

**Diabetic Retinopathy Clinical Research Network**  
**U1 Manuscript**

**Continued Ranibizumab With or Without Dexamethasone for Persistent  
Diabetic Macular Edema (Protocol U):  
A DRCR.net Phase 2 Randomized Clinical Trial**

**Technical Plan**

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION
1.0	Danni Liu	Michele Melia	9-25-2017	Initial version

## **OVERVIEW**

Protocol U is a short-term evaluation of combination intravitreal corticosteroid + anti-VEGF treatment for persistent central-involved diabetic macular edema following anti-VEGF therapy.

All potential study participants were required to participate in a 12-week run-in phase. In order to enter the run-in phase, all eligibility criteria were assessed and met. During the run-in phase, study eyes received 3 study ranibizumab 0.3mg injections approximately 4 weeks apart. At the end of the run-in phase, eligible study eyes that still met eligibility criteria were randomly assigned to one of the two treatment groups: A) sham + intravitreal ranibizumab 0.3mg [ranibizumab group], or B) dexamethasone intravitreal implant + intravitreal ranibizumab 0.3mg [combination group]. Study participants may have one or two study eyes. Study participants with two study eyes were randomly assigned to receive continued anti-VEGF therapy (ranibizumab) in one eye and dexamethasone intravitreal implant + ranibizumab in the other eye. Study duration was 24 weeks post randomization.

**The protocol statistical analysis plan can be found at this location:**

[\\Eros\sys\user\A\DRN\Protocols\Protocol U – Phase II Persistent DME Study\Statistics\SAP\Protocol U SAP 04-12-17 \(8-29-17\) ver 2 clean.docx](\\Eros\sys\user\A\DRN\Protocols\Protocol U – Phase II Persistent DME Study\Statistics\SAP\Protocol U SAP 04-12-17 (8-29-17) ver 2 clean.docx)

**The purpose of this document is to provide the specific analysis objectives and technical details for the protocol U primary manuscript.**

## **DATA SOURCE**

**Database used to create SAS data sets:** DRNnet\_U\_PrimManu\_21sep2017

Data validation: <\\Eros\sys\user\A\DRN\Protocols\Protocol U – Phase II Persistent DME Study\Statistics\Data Closeout\Documentation>

### **SAS master data sets**

Dataset creation: <\\Eros\sys\user\A\DRN\Protocols\Protocol U – Phase II Persistent DME Study\Master Dataset\Documentation>

Location: <\\Eros\sys\user\A\DRN\Protocols\Protocol U – Phase II Persistent DME Study\Master Dataset\Datasets\20170921Frz>

SAS master datasets utilized:

1. *eyes* – one record per eye (study eyes and non-study eyes)
  - a. Read in as *eyesRand*
    - i. Limited to randomized eyes only (*randEyeFlg*)
2. *visitEyes* – one record per completed visit per eye (study eyes and non-study eyes)
  - a. Read in as *visitEyesRand*
    - i. Limited to randomized eyes only (*randEyeFlg*)

3. *advEvents* – one record per adverse event
  - a. Including study eyes events, non-study eyes events, and systemic events
4. *intraInj* – one record per completed visit that included study treatment injection(s) (study eyes only)

## **VARIABLE CREATED**

All variable creation procedures were completed and documented in the master dataset creation program.

## **TECHNICAL DETAILS**

Note: All *P*-values will be reported using *New England Journal of Medicine* standards: 2 decimal places if  $P > 0.01$ , 3 decimal places for  $0.01 > P > 0.001$ , or if  $P < 0.001$ , then show  $< 0.001$ .

1. Describe recruitment and follow-up visit completion
  - a. Create a flow chart for the completion of follow-up during the entire study, including run-in phase and randomization phase [**Figure 1**]
  - b. Tabulate patient status
    - i. Report No. of patients and eyes enrolled into the study
    - ii. Report No. of patients and eyes randomized into the main trial
    - iii. Report No. of patients dropped during run-in phase by reasons entered on final status form (by reducing *eyes* dataset to patient-level)
  - c. Use *eyesRand* dataset to tabulate visit completion status on eye level by treatment group for each post randomization visit
    - i. Tabulate No. of eyes completed the visit
    - ii. Tabulate No. of eyes dropped in randomization phase
    - iii. Tabulate visit completion status at 24 weeks
      - Note: One patient (U044-0672) completed an out-of-window 24-week visit (211 days from randomization visit) and did not have any other visits within the analysis window. Since it was the last follow-up visit in this study, the patient's record was hard-coded as "dropped" for the purpose of analysis.
    - iv. Footnote how visit analysis windows were defined in the statistical analysis plan
2. Describe baseline characteristics among randomized eyes by treatment group [**Table 1**] using *eyesRand* dataset
  - a. No. (%)
    - i. Gender

