Diabetic Retinopathy Clinical Research Network

Peripheral Diabetic Retinopathy (DR) Lesions on Ultrawide-field Fundus Images and Risk of DR Worsening Over Time

Version 4.0

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INTRODUCTION

1.1 Fundus Images for Determining DR and DME Severity Level

Photographic documentation of the fundus has been the standard method for detecting and assessing severity levels of diabetic retinopathy (DR) and diabetic macular edema (DME) since the Early Treatment Diabetic Retinopathy Study (ETDRS) first utilized seven standard field 35-mm film stereoscopic color photographs and the modified Airlie House classification to demonstrate the characteristics and extent of clinically pertinent lesions of DR.[1, 2] ETDRS standardized grading of the presence and severity of multiple lesions, including hemorrhages and microaneurysms, venous caliber abnormalities or intraretinal microvascular abnormalities in each of the 7 standard fields yields an overall level of DR severity.

Photographic-documentation of DR lesions by well-established imaging and grading protocols allows standardization of DR assessment across a wide range of study sites. Many multicenter trials have utilized fundus images for grading DR severity. Seminal studies in DR have relied upon ETDRS-protocol stereoscopic fundus photography to record the extent and severity of DR lesions in their study participants. [3] The Diabetic Retinopathy Clinical Research Network (DRCR.net) has also utilized graded fundus images in order to establish DR severity for all of its studies in which DR worsening or severity has been a primary or important secondary outcome, and has shown good agreement between investigator grading of diabetic retinopathy severity level from clinical examination and that obtained by standardized grading of fundus photographs.[4]

Although the ETDRS protocol is well-validated and an established method of image acquisition of fundus photographs for DR severity level determination, it has several disadvantages including the use of film slides and the need for multiple images that are not always well tolerated by study participants exposed to the associated bright camera flashes. Thus, several modifications to the 7 standard field stereoscopic film imaging protocol have been evaluated, validated, and widely accepted for clinical research in DR, including the substitution of uncompressed, digital images for film images and the acquisition of fewer 45º to 60º wide angle images as compared with the ETDRS standard 30º to 35º 7 standard fields. [5-12]

1.2 Current DRCR.net Imaging Protocols

The current standard DRCR.net method of determining DR severity levels is to grade 7-field modified or 4-field wide angle stereoscopic digital photographs obtained from eyes of study participants after pupillary dilation.

The 7-field protocol consists of fields 1M, 2, and 3M, which are centered on the temporal edge of the optic nerve, the macula, and the temporal macula respectively, and fields 4-7 which capture the superotemporal, inferotemporal, superonasal, and inferotemporal quadrants, respectively (Figure 1). Images are taken on 30º to 35º settings depending on the fundus camera being utilized for image acquisition. An anterior segment image that provides a fundus reflex is also standard. In order to achieve an adequate stereoscopic effect, dilation of the pupil to at least 6 mm is recommended.

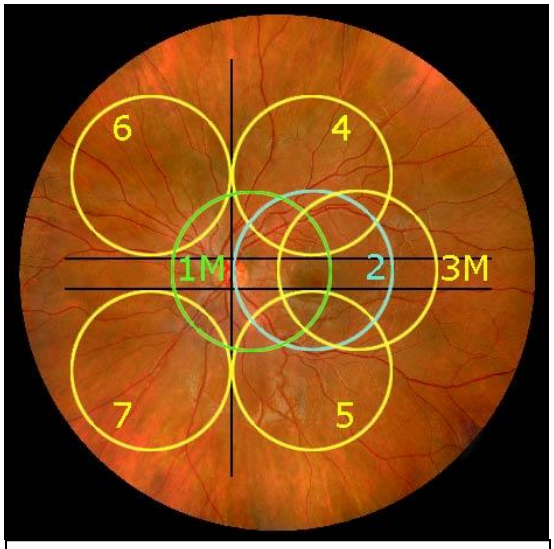

Figure 1. DRCR.net imaging protocol: modified ETDRS 7 standard fields
Photograph: http://eyephoto.ophth.wisc.edu/photography/tutorial/slide22.html

Obtaining 4 wide-angle stereoscopic fields requires a camera with a 45º to 60º view. The 4 fields consist of Field 1W located nasal to the disc, Field 2W centered temporal to the macular center, Field 4W located superotemporally and Field 5W located inferotemporally (Figure 2). A stereoscopic fundus reflex photograph also is taken in order to document media opacities.

There are disadvantages to both the 7-field and 4-field wide angle stereoscopic digital methods, including necessitating pupillary dilation in all study participants, at least 10-16 images/flashes per eye, the need to sometimes refocus between acquisition of different fields, and extensive training and certification of study imagers to ensure competence in field definition and image quality. In addition, there are areas of the mid peripheral and peripheral retina that are not covered by either the 7-field or 4-field wide angle methods

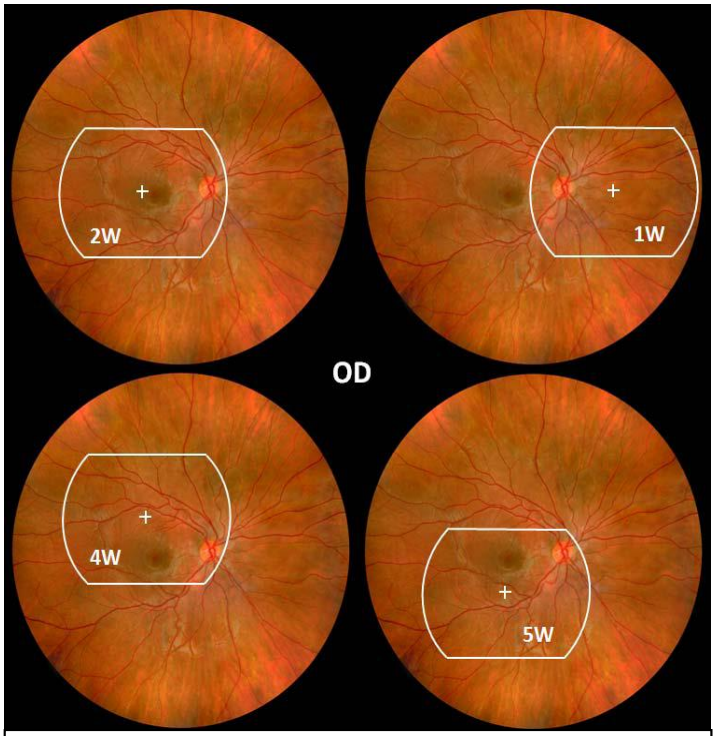

Figure 2. DRCR.net imaging protocol: Fields 1W, 2W, 4W, 5W of the right eye. Photograph: http://http:/eyephoto.ophth.wisc.edu/photography/PDFs/4W-D.pdf

