might be non-inferior or superior to ranibizumab; bevacizumab might appear as safe or not as safe.

Data from the DRCR.net study Protocol I and the BOLT study suggest that the two drugs potentially have similar efficacy for DME treatment. At one year after baseline, study participants who received 1.25 mg bevacizumab in the BOLT study had median improvement in visual acuity of 8 letters, compared with 9 letters in the study participants who received 0.5mg ranibizumab+deferred laser in the DRCR.net Protocol I. However, these results are not directly comparable especially given the small number of patients enrolled in the BOLT study.

There is also evidence, from two phase two studies comparing VEGF Trap-eye to ranibizumab (VIEW I and VIEW II) performed for treatment of neovascular AMD, that aflibercept may have a similar efficacy to ranibizumab for at least some types of retinal vascular pathology. In the

VIEW I and VIEW II trials, aflibercept was equivalent in efficacy to ranibizumab in stabilizing visual acuity (as measured by percentages of study participants with less than a 15 letter score visual acuity loss at week 52 as compared with baseline) in patients with neovascular AMD. In the combined analysis of VIEW 1 and VIEW 2, average visual acuity gain from baseline in the eyes treated with aflibercept 2 mg every eight weeks (after three initial monthly injections) was 8.4 and 7.6 letters at weeks 52 and 96, respectively, as compared with 8.7 and 7.9 letter score gains at weeks 52 and 96, respectively in the ranibizumab-treated eyes .[40] Although these results demonstrate equivalent efficacy for treatment of neovascular AMD with aflibercept and ranibizumab, aflibercept has not yet been directly compared to ranibizumab for treatment of DME and so it is unclear at this time whether the AMD aflibercept study results will be generalizable to diabetic retinal disease.

Although there is evidence to suggest that pegaptanib may have some efficacy in the treatment of DME, this compound may not have equal efficacy as compared with ranibizumab, bevacizumab or aflibercept for DME since it selectively inhibits the VEGF isoform 165 rather than serving as a nonspecific inhibitor of VEGF function. Although it is not possible to directly compare results across studies given different patient cohorts and treatment algorithms, the mean letter score improvements in the currently available studies may suggest a trend for less visual improvement with pegaptanib than with ranibizumab. Furthermore, pegaptanib appears to be less effective for the treatment of neovascular macular degeneration than ranibizumab. For these reasons, pegaptanib has not been selected for evaluation in this trial.

There is, however, excellent rationale to compare the efficacy and safety of ranibizumab, bevacizumab, and aflibercept for the treatment of DME. Considering there have been no studies directly comparing any of these three anti-VEGF treatments for DME a large prospective multicenter randomized trial is necessary to compare the effect of these agents.

1.1.7 Public Health Implications of Bevacizumab as an Alternative to Ranibizumab

In addition to the scientific evidence that suggests bevacizumab may have short-term efficacy in treating DME and potentially similar effects to ranibizumab on intraocular pathology in general, there is an additional, compelling socioeconomic reason to explore the use of bevacizumab as an alternative to ranibizumab in the treatment of DME. There is a considerable cost difference between the two agents. Although actual costs may vary across the nation and at different centers, it is estimated that a single dose of 0.5 mg ranibizumab costs approximately $1,950, a single dose of 0.3 mg ranibizumab costs approximately $1,200, a single dose of 2 mg aflibercept costs approximately $1,850, and a comparable dose of 1.25 mg bevacizumab costs approximately $50-$100 to prepare when compounded from a vial approved for metastatic colon cancer. Given the current recommended treatment regimen of monthly dosing, and the fact that multiple injections are generally needed for effective treatment of DME over the long term, the establishment of bevacizumab as a safe, effective, and much lower cost alternative to ranibizumab in the treatment of DME would have substantial implications for public policy in terms of future estimates of health care dollars devoted to anti-VEGF DME treatment, and might be extrapolated to anti-VEGF treatment for other causes of macular edema, such as retinal vein occlusions.[41, 42]

Although it was not formulated specifically for use within the eye, because of its availability and lower cost, bevacizumab is already currently in widespread clinical use for treatment of DME. It was first reported as being used off-label as an intravitreal injection to treat DME in 2006.[43]

Since then, the drug has been utilized extensively in the clinical treatment of recalcitrant DME despite the lack of FDA approval for this indication. More than 32% (N = 138) of retina specialists surveyed for the 2009 American Society of Retina Specialists (ASRS) Preferences and Trends Survey said that Avastin (bevacizumab) would be their first choice therapy for a patient with "diffuse, center-involved DME who had had one unsuccessful grid laser treatment."[44] Thus, a clinical trial that definitively answered whether bevacizumab could be used as a safe and efficacious alternative to ranibizumab could substantially impact nationwide practice patterns for treatment of DME by either validating the current use of bevacizumab or by demonstrating improved outcomes with ranibizumab treatment for DME.

Although the cost differential between ranibizumab and aflibercept is much smaller than that between bevacizumab and ranibizumab, the ability to substitute aflibercept for ranibizumab might still save public health care dollars when spread over multiple treatments for many patients, especially if dosing requirements are less frequent for aflibercept use. Aflibercept has not commonly been used outside of clinical trials for treatment of DME, however, the finding that visual acuity outcomes in DME are superior with aflibercept as compared with either bevacizumab or ranibizumab might substantially change standard care practice in management of DME.

1.1.8 Bevacizumab Dosing

The bevacizumab dose most commonly used in clinical practice in the United States is 1.25 mg in 0.05 ml. This dose was initially derived from consideration of the molecular weight and binding affinity differences between ranibizumab and bevacizumab, as well as the differences in presumed retinal penetration. It is estimated that 1.25 mg of bevacizumab may be roughly equivalent to 0.3 to 0.5 mg of ranibizumab in terms of the number and affinity of the binding sites that are delivered to the eye.[45] A 2.5 mg dose has also been used clinically, although dose ranging studies, including the DRCR.net Protocol H and the PACORES study have not found a substantial difference in treatment effect for DME between 1.25 and 2.5 mg doses.[15, 23]

1.1.9 Summary of Rationale for the Study

Although multiple studies have suggested that treatment with ranibizumab is safe and efficacious and superior to focal/grid laser alone for patients with center-involved DME, there may be barriers in place to widespread adoption of ranibizumab use given its high cost per dose and the need for multiple treatments over time. Prioritizing resources from a public health policy perspective could be easier if more precise estimates regarding the risks and benefits of other anti-VEGF therapies were available, especially when the difference in costs could be billions of dollars over just a few years. Thus, there is a clear rationale at this time to explore potential anti-VEGF alternatives to ranibizumab that might prove to be as or more efficacious, might deliver equally lasting or longer-lasting treatment effects, and cost substantially less. Of the potentially available alternative anti-VEGF agents for this trial, bevacizumab and aflibercept are the best candidates for a direct comparison study. Bevacizumab shares the most similar molecular structure, costs far less, and is widely available. Furthermore, there is already preliminary evidence to suggest that it may be efficacious in the treatment of DME and it is already being widely used for this indication. Although aflibercept is more expensive than the 0.3 mg dose of ranibizumab, evidence that supports equivalent efficacy of every 2 month dosing of aflibercept to 1 month dosing suggests that it may have the potential to decrease treatment burden and thus overall associated costs. If results from a comparative trial demonstrate improved efficacy or suggest similar efficacy of bevacizumab or aflibercept over ranibizumab, this information might

give clinicians scientific rationale to substitute either one of these drugs for ranibizumab in the treatment of DME, and might thereby have substantial implications for public policy in terms of future estimates of health care dollars and possibly number of treatments necessary for anti-VEGF treatment of diabetic macular disease.

Because of its availability and lower cost, bevacizumab is already currently in widespread clinical use for treatment of DME despite the lack of FDA approval for this indication. Thus, a clinical trial that suggested whether bevacizumab could be used as a safe and efficacious alternative to ranibizumab could substantially impact nationwide practice patterns for treatment of DME by either validating the current use of bevacizumab or by demonstrating improved outcomes with ranibizumab or aflibercept treatment for DME.

1.2 Study Objective

- The primary objective of the proposed research is to compare the efficacy and safety of (1) intravitreal aflibercept, (2) intravitreal bevacizumab, and (3) intravitreal ranibizumab when given to treat central-involved DME in eyes with visual acuity of 20/32 to 20/320.

1.3 Study Design and Synopsis of Protocol

A. Study Design

- Randomized, multi-center clinical trial.

B. Major Eligibility Criteria

- Age ≥18 years.
- Type 1 or type 2 diabetes.
- Central-involved DME in study eye (OCT CSF ≥250 µm on Zeiss Stratus or the equivalent on spectral domain OCT based on gender specific cutoffs) within eight days of randomization.
- Visual acuity letter score in study eye ≤ 78 and ≥24 (approximate Snellen equivalent 20/32 to 20/320) within eight days of randomization.
- No history of an anti-VEGF treatment for DME in the past 12 months in the study eye and no history of any other treatment for DME in the study eye at any time in the past four months (such as focal/grid macular photocoagulation, intravitreal or peribulbar corticosteroids).
    - Enrollment will be limited to a maximum of 25% of the planned sample size with any history of anti-VEGF treatment in the study eye. Once this number of eyes has been enrolled, any history of anti-VEGF treatment in the study eye will be an exclusion criterion.
- No history of major ocular surgery in the study eye within prior four months or anticipated within the next six months following randomization.

C. Treatment Groups

Study participants will be assigned randomly to one of the following three groups:

1) 2.0 mg intravitreal aflibercept
2) 1.25 mg intravitreal bevacizumab

3) 0.3 mg intravitreal ranibizumab

Study participants can have only one study eye. If both eyes are eligible at the time of randomization and one of the eyes has never received anti-VEGF treatment, that eye should be randomized. If both eyes are eligible and have previously received anti-VEGF treatment or both eyes have never received anti-VEGF then the study eye will be selected by the investigator and the participant before randomization. Further details on randomization are located in section 2.4.

The treatment schedule and criteria for retreatment are described in section four.

D. Sample Size

- The sample size is 660 study eyes (220 eyes per group)

E. Duration of Follow-up

- Duration of follow-up is 2 years with the primary outcome at one year

F. Follow-up Schedule

- Follow-up visits occur every four weeks up to the one year visit
- After one year, visits occur every 4 to 16 weeks depending on disease progression and treatment administered
- All participants will have follow-up visits at 1 and 2 years
- Participants will be requested to complete one optional visit 2-3days (+/- 1 day if the participant cannot return within 2-3 days) after either the first, second, or third injection

G. Main Efficacy Outcomes

Primary: Change in visual acuity from baseline to one year adjusted for baseline visual acuity.

Secondary:

- Change in visual acuity at four months
- Change in visual acuity at 2 years
- Number of intravitreal injections given per protocol
- Proportion of eyes with two and three line gains or losses in visual acuity
- Change in OCT central subfield thickness and retinal volume
- Proportion of eyes with OCT central subfield thickness of <250 µm on Stratus OCT (or spectral domain equivalent)
- Of eyes with non-prolific diabetic retinopathy at baseline, proportion of eyes with regression of retinopathy severity level
- Proportion receiving panretinal photocoagulation, vitrectomy, or vitreous hemorrhage
- Change in blood pressure 2-3 days (+/- 1 day) after an injection and at 1 year
- Change in albumin/creatinine ratio for microalbuminuria 2-3 days (+/- 1 day) after an injection and at 1 year

H. Main Safety Outcomes

- Injection-related: endophthalmitis, traction retinal detachment, rhegmatogenous

retinal detachment, retinal tear, cataract, intraocular hemorrhage, increased intraocular pressure.

- Ocular drug-related: inflammation, new or worsening traction retinal detachment, progression of traction retinal detachment from extramacular to macular.
- Systemic drug-related: hypertension events, kidney, gastrointestinal events, and cardiovascular events as defined by the Antiplatelet Trialists' Collaboration.