- ii. Race/ethnicity
    - iii. Diabetes type
    - iv. Use of insulin
    - v. Smoking status
    - vi. Participants with two study eyes
    - vii. Prior macular laser treatment for DME
    - viii. Prior anti-VEGF treatment for DME
    - ix. Prior intravitreal corticosteroid for DME
    - x. Prior PRP
    - xi. Lens status
    - xii. Randomization visual acuity letter score
    - xiii. Randomization central subfield thickness
    - xiv. Improvement in visual acuity and OCT during run-in phase
    - xv. Randomization diabetic retinopathy severity category on clinical exam
  - b. Median [IQR] (and mean  $\pm$  SD)
    - i. Age
    - ii. Duration of diabetes
    - iii. Hemoglobin A1c
    - iv. Arterial blood pressure
    - v. Body Mass Index
    - vi. Total number of prior anti-VEGF treatment for DME
    - vii. Intraocular pressure
    - viii. Randomization visual acuity letter score
    - ix. Change in visual acuity letter score from enrollment to randomization
    - x. Randomization central subfield thickness
    - xi. Change in central subfield thickness from enrollment to randomization
    - xii. Randomization retinal volume
  - c. Footnote No. of missing data (if any)
3. Describe the frequency of post randomization study treatment using *eyesRand* dataset  
**[Results – text]**
- a. No. of sham injections by injection procedures
    - i. Note: A protocol amendment was implemented which changed the sham injection procedure from applicators to needleless syringes (effective April 1<sup>st</sup>, 2016)
  - b. Mean No. of ranibizumab injections between randomization and 24 weeks by treatment group
  - c. % of eyes received second combination injections during the study among participants who completed at least one visit between 12 weeks and 20 weeks by treatment group
    - i. Note: injections administered at the 20-week visit will be included

- d. % of dexamethasone/sham injections performed on the same day as a ranibizumab injection by treatment group
  - e. Check number of eyes received more than 2 combination injections due to data entry error using *intraInj* dataset [**not in text**]
  - f. Check number of eyes completed 24 week visit but did not receive all required injections or received non-protocol treatment using *visitEyesRand* dataset [**not in text**]
  - g. No. (%) of eyes received non-protocol treatment for DME
4. Describe visual acuity at 24 weeks by treatment group
- a. Report descriptive statistics using observed data in *visitEyesRand* dataset [**Table 2**]
    - i. Visual acuity letter score at 24 weeks [**eFigure 1a**]
      - Mean  $\pm$  SD
      - Snellen equivalent (mean)
      - No. (%) of eyes 20/20 or better (letter score  $\geq 84$ ), 20/40 or better ( $\geq 69$ ), 20/200 or worse ( $\leq 38$ )
    - ii. Mean truncated change at 24 weeks from randomization [**eFigure 1b**]
      - Mean  $\pm$  SD
      - Median [IQR]
      - No. (%) of eyes  $\geq 15$  letters improvement,  $\geq 10$  letters improvement,  $\geq 10$  letters worsening,  $\geq 15$  letters worsening
      - Footnote No. of missing values and No. of values truncated at 3 standard deviation from the mean (if any) <sup>1</sup>
      - Review lens status for eyes improved or worsened on visual acuity at 24 weeks [**Discussion – text**]
    - iii. Area under the curve (AUC) between randomization and 24 weeks using truncated data [**Figure 2**]
      - Mean  $\pm$  SD
      - Median [IQR]
      - Note: AUC is computed only for those completing the 24 week follow-up visit.
  - b. Compare change in visual acuity at 24 weeks from randomization between treatment groups
    - i. Perform Markov Chain Monte Carlo multiple imputation<sup>2</sup> on missing data (100 imputations) for change in visual acuity at 24 weeks using all available visual acuity data at post randomization follow-up visits, accounting for treatment group assignment, randomization visual acuity value, laterality and improvement in visual acuity during run-in phase. Create flags for binary outcomes specified above based on imputed data.