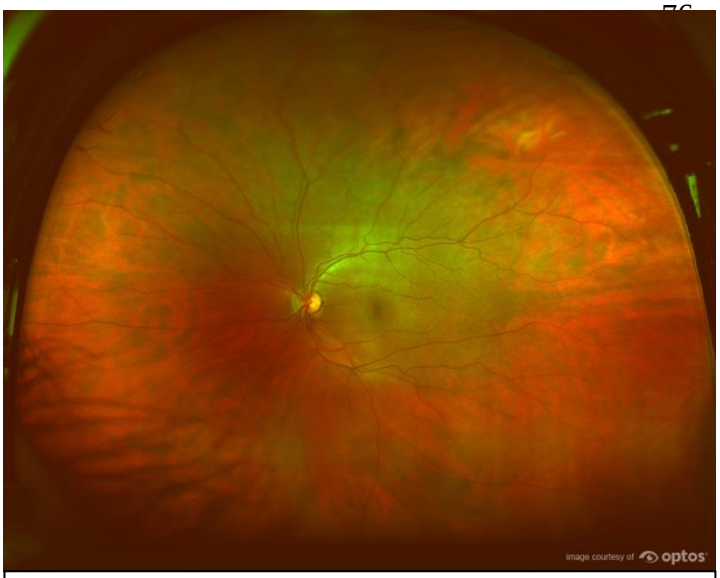

Figure 3. Optomap® 200º image Photograph: http://www.optos.com/en-us/Professionals/Image-library/Color-fundus-images/Healthy/

1.3 Ultrawide Field Fundus Imaging

Additional methods for determining DR severity levels have been developed to permit wide field imaging of the retinal periphery with fields encompassing more than 60 º, including the Pomerantzeff camera, the Retcam, the Panoret-1000™ camera, and wide angle contact lenses.[13] The Staurenghi lens is currently the most widely used wide angle contact lens and is designed for utilization with a scanning laser ophthalmoscope camera. However, this has not been widely adopted clinically because the use of a contact lens can be cumbersome and not well tolerated by patients. An additional non-contact wide field angiography imaging system has recently become commercially available with Spectralis Spectral Domain optical coherence tomography (SD OCT) (Heidelberg, Germany), although this system has not yet been widely used in either clinical or research settings, and currently is limited to infra-red, ICG, and fluorescein angiographic imaging and specifically cannot obtain color fundus photographic images.[13]

The Optomap® system (Optos, Scotland, UK) utilizes a non-contact scanning laser ophthalmoscope technology that allows ultrawide field (UWF), high definition color imaging of the retina with the potential to image more than 80% of the retina in a single view. Both 100º and 200º fields (Figure 3) can be obtained through an undilated pupil with excellent image quality. Optos technology utilizes low-powered 532 nm (green) and 633 nm (red) laser wavelengths that scan simultaneously, allowing review of the retinal substructures by their individual laser separations. The system also allows red-free imaging and fluorescein angiography. Ultrawide field images have been utilized for the detection and evaluation of multiple types of non-diabetic ocular pathology, including sickle cell retinopathy,[14] retinal detachment,[15] choroidal pigmented lesions,[16] giant retinal tear,[17] cytomegalovirus retinitis,[18] congenital hypertrophy of the retinal pigment epithelium,[19, 20] choroidal detachment,[21] and trauma-related injuries.[22]

Several small studies comparing Optomap® images to clinical exam and ETDRS-protocol photographs for the evaluation of DR severity have been published by the Ludwig Maximilian University group in Munich, Germany. One study comparing grading of DR severity using nonmydriatic Optomap® images versus dilated clinical ophthalmoscopic examination in 51 eyes of 51 diabetic patients, found good levels of agreement between the two modalities. Although, 9.8% of the images were ungradable.[23] Three independent readers graded each of the UWF images, resulting in unweighted kappa statistics for DR severity of 0.68, 0.68 and 0.51. A sensitivity of 94% and specificity of 100% was obtained for all three graders' assessment of more than mild non-proliferative diabetic retinopathy (NPDR) on the ultrawide-field images. Assessment of clinically significant macular edema on UWF images revealed sensitivities of 89-93% and specificities of 72-89%. A second study compared 200º images with ETDRS 7 field fundus photographs in 66 eyes of 34 patients. In the 48 sets that could be graded for both ETDRS and UWF images, kappas of 0.70 and 0.66 were obtained for agreement of DR severity level and 0.68 and 0.74 for DME severity.[24] A

third study also resulted in substantive agreement between UWF and ETDRS grading for both DR and for DME severity (Kappas = 0.79, 0.77 for DR and 0.73, 0.77 for clinically significant macular edema [CSME]). [24]

A small single-center imaging validation study was also conducted at the Beetham Eye Institute, Joslin Diabetes Center (BEI/JDC) to compare nonmydriatic Optomap® UWF images to mydriatic ETDRS-protocol 30º 7 standard field stereoscopic fundus film photographs for the grading of DR and DME. [24] Subjects underwent nonmydriatic 100º and 200º imaging, dilated ETDRS photography and dilated ophthalmoscopic examination by a masked retina specialist. Images were graded by two independent masked readers according to a strictly defined protocol. Each image was graded for presence and extent of specific diabetic lesions as well as for overall clinical DR severity. An independent masked retina specialist adjudicated any discrepancies. Unweighted (K) and weighted (KW) kappa statistics (linear scale) assessed agreement. Images from 200 eyes of 103 patients with type 1 or 2 diabetes were evaluated. By ETDRS photographs there was no DR in 25 (12.5%) eyes, mild NPDR in 47 (23.5%), moderate NPDR in 61 (30.5%), severe or very severe NPDR in 14 (7%), proliferative DR (PDR) in 52 (26%), and 1 (0.5%) eye was ungradable. No DME was present in 114 eyes (57%), non-clinically significant DME in 28 (14%), CSME in 47 (23.5%) and 11 (5.5%) images were ungradable. Exact agreement of DR severity grading between 100º images and ETDRS photographs occurred in 84% with agreement within one level in 91% (KW=0.85, K=0.79). Optomap® images exactly matched DR grading by clinical exam in 70% and were within one level in 93% (KW=0.77, K=0.61). Exact agreement with ETDRS photographs for DME graded on a 3-category scale (No DME, DME < CSME, CSME) occurred in 79% and was within 1 step for 91% of eyes (KW=0.66, K=0.60). Nonmydriatic UWF imaging time was significantly shorter than that of dilated ETDRS photographs, even when excluding dilation time (Mean $\pm$ SD: 170 ± 80 versus 370 ± 130 seconds, $p<0.0001$).

Results from these small studies evaluating UWF images for DR severity level assessment suggest that grading of undilated Optomap® images demonstrates high agreement with grading of dilated ETDRS photographs and assessment by dilated fundus examination in determining severity of DR and DME. In addition, the 100º and 200º images obtained on the Optos system cover more retinal area than is evaluated by current DRCR.net imaging protocols. If results from these single-center, smaller cohorts are confirmed in a broader diabetic population, Optos imaging may be applicable to both research and clinical settings with the additional benefits of faster imaging times and easier acquisition through an undilated pupil.

