

OPTICS Trial

Ocular Pathogen and Transcriptome Investigation using Comprehensive Sequencing

Statistical Analysis Plan

1. Administrative Information

Trial registration:	NCT05286203
Funder:	Research to Prevent Blindness (P0555674)
SAP Version:	Version 2, Updated 2024-08-05 A revision history for this document is included at the end.
Protocol Version:	Refers to Protocol Version 2.5
Contributors and Signatures:	Statisticians responsible: Benjamin Arnold ¹ Travis Porco ¹ Principal Investigators: Thuy Doan ¹ Jessica Shantha ¹

¹ Francis I. Proctor Foundation for Research in Ophthalmology,
University of California, San Francisco, USA

SAP Table of Contents

1. ADMINISTRATIVE INFORMATION	1
2. INTRODUCTION	4
2.1. BACKGROUND AND RATIONALE	4
2.2. OBJECTIVES	4
3. STUDY METHODS	5
3.1. TRIAL DESIGN	5
3.2. RANDOMIZATION	6
3.3. SAMPLE SIZE	6
3.4. STATISTICAL FRAMEWORK	6
3.5. STATISTICAL INTERIM ANALYSES AND STOPPING GUIDANCE	6
3.5.1 <i>Reporting to the DSMC</i>	6
3.5.2 <i>Safety monitoring</i>	7
3.6. TIMING OF THE FINAL ANALYSIS	7
3.7. TIMING OF OUTCOME ASSESSMENTS	7
4. STATISTICAL PRINCIPLES	8
4.1. CONFIDENCE INTERVALS AND <i>P</i> -VALUES	8
4.2. PROTOCOL DEVIATIONS	8
5. TRIAL POPULATION	8
5.1. SCREENING DATA	8
5.2. ELIGIBILITY	8
5.3. RECRUITMENT	9
5.4. WITHDRAWAL/FOLLOW-UP	9
5.5. BASELINE PATIENT CHARACTERISTICS	9
6. ANALYSIS	9
6.1. OUTCOME DEFINITIONS	9
6.1.1 <i>Main Outcomes</i>	9
6.1.2 <i>Other Outcomes</i>	10
6.2. ANALYSIS METHODS	10
6.2.1 <i>Proportion of patients with clinical improvement</i>	10
6.2.2 <i>Proportion of patients that received appropriate antimicrobial therapy</i>	11
6.2.3 <i>Provider certainty of belief that the patient has an infection</i>	11
6.2.4 <i>Patient quality of life</i>	11
6.2.5 <i>Performance of MDS compared to conventional diagnostics</i>	11
6.2.6 <i>Host and non-host genomic markers</i>	12
6.3. MISSING DATA	12
6.4. ADDITIONAL ANALYSES	12
6.5. HARMS	12
6.6. STATISTICAL SOFTWARE	12
7. REFERENCES	13
8. REVISION HISTORY	13

This SAP was organized following guidelines proposed in:

Gamble C, Krishan A, Stocken D, Lewis S, Juszczak E, Doré C, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *JAMA*. 2017;318: 2337–2343. [PMID: 29260229](#)

The companion computational notebook with underlying sample size calculations presented herein is entitled: `OPTICS-sample-size-power.Rmd/.html`; it is saved in the same directory as this document.

2. Introduction

2.1. Background and rationale

This document (Statistical Analysis Plan, SAP) describes the planned analysis and reporting for the clinical trial, Ocular Pathogen and Transcriptome Investigation using Comprehensive Sequencing (OPTICS). It includes specifications for the statistical analyses and tables to be prepared for the interim and final Clinical Study Report. This study is designed to compare metagenomic deep sequencing (MDS) versus standard of care testing for improvement of outcomes in intraocular infections. The content of this Statistical Analysis Plan meets the requirements stated by the US Food and Drug Administration and conforms to the American Statistical Association's Ethical Guidelines.