- ii. **Primary analysis:** Perform a linear mixed effects model for treatment group comparison of mean change in visual acuity at 24 weeks from randomization. (*%MlAnalyzeMeanMixed* macro)
  - Truncated change in visual acuity letter scores at 24 weeks will be fitted as the dependent variable, and the treatment group as the independent variable, adjusting for randomization visual acuity value, randomization stratification factors (laterality and improvement in visual acuity during run-in phase as defined in the protocol) by including each as a fixed effect in the model. A random subject effect will be included to adjust for the correlation between eyes of participants who have two randomized eyes (assuming a compound symmetry covariance structure).
    - Run the model on observed data to check model assumptions including residual analysis, goodness of fit and influence diagnostics [**not in text**]
    - Run the model on the imputed datasets and report the adjusted mean difference, 95% confidence interval, and *P*-value from treatment group comparison [**Table 2**]
  - **Assessment of confounding:** Impute the outcome accounting for additional pre-specified potential confounding factors, including age, duration of diabetes, HbA1c levels, retinal thickening on OCT, and diabetic retinopathy severity on clinical exam. All categorical covariates with more than 2 levels are collapsed to a binary covariate for modeling to aid convergence. Mimic primary analysis and obtain *P*-value for treatment group comparison.
    - Post hoc assessment: impute the data with additional factors including lens status (pseudophakic vs. phakic) and number of anti-VEGF injections (3 vs. more than 3 injections) within 20 weeks prior to run-in. Mimic the pre-specified analysis above by including each additional variable one at a time. [**Table 2 Footnote**]
      - Construct a scatter plot of number of prior anti-VEGF (as a continuous outcome) versus change in visual acuity at 24 weeks by treatment group to explore the potential relationship using observed data. [**not in text**]
  - **Per-protocol analysis** [**eTable 2**]
    - As specified in the statistical analysis plan, the per-protocol analysis will mimic the primary analysis limiting the cohort to 24-week completers who had available data and received all required injections at the completed visits without receiving any non-protocol treatment. No imputation will be performed.

- Report mean  $\pm$  SD and median [IQR] by treatment group, adjusted mean difference, 95% CI and *P*-value from the treatment group comparison
  - Sensitivity analysis [**not in text**]
    - All sensitivity analyses will mimic the primary analysis model using imputed data.
      - Pre-specified sensitivity analysis will be performed and stratified by sham injection procedure.
      - Post hoc sensitivity analysis will exclude all patients at Site 194.
        - Note: this was at recommendation of DSMC, after review of cases of ineligible participants enrolled at that site discovered at a site visit.
  - Subgroup analysis (%*subgrpMixed* macro):
    - Pre-specified subgroup analysis will evaluate the effects of following baseline factors: lens status [**eFigure 2**], improvement in visual acuity during run-in phase [**eFigure 3**], and improvement in OCT during run-in phase [**eFigure 4**]. [**eTable 3**]
    - Post hoc subgroup analysis will mimic the pre-specified subgroup analysis to examine the effect of the following baseline factors: randomization visual acuity (<64 vs.  $\geq$ 64 letters), and number of prior anti-VEGF injections within 20 weeks prior to run-in (3 vs. 4 or more injections). [**eTable 4**]
    - The subgroup analysis will mimic the primary analysis adding an interaction between subgroup and treatment to the primary mixed model using observed data only.
      - Report the *P*-value for the interaction term. Report mean  $\pm$  SD and median [IQR] by treatment group within each subgroup.
      - Report adjusted treatment group mean difference and 95% confidence interval from treatment group comparison within each subgroup. Only in the presence of a significant interaction term ( $P < 0.05$ ) the within-group *P*-value will be reported.
    - The effect of gender and race/ethnicity will be explored in the same way as the subgroup factors listed above. [**not in text**]
- iii. Secondary outcomes [**Table 2**]:
- Binary outcomes: Perform binomial regression models with adjustment for same covariates in the primary analysis and use

generalized estimating equations to account for correlation between eyes of bilateral participants. All will be analyzed using imputed data (*%MIAnalyzeBinary* macro).