Peripheral Lesions on Ultrawide-field Imaging and Progression of Non-proliferative Diabetic Retinopathy

Another benefit of UWF imaging is the ability to find far peripheral lesions that are outside the range of standard ETDRS fields. Studies of UWF fundus images have demonstrated that peripheral DR lesions can be identified that are not present within the ETDRS fields, including retinal nonperfusion and neovascularization in diabetic eyes.[25] Additional data from the BEI/JDC suggest that these peripheral lesions have implications for diagnosing more severe DR.[26] Peripheral lesions were present in more than half the eyes imaged (54%) and were more prominent outside the standard ETDRS 7 fields in 30-40% of eyes and these lesions suggested a more severe DR level in 10% of eyes. Another study that examined an independent sample of 502 eyes imaged with nonmydriatic 100º and 200º UWF images found similarly that the distribution of peripheral lesions outside ETDRS fields suggested a more severe DR level in 9.0% (N = 45) of eyes. [24]

Rates of DR worsening from baseline DR levels were well established in early seminal studies, but these were based solely on lesions within the standard ETDRS fields.[1] Improved ability to reliably identify peripheral DR lesions outside the standard ETDRS 7 fields on UWF images may have implications for how we assess risk of future DR worsening or improvement. If lesions identifiable only on UWF imaging improve our ability to predict rates of worsening or improvement of diabetic eye complications, this information could be highly valuable in clinical and research decisions as to how patients are followed and managed.

Pilot data from the Joslin Diabetes Center suggest that peripheral lesions on UWF images may serve as biomarkers of faster worsening of DR severity level. In 121 eyes with NPDR at baseline, UWF imaging was used at baseline and data on DR worsening were gathered from clinic records over 3 years. The absence of baseline, predominantly, peripheral lesions was associated with risk reductions of 62%, 67% and 73% for future DR worsening at 1, 2 and 3 years respectively.[26] Preliminary BEI/JDC data from a subset of 109 eyes in an ongoing study with repeat mydriatic ETDRS 7 standard fields, obtained on average 4 years after baseline UWF imaging, revealed higher rates of DR worsening over time in eyes with predominantly peripheral lesions at baseline, which was associated with a >5 fold increase in ≥2 step progression of DR severity level at year 4 (11% versus 34%, $P = 0.005$).[27]

1.4 Ultrawide-field Fluorescein Angiography

In addition to standard fundus photographs, UWF imaging technology allows broader views of the retinal periphery during fluorescein angiography (FA). Applications of fluorescein angiography for evaluation and management of diabetic retinopathy includes identification of leakage from microaneurysms and retinal neovascularization and, delineation of areas of non-perfusion. An early study utilizing UWF FA found 3.9 times more nonperfusion and 1.9 times more neovascularization on UWF FA images than conventional 7 standard field images.[25] In addition, reports of UWF FA have identified peripheral areas of retinal vascular staining and leakage in diabetic eyes,[28] although it is not yet clear how these abnormalities relate to central pathology and vision loss. Some authors have reported using UWF FA to guide treatment of peripheral laser photocoagulation for either DR or retinal vein occlusion.[29] Nonetheless, many questions remain as to how to standardize acquisition and analysis of UWF FAs as well as how to utilize the findings from UWF FA to guide evaluation and management of patients with diabetic and other ocular pathology. This study will include the acquisition of UWF FA to determine whether the presence or severity of peripheral non-perfusion as evaluated on UWF FA is associated with increased rates of DR or DME worsening over time beyond what is seen on modified 7-field stereoscopic digital photographs or beyond what is seen on UWF fundus color photographs.

1.5 Association of Diabetic Retinopathy, Nephropathy and Cardiovascular Complications

In addition to retinal disease, persons with diabetes are at increased risk of other systemic vascular complications including kidney and cardiovascular disease. Previous studies have suggested a positive correlation between increasing severity of diabetic retinopathy and both nephropathy[30] and cardiovascular disease.[31] Given our ability to visualize blood vessels in the retina using non-invasive photographs, aspects of retinal anatomy aside from overall DR severity level may provide insight into the development of complications in the kidney and cardiovascular system. Therefore, this study will also explore whether parameters on UWF field fundus photography and UWF FA are associated with evidence of end organ damage in the kidney and cardiovascular system and to see if such changes in the retina predict incident end organ damage or progression of prevalent end organ damage. We hypothesize that more severe extent of diabetic retinopathy lesions and retinal

nonperfusion evaluated on UWF color photographs and fluorescein angiography will be significantly associated with increased prevalence and incidence of diabetic nephropathy and/or cardiovascular disease. If results from this study demonstrate that changes in retinal vascular parameters reflect systemic micro and macrovascular pathology, results from this project could substantively impact the evaluation and management of patients with diabetes. The identification of retinal vascular characteristics as biomarkers of systemic diabetic complications would allow more effective clinical risk stratification and provide alternative methods for clinical research assessment of diabetic vascular disease.

1.6 Summary of Protocol Rationale

This study will investigate the association between peripheral retinal lesions and the likelihood of DR worsening or improvement over time and provide a comparison of UWF images to current DRCR.net protocol 7 standard field fundus photographs for grading severity levels of DR and DME. Ultrawide field fundus images offer the ability to evaluate areas of the retinal far periphery that are not covered by the 7 standard fields. If peripheral retinal lesions identified on UWF images improve our ability to predict likelihood of DR severity level worsening or improvement, this not only might change ways in which patients at risk for diabetic eye complications are evaluated and followed, but also offer new insights into mechanisms for changes in retinal pathology. Even if identification of peripheral lesions does not provide increased ability to predict DR outcomes, if UWF images are comparable for DR and DME assessment to those obtained by current DRCR.net protocol, the ability to substitute UWF imaging for our current methods offers several potential benefits, including imaging portions of the retina that are not evaluated by the current protocol, decreases in the number of images taken with consequent increased imaging speed and study participant exposure to fewer light flashes, reduced requirements for imager training and reduced image reading time. These benefits could translate into increased patient participation in DRCR.net studies, improved ability to evaluate severity of DR and DME, greater ease and comfort for study participants in obtaining fundus photographs, and substantial savings in time and cost related to image acquisition and imager training and certification. Thus, the results from this protocol could potentially have wide ranging influence on future clinical trial protocols developed for participants with DR and DME.

1.7 Study Objective

Primary Objective:

1. To assess whether evaluation of the retinal far periphery on UWF images improves the ability to assess DR and predict rates of DR worsening over time as compared with evaluation only of the area within the 7 standard ETDRS fields.

This objective will be accomplished through the following specific analyses:

1. Assessment of whether any predominance versus no predominance of diabetic retinopathy lesions (see definition section 1.7) in any field of the retinal periphery (lesions located primarily outside versus primarily within the 7 standard ETDRS fields) on UWF images is associated with rates of DR worsening over time
2. Redefining diabetic retinopathy severity grading level based on the status of the periphery and assessing whether differences in severity level assessment between grading with or without inclusion of peripheral findings is associated with rates of DR worsening over time.
3. Evaluating how often mydriatic 200º UWF digital photographs are comparable to mydriatic DRCR.net protocol modified 7-field stereoscopic digital photographs for the grading and

assessment of DR, and whether grading of the UWF photographs can be reliably used as an outcome variable in future clinical trials.