The following documents were reviewed in preparation of this Statistical Analysis Plan:

- OPTICS Protocol
- ICH Guidance on Statistical Principles for Clinical Trials
- JAMA Guidelines for the content of Statistical Analysis Plans ([PMID: 29260229](#))

The planned analyses described in this SAP will be included in future manuscripts. Exploratory analyses not necessarily identified in this Statistical Analysis Plan may be performed to support the analysis. Unplanned analyses not delineated in this Statistical Analysis Plan will be documented as such in the final Clinical Study Report and manuscripts.

This document will be reviewed prior to the enrollment of patients. All subsequent changes will be indicated by detailed change log in the Appendix.

The following individuals have contributed to this Plan: T. Doan, J. Shantha, B. Arnold, T. Porco, and N. Acharya.

2.2. Objectives

The overall objectives of this study are to rigorously determine if MDS changes treatment, clinical diagnosis and improves clinical outcomes for patients with ocular infections.

Specific Aim 1: To determine if metagenomic deep sequencing (MDS) will improve treatment for patients with presumed ocular infections in a multicenter, evaluator-masked, randomized controlled trial.

Specific Aim 2: To determine if metagenomic deep sequencing (MDS) will alter clinical outcomes for patients with presumed ocular infections.

Specific Aim 3: To determine the performance of MDS compared to conventional diagnostics

Specific Aim 4: To determine the quality-of-life impact on patients.

We hypothesize that providers with access to MDS early in the study will have improved clinical decision making and provide the correct treatment based on pathogen detected as reflected by their treatment decision.

We hypothesize that patients with access to MDS early in the study will have improved clinical outcomes (decreased inflammation and/or improved vision) compared to patients with access to traditional testing only.

We hypothesize that host gene analysis will accurately discriminate between categories of infection (viral versus bacterial versus fungal versus non-infectious) and that non-host gene analysis will identify resistance, allowing for precision care and prompt transition to appropriate therapy.

3. Study Methods

3.1. Trial design

OPTICS is a multicenter, evaluator-masked, parallel group trial with 1:1 randomization. Patients will be enrolled at up to six study centers and will proceed through Phase I of the trial for the first 2 weeks. Patients who have not had a pathogen identified and who have not had complete resolution of their inflammation will proceed to Phase II, where they will be randomized (Figure 1).