- When binomial regression model failed to converge, a hierarchy is used to remove the covariates: laterality → improvement in visual acuity during run-in phase → randomization visual acuity letter score.
  - This hierarchy was chosen based on judgment regarding theorized existence and strength of association with the outcome (least important → most important)
- Report the observed No. (%) by treatment group, adjusted difference in proportions, 95% confidence interval, and *P*-value from treatment group comparisons
- AUC outcome: Mimic the linear mixed effect model in the primary analysis limiting the cohort to 24-week completers. No imputation will be performed.
  - Report mean ± SD and median [IQR] by treatment group, adjusted mean difference, 95% confidence interval, and *P*-value from treatment group comparison.

5. Describe OCT Central Subfield Thickness at 24 weeks by treatment group

a. Report descriptive statistics using observed data in *visitEyesRand* dataset [Table 2]

i. Central subfield thickness at 24 weeks

- Mean ± SD
- Median [IQR]
- No. (%) of eyes below gender and OCT machine-specific values
  - Defined as <290 in women and <305 in men in Zeiss Cirrus; <305 in women and <320 in men in Heidelberg Spectralis
  - No imputation will be performed for this outcome. The analysis will include observed data only.

ii. Mean truncated change at 24 weeks from randomization

- Mean ± SD
- Median [IQR]
- No. (%) of eyes ≥ 1 logOCT step improvement, ≥ 2 logOCT steps improvement, ≥ 1 logOCT step worsening, ≥ 2 logOCT steps worsening
- Footnote No. of missing values and No. of values truncated at 3 standard deviation from the mean (if any) <sup>1</sup>

- iii. Area under the curve (AUC) between randomization and 24 weeks using truncated data [**Figure 3**]
  - Mean  $\pm$  SD
  - Median [IQR]
  - Note: AUC is computed only for those completing the 24 week follow-up visit.
- b. Compare change in central subfield thickness (CST) at 24 weeks from randomization between treatment groups
  - i. Perform Markov Chain Monte Carlo multiple imputation<sup>2</sup> on missing data (100 imputations) for change in CST at 24 weeks using all available OCT CST data at post randomization follow-up visits, accounting for treatment group assignment, randomization visual acuity value, laterality and improvement in OCT CST during run-in phase. Create flags for binary outcomes specified above based on imputed data.
  - ii. **Primary analysis**: Perform a linear mixed effects model for treatment group comparison of mean change in CST at 24 weeks from randomization (*%MlAnalyzeMeanMixed* macro).
    - Truncated changes in CST at 24 weeks will be fitted as the dependent variable, and the treatment group as the independent variable, adjusting for randomization OCT CST, randomization stratification factors (laterality and improvement in OCT CST during run-in phase as defined in the protocol) by including each as a fixed effect in the model. A random subject effect will be included to adjust for the correlation between eyes of participants who have two randomized eyes (assuming a compound symmetry covariance structure).
      - Run the model on observed data to check model assumptions including residual analysis, goodness of fit and influence diagnostics [**not in text**]
      - Run the model on the imputed datasets and report the adjusted mean difference, 95% confidence interval, and *P*-value from treatment group comparison [**Table 2**]
    - Assessment of confounding: Impute the outcome accounting for additional pre-specified potential confounding factors, including age, duration of diabetes, HbA1c levels, and diabetic retinopathy severity on clinical exam. All categorical covariates with more than 2 levels are collapsed to a binary covariate for modeling to aid convergence. Mimic primary analysis and obtain *P*-value for treatment group comparison. [**not in the text**]
    - Per-protocol analysis [**eTable 2**]
      - As specified in the statistical analysis plan, the per-protocol analysis will mimic the primary analysis limiting the cohort to 24-week completers who had available data and received

all required injections at the completed visits without receiving any non-protocol treatment. No imputation will be performed.

- Report mean  $\pm$  SD and median [IQR] by treatment group, adjusted mean difference, 95% CI and *P*-value from the treatment group comparison
- Sensitivity analysis [not in text]
  - All sensitivity analyses will mimic the primary analysis model using imputed data.
    - Pre-specified sensitivity analysis will be performed and stratified by sham injection procedure.
    - Post hoc sensitivity analysis will exclude all patients at Site 194.
      - Note: this was at recommendation of DSMC, after review of cases of ineligible participants enrolled at that site discovered at a site visit.
- Subgroup analysis (%subgrpMixed macro) [eTable 3]:
  - Pre-specified subgroup analysis will evaluate the effects of following baseline factors: lens status [eFigure 5], improvement in visual acuity during run-in phase [eFigure 6], and improvement in OCT during run-in phase [eFigure 7].
  - The subgroup analysis will mimic the primary analysis adding an interaction between subgroup and treatment to the primary mixed model using observed data only.
    - Report the *P*-value for the interaction term. Report mean  $\pm$  SD and median [IQR] by treatment group within each subgroup.
    - Report adjusted treatment group mean difference and 95% confidence interval from treatment group comparison within each subgroup. Only in the presence of a significant interaction term ( $P < 0.05$ ) a within-subgroup *P*-value will be reported.
  - The effect of gender and race/ethnicity will be explored in the same way as the subgroup factors listed above. [not in text]

iii. Secondary outcomes [Table 2]:

- Binary outcomes: Perform binomial regression models with adjustment for same covariates in the primary analysis and use generalized estimating equations to account for correlation between eyes of bilateral participants. All outcomes will be

analyzed using imputed data except for the gender and OCT machine-specific outcome (**%MIAnalyzeBinary** macro).

- When binomial regression model failed to converge, a hierarchy is used to remove the covariates: laterality → improvement in OCT CST during run-in phase → randomization OCT CST.
  - This hierarchy was chosen based on judgment regarding theorized existence and strength of association with the outcome (least important → most important)
- Barnard's unconditional exact test will be used when binomial regression model failed to converge without any covariates (**%MIAnalyzeBarnards** macro).
- Report the observed No. (%) by treatment group, adjusted difference in proportions, 95% confidence interval, and *P*-value from treatment group comparisons
- AUC outcome: Mimic the linear mixed effect model in the primary analysis limiting the cohort to 24-week completers. No imputation will be performed.
  - Report mean ± SD and median [IQR] by treatment group, adjusted mean difference, 95% confidence interval, and *P*-value from treatment group comparison.

6. Summarize safety events occurred at any time during randomization phase

a. Ocular adverse events [**eTable 5**]

i. Tabulate No. (%) of eyes experienced at least one event by treatment group using **eyesRand** dataset

- At least one ocular adverse events in the study eye
- Increased intraocular pressure (IOP) events (a composite outcome, defined as any of the below). Each component will also be reported separately.
  - IOP increased  $\geq 10$  mmHg from randomization at any visit
  - IOP  $\geq 30$  mmHg at any visit
  - Received ocular anti-hypertensives
- Endophthalmitis
- Inflammation
- Any retinal detachment (a composite outcome, defined as any of the below). Each component will also be reported separately.
  - Traction retinal detachment
  - Rhegmatogenous retinal detachment
  - Unspecified retinal detachment
- Retinal tears

- Retinal hemorrhage
- Vitreous hemorrhage
- Cataract extractions
- Glaucoma surgery
- Received post-injection treatment to lower IOP
- Migration of dexamethasone implant to the anterior chamber and subsequent corneal complications (combination group only)
  - Note: There is no specific MedDRA term for this type of adverse event. Instead, perform a manual check by searching for keywords “migration” and “anterior chamber” in the description field on the AE form, limiting to records in randomized eyes at post randomization.
- ii. Compare the frequency between treatment groups using Fisher’s exact test and report *P*-value.
- iii. Footnote No. of patients experienced multiple types of ocular adverse events as listed above (if any)
- b. Systemic adverse events
  - i. All systemic adverse events will be reported by treatment group: combination group (unilateral participants), ranibizumab group (unilateral participants), and bilateral participants.
  - ii. Reduce ***eyesRand*** dataset to patient-level as a temporary dataset ***ptRand***
  - iii. Tabulate No. (%) of participants experienced at least one event [**eTable 6**]
    - At least one systemic adverse event
    - At least one serious systemic adverse event
    - At least one hospitalization
    - Death
    - APTC cardiovascular/cerebrovascular event (a composite outcome)
      - Non-fatal myocardial infarction
      - Non-fatal stroke
      - Death of vascular or unknown cause
  - iv. Compare frequency among unilateral participants between treatment groups using Fisher’s exact test and report *P*- value. [**eTable 6**]
  - v. Tabulate No. (%) of participants had at least one adverse event in each MedDRA system organ class [**eTable 7**]

## References

1. Tukey JW. The Future of Data Analysis. *Ann. Math. Statist.* 1962; 33(1):1-67.  
<https://projecteuclid.org/euclid.aoms/1177704711#ui-tabs-1>. Accessed 21 September 2017.

2. Schafer J. Multiple Imputation: A Primer. *Statistical Methods in Medical Research*. 1999(8):3-15.