4. Determining whether extent and location (peripheral versus posterior) of nonperfusion on UWF fluorescein angiograms is associated with baseline DR and DME severity as well as rates of DR and DME worsening over time

Secondary Objective:

To explore whether the prevalence and severity of diabetic nephropathy or cardiovascular disease at baseline and the incidence of these findings over time (4 years) is associated with

- The severity and location (within and peripheral to the standard ETDRS 7 fields) of classic non-proliferative diabetic retinopathy lesions including hemorrhages and microaneurysms, venous beading, intraretinal microvascular anomalies, and neovascularization) identified on UWF FA and UWF fundus photos.
- The extent of peripheral non-perfusion on UWF fluorescein angiography

1.8 Definitions

1. **Ultrawide field**: Fundus photography field that is 100º or more
2. **Peripheral Lesions**: Lesions located outside of the modified ETDRS 7-standard fields
3. A *lesion* (each of the following lesions is graded separately: hemorrhages/microaneurysms, venous beading, intraretinal microvascular abnormalities and neovascularization elsewhere) is **predominantly peripheral** in a specific *field* (fields 3-7 graded separately) if more than 50% of the lesions are in the retinal periphery compared with within the modified ETDRS fields taking into account the number and extent.
4. A *lesion* is **uniformly distributed** in a specific *field* if the severity of lesion (taking into account number and extent) is approximately equivalent both within and outside the ETDRS field
5. An *eye* has predominantly peripheral lesions if any one of the lesions graded in any field is predominantly peripheral.

1.9 Study Design and Synopsis of Protocol

A. Study Design

Prospective, observational longitudinal study

B. Major Eligibility Criteria

- Age >=18 years.
- Type 1 or type 2 diabetes
- Ability to cooperate with imaging procedures
- At least one eye with each of the following:
    a. No known substantial media opacities that would preclude successful imaging
    b. No history of panretinal (scatter) photocoagulation (PRP) and PRP is not anticipated for 6 months following study enrollment.

c. No history of treatment with intravitreal agents over the prior 12 months and treatment is not anticipated for the next 6 months

   i. Enrollment of eyes with any prior intravitreal anti-VEGF or steroid for DME will be limited to 50% of the cohort.

   ii. Macular edema involving the central subfield on OCT or clinical exam is an exclusion

d. Non-proliferative diabetic retinopathy (ETDRS level 35- level 53) on clinical exam and based on modified 7 field ETDRS grading, without the use of ultrawide-field imaging.

Participants may have 1 or 2 study eyes.

C. Sample Size

At least 350 participants are expected to be enrolled in this study. At least 175 participants with predominantly peripheral lesions and at least 175 participants without predominantly peripheral lesions will be enrolled. In order to achieve a minimum of 175 participants in each of these primary cohorts, over enrollment of one of the groups may be necessary. Within each primary cohort, participant enrollment will be stratified so that there will be approximately 70 eyes (~40%) of the cohort with mild NPDR (ETDRS levels 35), approximately 70 eyes (~40%) with moderate or moderately severe NPDR (ETDRS levels 43-47) and approximately 35 eyes (~20%) with severe NPDR (ETDRS level 53). Throughout the study, the distribution of DR severity levels will be evaluated and enrollment may be tailored to add balance between the strata. In addition, to ensure sufficient numbers in each retinopathy severity group as outlined above, over enrolment of a retinopathy severity group may be necessary. Retinopathy levels will be based on the ETDRS 7-modified field photographs. Primary analyses will be adjusted for baseline level of retinopathy based on the modified 7 field grading.

D. Protocol Summary

The participant cohort will consist of individuals with type I and type II diabetes with NPDR (ETDRS level 35- level 53) based on modified 7 field ETDRS grading and without central involved DME in at least one eye.

Visits will occur annually for a total of four years. During each study visit, participants will receive a comprehensive dilated eye examination and will have 200º mydriatic UWF fundus images taken using the Optos system for each eye. Modified 7 standard field color digital photographs will be acquired following a DRCR.net protocol at baseline only to be compared with Optos images. Ultrawide-Field fluorescein angiography will also be obtained at baseline, 1 year, and 4 years. The DRCR.net protocol images will be obtained by a study imager certified by the DRCR.net for that imaging protocol. The images will be sent to the DRCR.net Coordinating Center (uploaded through the website as available) and sent to the reading center for further evaluation.

E. Schedule of Study Visit and Examination Procedures

	Baseline	**1 year**	**2 year**	**3 year**	**4 year**	

Visit and Visit Window		**± 2 month**	**± 3 month**	**± 3 month**	**± 3 months**	Phone Call 6, 18, 30, and 42 months **(± 1 mo)**
Best corrected visual acuity	X	X	X	X	X	
Eye Exam	X	X	X	X	X	
DRCR.net 7- modified field Fundus Photography[a]	X					
UWF Imaging[b]	X	X	X	X	X	
UWF Fluorescein Angiography[b]	X	X			X	
Spectral Domain OCT[c]	X	X	X	X	X	
Blood collection (HbA1c/eGFR)[d,]	X	X	X	X	X	
BP	X	X	X	X	X	
Urine Sample[e]	X	X	X	X	X	
Medical Conditions Assessment	X	X	X	X	X	X
OCT Angiography[b,f]		X	X	X	X	

a. Analyses will be performed on the comparison between DR severity grading from 7 modified fields and the UWF at the baseline visit. If the two modalities are not sufficiently comparable within the 7 fields, 7 field photos will also be obtained annually.

b. UWF Imaging, FA, and OCT must also be performed prior to the initiation of PRP, any intravitreal treatment with anti-VEGF or steroid agents, or vitrectomy. OCT angiography will also be performed prior to initiating treatment, at sites with OCT angiography capabilities.

c. Includes macular thickness and choroidal thickness scans

d. Blood collection must occur prior to any intravitreous injection

e. Must be obtained prior to fluorescein angiography. Albumin and creatinine will be measured.

f. Only at sites with OCT angiography capabilities.

F. Outcomes

a. Longitudinal Analysis

i. The primary outcome of this study is the relative risk of 2 or more step worsening of DR severity over 4 years in the groups with and without any predominantly peripheral lesions on UWF images at baseline. Diabetic retinopathy severity at baseline and follow up visits

will be defined as the ETDRS DR severity score based on the area of the 7-modified fields from the UWF images. Eyes receiving PRP will be considered an event for the primary DR worsening outcome regardless of starting retinopathy level.

ii. Secondary analysis will explore additional risk factors including: type of peripheral lesions, location of peripheral lesions, presence or absence of peripheral lesions, whether DR severity level is different within the 7-modified fields compared with UWF images, and extent of peripheral or posterior non-perfusion on fluorescein angiography

iii. Secondary analysis will also explore correlation between risk factors listed above and estimated glomerular filtration rate (eGFR), urine albumin–to-creatinine ratio (ACR), and cardiovascular events.

iv. Secondary outcomes include worsening to PDR, improvement of DR severity level, improvement, worsening, or development of DME, and development of vitreous hemorrhage, PRP initiation, and development of peripheral lesions. Secondary analyses will evaluate risk factors for these secondary outcomes, parallel to risk factor analyses for the primary outcome.

b. **Cross Sectional Analysis**

i. Level of agreement between DR or DME severity as graded on UWF versus DRCR.net protocol images

ii. Percent and type of peripheral lesions identified on UWF images not seen on DRCR.net protocol images

iii. Percent of time peripheral lesions seen on UWF images outside the 7 standard fields could change level of ETDRS DR severity

iv. Correlation between peripheral and posterior nonperfusion on the UWF FA

v. Extent of peripheral and posterior nonperfusion on the UWF FA and association with baseline DR and DME severity level

vi. Compare clinician assessment of diabetic retinopathy severity and the reading center assessment of diabetic retinopathy severity on UWF photos.

vii. Correlation between baseline NPDR level and eGFR and urine albumin–to-creatinine ratio (ACR).

viii. Correlation between baseline NPDR level and cardiovascular events

1.10 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 (Modified 7 Standard Field Color- Digital, Optomap® Photography and Fluorescein Angiography Manuals) provide details of the imaging procedures.