I. Schedule of Study Visits and Examination Procedures

Visit	0	4w-48w	52w	Between 52w-104w Visits Every 4-16w*	104w
E-ETDRS best corrected visual acuity [a]	X	X	X	X	X
OCT [b]	X	X	X	X	X
Eye Exam [c]	X	X	X	X	X
7-field Fundus Photography [d]	X		X		X
Blood pressure	X	X[f]	X		
Hemoglobin A1c [e]	X				
Urine Sample	X	X[f]	X		

A medical history will be elicited at baseline and an updated history at each visit. Concomitant medications will be recorded at baseline and updated at each visit. Adverse events will be recorded at each visit.

a= both eyes at each visit; includes protocol refraction in study eye at each visit. Protocol refraction in nonstudy eye is only required at baseline, 52 week and 104 week visits. E-ETDRS refers to electronic ETDRS testing using the Electronic Visual Acuity Tester that has been validated against 4-meter chart ETDRS testing.

b=study eye only

c=both eyes at baseline, 52 weeks and 104 weeks; study eye only (and nonstudy eye if treated with study drug) at all other follow-up visits. Includes slit lamp exam (including assessment of lens), measurement of intraocular pressure, and dilated ophthalmoscopy.

d=digital 7-fields or 4WF; study eye only

e=does not need to be repeated if Hemoglobin A1c is available from within the prior 3 months. If not available, can be performed within 3 weeks after randomization.

f=Participants will be asked to return for an optional visit 2-3 days (+/- 1 day) after the baseline injection to obtain a blood pressure measurement and urine sample. If the participant is unable or unwilling to return after the baseline injection he/she will be asked to return for an optional visit 2-3 (+/- 1 day) days after either of the next 2 injections to have the blood pressure measured and urine sample collected. Blood pressure will also be obtained at the first 4 week protocol visit after the post-injection blood pressure was obtained.

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 procedure manuals, Photography Testing Procedures Manual, and Study Procedures Manual) provide details of the examination procedures and intravitreal injection procedure.

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.

CHAPTER 2.
STUDY PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Identifying Eligible Study Participants and Obtaining Informed Consent

A minimum of 660 study participants are expected to be enrolled, with a goal to enroll an appropriate representation of minorities. Study participants can have only one study eye. 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 potential study participants who are considered potentially eligible for the study based on a routine-care exam, the study protocol will be discussed with the potential study participant by a study investigator and clinic coordinator. The potential study participant will be given the Informed Consent Form to read. Potential study participants will be encouraged to discuss the study with family members and their personal physician(s) before deciding whether to participate in the study.

Consent may be given in two stages (if approved by the IRB of the potential study participant). 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 participant: one for the study participant to give consent for the completion of the screening procedures and one for the study participant to give 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 or not. Thus, the investigator must not proceed to enroll an individual until he or she is convinced that the individual is eligible and will accept assignment to any of the three treatment groups.

2.2 Study Participant Eligibility Criteria

2.2.1 Participant-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 DME is so rare in this age group that the diagnosis of DME 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. Significant renal disease, defined as a 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).
    - *Individuals in poor glycemic control who, within the last four months, initiated intensive insulin treatment (a pump or multiple daily injections) or plan to do so in the next four months should not be enrolled.*
7. Participation in an investigational trial within 30 days of randomization that involved treatment with any drug that has not received regulatory approval for the indication being studied at the time of study entry.
    - *Note: study participants cannot receive another investigational drug while participating in the study.*
8. Known allergy to any component of the study drug.
9. 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.*
10. Myocardial infarction, other acute cardiac event requiring hospitalization, stroke, transient ischemic attack, or treatment for acute congestive heart failure within 4 months prior to randomization.
11. Systemic anti-VEGF or pro-VEGF treatment within four months prior to randomization or anticipated use during the study.
    - *These drugs cannot be used during the study.*
12. For women of child-bearing potential: pregnant or lactating or intending to become pregnant within the next 24 months.
    - *Women who are potential study participants should be questioned about the potential for pregnancy. Investigator judgment is used to determine when a pregnancy test is needed.*
13. Individual is expecting to move out of the area of the clinical center to an area not covered by another clinical center during the first 12 months of the study.

2.2.2 Study Eye Criteria

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

Study participants can have only one study eye. If both eyes are eligible at the time of randomization, the eye without previous intravitreal anti-VEGF treatment will be randomized. If both eyes have previously received intravitreal anti-VEGF or neither eye has previously received

intravitreal anti-VEGF, the study eye will be selected by the investigator and the participant before randomization.

The eligibility criteria for a study eye are as follows:

Inclusion

a. Best corrected E-ETDRS visual acuity letter score ≤ 78 (i.e., 20/32 or worse) and ≥ 24 (i.e., 20/320 or better) within eight days of randomization.

b. On clinical exam, definite retinal thickening due to diabetic macular edema involving the center of the macula.

c. Diabetic macular edema present on OCT (central subfield thickness on OCT ≥250 µm on Zeiss Stratus or the equivalent on spectral domain OCTs based on gender specific cutoffs), within eight days of randomization.

   - *Investigator must verify accuracy of OCT scan by ensuring it is centered and of adequate quality (for Zeiss Stratus, standard deviation of center point thickness should be ≤ 10% of the center point thickness and signal strength should be ≥ 6)*

d. Media clarity, pupillary dilation, and individual cooperation sufficient for adequate fundus photographs.

Exclusions

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

e. Macular edema is considered to be due to a cause other than diabetic macular edema.
   - *An eye should not be considered eligible if: (1) the macular edema is considered to be related to ocular surgery such as cataract extraction or (2) clinical exam and/or OCT suggest that vitreoretinal interface abnormalities (e.g., a taut posterior hyaloid or epiretinal membrane) are the primary cause of the macular edema.*

f. An ocular condition is present such that, in the opinion of the investigator, visual acuity loss would not improve from resolution of macular edema (e.g., foveal atrophy, pigment abnormalities, dense subfoveal hard exudates, nonretinal condition).

g. An ocular condition is present (other than diabetes) that, in the opinion of the investigator, might affect macular edema or alter visual acuity during the course of the study (e.g., vein occlusion, uveitis or other ocular inflammatory disease, neovascular glaucoma, etc.).

h. Substantial cataract that, in the opinion of the investigator, is likely to be decreasing visual acuity by three lines or more (i.e., cataract would be reducing acuity to 20/40 or worse if eye was otherwise normal).

i. History of an anti-VEGF treatment for DME in the past 12 months or history of any other treatment for DME at any time in the past four months (such as focal/grid macular photocoagulation, intravitreal or peribulbar corticosteroids).

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

j. History of pan-retinal photocoagulation within four months prior to randomization or anticipated need for pan-retinal photocoagulation in the six months following randomization.

k. History of anti-VEGF treatment for a disease other than DME in the past 12 months.

l. History of major ocular surgery (including vitrectomy, cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior four months or anticipated within the next six months following randomization.

m. History of YAG capsulotomy performed within two months prior to randomization.

n. Aphakia.

o. Exam evidence of external ocular infection, including conjunctivitis, chalazion, or significant blepharitis.

2.2.3 Non-Study Eye Criteria

If anti-VEGF treatment is indicated for any condition in the non-study eye at anytime during the study, the investigator must be willing to use the randomized anti-VEGF drug on the non-study eye. If the non-study eye is being treated with an anti-VEGF drug for any condition at the time of randomization, then the investigator and patient must be willing to switch the anti-VEGF drug currently being used to the randomized anti-VEGF drug assigned to the study eye. If the investigator or patient is unwilling to change anti-VEGF treatment in the non-study eye the patient should not be enrolled. Study participants will be masked to the treatment assignment of both the study and non-study eyes.

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

The following procedures are needed to assess eligibility and/or to serve as baseline measures for the study.

- If a procedure has been performed (using the study technique and by study certified personnel) as part of usual care, it does not need to be repeated specifically for the study if it was performed within the defined time windows specified below.
- The testing procedures are detailed in the DRCR.net Visual Acuity-Refraction Testing Procedures Manual, Photography Testing Procedures Manual, and Study Procedures Manual. Visual acuity testing, ocular exam, fundus photography, and OCT will be performed by DRCR.net certified personnel.
- The fundus photographs will be sent to the Fundus Photograph Reading Center for grading
- OCTs meeting DRCR.net criteria for manual grading will be sent to the Duke Reading Center but study participant eligibility is determined by the site (i.e., individuals deemed eligible by the investigator will be randomized without pre-randomization Reading Center confirmation).

1. Electronic-ETDRS visual acuity testing at 3 meters using the Electronic Visual Acuity Tester (including protocol refraction) in each eye. *(within eight days prior to randomization)*
    - *This testing procedure has been validated against 4-meter ETDRS chart testing.*[46]
2. OCT on study eye *(within eight days prior to randomization)*
    - *Both spectral domain and time domain machines are permitted*
    - *For a given study participant, the same machine type should be used for the duration of the study, unless circumstances do not permit (e.g., replacement of damaged machine). If a switch is necessary, the same machine type should be used for the remainder of the study.*
3. Ocular examination on each eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy *(within 21 days prior to randomization)*
4. ETDRS protocol 7-modified or 4-wide field stereoscopic fundus photography in the study eye (fields 1M, 2, 3M, 4, 5, 6, 7 and red reflex). *(within 21 days prior to randomization)*
5. Measurement of blood pressure *(see study procedures manual for collection procedure.)*
6. Laboratory Testing- Urine Sample
    - *A urine sample must be collected. See study procedures manual for collection procedure.*
7. Laboratory Testing- Hemoglobin A1c
    - *Hemoglobin A1c does not need to be repeated if available in the prior three months. If not available at the time of randomization, the individual may be enrolled but the test must be obtained within three weeks after randomization.*

2.4 Enrollment/Randomization of Eligible Study Participants

Study participants can have only one study eye.

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 baseline injection must be given on the day of randomization; therefore, a study participant should not be randomized until this is possible.
3. Randomization is completed on the DRCR.net website.
    - The study eye will be randomly assigned (stratified by site and visual acuity: ≥ 66 letter score/ ≤ 65 letter score) with equal probability to receive either:
        - 2.0 mg intravitreal aflibercept
        - 1.25 mg intravitreal bevacizumab
        - 0.3 mg intravitreal ranibizumab

CHAPTER 3.
TREATMENT REGIMENS

3.1 Introduction

The study eye is assigned to one of the three treatment groups.

The treatment groups are as follows:

- 2.0 mg intravitreal aflibercept
- 1.25 mg intravitreal bevacizumab
- 0.3 mg intravitreal ranibizumab

The initial injection will be given on the day of randomization.

Treatment procedures are described below. The timing and criteria for retreatment are outlined in chapter 4.

3.2 Intravitreal Injections

3.2.1 Intravitreal Aflibercept Injection (Eylea)

Eylea (intravitreal aflibercept injection) is made by Regeneron and is approved by the FDA for the treatment of neovascular age-related macular degeneration.

Study eyes assigned to receive aflibercept will receive a dose of 2.0 mg in 0.05 cc. The physical, chemical, and pharmaceutical properties and formulation of aflibercept are provided in the Clinical Investigator's Brochure. Aflibercept for the study and non-study eye will be distributed by the Network.

3.2.2 Bevacizumab (Avastin)

Bevacizumab (Avastin) is made by Genentech, Inc. and is approved by the FDA for the treatment of metastatic colorectal cancer as well as the treatment of non-squamous non-small cell lung cancer, glioblastoma, and metastatic renal cell carcinoma.

Study eyes assigned to receive bevacizumab will receive a dose of 1.25 mg provided by a single compounding pharmacy identified by the Network and distributed by the Network. Avastin for the non-study eye for participants with study eyes assigned to Avastin will be distributed by the Network. The volume of the injection will be 0.05 cc. The physical, chemical, and pharmaceutical properties and formulation of ranibizumab are provided in the Clinical Investigator's Brochure.

3.2.3 Ranibizumab (Lucentis™)

Ranibizumab (Lucentis™) is made by Genentech, Inc. and is approved by the FDA for the treatment of DME in a dose of 0.3 mg. A 0.5 mg dose of ranibizumab is also FDA approved for age-related macular degeneration and macular edema secondary to retinal vein occlusion.