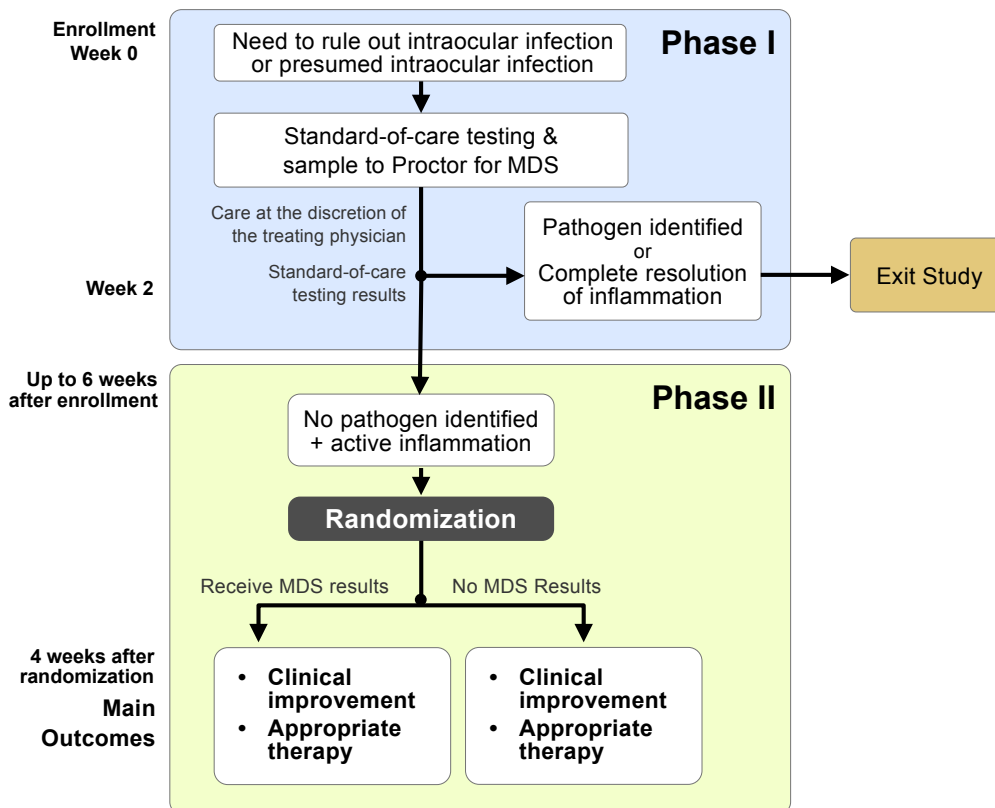


Figure 1. Overview of the OPTICS trial.

3.2. Randomization

Randomization Units. The randomization unit for this trial will be the patient.

Stratification. Randomization will be stratified by center (up to six centers). We propose to use permuted block randomization within centers, with blocks of size 4 and 6 (with proportions 0.8 and 0.2), respectively. The randomization sequence will be generated in R. Randomization will be conducted at UCSF by the trial's biostatistician.

Allocation concealment and masking. Each center will be provided with consecutive unique IDs, and randomization assignments (Disclose MDS results, and no MDS results). The trial will use the REDCap randomization module to provide each participating center with a patient ID and assigned treatment upon subject enrollment. Outcome assessors will be masked to participant assignment. Treating clinicians will not be masked due to the nature of the intervention.

3.3. Sample size

We assumed that 40 patients will enter Phase II of the trial and will be randomized and up to 10% loss to follow-up over the 4-week follow-up period (36 patients completing the trial). With equal allocation (1:1) and 18 patients per group, the trial will have 80% power to detect a 44% improvement in control of inflammation (from 40% improvement in standard of care to 84% improvement in the MDS group).

To detect a 20% improvement in control of inflammation (from 40% to 60%) with 80% power, the trial would need 194 patients to complete Phase II (97 per group). Assuming 10% loss to follow-up, that would require enrollment of approximately 110 patients per group, or 220 total.

3.4. Statistical framework

OPTICS is a superiority trial. The two groups will be compared on an intention to treat basis, with patients analyzed as they are randomized.

3.5. Statistical interim analyses and stopping guidance

3.5.1 Reporting to the DSMC

A Data and Safety Monitoring Committee (DSMC) will review any interim materials. Since the initial study will be a pilot study, we propose no interim stopping rules for efficacy or futility.

Interim monitoring will focus on protocol deviations and patient safety.

Interim reports for the DSMC will be prepared by the data analytic team. These reports will include (a) recruitment overall, and by study site, (b) compliance, and (c) retention. The reports will also list study outcomes and all adverse outcomes, including deaths. The DSMC will determine the database closure dates for each report in advance; archival copies of the (a) main database and (b) study analysis file as they exist at the time of each report will be maintained. All reports will be sent using secure email to the members of the DSMC at least one week prior to each meeting.

Electronic copies of interim reports will be maintained on an encrypted and restricted access directory on Box, with access granted only to the trial's biostatistician, analyst, and members of the DSMC. Any printed (hard copy) interim reports used for in-person meetings will be labeled clearly as confidential, bound so that the contents are not visible from the outside, and labeled with the name of each person authorized to receive it. Reports will be kept in possession of the primary biostatistician and research data analyst and only delivered in person or by encrypted email; reports not delivered due to absences are to be shredded. In addition, redacted versions of the interim reports will be prepared which contain no masked study information, and which are suitable for restricted distribution to other personnel on an as-needed basis. All hard copies will be destroyed at the end of each meeting.