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 participants to be enrolled by a site.

STUDY PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Identifying Eligible Participants and Obtaining Informed Consent

Approximately 350 participants are expected to be enrolled in this study. At least 175 participants with predominantly peripheral lesions and at least 175 participants without predominantly peripheral lesions will be enrolled. In order to achieve a minimum of 175 participants in each of these primary cohorts, over enrollment of one of the groups may be necessary. Within each primary cohort, participant enrollment will be stratified so that there will be approximately 70 eyes (~40%) of the cohort with mild NPDR (ETDRS levels 35), approximately 70 eyes (~40%) with moderate or moderately severe NPDR (ETDRS levels 43-47) and approximately 35 eyes (~20%) with severe NPDR (ETDRS level 53). Throughout the study, the distribution of DR severity levels will be evaluated and enrollment may be tailored to add balance between the strata. In addition, to ensure sufficient numbers in each retinopathy severity group as outlined above, over enrolment of a retinopathy severity group may be necessary. Retinopathy levels will be based on the ETDRS 7-modified field photographs. In addition, enrollment of eyes with any prior intravitreal anti-VEGF or steroid for DME will be limited to only 50% of the cohort.

Potential eligibility will be assessed as part of a routine-care examination. For subjects who are eligible for the study, the study protocol will be discussed with the patient by a study investigator and clinic coordinator. Prior to completing any procedures or collecting any data that are not part of usual care, informed consent will be obtained.

2.2 Subject Eligibility and Exclusion Criteria

2.2.1 Eligibility Criteria

1. Age >= 18 years
   - *Potential participants <18 years old are not being included because advanced diabetic retinopathy is so rare in this age group that the diagnosis of diabetic retinopathy may be questionable.*
2. Diagnosis of diabetes mellitus (type 1 or type 2).
   - Any one of the following will be considered sufficient evidence that diabetes is present:
     - *Current regular use of insulin for the treatment of diabetes*
     - *Current regular use of oral antihyperglycemia agents for the treatment of diabetes*
     - *Documented diabetes by ADA and/or WHO criteria (see Site Coordinator Manual)*
3. Able and willing to provide informed consent.
4. Ability to cooperate with imaging procedures

<u>Exclusion</u>

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

5. History of chronic renal failure requiring dialysis or kidney transplant.
6. A condition that, in the opinion of the investigator, would adversely affect the participant's ability to comply with the follow-up regimen.

7. Initiation of intensive insulin treatment (a pump or multiple daily injections) within 4 months prior to enrollment or plans to do so in the next 4 months.

8. Participation in an investigational trial within 30 days of enrollment that involved treatment with any systemic drug therapy or drug therapy that affects the study eye.
   - *Note: study participants can receive another investigational drug while participating in the study if it is not systemic drug therapy and if treatment does not affect the study eye.*

9. Systemic anti-VEGF or pro-VEGF treatment within 4 months prior to enrollment.
   - *These drugs should not be used during the study.*

10. Individual is expecting to move out of the area of the clinical center to an area not covered by another clinical center during the next 24 months.

2.2.2 Study Eye Criteria:

The study participant must have at least one eye meeting all of the inclusion criteria listed below.

The eligibility criteria for a study eye are as follows (both eyes will be considered study eyes if both meet the eligibility criteria at the time of enrollment):

1. No substantial non-diabetic intraocular pathology, including age-related macular degeneration or other conditions that could lead to ocular neovascularization

2. Pupillary dilation is adequate for DRCR.net protocol 7 standard field acquisition (at least 4mm or wider).

3. No known substantial media opacities that would preclude successful imaging

4. Primary intraocular pathology is diabetic retinopathy in the judgment of the enrolling investigator.

5. Non-proliferative diabetic retinopathy (ETDRS level 35- level 57) on clinical exam and based on modified 7 field ETDRS grading, without the use of ultrawide-field imaging.
   - *Note: An eye with only peripheral NV (NV outside the area captured by the modified 7 field EDTRS imaging) can be enrolled if treatment is not anticipated within 6 months.*
   - Within each primary cohort, participant enrollment will be stratified so that there will be ~40% of the cohort with mild NPDR (ETDRS levels 35), ~40% with moderate or moderately severe NPDR (ETDRS levels 43-47) and ~20% with severe NPDR (ETDRS level 53). Throughout the study, the distribution of DR severity levels will be evaluated and enrollment may be tailored to add balance between the strata.
   - Final determination of study eye eligibility is dependent on Reading Center confirmation that the diabetic retinopathy severity level is between 35 and 53. If the Reading Center determines that the baseline retinopathy severity level is outside of the above range, the participant will not continue follow-up in the study. If the Reading Center judges the baseline fundus photo to be ungradable, the subject will be asked to revisit the clinic and have the image repeated as soon as possible.

6. No history of panretinal (scatter) photocoagulation (PRP) and PRP not anticipated for 6 months following study enrollment.

7. No prior history of vitrectomy
8. No treatment with an intravitreal agents over the prior 12 months and intravitreal treatment is not anticipated for the next 6 months
    - Note: Enrollment of eyes with any prior intravitreal anti-VEGF or steroid for DME will be limited to only 50% of the cohort.
9. No macular edema involving the central subfield on clinical exam or on Spectral Domain OCT defined as:
    - Zeiss Cirrus: < 290 µm in women, and < 305µm in men
    - Heidelberg Spectralis: < 305µm in women, and < 320µm in men
10. No history of major ocular surgery (cataract extraction, scleral buckle, any intraocular surgery, etc.) within prior 4 months or anticipated within the next 6 months following study enrollment.

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 including cardiovascular symptoms and events, 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 Procedures Manuals. Visual acuity testing, ocular exam, fundus photography, fluorescein angiography and OCT will be performed by DRCR.net certified personnel.
- The fundus photographs and fluorescein angiograms will be sent to a reading center for grading.
- OCTs meeting DRCR.net criteria for manual grading will be sent to a reading center.