Study eyes assigned to receive ranibizumab will receive a dose of 0.3 mg in 0.05 cc. The physical, chemical, and pharmaceutical properties and formulation of ranibizumab are provided in the Clinical Investigator's Brochure. If the study eye is assigned to ranibizumab and the non-study eye is being treated for DME, then study provided 0.3 mg ranibizumab must be used for

the treatment of the non-study eye. If the study eye is assigned to ranibizumab and the non-study eye is being treated for a condition other than DME, then study provided 0.5 mg ranibizumab must be used for treatment of the non-study eye. Both 0.3 mg ranibizumab for study eye injections and non-study eye injections for the treatment of DME, and 0.5 mg ranibizumab for treatment of conditions other than DME in the non-study eye will be distributed by the Network.

3.2.4 Intravitreal Injection Technique

The injection is preceded by a povidone iodine prep of the conjunctiva. Antibiotics in the pre-, peri-, or post-injection period are not necessary but can be used at investigator discretion if such use is part of his/her usual routine.

The injection will be performed using sterile technique. The full injection procedure is described in a DRCR.net Study Procedures Manual, including procedures to be followed when the fellow eye is receiving an injection as part of standard care for DME.

3.2.5 Deferral of Injections Due to Pregnancy

Female study participants must be questioned regarding the possibility of pregnancy prior to each injection. In the event of pregnancy, study injections must be discontinued.

3.2.6 Delay in Giving Injections

If a scheduled injection is not given by the end of the visit window, it can still be given up to one week prior to the next visit window opening. If it is not given by that time, it will be considered missed.

If an injection is given late, the next scheduled injection should occur no sooner than three weeks after the previous injection.

3.2.7 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, the non-study eye must be treated with bevacizumab if the study eye is randomized to bevacizumab, or ranibizumab if the study eye is randomized to ranibizumab, or aflibercept if the study eye is randomized to aflibercept. If the study eye is assigned to ranibizumab and the non-study eye is being treated for DME, then study provided 0.3 mg ranibizumab must be used for the treatment of the non-study eye. If the study eye is assigned to ranibizumab and the non-study eye is being treated for a condition other than DME, then study provided 0.5 mg ranibizumab must be used for the treatment of the non-study eye. When to treat the non-study eye with intravitreal anti-VEGF is at investigator discretion. However, if intravitreal anti-VEGF treatment is planned on the same day as an intravitreal 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 different intravitreal anti-VEGF injection than described above is desired in the non-study eye, a discussion with the Protocol Chair is required first.

3.3 Focal/Grid Photocoagulation

If focal/grid photocoagulation is warranted (see criteria section 4.3.2), the laser treatment 'session' should generally be completed in a single 'sitting'. The photocoagulation treatment technique, as described below, is a modification of the ETDRS technique and is the treatment approach that is commonly used in clinical practice. Use of fluorescein angiography to direct the

treatment is at the discretion of the investigator. Laser treatment following an injection, if needed, will be based on the pre-injection macular appearance.

Burn Characteristic	Focal Photocoagulation (non-PASCAL guidelines)* (DRCR.net focal/grid laser technique)
Direct Treatment	Directly treat all leaking microaneurysms in areas of retinal thickening between 500 and 3000 µm from the center of the macula (although may treat between 300 and 500 µm of macula if central-involved edema persists after initial focal photocoagulation, but generally not if the visual acuity is better than 20/40)
Change in MA Color with Direct Treatment	Not required, but at least a mild gray-white burn should be evident beneath all microaneurysms
Spot Size for Direct Treatment	50 µm
Burn Duration for Direct Treatment	0.05 to 0.1 sec
Grid Treatment	Applied to all areas with edema not associated with microaneurysms. If fluorescein angiography is obtained, grid is applied to areas of edema with angiographic nonperfusion when judged indicated by the investigator.
Area Considered for Grid Treatment	500 to 3000 µm superiorly, nasally and inferiorly from center of macula 500 to 3500 µm temporally from macular center No burns placed within 500 µm of disc
Burn Size for Grid Treatment	50 µm
Burn Duration for Grid Treatment	0.05 to 0.1 sec
Burn Intensity for Grid Treatment	Barely visible (light gray)
Burn Separation for Grid Treatment	2 visible burn widths apart
Wavelength (Grid and Direct Treatment)	Green to yellow wavelengths

**Additional guidelines for performing laser treatment using the PASCAL are included in the Procedure Manual.*

Note:

- *The investigator may choose any laser wavelength for photocoagulation within the green to yellow spectrum. The wavelength used will be recorded.*
- *Lenses used for the laser treatment cannot increase or reduce the burn size by more than 10%. The Procedure Manual contains a listing of acceptable lenses.*

CHAPTER 4.
FOLLOW-UP VISITS AND TREATMENT

4.1 Visit Schedule

The schedule of protocol-specified follow-up visits is as follows:

First Year

- Visits every 4±1 weeks (with a minimum of 21 days between visits) through 1 year

Year 2

- Visits every 4±1 weeks (with a minimum of 21 days between visits) as long as intravitreal injections are given
- Otherwise, visits every 4 to 16 weeks (±1 week windows)
  - *Note: The first two times an injection is deferred, the subject will return in 4 weeks for re-evaluation. If deferral continues, the subject will return in 8 weeks for re-evaluation before beginning the every 16 week schedule.*

Additional visits may occur as required for usual care of the study participant.

4.2 Testing Procedures

The following procedures will be performed at each protocol visit unless otherwise specified. A grid in section 1.3 summarizes the testing performed at each visit.

Visual acuity testers and OCT technicians will be masked to treatment group at the annual visits (including the primary outcome visit at 1 year). Study participants will be masked to their treatment group assignment; however, it is possible that study participants may become unmasked to treatment group assignment when discussing non-study eye anti-VEGF treatment after consultation with the Protocol Chair. The investigators and the study coordinators will not be masked to the treatment group assignment.

1. E- ETDRS visual acuity testing in each eye (best corrected).
   - A protocol refraction in the study eye is required at all protocol visits. Refraction in the nonstudy eye is only required at the 1 and 2 year visits. When a refraction is not performed, the most-recently performed refraction is used for the testing.
2. OCT on the study eye
   - Both spectral domain and time domain machines are permitted. For a given study participant, the same machine type should be used for the duration of the study, unless circumstances do not permit (e.g., replacement of damaged machine). If a switch is necessary, the same machine type should be used for the remainder of the study.
3. Ocular exam on both eyes at the annual visits and study eye only at all other follow-up visits, including slit lamp examination, lens assessment, measurement of intraocular pressure and dilated ophthalmoscopy. Non-study eyes that have received intravitreal anti-VEGF during the study will also receive an ocular exam for safety assessment.
4. Fundus photographs (7-modified or 4-wide fields at annual visits on the study eye only).
5. A blood pressure measurement and urine sample will be collected 2-3 days (+/- 1 day if the participant cannot return in 2-3 days) after the baseline injection. If the participant is unable

or unwilling to return after the baseline injection the participant will be asked to return after either of the next 2 injections to have blood pressure measured and urine sample collected. Blood pressure will also be obtained at the first 4 week protocol visit after the post-injection blood pressure was obtained. Blood pressure measurement and urine sample will also be obtained at the 1 year visit. Although encouraged, this additional visit is optional. See the study procedures manual for more details on urine collection and blood pressure measurement.

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 retreatment.

Testing procedures at unscheduled visits are at investigator discretion. However, it is recommended that procedures that are performed should follow the standard DRCR.net protocol for each procedure.

4.3 Treatment During Follow Up

The treatment groups are as follows:

- 2.0 mg intravitreal aflibercept
- 1.25 mg intravitreal bevacizumab
- 0.3 mg intravitreal ranibizumab

All OCT retreatment criteria outlined in the following sections 4.3.1-4.3.2 are based on time domain values. When spectral domain machines are used, the criteria will be adjusted according to the equivalent value for the given machine based on gender specific cutoffs.

4.3.1 Intravitreal Injection Re-Treatment

At the baseline visit all three groups will receive an intravitreal injection according to their assigned treatment group. After the initial injection each eye will be treated according to retreatment protocol. 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 OCT central subfield thickness is <250µ and visual acuity is 20/20 or better, the injection will be deferred. If the eye has not improved or worsened for at least 2 consecutive 4-week visits and OCT central subfield thickness is ≥250µ or visual acuity is worse than 20/20, the following will be done:

- Prior to the 24-week visit, an injection will be given.
- At and after the 24-week visit, the injection will be deferred.

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

4.3.2 Focal/Grid Laser Treatment at and after 24-week Follow-Up Visit

In general, focal/grid laser will be initiated at or after the 24 week visit if 1) the OCT central subfield thickness is ≥250µ 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 <250 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 details.

CHAPTER 5.
MISCELLANEOUS CONSIDERATIONS IN FOLLOW-UP

5.1 Endophthalmitis

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

5.2 Surgery for Vitreous Hemorrhage and Other Complications of Diabetic Retinopathy

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

5.3 Panretinal (Scatter) Photocoagulation (PRP)

PRP can be given if it is indicated in the judgment of the investigator. Individuals are not eligible for this study if, at the time of randomization, it is expected that they will need PRP within six months. In general, PRP should not be given if the study participant has less than severe non-proliferative diabetic retinopathy. In general, PRP should be given promptly for previously untreated eyes exhibiting proliferative diabetic retinopathy with high-risk characteristics and can be considered for persons with non high-risk proliferative diabetic retinopathy or severe non-proliferative diabetic retinopathy. Guidelines for PRP are given below.

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

Size (on retina)	Spot size is 500 µm (e.g. argon laser using 200 µm with Rodenstock lens [or equivalent] or 500 µm with three mirror contact lens).
Exposure	Recommendation of 0.1 seconds, 0.05 to 0.2 allowed.
Intensity	Mild white (i.e. 2+ to 3+ burns).
Distribution	Edges one burn width apart.
No. of sessions/sittings	One to three.
Nasal proximity to disk	No closer than 500 µm.
Temp. proximity to center	No closer than 3000 µm.
Superior/inferior limit	No further posterior than one burn within the temporal arcades.
Extent	Arcades (~3000 µm from the macular center) to at least the equator.
Total number 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 panretinal photocoagulation session. Similarly, there may be clinical situations where 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 DRCR.net procedure manual.

5.4 Treatment of Macular Edema in Nonstudy Eye

Treatment of DME using an anti-VEGF agent in the nonstudy eye is described in section 3.2.7. Non anti-VEGF treatment for DME in the non-study eye is at investigator discretion.

5.5 Diabetes Management

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

5.6 Study Participant Withdrawal and Losses to Follow-up

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

A goal for the study is to have as few losses to follow-up as possible. The DRCR.net 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 protocol visits will be performed. Study participants who have an adverse effect attributable to a study treatment or procedure will be asked to continue in follow-up until the adverse event has resolved or stabilized.

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

5.7 Discontinuation of Study

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

5.8 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 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 participants.

5.9 Study Participant Reimbursement

The study will be providing the study participant with a $25 gift card per completed protocol visit to cover travel and other visit-related expenses. If the participant completes the optional visit 2-3 days after either their first, second, or third injection the participant will receive a $50 gift card for completing the visit. Additional travel expenses will be paid in cases for 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.

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 Coordinating Center 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 (including treatment of the non-study eye with study 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 or 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 Clinical Investigator's Brochures.

Serious or unexpected adverse events must be reported to the Coordinating Center immediately via completion of the online serious adverse event form. If the study participant required hospitalization, the hospital discharge summary must also be sent to the Coordinating Center.

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 reporting serious study-related adverse events and abiding by any other reporting requirements specific to their Institutional Review Board.

6.4 Data and Safety Monitoring Committee Review of Adverse Events

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

6.5 Risks

6.5.1 Potential Adverse Effects of Study Drugs

6.5.1.1Ranibizumab

Ranibizumab is well tolerated in people. More than 5000 individuals have been treated with injections of ranibizumab in clinical studies to date, however the full safety profile with long-term injections is not yet known. Some participants in ongoing clinical studies have developed

inflammation in the eye (uveitis) which can be treated with anti-inflammatory drops. Increased eye pressure leading to glaucoma or cataract has also resulted from injections of ranibizumab. Other ocular adverse events that have occurred in ongoing clinical studies are believed to be due to the intravitreal injection itself and not the study drug (Section 6.5.2).