We anticipate that the DSMC may request to review reports with the study arms distinguished. In this case, the DSMC will choose whether we will (a) provide labels for MDS and Standard of Care at all times to all members, or (b) label arms randomly as A or B (with the option of unmasking/unblinding at any time).

3.5.2 Safety monitoring

Adverse events will be reported within 24 hours to the medical monitor. The medical monitor will make a determination of relatedness to the study intervention. Adverse event narratives will always be reported to the DSMC, with the reporting format and timing to be decided at the first meeting.

We will tabulate and report adverse events in manuscripts and publications, by study arm. Statistical comparisons between arms will be reported internally at the discretion of the investigators and the DSMC, but we do not propose to report such comparisons in research communications. Trials are often underpowered with respect to such comparisons, and failure to find statistical evidence of a difference may be misleadingly reassuring. Additional information is provided below.

The trial may be terminated for reasons of harm at the judgment of the DSMC. Several endpoints will be examined, including serious adverse events as described in the protocol. While the analysis would consider maldistribution of predictive factors such as age, it is recognized that ethical considerations require careful considerations of statistical tests as well as qualitative judgments in the light of experience. Any additional analyses required by the DSMC will be reported as needed.

3.6. Timing of the final analysis

The final analysis will be completed after all enrolled patients complete their 4-week follow-up visit during Phase II of the trial.

3.7. Timing of outcome assessments

Protocol Section 4.9 includes details regarding the trial's outcome measurements. The trial's two main outcomes, appropriate treatment and improvement in inflammation, will be assessed in each patient 4 weeks after they are randomized in Phase II (Figure 1, above).

Visit window for main outcomes: Patients whose outcomes are measured within 7 days of their 4-week time point will be included in the primary analysis.

4. Statistical Principles

4.1. Confidence intervals and *P*-values

The trial will compare groups using two-sided tests and confidence intervals at a conventional type 1 error (alpha) rate of 0.05, i.e., $P=0.05$ and 95% confidence levels.

Measurement of host and non-host genomic markers will yield a large number of comparisons in purely exploratory analyses. To control our False Discovery Rate (FDR) We propose to use a 5% Benjamini-Hochberg FDR approach [1], as described below in section 6 analysis methods.

4.2. Protocol deviations

The incidence of deviations from the inclusion and exclusion criteria will be summarized using counts and percentages, and the treatment groups compared for the overall frequency of deviations using the Fisher's exact test. Similar deviations will be grouped into general categories of deviations for a more condensed summary. A listing of deviations by participant will also be produced. Any major deviations from the protocol will be listed and/or summarized, including, but not limited to, participants who:

- Never received diagnostic testing
- Were subsequently found to be ineligible for the study
- Never returned for a follow-up visit

The number and percentage of randomized participants that actually receive the study intervention and permanently discontinue the study interventions will be summarized. A summary of study participants randomized by site will also be provided. Randomization groups will be compared for the proportion and reason for study intervention discontinuation using Fisher's Exact test. A summary of participant status at the end of the study period will also be generated with categories including lost to follow-up.

5. Trial Population

5.1. Screening data

We will report the number of patients screened and characteristics to the extent they are available to assess representativeness of the enrolled study population.

5.2. Eligibility

See Section 5 of the trial protocol for eligibility criteria.

5.3. Recruitment

See section 6.1 of the study protocol. We will report the number of participants screened, enrolled, randomized, and measured at the primary end point, along with reasons for exclusion at each step following CONSORT guidelines

5.4. Withdrawal/follow-up

We will report the proportion of patients in both study arms who withdraw from the trial, along with reasons. We will report this information in the trial's CONSORT flow chart. Patients who are enrolled in Phase II (see Study Diagram) but withdraw prior to the primary endpoint will not be included in the analysis of the main outcomes.

5.5. Baseline patient characteristics

We will summarize patient characteristics by arm, including: age, sex, laterality, anatomical location of inflammation, and race.