1. E-ETDRS visual acuity testing at 3 meters using the Electronic Visual Acuity Tester (including protocol refraction) in each eye
2. Ocular examination on each eye including dilated ophthalmoscopy
3. Spectral Domain OCT using Zeiss Cirrus or Heidelberg Spectralis OCT machine on both eyes
4. ETDRS protocol 7 modified-field digital stereoscopic fundus photography in both eyes (within 21 days of enrollment)
5. Ultrawide field images using the Optos Optomap software - includes color and red free images (within 21 days of enrollment)
6. Digital fluorescein angiogram (FA) using the Ultra-wide field imaging device (within 21 days of enrollment)
7. Blood pressure measurement

8. Laboratory Testing – Urine Sample

- A urine sample will be collected for measurement of albumin and creatinine. See study manual for collection procedure.

9. Laboratory Testing- HbA1c. –Blood collection

- A blood sample less than 15 mL will be obtained to measure HbA1c and eGFR. See study manual for collection procedure.

FOLLOW-UP

3.1 Visit Schedule

Each participant will have protocol specific follow-up visits at 12 months (± 2 months) and at 2, 3, and 4 years (± 3 months). Additional visits may occur as required for usual care of the study participant.

3.2 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.

1. Visual Acuity:
    - A protocol refraction followed by E-ETDRS visual acuity testing in both eyes (best corrected).
2. Ocular examination on each eye including dilated ophthalmoscopy
3. OCT using Zeiss Cirrus or Heidelberg Spectralis OCT machine on both eyes
4. Ultrawide field images using the Optos Optomap software (includes color and red free images)
5. Digital fluorescein angiogram (FA) using the Ultrawide field imaging device at 1 year and 4 year visits only.
6. OCT angiography on both eyes
    - Only obtained by a subset of sites with OCT angiography capabilities. If a site has OCT angiography systems from more than one manufacturer, the images should be obtained on each system available.
7. Blood pressure measurement
8. Laboratory Testing – Urine Sample
    - A urine sample must be collected. See study manual for collection procedure.
9. Laboratory Testing –Blood collection
    - A blood sample less than 15 mL will be obtained to measure HbA1c and eGFR. See study manual for collection procedure.

In addition, a phone call is completed at 6, 18, 30, and 42 months (± 1 month) to collect medical conditions that occurred in the prior 6 months.

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

Testing procedures at usual care, non-protocol visits, are at investigator discretion. However, UWF fundus photographs, UWF FA, and OCT should be obtained prior to initiation of PRP, any intravitreal treatment with anti-VEGF or steroid agents, or vitrectomy, if performed. OCT angiography should also be obtained prior to treatment at sites with OCT angiography capabilities.

3.2.1 ETDRS Protocol 7 Modified-field Digital Photos During Follow-up

After the baseline images are collected, analyses will be performed comparing DR severity grading based on ETDRS 7 modified fields and the UWF. If the two modalities are not sufficiently comparable within the 7 modified fields for DR severity grading, ETDRS 7 modified fields

photographs will also be obtained annually. Otherwise the UWF images will be used to assess retinopathy severity within the 7 field area by using a template overlay to standardize grading of images. Details of sufficient comparability will be defined in the detailed statistical analysis plan.

STUDY PROCEDURES

4.1 Imaging Procedures

The 200º UWF images should be obtained first and acquired after pupillary dilation. Pupil dilation should be checked prior to the DRCR.net protocol imaging and if pupil is not dilated to at least 5 mm, reapplication of dilating drops should be considered. The DRCR.net Optomap® Photography Manual details the procedures involved in obtaining 200º Optomap® images and submitting the images to the DRCR.net Coordinating Center. At baseline, modified 7 standard field photographs of the study eye will be taken as per DRCR.net protocol in order to establish level of agreement between grading of UWF images and DRCR.net modified 7 standard fields for DR and DME severity. If level of agreement for DR severity between the two imaging methods is substantial or better (as suggested by previous studies [5-12]), only UWF images may be obtained at subsequent follow-up visits at the discretion of a committee that will review results from this interim data analysis, since an overlay of the ETDRS 7 standard fields on the UWF images will allow evaluation of both standard ETDRS and more peripheral retinal areas. The DRCR.net Modified 7 Standard Field Color- Digital Photography Manuals details the procedures involved in obtaining the DRCR.net protocol images and submitting these images to the DRCR.net Coordinating Center.

Details on acquisition of the UWF FA will be provided in the procedure manual. FA will be obtained on both eyes. If the participant has only one study eye the study eye will be the transit (rapid series) eye with late phase images only obtained on the fellow eyes. If both eyes are study eyes, then the transit eye should be the right eye unless the investigator or imager can justify that if the left eye was the transit eye that the image quality for either or both eyes would be markedly superior.

The DRCR.net protocol images (7 standard field images) and UWF images will be obtained by a fundus photographer specifically certified by the DRCR.net for these imaging procedures. The images will be sent to the DRCR.net Coordinating Center (uploaded through the website as available) and may be sent to a reading center for further evaluation. During image grading, a map of the ETDRS 7 standard fields will be placed as an overlay on each UWF image with peripheral areas outside the ETDRS fields darkened so that extent and severity of DR lesions can be graded separately for the areas within and outside the ETDRS fields.

OCT will be performed by DRCR.net certified personnel. Only spectral 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. The images will be sent to the DRCR.net Coordinating Center (uploaded through the website as available) and may be sent to a reading center for further evaluation.

Details on OCT angiography acquisition, including which fields to collect on a given OCT angiography system, are documented in the procedure manual. Images may be sent to a reading center for further evaluation.

Each digital image must be evaluated to be of adequate quality for submission, according to the study procedures. If photograph quality is judged substandard by the operator, then the imaging should be repeated until a good quality image is obtained.

4.2 Other Procedures

Historical information will be collected, including demographics, prior treatment for diabetic retinopathy, standard office visual acuity, prior cardiovascular events, and medications. Procedures for obtaining a urine and blood sample will be detailed in the study manual.

MISCELLANEOUS CONSIDERATIONS IN FOLLOW-UP

5.0 Treatment of Diabetic Retinopathy and Macular Edema

Treatment of diabetic retinopathy and/or DME is at investigator discretion including initiation of PRP or anti-VEGF treatment. However, the first time PRP, intravitreal anti-VEGF or steroid treatment, or vitrectomy is performed, the study procedures (procedures as performed for 1 year visit) should be performed. After treatment is administered, study participants will continue to follow-up as per the original study schedule through the full 4 year duration of the study.

5.1 Risks and Benefits

The procedures in this study are part of daily ophthalmologic practice in the United States and pose few known risks. Dilating eye drops will be used as part of the exam. There is a small risk of inducing a narrow-angle glaucoma attack from the pupil dilation. However, all participants will have had prior pupil dilation usually on multiple occasions and therefore the risk is extremely small. Fundus photographs have bright lights associated with the camera flashes with can be uncomfortable for study participants, but these carry no known risk to the eye or vision.
For the blood draw and fluorescein injection, there is a small risk of discomfort, bruising, or phlebitis at the site of the injection. Both the skin and urine are expected to turn yellow/orange for up to 24 hours after the injection of fluorescein dye. Patients occasionally experience lightheadedness or nausea after dye injection which are usually transient and resolve after a few minutes without further intervention. An allergic reaction to the dye used to do the fluorescein angiography imaging is rare. A rash or pruritus (itching) can develop, but true anaphylactic reactions are very rare.

The participant is not expected to receive direct benefit from study participation.