Some study participants have experienced systemic adverse events that may possibly be related to ranibizumab. There is evidence that intravitreally administered ranibizumab is associated with a decrease in serum VEGF concentrations, but it has not been established whether this decrease results in clinically significant adverse events.[25] Until cumulative safety data are analyzed, precise incidence figures are unknown and a causal relationship cannot be ruled out. These include arterial thromboembolic events and other events potentially related to systemic VEGF inhibition. In a phase IIIb study to evaluate the long-term safety and efficacy of ranibizumab (The Safety Assessment of Intravitreous Lucentis for AMD (SAILOR trial), which randomized patients with wet age-related macular degeneration to 0.5 mg ranibizumab or 0.3 mg ranibizumab, there was a higher rate of cerebrovascular stroke in the group that received the higher drug dose (1.2 vs. 0.7%), although this trend did not achieve statistical significance.[47] It appeared that patients who had a prior history of stroke may be at greater risk for having a stroke after receiving ranibizumab, although there was a low incidence of stroke overall in this group.

Additional data regarding systemic safety of ranibizumab in a diabetic population is also available from the DRCR.net Protocol I primary results.[13] This study enrolled a combined total of 375 patients in the two ranibizumab arms, who received an average of eight to nine intravitreal injections of 0.5 mg ranibizumab over the first year of treatment. There was no indication of an increased risk of cardiovascular or cerebrovascular events in the ranibizumab-treated study participants as compared with the triamcinolone-treated study participants or study participants who received no intravitreal drug. Indeed, lower rates of cardiovascular events, as defined by the Antiplatelet Trialists' Collaboration, were seen in the ranibizumab groups as compared with the sham group at both one (3% versus 8%) and two (5% versus 12%) years. In the RISE and RIDE studies, ranibizumab therapy was also well-tolerated overall, although the rate of Antiplatelet Trialists' Collaboration events was slightly higher in the 0.3 mg (5.6%) and 0.5 mg (7.2%) groups as compared with the sham group (5.2%) in the pooled RISE and RIDE results. Deaths were also more frequent in the ranibizumab groups (0.8% and 1.6% of sham and 2.4-4.8% of ranibizumab treated patients) in these trials.[16] The rate of non-fatal cerebrovascular events in this pooled analysis was higher in the 0.5mg group (2%) than in the sham (1.2%) or 0.3mg group (0.8%) but the rate of non-fatal myocardial infarctions was similar across treatment groups (2.8%, 2.8% and 2.4% in the sham, 0.3mg and 0.5mg groups, respectively).

There may be side effects and discomforts that are not yet known. Long-term studies in animals have not been performed to evaluate the carcinogenic potential of ranibizumab or its effect on fertility.

6.5.1.2 Bevacizumab

In a meta-analysis performed by Genentech, Inc on all clinical trial results using intravenously administered bevacizumab (usually dose 5 mg/kg every 14 days), it was found that study participants were at an increased risk for certain adverse events, some of which were potentially fatal. These included wound healing complications, bowel perforation, hemorrhage, stroke, myocardial infarction, hypertension, congestive heart failure, and proteinuria. Warnings and precautions included in the bevacizumab package insert for intravenously administered drug fall

under the categories of gastrointestinal perforations, surgery and wound healing complications, hemorrhage, non-gastrointestinal fistula formation, arterial thromboembolic events, hypertension, reversible posterior leukoencephalopathy syndrome, proteinuria, infusion reactions and ovarian failure.[19]

In contrast, available data suggest that intravitreally-administered bevacizumab in substantially smaller doses (1.25 or 2.5 mg) appears to have a good safety profile with regard to ocular and systemic adverse events. No increased rates of thromboembolic events or death in bevacizumab versus control groups have been reported in smaller, prospective randomized studies including the DRCR.net Protocol H or the BOLT study.[18] Retrospective, observational data from larger patient groups also does not appear to indicate an increased risk of ocular or systemic events with intravitreal bevacizumab treatment. In 2006, an internet-based survey of 70 international sites from 12 countries was reported that described outcomes after 7,113 injections given to 5,228 patients. Rates were 0.21% or less for each category of doctor-reported adverse events, including blood pressure elevation, transient ischemic attack, cerebrovascular accident, death, endophthalmitis, retinal detachment, uveitis, or acute vision loss.[27] The PACORES group reported 12 month safety of intravitreal injections of 1.25 and 2.5 mg doses of bevacizumab given for a variety of conditions in a large group of study participants including 548 patients with diabetes.[28] A total of 1,174 patients were followed for at least 1 year. Systemic adverse events were reported in 1.5% (N = 18), including elevated blood pressure in 0.6% (7), cerebrovascular accidents in 0.5% (6), myocardial infarctions in 0.4% (5), iliac artery aneurysms in 0.2% (2), toe amputations in 0.2% (2), and deaths in 0.4% (5) of patients. The overall mortality rate of diabetic patients in this study was low at 0.55% (3/548). Ocular complication were reported as bacterial endophthalmitis in 0.2% (7), traction retinal detachments in 0.2% (7), uveitis in 0.1% (4), and a single case each of rhegmatogenous retinal detachment and vitreous hemorrhage.

Recently reported results from the CATT Research Group also suggest that intravitreal bevacizumab is well tolerated. At one year, four of 286 participants (1.4%) in the monthly bevacizumab group had died and 11 of 300 participants (3.7%) in the bevacizumab given as needed group had died. Arteriothrombolic events occurred at a rate of 2.1% and 2.7% in the monthly bevacizumab and as needed bevacizumab groups, respectively. Venous thrombotic events occurred at rates of 1.4% and 0.3% in the monthly bevacizumab and as needed bevacizumab groups, respectively. Endophthalmitis occurred after 0.07% of injections in patients treated with bevacizumab. Although a higher rate of serious systemic adverse events was present in the bevacizumab group as compared with the ranibizumab group, the excess events in the bevacizumab group were primarily hospitalizations due to events not previously attributed to anti-VEGF treatment.[39] Differences in rates were largest for hospitalizations for infections (e.g., pneumonia and urinary tract infections) and gastrointestinal disorders (e.g., hemorrhage and nausea and vomiting). Two year follow-up safety data from the CATT study did not reveal significant differences in rates of arterial thromboembolic events or death between bevacizumab and ranibizumab treated participants. Overall rates of serious adverse events, however, were higher among bevacizumab-treated patients (39.9%) than ranibizumab-treated patients (31.7%), with the greatest imbalance in gastrointestinal disorders not previously linked to anti-VEGF therapy.[24] In contrast, at 1 year in the IVAN study, fewer arteriothrombotic events or heart failure cases were seen in the bevacizumab treated group and there was no difference in the percentage of patients experiencing serious adverse events between the bevacizumab and ranibizumab treatment groupS.[25]

As noted in the introduction, bevacizumab has been given intravitreally to several thousand patients with age-related macular degeneration or diabetic macular edema in doses generally of 1.25 or 2.5 mg per injection (a fraction of the systemic dose). There have not been consistent reports suggestive of adverse systemic effects of the drug. This likely rules out serious systemic events being common but does not rule out the possibility of such events occurring rarely. Patients with diabetes are at increased risk for myocardial infarction, stroke, and renal disease. Thus, if a study participant develops a cardiovascular or renal problems, it may be due to the vascular effects of diabetes and other systemic factors and not related to bevacizumab. It is likely that only in a large study comparing adverse event rates between a bevacizumab-treated group and a control group will it be possible to determine if there is an excess of systemic adverse events with bevacizumab. At this time, we believe the chances of a serious systemic effect of bevacizumab are very small. However, we cannot rule out this possibility and there is evidence that systemic concentrations of VEGF may be reduced to an even greater extent with intravitreal bevacizumab as compared with ranibizumab treatment.[25] In view of the large number of eyes treated with bevacizumab injections, it also seems unlikely that the drug has a deleterious effect on the retina or other parts of the eye.

6.5.1.3 Aflibercept

Very limited data are available for the use of aflibercept in diabetic cohorts, and published results are available for short duration follow-up of only 24 weeks. The DA VINCI study, evaluating aflibercept for treatment of DME reported common adverse events that were consistent with those previously seen with intravitreal injections. In all aflibercept-treated eyes, these included conjunctival hemorrhage (18.9%), intraocular pressure increase (9.7%), eye pain (8.6%), ocular hyperemia (6.3%) and vitreous floaters (5.1%). Two cases of endophthalmitis were reported in aflibercept treated eyes, one of which was culture negative. An additional case of uveitis that was treated as endophthalmitis was also seen with aflibercept treatment. The percentages of aflibercept-treated patients with arterial thromboembolic events in the DA VINCI study were 1.1% for myocardial infarction and 1.1% for cerebrovascular accident. None of the laser-treated patients had myocardial infarctions or cerebrovascular accidents. Serious adverse hypertensive events were reported for 9.7% of the combined aflibercept group as compared with 6.8% of the laser treated group. In the combined analysis of the VIEW 1 and VIEW 2 studies, the rates of Anti-Platelet Trialist arterial thrombolic events were 3.2% and 3.3% in the ranibizumab and the combined aflibercept groups, respectively.[40]

6.5.2 Potential Adverse Effects of Intravitreal Injection

Rarely, the drugs used to anesthetize the eye before the 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 intravitreal injection. Mild discomfort, ocular hyperemia, increased lacrimation, discharge 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 one percent.

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

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 one percent.

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 one percent.

6.5.3 Risks of Laser Photocoagulation Treatment

Serious complications from laser treatment are rare. They occur in less than one in 1,000 cases. These include damage to the macula, bleeding inside the eye, immediate or delayed increase in pressure inside the eye, damage to the optic nerve, damage to the iris, damage to the lens or an intraocular lens, retinal hole, blindness, and loss of the eye. If a laser burn occurs too near the center of vision, a scotoma could develop. After several years, the scars caused by the laser may enlarge and cause vision to decrease.

Anesthetic drops and a contact lens may be used as a part of the laser procedure. Risks include allergic reaction, infection, and corneal abrasion (scratch on the clear front surface of the eye). If any of these problems occur, they usually clear up rapidly.

In some cases retrobulbar or peribulbar injection may be used to anesthetize the eye and to reduce eye movements. Complications of retrobulbar and peribulbar injections are rare. They include, but are not limited to, the following: retrobulbar hemorrhage (bleeding behind your eyeball); perforation of the eye by the needle; damage to the optic nerve; diplopia lasting up to 24 hours or more; ptosis lasting up to 24 hours or more; difficulty speaking or breathing; lightheadedness/syncope/vasovagal response; allergy to any components of the injection; life threatening response due to the spread of anesthesia to the brain stem, resulting in seizures, drowsiness, confusion, loss of ability to talk, convulsions, stoppage of breathing, or stoppage of heartbeat. All of these complications are rare.

6.5.4 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 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.

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 completion of the study. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

7.1 Sample Size

The primary analysis will consist of three two-group comparisons of mean change in visual acuity at one year.

7.1.1 Ranibizumab Group Projection

The one-year change in visual acuity data for the ranibizumab group can be estimated using data from the ranibizumab+deferred laser group in DRCR.net Protocol I (Table 1). These eyes had the same visual acuity and OCT central subfield eligibility criteria as the current study. Based on these data, a standard deviation of visual acuity change adjusted for baseline visual acuity of 11.4 was used to estimate sample size for this study.

Table 1. Protocol I Ranibizumab+Deferred Laser Group One Year Visual Acuity Data	
N	173
Standard Deviation Visual Acuity change (95% CI)	11.2 (10.0, 12.5)
Correlation between baseline visual acuity and visual acuity change (95% CI)	-0.45 (-0.33, -0.56)
Standard Deviation of Visual Acuity Change adjusted for baseline (95% CI)	10.2 (9.2, 11.4)

7.1.2 Visual Acuity Differences Between Treatment Groups

This study will be powered to detect a difference between treatment groups if the true difference between the groups is a visual acuity letter score of 4 or more.

7.1.3 Power Estimation

1. Power Estimation for Primary Outcome

A sample size of 660 eyes (220 eyes per group) was selected. With this sample size, the power for the largest treatment difference was estimated to be 90%, under the following assumptions:

In estimating the power, the following assumptions were made:

- Standard error of change in letter score adjusted for baseline letter score = 11.4
- Overall Type 1 error rate controlling for three multiple comparisons = 0.049 (2-sided), after adjusting for total alpha spending of 0.001 for DSMC data review. The Hochberg adjustment will be used to control the overall type 1 error rate for the multiple comparisons
- Largest treatment group difference in change in visual acuity = 4 letters
- Sample Size: N = 220 per group
- Loss to Follow-up at one year: 7.5%

Additional Assumptions:

- Without loss of generality, take group X to have the lowest visual acuity among the 3 groups.

- Without loss of generality, take group Z to have the highest visual acuity among the 3 groups.
- Fix the difference Z – X at 4 letters.

Because the Hochberg procedure is being used, the power to reject the pairwise comparison X vs. Z also depends on where the intermediate group, Y, falls.

Given these assumption, power calculations included in the table below, have been performed under worst-case and best-case scenarios:

Scenario	**Reject Any Pairwise Comparison**	**Reject Largest Difference** (Z – X = 4 letters)
Worst Case: (Y is at the midpoint of X and Z) Treatment Z – Treatment Y = 2 letters Treatment Y - Treatment X = 2 letters	89%	88%
Best Case: (Y = Z)* Treatment Z – Treatment Y = 0 letters Treatment Y – Treatment X = 4 letters	96%	90%

* By symmetry, the same best-case power is also achieved when Y = X and Treatment Z – Treatment Y = 4 letters.

The true power will be slightly higher as the sample size is adjusted for 7.5% lost to follow-up whereas the primary analysis (see section 7.2.1) will employ multiple imputation methods to include all study eyes.

7.2 Statistical Analysis Plan

7.2.1 Primary Outcome

The primary analysis will consist of three two-group comparisons of change in visual acuity at the one-year follow-up visit, using analysis of covariance to adjust for baseline visual acuity. The Hochberg approach will be used to control the Type 1 error.

The primary analysis will be an intent-to-treat analysis that includes all randomized eyes, according to the treatment group assignment at randomization.

A per-protocol analysis will be conducted in which any eye receiving an alternative treatment will be excluded. Additional per-protocol analyses will exclude eyes receiving alternative treatment and eyes for which there was a protocol deviation either for injecting the eye when deferral was required, or deferring when an injection was required, and will use multiple imputation methods to impute an outcome visual acuity based on visual acuities obtained up to an including the visit of the treatment deviation for these eyes. If the results of the methods differ, exploratory analyses will be performed to evaluate the factors that have contributed to the differences.

Note: Focal/grid laser is part of the protocol treatment regime and is not considered alternate treatment.

The intent-to-treat analysis is considered the primary analysis. If the intent-to-treat and per-protocol analyses yield the same results, the per-protocol analysis will be used to provide supportive evidence of the magnitude of treatment effect among patients who received the treatment. If the results of the two methods differ, exploratory analyses will be performed to evaluate the factors that have contributed to the differences.

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 regression models by including baseline covariates related to the patient (age) and study eye (visual acuity, retinal thickening on OCT, and prior treatment for DME). Additional variables that are associated with the outcome will be included if there is an imbalance in the variables between groups.

Pre-planned subgroup analyses will be described in the detailed Statistical Analysis Plan and include stratification by presence of central-subfield involved DME, visual acuity, central subfield thickness, and prior DME treatment history. 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 analyses.

Longitudinal analyses also will be conducted to assess trends in visual acuity over time.

The number of subjects per center is small for many centers therefore center effects will not be included in the statistical model; however for centers with a large number of subjects the treatment effect will be assessed. If a positive overall effect of treatment is found, heterogeneity of treatment effect across centers will be explored using random center effects.

7.2.2 Secondary Outcomes

The treatment groups will be compared on the following key secondary outcomes of interest at the 1 year visit:

- Number of intravitreal injections given per protocol
- Proportion of eyes receiving focal/grid laser treatment and number of treatment sessions
- Proportion of eyes with 2 and 3 line gains or losses in visual acuity
- Change in OCT central subfield thickness and retinal volume
- Proportion of eyes with OCT central subfield thickness of <250 μm on Stratus OCT (or spectral domain equivalent)
- Of eyes with NPDR at baseline, proportion of eyes with improvement of retinopathy severity level
- Proportion receiving PRP, vitrectomy, or vitreous hemorrhage

Binary outcomes will be analyzed using logistic regression models adjusting for baseline factors where appropriate. Continuous outcomes will be analyzed using an analysis of covariance model adjusting for baseline measures where appropriate. All linear model assumptions will be verified including linearity, normality of residuals, and homoscedasticity. If model assumptions are not met data transformation or a nonparametric analysis will be considered.

Additional secondary analyses mimicking the primary and secondary outcomes at 52 weeks will be conducted at 16 weeks (the time point at which deferral of injection first becomes an option).

7.2.3 Safety Analysis Plan

Adverse events will be categorized as study eye, nonstudy eye, and systemic. The events will be tabulated separately for the three treatment groups. Adverse events of interest will include:

- Injection-related: endophthalmitis, traction retinal detachment, rhegmatogenous retinal detachments, retinal tears, cataract, intraocular hemorrhage, increased intraocular pressure
- Ocular drug-related: inflammation, new or worsening traction retinal detachment, progression of traction retinal detachment from extramacular to macular
- Systemic drug-related: hypertension events, kidney, gastrointestinal events, and cardiovascular events as defined by the Antiplatelet Trialists' Collaboration
    - *The primary systemic safety analysis will include all randomized eyes analyzed in the randomly assigned treatment group. Additional analysis will stratify systemic adverse event outcomes by fellow eye intravitreal anit-VEGF treatment during the study.*
    - Change in blood pressure 2-3 days (+/- 1 day) after an injection and at 1 year will be analyzed
    - Change in albumin/creatinine ratio for microalbuminuria 2-3 days (+/- 1 day) after an injection and at 1 year will be analyzed

Further definitions of the events for analysis and the analytic approach will be provided in the detailed statistical analysis plan.

7.2.4 Additional Tabulations and Analyses

The following will be tabulated according to treatment group:

- Baseline demographic and clinical characteristics
- Visit completion rate for each visit
- Protocol deviations

7.2.5 Interim Monitoring Plan

A formal plan for interim monitoring will be established in consultation with the Data and Safety Monitoring Committee.

CHAPTER 8. REFRENCES

1. Centers for Disease Control and Prevention. State-specific incidence of diabetes among adults--participating states, 1995-1997 and 2005-2007. *MMWR Morb Mortal Wkly Rep.* 2008;57(43):1169-73.

2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract.* 2010;87(1):4-14.

3. Kempen JH, O'Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. *Arch Ophthalmol.* 2004;122(4):552-63.

4. Klein R, Klein BE, Moss SE, Davis MD, DeMets DL. The Wisconsin epidemiologic study of diabetic retinopathy IV. Diabetic macular edema. *Ophthalmology.* 1984;91(12):1464-74.

5. Ferris F, Patz A. Macular edema: a complication of diabetic retinopathy. *Surv Ophthalmol.* 1984;28 (suppl)(May):452-61.

6. Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema: ETDRS report number 4. *Int Ophthalmol Clin.* 1987;27(4):265-72.

7. Ferris FL, 3rd, Patz A. Macular edema. A complication of diabetic retinopathy. *Surv Ophthalmol.* 1984;28 (suppl)(May):452-61.

8. Antcliff RJ, Marshall J. The pathogenesis of edema in diabetic maculopathy. *Semin Ophthalmol.* 1999;14(4):223-32.

9. Aiello LP, Bursell SE, Clermont A, et al. Vascular endothelial growth factor-induced retinal permeability is mediated by protein kinase C in vivo and suppressed by an orally effective beta-isoform-selective inhibitor. *Diabetes.* 1997;46:1473-80.

10. Early Treatment Diabetic Retinopathy Study Research Group. Photocoagulation for diabetic macular edema. Early Treatment Diabetic Retinopathy Study report number 1. *Arch Ophthalmol.* 1985;103(12):1796-806.

11. Diabetic Retinopathy Clinical Research Network. A randomized trial comparing intravitreal triamcinolone acetonide and focal/grid photocoagulation for diabetic macular edema. *Ophthalmology.* 2008;115(9):1447-9, 9 e1-10.

12. Diabetic Retinopathy Clinical Research Network. Three-year follow up of a randomized trial comparing focal/grid photocoagulation and intravitreal triamcinolone for diabetic macular edema. *Arch Ophthalmol.* 2009;127(3):245-51.

13. Diabetic Retinopathy Clinical Research Network. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology.* 2010;117(6):1064-77 e35.

14. Nguyen QD, Shah SM, Heier JS, et al. Primary end point (six months) results of the ranibizumab for edema of the macula in diabetes (READ-2) study. *Ophthalmology.* 2009;116(11):2175-81 e1.

15. Diabetic Retinopathy Clinical Research Network, Scott IU, Edwards A, et al. A phase II randomized clinical trial of intravitreal bevacizumab for diabetic macular edema. *Ophthalmal.* 2007;114(10):1860-7.

16. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for Diabetic Macular Edema: Results from 2 Phase III Randomized Trials: RISE and RIDE. *Ophthalmology.* 2012;119(4):789-801.

17. Mitchell P, Bandello F, Schmidt-Erfurth U, et al. The RESTORE study ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology.* 2011;118(4):615-25.

18. Michaelides M, Kaines A, Hamilton RD, et al. A prospective randomized trial of intravitreal bevacizumab or laser therapy in the management of diabetic macular edema (BOLT study) 12-month data: report 2. *Ophthalmology.* 2010;117(6):1078-86 e2.

19. Genentech I. AVASTIN (bevacizumab) injection, solution http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=14571. Accessed Accessed: 12 July 2010.

20. FDA begins process to remove breast cancer indication from Avastin label http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm237172.htm. Accessed Accessed June 1, 2009.

21. Cunningham ET, Jr., Adamis AP, Altaweel M, et al. A phase II randomized double-masked trial of pegaptanib, an anti-vascular endothelial growth factor aptamer, for diabetic macular edema. *Ophthalmology.* 2005;112(10):1747-57.

22. Gragoudas ES, Adamis AP, Cunningham ET, Feinsod M, Guyer DR, the VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. *N Engl J Med.* 2004;351(27):2805-16.

23. Arevalo JF, Sanchez JG, Fromow-Guerra J, et al. Comparison of two doses of primary intravitreal bevacizumab (Avastin) for diffuse diabetic macular edema: results from the Pan-American Collaborative Retina Study Group (PACORES) at 12-month follow-up. *Graefes Arch Clin Exp Ophthalmol.* 2009;247(6):735-43.

24. Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and Bevacizumab for Treatment of Neovascular Age-Related Macular Degeneration: Two-Year Results. *Ophthalmology.* 2012;E-Press.

25. Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus Bevacizumab to Treat Neovascular Age-related Macular Degeneration: One-Year Findings from the IVAN Randomized Trial. *Ophthalmology.* 2012.

26. Curtis LH, Hammill BG, Schulman KA, Cousins SW. Risks of mortality, myocardial infarction, bleeding, and stroke associated with therapies for age-related macular degeneration. *Arch Ophthalmol.* 2010;128(10):1273-9.

27. Fung AE, Rosenfeld PJ, Reichel E. The International Intravitreal Bevacizumab Safety Survey: using the internet to assess drug safety worldwide. *Br J Ophthalmol.* 2006;90(11):1344-9.

28. Wu L, Martinez-Castellanos MA, Quiroz-Mercado H, et al. Twelve-month safety of intravitreal injections of bevacizumab (Avastin): results of the Pan-American Collaborative Retina Study Group (PACORES). *Graefes Arch Clin Exp Ophthalmol.* 2008;246(1):81-7.

29. Gonzales CR for the VEGF Inhibition Study in Ocular Neovascularization (VISION) Clinical Trial Group. Macugen in the treatment of diabetic macular edema: results of the phase 2 trial. *2005 ASRS scientific paper presentation, Montreal, Quebec, Canada.* 2005.