5.2 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.

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

Study participants who withdraw will be asked to have a final closeout visit at which the testing described for the protocol visits will be performed.

5.3 Discontinuation of Study

The study may be discontinued by the Executive Committee prior to the preplanned completion of follow-up for all study participants.

5.4 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 may be sent 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.

5.5 Subject Reimbursement

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

STATISTICAL CONSIDERATIONS

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.

6.1 Primary Outcome

The primary outcome of this study is a 2 or more step worsening of DR severity over 4 years. Diabetic retinopathy severity at baseline and follow up visits will be defined as the ETDRS DR severity score based on the area of the 7-modified fields from the UWF images. Eyes receiving PRP will be considered an event for the primary DR worsening outcome regardless of starting retinopathy level.

6.1.1 Primary Outcome Analysis

The primary analysis will involve computing the relative risk and 95% confidence interval of a 2 step worsening in DR severity comparing eyes with and without predominately peripheral lesions at baseline, using the Cox proportional hazard model, adjusting for baseline DR severity and whether the participant has one or two study eyes. A robust sandwich estimate of the covariance matrix will be used to account for correlation within participants who have both eyes studied.

Kaplan-Meier curves will be used to evaluate the proportional hazards assumption for peripheral lesion status and baseline DR severity. In addition, the proportional hazards assumption will be tested by adding the factor by time interaction terms to the proportional hazards model. If the proportional hazards assumption is violated for baseline DR severity, stratification will be used to adjust for baseline severity. If the proportional hazards assumption for peripheral lesion status is violated, alternative methods of analysis to the Cox proportional hazards model will be explored. Due to the discrete time data the exact method for ties will be used.

Data of study participants who are lost to follow up without 2 or more steps of DR severity worsening will be censored on the date of the last visit. For the participants who initiated anti-VEGF or steroid treatment, data will be censored after the visit at which treatment is initiated. Several analytical methods will be employed to explore the potential problem of informative censoring, including imputation techniques for missing data, sensitivity analyses to mimic best and worse-case scenarios, and use of published models that deal with informative censoring.

Eyes that are not eligible based on diabetic retinopathy severity after reading center assessment, will not be included in longitudinal analyses.

6.1.2 Risk Factors

The following potential risk factors for the development of 2 step DR worsening will be assessed:

- Primary Risk Factor of Interest as indicated above: Eyes without predominantly peripheral lesions/eyes with predominantly peripheral lesions
- Presence or absence of peripheral lesions
- Whether the diabetic retinopathy severity level is different when graded within the modified 7 fields compared with the UWF image.
- Type of peripheral lesions
- Location of peripheral lesions
- Extent of peripheral or posterior non-perfusion on fluorescein angiography
- Peripheral DR lesions identified on fluorescein angiography that are not visualized on the color photographs
- Age

- Diabetes Type
- Duration of diabetes
- Blood Pressure
- HbA1c
- Urine albumin/creatinine ratio (ACR)
- Estimated glomerular filtration rate (eGFR)

A univariate assessment of the relationship between the outcomes and each risk factor will be performed using Cox proportional hazards model. Descriptive data for the risk factors from the Kaplan-Meier analysis will be presented overall and also stratified by baseline retinopathy level.

A Cox proportional hazards model including those factors with any evidence of association will be used to evaluate the association of factors with 2 step worsening while controlling for other factors. Factors that are not stable over follow-up will be included as time-dependent variables. The assumption of a linear relationship between hazard and continuous variables will be assessed by fitting an alternate model categorizing continuous variables and examining coefficients for linear trend. If the linearity assumption is violated, the variable will be transformed, or categorized for analysis. The proportional hazards assumption will be tested as described for the primary analysis, and alternative analytic methods will be explored if there is evidence that the proportional hazards assumption is violated.

6.1.3 Secondary Outcomes

The following lists secondary outcomes that will be assessed. The methods for secondary analyses including evaluation of risk factors will be parallel to methods described above for the primary analyses.

Secondary outcomes include:

- Proportion of eyes developing PDR
- Proportion of eyes with 1 or 2 step improvement of diabetic retinopathy
- Proportion of eyes receiving PRP
- Proportion of eyes developing vitreous hemorrhage
- Proportion of eyes receiving PRP or developing vitreous hemorrhage
- Proportion of eyes developing DME
- In eyes with DME at baseline, proportion with improvement or worsening of DME
- Proportion of eyes developing peripheral lesions

Secondary outcome also will be assessed on a participant level for participants with two study eyes.

6.1.4 Cross-Sectional Analyses

The following key outcomes will be assessed at baseline and follow-up where applicable.

Agreement on DR severity between UWF images and DRCR.net protocol fundus photographs

Level of DR severity identified on UWF images and DRCR.net protocol fundus photographs will be cross-tabulated, and agreement between the two will be assessed by calculation of both unweighted kappa and weighted kappa values. The agreement between UWF images and on fundus photograph grading will also be assessed by calculation of sensitivity/specificity percentages and positive/negative predictive values using various cutoffs in DR severity, e.g. proliferative versus non-proliferative disease. The 7 modified field images will be considered the gold standard image. Images that are classified as ungradable will be excluded from these analyses; however, the

classification of the UWF and protocol fundus images as ungradable will be cross-tabulated for descriptive purposes.

Peripheral lesions identified on UWF images outside the 7 standard fields

Percentage and type of peripheral lesions (hemorrhages/microaneurysms, venous beading, intraretinal microvascular abnormalities and neovascularization elsewhere) will be tabulated and frequency of peripheral lesions on UWF images that affect level of DR severity will be reported. The analysis will only include data from eyes with gradable UWF images *and* gradable DRCR.net protocol fundus photographs.

Agreement on DR severity between UWF images and clinician assessment of DR severity

Level of DR severity identified on UWF images and by clinician assessment on clinical exam will be cross-tabulated, and agreement between the two will be assessed by calculation of both unweighted kappa and weighted kappa values. The agreement between UWF images and by clinical exam will also be assessed by calculation of sensitivity/specificity percentages and positive/negative predictive values using various cutoffs in DR severity, e.g. proliferative versus non-proliferative disease. Images that are classified as ungradable will be excluded from the analysis.

Relationship between Peripheral and Posterior nonperfusion on UWF

The correlation between peripheral and posterior nonperfusion on the UWF FA will be evaluated. In addition, association between the extent of peripheral and posterior non-perfusion on the UWF FA and baseline diabetic retinopathy and DME severity will be evaluated.

Relationship between diabetic retinopathy and kidney and cardiovascular outcomes

For continuous outcomes (eGFR and urinary albumin/creatinine ratio) an analysis of covariance (ANCOVA) model will be used to assess the association between each UWF risk factor in section 6.1.2 (explanatory variable) and the continuous organ system outcomes. Models will be adjusted for the baseline level of the organ system outcome measurement. In addition, potential confounding factors will be assessed including age, gender, diabetes type, smoking history, socioeconomic status, body-mass index, duration of diabetes, and HbA1c. The association between UWF risk factors and organ measurements will be assessed cross-sectionally and the relationship between changes in a risk factor and changes in an organ measurement will be assessed over time. 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. Median and interquartile ranges and/or means and standard deviations will be reported to describe the distribution of the data and 95% confidence intervals will be constructed where appropriate. For ocular outcomes, generalized estimating equations will be used to adjust for the correlation between eyes of patients who have two study eyes.