30. Phase 3 study showed MACUGEN improved vision over standard of care in patients with diabetic macular edema. http://multivu.prnewswire.com/mnr/pfizer/43437/. Accessed Accessed: 12 June 2010.

31. Do DV, Nguyen QD, Shah SM, et al. An exploratory study of the safety, tolerability and bioactivity of a single intravitreal injection of vascular endothelial growth factor Trap-Eye in patients with diabetic macular oedema. *Br J Ophthalmol.* 2009;93(2):144-9.

32. Do DV, Schmidt-Erfurth U, Gonzalez VH, et al. The DA VINCI Study: phase 2 primary results of VEGF Trap-Eye in patients with diabetic macular edema. *Ophthalmology.* 2011;118(9):1819-26.

33. US FDA approves EYLEA (aflibercept) injection for the treatment of we age-related macular degeneration. http://www.press.bayer.com/baynews/baynews.nsf/0/EB1AAB6D8405A0BDC125794A0056C856. Accessed accessed January 1, 2012.

34. Regeneron Pharmaceuticals I. VEGF TRAP-EYE (aflibercept ophthalmic solution) Ophthalmologic Drugs Advisory Committee Briefing Document. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/DermatologicandOphthalmicDrugsAdvisoryCommittee/UCM259143.pdf. Accessed Accessed January 17, 2012.

35. Klettner A, Roider J. Comparison of bevacizumab, ranibizumab, and pegaptanib in vitro: efficiency and possible additional pathways. *Invest Ophthalmol Vis Sci.* 2008;49(10):4523-7.

36. Costa R, Carneiro A, Rocha A, et al. Bevacizumab and ranibizumab on microvascular endothelial cells: A comparative study. *J Cell Biochem.* 2009;108(6):1410-7.

37. Gharbiya M, Giustolisi R, Allievi F, et al. Choroidal neovascularization in pathologic myopia: intravitreal ranibizumab versus bevacizumab--a randomized controlled trial. *Am J Ophthalmol.* 2010;149(3):458-64 e1.

38. Stepien KE, Rosenfeld PJ, Puliafito CA, et al. Comparison of intravitreal bevacizumab followed by ranibizumab for the treatment of neovascular age-related macular degeneration. *Retina.* 2009;29(8):1067-73.

39. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2011;364(20):1897-908.

40. Two year results of phase 3 studies with EYLEA (aflibercept) injection in wet AMD show sustained improvement in visual acuity. http://www.prnewswire.com/news-releases/two-year-results-of-phase-3-studies-with-eylea-aflibercept-injection-in-wet-amd-show-sustained-improvement-in-visual-acuity-135011348.html. Accessed 13 February 2012.

41. Campochiaro PA. Safety and efficacy of intravitreal ranibizumab (Lucentis) in patients with macular edema secondary to branch retinal vein occlusion: The BRAVO Study. *Retina Congress*. New York, NY.; 2009.

42. Brown DM. Safety and efficacy of intravitreal ranibizumab (Lucentis) in patients with macular edema secondary to central retinal vein occlusion: The CRUISE Study. *Retina Congress*. New York, NY.; 2009.

43. Haritoglou C, Kook D, Neubauer A, et al. Intravitreal bevacizumab (Avastin) therapy for persistent diffuse diabetic macular edema. *Retina.* 2006;26(9):999-1005.

44. ASRS. PAT Survey. http://www.asrs.org/home/communicate_and_educate/pat_survey/2009/. Accessed March 25, 2010.

45. Rosenfeld PJ, Fung AE, Puliafito CA. Optical coherence tomography findings after an intravitreal injection of bevacizumab (avastin) for macular edema from central retinal vein occlusion. *Ophthalmic Surg Lasers Imaging.* 2005;36:336-9.

46. Beck RW, Moke PS, Turpin AH, et al. A computerized method of visual acuity testing: adaptation of the early treatment of diabetic retinopathy study testing protocol. *Am J Ophthalmol.* 2003;135(2):194-205.

47. Boyer DS, Heier JS, Brown DM, Francom SF, Ianchulev T, Rubio RG. A Phase IIIb study to evaluate the safety of ranibizumab in subjects with neovascular age-related macular degeneration. *Ophthalmology.* 2009;116(9):1731-9.

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APPENDIX 1

Ancillary Study: Assessment of Plasma VEGF Concentrations after Intravitreal Anti-VEGF Therapy for Diabetic Macular Edema

CHAPTER 1: BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.0 Background Information

1.1 Systemic Serious Adverse Events Associated with Intravitreal Anti-VEGF Therapy

The evaluation of systemic safety profiles is an important facet to the comparison of different, intravitreally administered anti- vascular endothelial growth factor (VEGF) agents since VEGF is known to have a widespread role in normal physiologic processes involving angiogenesis. Thus, the systemic blockade of VEGF could theoretically have wide-ranging adverse systemic effects. Indeed, in a meta-analysis performed by Genentech, Inc on all clinical trial results using intravenously administered bevacizumab (usually dosed 5 mg/kg every 14 days), it was found that study participants were at an increased risk for certain serious adverse events, some of which were potentially fatal. These included wound healing complications, bowel perforation, hemorrhage, stroke, myocardial infarction, hypertension, congestive heart failure, and proteinuria.[1] Despite these concerning results from cohorts undergoing systemic administration of anti-VEGF treatment, it appears that intravitreally administered anti-VEGF therapy is generally well-tolerated. Anti-VEGF treated study participants in ophthalmic trials have not appeared to have higher rates of thromboembolic events than their non-anti-VEGF treated counterparts.

1.2 Ranibizumab

More than 5000 individuals have been treated with injections of ranibizumab in clinical studies to date, however the full safety profile with long-term injections is not yet known. Some study participants have experienced systemic adverse events that may possibly be related to ranibizumab. There is evidence that intravitreally administered ranibizumab is associated with a decrease in serum VEGF concentrations, but it has not been established whether this decrease results in clinically relevant increases in serious systemic adverse events.[2] Until cumulative safety data are analyzed, precise incidence figures are unknown and a causal relationship cannot be ruled out. These include arterial thromboembolic events and other events potentially related to systemic VEGF inhibition. In a phase IIIb study to evaluate the long-term safety and efficacy of ranibizumab (The Safety Assessment of Intravitreous Lucentis for AMD (SAILOR trial), which randomized patients with neovascular age-related macular degeneration to 0.5 mg ranibizumab or 0.3 mg ranibizumab, there was a higher rate of cerebrovascular stroke in the group that received the higher drug dose (1.2 vs. 0.7%), although a statistically significant difference was not identified.[3] It appeared that patients who had a prior history of stroke may be at greater risk for having a stroke after receiving ranibizumab, although there was a low incidence of stroke overall in this group. A similar trend was noted in the MARINA study but not in the ANCHOR study.

Additional data regarding systemic safety of ranibizumab in a diabetic population is also

available from the DRCR.net Protocol I primary results.[4] This study enrolled a combined total of 375 patients in the two ranibizumab arms, who received an average of eight to nine intravitreal injections of 0.5 mg ranibizumab over the first year of treatment. There was no indication of an increased risk of cardiovascular or cerebrovascular events in the ranibizumab-treated study participants as compared with the triamcinolone-treated study participants or study participants who received no intravitreal drug. Indeed, lower rates of cardiovascular events, as defined by the Antiplatelet Trialists' Collaboration, were seen in the ranibizumab groups as compared with the sham group at both one (3% versus 8%) and two (5% versus 12%) years. In the RISE and RIDE studies, ranibizumab therapy was also well-tolerated overall, although the rate of Antiplatelet Trialists' Collaboration events was slightly higher in the 0.3 mg (5.6%) and 0.5 mg (7.2%) groups as compared with the sham group (5.2%) in the pooled RISE and RIDE results. Deaths were also more frequent in the ranibizumab groups (0.8% and 1.6% of sham and 2.4-4.8% of ranibizumab treated patients) in these trials.[5] The rate of non-fatal cerebrovascular events in this pooled analysis was higher in the 0.5mg group (2%) than in the sham (1.2%) or 0.3mg group (0.8%) but the rate of non-fatal myocardial infarctions was similar across treatment groups (2.8%, 2.8% and 2.4% in the sham, 0.3mg and 0.5mg groups, respectively).

1.3 Bevacizumab

Despite the data demonstrating higher rates of systemic adverse events with systemically administered bevacizumab for treatment of cancer, available data suggest that intravitreally-administered bevacizumab in substantially smaller doses (1.25 or 2.5 mg) appears to have a good safety profile with regard to ocular and systemic adverse events. No increased rates of thromboembolic events or death in bevacizumab versus control groups have been reported in smaller, prospective randomized studies including the DRCR.net Protocol H or the BOLT study.[6] Retrospective, observational data from larger patient groups also does not appear to indicate an increased risk of ocular or systemic events with intravitreal bevacizumab treatment. In 2006, an internet-based survey of 70 international sites from 12 countries was reported that described outcomes after 7,113 injections given to 5,228 patients. Rates were 0.21% or less for each category of doctor-reported adverse events, including blood pressure elevation, transient ischemic attack, cerebrovascular accident, death, endophthalmitis, retinal detachment, uveitis, or acute vision loss.[7] The PACORES group reported 12 month safety of intravitreal injections of 1.25 and 2.5 mg doses of bevacizumab given for a variety of conditions in a large group of study participants including 548 patients with diabetes.[8] A total of 1,174 patients were followed for at least 1 year. Systemic adverse events were reported in 1.5% (N = 18), including elevated blood pressure in 0.6% (7), cerebrovascular accidents in 0.5% (6), myocardial infarctions in 0.4% (5), iliac artery aneurysms in 0.2% (2), toe amputations in 0.2% (2), and deaths in 0.4% (5) of patients. The overall mortality rate of diabetic patients in this study was low at 0.55% (3/548). Ocular complication were reported as bacterial endophthalmitis in 0.2% (7), traction retinal detachments in 0.2% (7), uveitis in 0.1% (4), and a single case each of rhegmatogenous retinal detachment and vitreous hemorrhage.

Recently reported results from the CATT Research Group also suggest that intravitreal bevacizumab is well tolerated. At one year, four of 286 participants (1.4%) in the monthly bevacizumab group had died and 11 of 300 participants (3.7%) in the bevacizumab given as needed group had died. Arteriothrombolic events occurred at a rate of 2.1% and 2.7% in the monthly bevacizumab and as needed bevacizumab groups, respectively. Venous thrombotic events occurred at rates of 1.4% and 0.3% in the monthly bevacizumab and as needed bevacizumab groups, respectively. Endophthalmitis occurred after 0.07% of injections in

patients treated with bevacizumab. Although a higher rate of serious systemic adverse events was present in the bevacizumab group as compared with the ranibizumab group, the excess events in the bevacizumab group were primarily hospitalizations due to events not previously attributed to anti-VEGF treatment.[9] Differences in rates were largest for hospitalizations for infections (e.g., pneumonia and urinary tract infections) and gastrointestinal disorders (e.g., hemorrhage and nausea and vomiting). Two year follow-up safety data from the CATT study did not reveal significant differences in rates of arterial thromboembolic events or death between bevacizumab and ranibizumab treated participants. Overall rates of serious adverse events, however, were higher among bevacizumab-treated patients (39.9%) than ranibizumab-treated patients (31.7%), with the greatest imbalance in gastrointestinal disorders, most of which were not previously linked to anti-VEGF therapy.[10] In contrast, at 1 year in the IVAN study, fewer arteriothrombotic events or heart failure cases were seen in the bevacizumab treated group and there was no difference in the percentage of patients experiencing serious adverse events between the bevacizumab and ranibizumab treatment groups.[2]

Bevacizumab has been given intravitreally to several thousand patients with age-related macular degeneration or diabetic macular edema in doses generally of 1.25 or 2.5 mg per injection (a fraction of the systemic dose). There have not been consistent reports suggestive of adverse systemic effects of the drug. This likely rules out serious systemic events being common but does not rule out the possibility of such events occurring rarely. Patients with diabetes are at increased risk for myocardial infarction, stroke, and renal disease. Thus, if a study participant develops a cardiovascular or renal problems, it may be due to the vascular effects of diabetes and other systemic factors and not related to bevacizumab.

1.4 Aflibercept

Very limited data are available for the use of aflibercept in diabetic cohorts, and published results are available for short duration follow-up of only 24 weeks. The percentages of aflibercept-treated patients with arterial thromboembolic events in the phase 2 DA VINCI study, which evaluated aflibercept as treatment for DME, were 1.1% for myocardial infarction and 1.1% for cerebrovascular accident. None of the laser-treated patients had myocardial infarctions or cerebrovascular accidents. Serious adverse hypertensive events were reported for 9.7% of the combined aflibercept group as compared with 6.8% of the laser treated group. In the combined analysis of the VIEW 1 and VIEW 2 studies (in which aflibercept was given for treatment of neovascular age-related macular degeneration), the rates of Anti-Platelet Trialist arterial thrombolic events were 3.2% and 3.3% in the ranibizumab and the combined aflibercept groups, respectively.[11]

1.5 Scientific Rationale for Evaluation of VEGF Plasma Concentrations after Intravitreal Anti-VEGF Therapy

Assuming efficacy for DME treatment is equivalent, an increased risk of systemic toxicity with one anti-VEGF drug as compared to another would have serious implications for choosing one anti-VEGF regimen over another. At this time, based on the available data, the chance of a large, serious systemic effect of any of the intravitreally administered anti-VEGF treatments appears to be small. However, given the relatively low event rates of some of the adverse events of concern, including myocardial infarction and stroke, it is likely that only in a very large study comparing adverse event rates between anti-VEGF-treated groups would it be possible to determine if there is an excess of systemic adverse events with any one anti-VEGF drug as compared to the others.

Some insight into whether or not one anti-VEGF agent is more likely to cause systemic adverse effects than another might be gained from evaluating systemic concentrations of VEGF in the plasma after intravitreally administered anti-VEGF therapy. It is plausible that agents that result in a greater effect on plasma VEGF concentrations might also be more likely to lead systemic safety complications, although this hypothesis has not been confirmed to date.

Multiple studies have demonstrated that there are reductions in plasma concentrations of VEGF after intravitreal treatment with anti-VEGF therapy, whether for neovascular age related macular degeneration or diabetic eye disease.[12-15] There is additional evidence that VEGF plasma concentrations may be reduced to a different extent with different anti-VEGF agents. The one year findings from the (IVAN) study demonstrated that plasma VEGF levels were lower at one year with intravitreal bevacizumab as compared with ranibizumab treatment (151 versus 83 pg/mL).[2] Interestingly, however, arteriothrombotic events or heart failure were more common in the ranibizumab than the bevacizumab treated group (0.7% vs. 2.9%; odds ratio, 0.23; 95% CI, 0.05 to 1.07; $P = 0.03$). [2]

Even if plasma VEGF concentrations are not directly linked to the occurrence of systemic VEGF-inhibition related adverse events, the assessment of plasma VEGF concentrations might still be worthwhile if these measurements can be shown to be highly correlated with ocular anatomic or functional outcomes. The ability to predict visual acuity or retinopathy severity outcomes through peripheral blood sampling would be highly valuable and potentially lead to changes in the way in which patients are counseled, followed and treated as well as in methods of early efficacy evaluation of novel therapies for DR or DME.

1.6 Summary of Rationale for the Study

Anti-VEGF treatment is the current first-line therapy for center-involved DME with visual impairment. Because multiple anti-VEGF treatment options are currently commercially available but phase III trial data is only available for ranibizumab, a DRCR.net comparative efficacy study assessing aflibercept, bevacizumab, and ranibizumab as treatment for DME is currently in late stage planning. An important part of the evaluation of these three agents will be the assessment of safety concerns, particularly rates of systemic serious adverse events including stroke and myocardial infarction. Because rates of serious adverse events may be low overall, the study may lack the requisite power to find a small or modest difference in systemic events between the treatment groups. The assessment of VEGF plasma concentrations could provide a more sensitive measure of the ability of each of these anti-VEGF agents to affect systemic VEGF differentially and might conceivably be associated with differing rates of systemic adverse events. It might also be useful to explore the relationship between systemic concentrations of VEGF and visual acuity and diabetic retinopathy progression/regression in order to determine whether plasma VEGF concentration is a useful biomarker of ocular outcomes.

CHAPTER 2: ASSESSMENT OF PLASMA VEGF CONCENTRATIONS AFTER INTRAVITREAL ANTI-VEGF THERAPY FOR DIABETIC MACULAR EDEMA

2.1 Study Objective

The primary objective of the proposed research is to assess and compare changes in plasma concentrations of VEGF after intravitreal treatment with ranibizumab versus bevacizumab versus aflibercept for diabetic macular edema.

2.2 Eligibility Criteria and Informed Consent

All study participants in the DRCR.net Protocol T will be eligible for participation in this ancillary study. Enrollment into this ancillary study will be a maximum of 660 study participants since this is the total expected to enroll in the main trial. However participation in the ancillary study is not a requirement for participation in the primary trial and therefore, participants and clinical centers can opt out of the plasma collection ancillary study. It is expected that sites with the capability will participate in this ancillary study.

Prior to obtaining a blood sample, written informed consent will be obtained.

2.3 Sample Collection Time points

Visit	**0**	**4w**	**52w**	**104w**
Blood sample for plasma collection (prior to any injection)	X	X	X	X

2.4 Collection, Processing, Handling, and Shipment Procedures

In general, blood will be collected using a CTAD tube, prior to an injection being given (if an injection is to be given per protocol at that visit). The filled tube will be centrifuged to separate the plasma. Plasma will be transferred into plasma collection tubes using a pipette. The plasma sample will be shipped to the Fred Hutchinson Cancer Research Center on dry ice.

Refer to the DRCR.net Study Procedures Manual for the full collection, processing, handling, and shipping procedure.

2.5 Analysis

The VEGF concentration in the plasma samples will be measured using a validated assay to be determined. The primary analysis will compare the average VEGF concentration between the 3 treatment groups at the 52 week visit.

REFERENCES

1. Genentech I. AVASTIN (bevacizumab) injection, solution http://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm?id=14571. Accessed Accessed: 12 July 2010.

2. Chakravarthy U, Harding SP, Rogers CA, et al. Ranibizumab versus Bevacizumab to Treat Neovascular Age-related Macular Degeneration: One-Year Findings from the IVAN Randomized Trial. *Ophthalmology.* 2012.

3. Boyer DS, Heier JS, Brown DM, Francom SF, Ianchulev T, Rubio RG. A Phase IIIb study to evaluate the safety of ranibizumab in subjects with neovascular age-related macular degeneration. *Ophthalmology.* 2009;116(9):1731-9.

4. Diabetic Retinopathy Clinical Research Network. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology.* 2010;117(6):1064-77 e35.

5. Nguyen QD, Brown DM, Marcus DM, et al. Ranibizumab for Diabetic Macular Edema: Results from 2 Phase III Randomized Trials: RISE and RIDE. *Ophthalmology.* 2012;119(4):789-801.

6. Michaelides M, Kaines A, Hamilton RD, et al. A prospective randomized trial of intravitreal bevacizumab or laser therapy in the management of diabetic macular edema (BOLT study) 12-month data: report 2. *Ophthalmology.* 2010;117(6):1078-86 e2.

7. Fung AE, Rosenfeld PJ, Reichel E. The International Intravitreal Bevacizumab Safety Survey: using the internet to assess drug safety worldwide. *Br J Ophthalmol.* 2006;90(11):1344-9.

8. Wu L, Martinez-Castellanos MA, Quiroz-Mercado H, et al. Twelve-month safety of intravitreal injections of bevacizumab (Avastin): results of the Pan-American Collaborative Retina Study Group (PACORES). *Graefes Arch Clin Exp Ophthalmol.* 2008;246(1):81-7.

9. Martin DF, Maguire MG, Ying GS, Grunwald JE, Fine SL, Jaffe GJ. Ranibizumab and bevacizumab for neovascular age-related macular degeneration. *N Engl J Med.* 2011;364(20):1897-908.

10. Martin DF, Maguire MG, Fine SL, et al. Ranibizumab and Bevacizumab for Treatment of Neovascular Age-Related Macular Degeneration: Two-Year Results. *Ophthalmology.* 2012;E-Press.

11. Two year results of phase 3 studies with EYLEA (aflibercept) injection in wet AMD show sustained improvement in visual acuity. http://www.prnewswire.com/news-releases/two-year-results-of-phase-3-studies-with-eylea-aflibercept-injection-in-wet-amd-show-sustained-improvement-in-visual-acuity-135011348.html. Accessed 13 February 2012.

12. Carneiro AM, Costa R, Falcao MS, et al. Vascular endothelial growth factor plasma levels before and after treatment of neovascular age-related macular degeneration with bevacizumab or ranibizumab. *Acta Ophthalmol.* 2012;90(1):e25-30.

13. Ma Y, Zhang Y, Zhao T, Jiang YR. Vascular endothelial growth factor in plasma and vitreous fluid of patients with proliferative diabetic retinopathy patients after intravitreal injection of bevacizumab. *Am J Ophthalmol.* 2012;153(2):307-13 e2.

14. Qian J, Lu Q, Tao Y, Jiang YR. Vitreous and plasma concentrations of apelin and vascular endothelial growth factor after intravitreal bevacizumab in eyes with proliferative diabetic retinopathy. *Retina.* 2011;31(1):161-8.

15. Matsuyama K, Ogata N, Matsuoka M, Wada M, Takahashi K, Nishimura T. Plasma levels of vascular endothelial growth factor and pigment epithelium-derived factor before and after intravitreal injection of bevacizumab. *Br J Ophthalmol.* 2010;94(9):1215-8.

APPENDIX 2

Extension Study: 5 year Follow-Up

CHAPTER 1. BACKGROUND INFORMATION AND STUDY SYNOPSIS

1.1 Background and Rationale

1.1.1 Background – Protocol T

Between August 2012 and August 2013 DRCR.net randomized 660 participants into Protocol T, a comparative effectiveness randomized trial of aflibercept, bevacizumab, or ranibizumab for eyes with decreased visual acuity from diabetic macular edema. The trial reported primary 1-year results in 2015 and the final 2-year results in 2016. Participants were followed for a total of 2 years. Treatment with the assigned anti-VEGF continued through 2 years based on a structured retreatment algorithm. The two-year visit was completed by 578 (88%) participants. Of the 82 participants who did not complete the 2 year visit, 24 had died.

The median numbers of injections were 5, 6, and 6 in year 2 and 15, 16, and 15 over 2 years in the aflibercept, bevacizumab, and ranibizumab groups, respectively. At the 2 year visit, eyes with worse baseline VA (20/50 to 20/320) (N=284) had a mean visual acuity improvement of 18.1, 13.3, and 16.1 letters, respectively (aflibercept vs. bevacizumab, P = 0.02; aflibercept vs. ranibizumab, P = 0.18; ranibizumab vs. bevacizumab, P = 0.18). In eyes with better baseline VA (20/32 to 20/40) (N=293), mean improvement at 2 years was 7.8, 6.8, and 8.6 letters, respectively (P > 0.10, for pairwise comparisons). In eyes with worse baseline visual acuity 25% of eyes in the aflibercept group, 54% in the bevacizumab group, and 34% in the ranibizumab group had center-involved DME on OCT at 2 years. Among eyes with better baseline visual acuity 33%, 63%, and 36% of eyes, respectively had center-involved DME on OCT. Anti-Platelet Trialists' Collaboration (APTC) events occurred in 5% with aflibercept, 8% with bevacizumab, and 12% with ranibizumab (P=0.047).

In conclusion, all 3 anti-VEGF groups showed VA improvement from baseline to 2 years with a decreased number of injections in year 2. Visual acuity outcomes were similar for eyes with better baseline VA. Among eyes with worse baseline VA, aflibercept had superior 2-year VA outcomes compared with bevacizumab, but superiority of aflibercept over ranibizumab, noted at 1 year, was no longer identified at 2 years. Higher APTC event rates with ranibizumab over 2 years warrant continued evaluation in future trials.