For binary outcomes (e.g. cardiac event) a logistic regression model will be used to assess the association between each UWF risk factor in section 6.1.2 (explanatory variable) and the binary organ system outcome. Models will be adjusted for the baseline level of the organ system outcome measurement or history of the disease as relevant. In addition, potential confounding factors will be assessed including age, gender, diabetes type, smoking history, and socioeconomic status, body-mass index, duration of diabetes, and HbA1c. The association between UWF risk factors and organ outcomes will be assessed cross-sectionally and the relationship between changes in a risk factor and the outcome will be assessed over time. For ocular outcomes, generalized estimating equations will be used to adjust for the correlation between eyes of patients who have two study eyes.

For the participants who initiated anti-VEGF treatment, data will be censored after the visit at which treatment is initiated. Several analytical methods will be employed to explore the potential problem of informative censoring, including imputation techniques for missing data, sensitivity analyses to mimic best and worse-case scenarios, and use of published models that deal with informative censoring.

6.1.5 Additional Analysis

Baseline demographic and clinical characteristics will be tabulated for each group.

6.1.6 Fellow Eyes

Data will be collected on both eyes of each participant even if only one eye is eligible. It is unknown what the distribution of retinopathy severity, diabetic macular edema, prior DME treatment, or prior PRP treatment will be in fellow eyes. If sufficient numbers exits, exploratory analyses will be conducted in fellow eyes in the following subgroups of interest:

- Eyes with diabetic retinopathy on UWF but no diabetic retinopathy in the modified 7-fields
- Eyes beginning or in the midst of anti-VEGF treatment
- Eyes with DME that are not beginning anti-VEGF treatment
- Eyes with PDR that have not previously received PRP
- Eyes status post PRP

6.2 OCT Angiography Ancillary Study

At a subset of sites with OCT angiography capabilities, images will be taken at all follow-up visits. Features evident on OCT angiography alone will not be used for the primary outcome determination. Exploratory analyses of OCT angiography may be completed, including but not limited to:

- Comparison with current imaging modalities for detection of diabetic retinopathy pathology.
- Identification of biomarkers at baseline that may predict retinopathy progression.
- Comparison of different OCT angiography systems at sites with more than one available.

6.3 Sample Size Estimation

The data from Silva et al on 109 eyes was used for the estimation of the proportion of eyes that will worsen 2 steps or more on ETDRS DR Severity scales over 4 years, and relative risk according to peripheral lesion status.[27] The proportion of eyes without predominantly peripheral lesions at baseline UWF imaging that will worsen 2 steps or more based on the preliminary data in the BEI/JDC study is 11% (95% confidence interval 3% to 20%) and the proportion of eyes with predominantly peripheral lesions at baseline that worsen 2 steps or more is approximately 34% (95% CI: 22% to 46%), for an estimated relative risk of 3.0 (95% CI: 1.3 to 6.9).

Table 1 shows per group sample size estimates needed to detect a relative risk that differs from 1, based on the logrank test, under varying assumptions for 4 year rates of DR severity worsening with a type 1 error rate of 0.05 (2-sided) and 90% power, assuming an exponential survival time distribution with equal numbers of subjects in the predominantly peripheral lesion and the without predominantly peripheral lesion groups and 15% loss to follow up over 4 years in each of the groups.

Table 1: Sample Size per Group Under Various Assumptions of Outcome Rates for DR Severity Worsening Over 4 Years

Predominantly Peripheral lesion at baseline	Without predominantly peripheral lesion at baseline				
	0.25	**0.20**	**0.15**	**0.10**	**0.05**
0.70	31	24	20	16	13
0.60	48	35	27	20	16
0.50	87	57	40	28	20
0.35	466	193	99	58	35
0.30	1745	402	163	82	46
0.25	--	1477	332	**130**	62
0.20	--	--	1183	255	95

Additional data that will aid in refining outcome estimates will be analyzed as it becomes available.

If we assume that 10% (point estimate) of the without predominantly peripheral lesions group will worsen 2 steps or more on DR severity over 4 years and 25% of the eyes predominantly with peripheral lesions will worsen (a relative risk of 2.5), a sample of N = 260 (130 per group) provides 90% power for a two-sided test of relative risk equal to 1 with type I error rate of 5%. This sample size includes adjustment for 15% lost to follow-up or initiating anti-VEGF. As some participants will contribute 2 study eyes, and the primary analysis will adjust for baseline DR severity, it is expected that actual power will be higher than 90% for the primary analysis. The sample size has been conservatively increased to 350 participants to account for the uncertainty in the estimations. This sample size will include at least 175 participants with predominantly peripheral lesions and at least 175 participants without predominantly peripheral lesions. In order to achieve a minimum of 175 participants in each of these primary cohorts, over enrollment of one of the groups may be necessary.

6.3.1 Detectable Relative Risks

Table 2 provides the detectable hazard ratios of worsening 2 steps or more over 4 years on ETDRS DR severity scales in eyes with peripheral lesions at baseline vs. eyes without peripheral lesions at baseline as a function of the hazard in the group without peripheral lesions, with a sample size of 175 in each group (assuming 15% lost to follow-up).

Table 2: Detectable Hazard Ratio of worsening 2 steps in eyes with peripheral lesions to without peripheral lesions with 90% or greater power

Outcome rate at 4 years in eyes without peripheral lesions	**Hazard in eyes without peripheral lesions**	**Detectable hazard ratio of 2 step worsening with N=175/group**
0.05	0.013	3.2
0.10	0.026	2.5
0.15	0.041	2.2
0.20	0.056	2.0
0.25	0.071	1.9

The study will have a minimum of 90% power to detect hazard ratio between 1.9 and 3.2 for assessing the association between the risk of worsening 2 steps or more with presence of peripheral lesions, depending on the 4 year outcome rate in the eyes without peripheral lesions.

Table 3. Detectable hazard ratio as a function of outcome rate in eyes without risk factor and proportions with and without risk factor

Outcome rate at 4 years in eyes without risk factor	**Hazard of Outcome in Eyes without risk factor**	Detectable Hazard Ratio with Total sample size = 350 subjects		
		W/ factor – 75% (n=262) W/o factor – 25% (n=88)	W/ factor – 33% (N=234) W/o factor – 67% (n=116)	W/ factor – 50% (n=175) W/o factor – 50% (n=175)
0.05	0.013	4.2	3.7	3.2
0.10	**0.026**	**3.0**	**2.7**	**2.5**
0.15	0.041	2.5	2.3	2.2
0.20	0.056	2.3	2.1	2.0
0.25	0.071	2.2	2.0	1.9

For other risk factors, whose study population prevalence will not necessarily be 50%, with a sample size of 350 subjects and 15% loss to follow up, the study will have a minimum of 90% power to detect hazard ratio between 1.9 and 4.2 for the range of likely hazard and risk factor prevalence combinations shown in Table 3.

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