1.1.2 Available Data on Longer Term Outcomes

There have been several published trials on the effects of anti-VEGF therapy on DME beyond 2 years. DRCR.net Protocol I demonstrated that on average eyes initially treated with ranibizumab maintained vision gains obtained by the first year through 5 years with minimal treatment after 3 years. At 5 years, the ranibizumab and deferred laser group gained an average of 9.8 letters from baseline even though the median number of injections in year 4 was 1 and year 5 was 0. Eyes in the ranibizumab and prompt laser group gained an average of 7.2 letters from baseline, even though the median number of injections in years 4 and 5 was 0.[1]

RISE and RIDE showed visual acuity (VA) outcomes seen at month 24 in ranibizumab groups were consistent through month 36 with continued monthly ranibizumab dosing. In RIDE, the mean number of ETDRS letters change from baseline at month 24 versus change from baseline at month 36 in patients randomized to 0.3 mg ranibizumab was 10.9 versus 10.6. In RISE, 12.5 versus 13.2.[2 2]

Results from the RIDE/RISE open label extension study, during which participants who participated in the core studies were treated with ranibizumab on a pro re nata basis for DME, also demonstrated maintenance of visual gains from the first 3 years of treatment despite reduced frequency dosing.[3]

The VIVID and VISTA studies, which assessed efficacy of intravitreous aflibercept for DME treatment, demonstrated that participants' 148 week and 100 week visual outcomes were consistent with the 52-week visual outcomes. . The mean BCVA gain from baseline to week 148 was 10.4 and 10.5 for the intravitreous aflibercept 2q4 regimen and 2q8, respectively in VISTA and 10.3 and 11.7 in VIVID.[4] The mean BCVA gain from baseline to week 100 was 11.5 and 11.1 for the intravitreous aflibercept 2q4 regimen and 2q8, respectively in VISTA and 11.4 and 9.4 in VIVID.[5] The mean BCVA gain from baseline to week 52 in VISTA was 12.5 and 10.7 and 10.5 and 10.7 in VIVID, in 2q4 and 2q8 groups respectively.[6]

1.1.3 Protocol T Beyond 2 Years

Bringing back Protocol T patients to complete one five year visit will provide information on treatment course, changes in visual acuity and macular edema after protocol specific treatment was stopped. Patients who were randomized in Protocol T will be asked to return to one of the approximately 80 Protocol T clinical sites currently active in the Network for an eye exam, visual acuity, OCT, and fundus photography approximately 5 years from initial randomization date. Medical history, diabetic retinopathy and DME treatment history will be collected.

1.1.4 Estimation of Number of Participants in Follow-up Study

83* of the original 88 clinical sites were surveyed to assess potential availability of participants. Of the 588 participants at the survey sites who were not known to be deceased prior to 2 years, 364 (62%) participants were seen at the practice within the prior 6 months and 395 (67%) were seen within the prior 12 months. The percentage of participants who died during the clinical trial was 2% in both the first and second year. Assuming that trend increases slightly over years 3-5, we can roughly expect approximately 10% of the participants alive at the end of the 2-year randomized trial to have died between years 3-5. Beginning with 588 participants alive at the end of the Protocol T clinical trial at active DRCR.net sites, applying a 10% death rate and 80% participation rate results in approximately 425 participants. However, since some sites may decline participation in the extension study, the goal is to include at least 400 participants in the extension study.

**5 sites have been dropped from the Network since Protocol T completed*

1.2 Study Objectives

The primary objective is to perform descriptive analyses for the following:

- Visual acuity outcomes at 5 years
- DME outcomes at 5 years
- Types of DME treatments used since 2 year study visit
- Frequency of DME agents used since 2 year study visit
- Treatments for diabetic retinopathy since 2 year study visit
- Diabetic retinopathy outcomes at 5 years
- APTC events occurring in participants since 2 year study visit

Secondary analyses will include original treatment group comparisons for the following:

- Visual acuity outcomes at 5 years
- DME outcomes at 5 years
- Diabetic retinopathy outcomes at 5 years

1.3 Study Design and Synopsis of Protocol

A. Study Design

- Cohort study

B. Eligibility Criteria

- Study eyes of previously randomized participants in Protocol T

C. Visits and Procedures

Participants would return for one follow-up visit 5 years (± 6 months) from original randomization for the following procedures to be performed:

- Assessment of DME and diabetic retinopathy treatments since 2 year visit
- Ocular and medical history since 2 year visit
- Best-corrected E-ETDRS visual acuity
- Comprehensive, dilated eye exam
- Optical coherence tomography
- HbA1c
- Color fundus photographs
- Assessment of APTC events since the 2 year visit

D. Sample Size

Sample size is expected to be between 400 and 425 participants.

CHAPTER 2. ELIGIBILITY AND INFORMED CONSENT

2.1 Eligibility

All participants randomized in Protocol T will be eligible for this extension follow-up study.

2.2 Informed Consent

Study participants randomized in Protocol T will be asked to return to one of the Protocol T clinical sites to complete a 5-year follow up visit. Prior to performing any study procedures, the participant will be asked to sign an informed consent form. If the participant does not sign the informed consent form, they will not participate in this study extension and no study procedures will be completed.

2.3 Patient Recruitment

All patients who were randomized in the Protocol T clinical trial who are not known to be deceased will be targeted for recruitment, including those who did not complete their 1 or 2 year visit in the clinical trial. Currently active DRCR.net clinical sites that participated in Protocol T will be asked to participate in this extension study. Participants from sites who decline to participate will not be included. Clinical coordinators from participating sites will contact the patients who were randomized at their site to invite participation either in-person while the patient is undergoing routine care at the DRCR.net clinical site, or by letter or telephone if the patient is not receiving eye care at the clinical center. Coordinators will make use of additional contact information provided by the patient at entry into Protocol T to attempt to reach the patient.

CHAPTER 3. FOLLOW-UP VISIT

3.1 Visit Schedule

Each willing participant will have one follow-up study visit, approximately 5 years (±6 months) after original randomization.

3.2 Testing Procedures

The testing procedures will be the same as in the Protocol T clinical trial and are detailed in the DRCR.net Procedures Manuals. Visual acuity testing, ocular exam, fundus photography, and OCT will be performed by DRCR.net certified personnel. When feasible, visual acuity, OCT, and fundus photography should be performed by personnel masked to the participant's original treatment group assignment.

The following procedures will be performed.

6. Assessment of DME and diabetic retinopathy treatments since 2 year (or last) visit for the study eye
7. Ocular and medical history since 2 year visit
8. Best corrected E-ETDRS visual acuity testing (including protocol refraction) in each eye
9. Ocular examination on each eye including slit lamp, measurement of intraocular pressure, lens assessment, and dilated ophthalmoscopy
10. Spectral domain OCT on study eye using Zeiss Cirrus or Heidelberg Spectralis
    - The same OCT machine used at the 2 year visit should be used if possible
11. Digital fundus photographs of the study eye
    - 4 wide field and 7 modified field images are preferred
    - Ultrawide field images are accepted only if 4 wide and 7 modified are not available
12. Hemoglobin A1c
    - *HbA1c does not need to be repeated if available in the prior 12 months.*

CHAPTER 4. ADVERSE EVENTS

4.1 Adverse Events/Risks

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.

4.2 Adverse Event Reporting

A complete adverse event history between 2 and 5 years will not be collected. Participants will be asked about the occurrence of specific events (e.g. heart attack and stroke). Where possible, clinical documentation will be obtained for reported heart attack and strokes. Where possible, death records will be obtained for participants who died between 2 and 5 years.

CHAPTER 5. PARTICIPANT PAYMENTS

The study will be providing the study participant with a $50 merchandise or money card for the completed visit. Additional travel expenses will be paid in select cases for study participants with higher expenses.

CHAPTER 6. STATISTICAL METHODS

The approach to statistical analyses is summarized below. A detailed statistical analysis plan will be written and finalized prior to the completion of the study.

6.1 Main Analyses

The primary objective is to perform descriptive analyses for the full cohort for the following:

- Types of DME treatments used since 2 year study visit
  - Types of anti-VEGF injections
  - Focal/grid laser treatments
  - Types of other DME treatments
- Frequency of DME agents used since 2 year study visit
- Treatments for diabetic retinopathy since 2 year study visit
- Visual acuity outcomes at 5 years
  - Mean change in visual acuity from baseline and from 2 year (or last) visit (primary VA outcome)
  - Proportion of eyes with 2 and 3 or more line gains or losses in visual acuity from baseline and from 2 year (or last) visit.
  - Distribution of visual acuity levels at 5 years
- DME outcomes at 5 years
  - Mean change in OCT from baseline and from 2 year (or last) visit (primary OCT outcome)
  - Proportion of eyes with OCT central subfield thickness < 250 μm on Zeiss Stratus or the equivalent on spectral domain OCT based on gender specific cutoffs at 5 years
- Diabetic retinopathy outcomes at 5 years
  - Diabetic retinopathy severity on fundus photos at 5 years
  - Improvement in diabetic retinopathy from baseline and from 2 years
  - Worsening in diabetic retinopathy from baseline and from 2 years

Secondary analyses will include original treatment group comparisons for the following:

- Visual acuity outcomes at 5 years (see outcomes above)
- DME outcomes at 5 years (see outcomes above)
- Diabetic retinopathy outcomes at 5 years (see outcomes above)

Note: If there are a substantial number of participants in all three treatment groups who were solely treated with their randomized treatment during the 5 years following randomization, a sensitivity analysis will be performed including only those participants.

Analyses will consist of three two-group comparisons. Within each outcome, the Hochberg approach will be used to control the Type 1 error. Binary outcomes will be analyzed using logistic regression models adjusting for baseline factors where appropriate. Continuous outcomes will be analyzed using an analysis of covariance model adjusting for baseline measures where appropriate. All linear model assumptions will be verified including linearity, normality of residuals, and homoscedasticity. If model assumptions are not met data transformation or a nonparametric analysis will be considered.

Subgroup analyses mirroring all analyses described above will be performed for participants with baseline visual acuity 20/32 to 20/40 and participants with baseline visual acuity 20/50 or worse.

Missing data will be excluded.

Since treatment for DME and DR between 2 and 5 years was at investigator discretion and may or may not have been the same treatment as initially randomly assigned, treatment during this stage will be considered when interpreting the results.

6.2 Safety Analyses

The frequency of the event occurring at least once per participant will be calculated.

- Death
- Cardiovascular/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)

  Notes: Transient ischemic attacks, angina, and possible MI or stroke are not counted. 'Nonfatal' MI or stroke require that the patient is alive at the end of the study. If not, only the death is counted.

Original treatment group comparisons will be performed as described above. Cumulative event rates by year will be reported. Since treatment between 2 and 5 years is at investigator discretion, interpretation of safety analyses will proceed with caution.

CHAPTER 7. REFERENCES

1. Bressler SB, Glassman AR, Almukhtar T, et al. Five-Year Outcomes of Ranibizumab With Prompt or Deferred Laser Versus Laser or Triamcinolone Plus Deferred Ranibizumab for Diabetic Macular Edema. *Am J Ophthalmol.* 2016;164:57-68.

2. Brown DM, Nguyen QD, Marcus DM, et al. Long-term outcomes of ranibizumab therapy for diabetic macular edema: the 36-month results from two phase III trials: RISE and RIDE. *Ophthalmology.* 2013;120(10):2013-22.

3. Boyer DS, Nguyen QD, Brown DM, Basu K, Ehrlich JS. Outcomes with As-Needed Ranibizumab after Initial Monthly Therapy: Long-Term Outcomes of the Phase III RIDE and RISE Trials. *Ophthalmology.* 2015;122(12):2504-13.e1.

4. Heier JS, Korobelnik JF, Brown DM, et al. Intravitreal Aflibercept for Diabetic Macular Edema: 148-Week Results from the VISTA and VIVID Studies. *Ophthalmology.* 2016;123(11):2376-85.

5. Brown DM, Schmidt-Erfurth U, Do DV, et al. Intravitreal Aflibercept for Diabetic Macular Edema: 100-Week Results From the VISTA and VIVID Studies. *Ophthalmology.* 2015.

6. Korobelnik JF, Do DV, Schmidt-Erfurth U, et al. Intravitreal aflibercept for diabetic macular edema. *Ophthalmology.* 2014;121(6):2247-54.