6. Analysis

6.1. Outcome definitions

6.1.1 Main Outcomes

The trial will have two main outcomes:

Clinical outcome. The proportion of patients with clinical improvement at the final visit compared to the enrollment visit. An improvement is defined as any of the following criteria:

- 2-step decrease in level of inflammation (anterior chamber cells or vitreous haze) or decrease to grade 0
- Inflammatory retinal/choroidal lesions reducing in size
- 20% improvement of macular thickness on OCT

In the absence of the above findings, then

- >10 letter improvement on Snellen or ETDRS chart

Process outcome. The proportion of patients that received the appropriate antimicrobial therapy. This outcome will be measured using an independent expert panel that reviews each patient's clinical information alongside the patient's MDS results. Details of how the panel will review information and the sequence in which the information will be presented to the panel is included in Protocol section 6.8. The measurement will be collected for every patient and will be summarized as a binary (Yes/No) response for each patient.

Outcome	Timing	Specific Measurement	Data Presentation/Comparison
Appropriate Antimicrobial	Expert panel assessment after the final visit (all data through 7-10 weeks)	Proportion of patients that received the appropriate antimicrobial therapy, survey	Between arms
Clinical Improvement	Final visit (7-10 weeks)	Proportion of patients with clinical improvement	Between arms

6.1.2. Other Outcomes

The trial will measure six additional, secondary outcomes:

Outcome	Timing	Specific Measurement	Data Presentation/Comparison
Appropriate Immunosuppression	Expert panel assessment after the final visit (all data through 7-10 weeks)	Proportion of patients that received the appropriate immunosuppression therapy, survey	Between arms
Provider Certainty that patient had infection	Randomization, Final visit (7-10 weeks)	Physician Survey provider certainty of belief score	Between arms
Impact of MDS	Randomization, Final visit (7-10 weeks)	Physician Survey	Descriptive summary
Infection Status	Data compiled from all visits	Results: MDS, culture, conventional molecular diagnostics (e.g. PCR)	Latent class analysis
Patient QOL	Randomization, Final visit (7-10 weeks)	NEI VFQ QOL Score	Between arms
Physician QOL	Randomization, Final visit (7-10 weeks)	WHOQOL-BREF	Descriptive summary

6.2. Analysis methods

6.2.1 Proportion of patients with clinical improvement

The outcome Y_i is a binary outcome that indicates whether patient i showed clinical improvement, assessed 4 weeks after randomization. Assignment to inclusion of MDS information in patient care, A_i , will be equal to 1 in the MDS group and 0 if they are in the standard of care group. We will estimate the proportion of patients in each group who had clinical improvement and will estimate the difference in proportions and 95% confidence interval using a generalized linear model with binomial error, identity link, and indicator variables for k clinical sites (used to stratify the randomization), $I(S_i = k)$, where S_i is the site for patient:

$$P(Y_i|A_i, S_i) = \alpha + \beta A_i + \sum_k \gamma_k I(S_i = k).$$

The P -value for differences between groups will be the test that the difference in proportions is different from zero $\hat{\beta} \neq 0$.

In the main outcome analyses and all other comparisons between randomized groups proposed below, since randomization is stratified by clinical center inclusion of indicator variables for center, $\sum_k \gamma_k I(S_i = k)$, is appropriate [2,3]. However, given the small size of the trial and the potentially small number of patients enrolled at each clinical center, it is possible that including indicator variables for center will result in too much stratification in the analysis. In this event, we will exclude those variables, which will make the analysis conservative with respect to the size of the standard errors and width of the 95% confidence intervals [2,3].

We will also fit an adjusted model that includes covariates age and sex, which may increase power if age and sex are strongly associated with the outcome and/or control for any chance imbalance between groups by age or sex despite randomization.

$$P(Y_i|A_i, S_i) = \alpha + \beta_1 A_i + \beta_2 Sex + \beta_3 Age + \sum_k \gamma_k I(S_i = k).$$

Given the small size of the trial we do not propose any pre-specified subgroup analyses for the main outcomes or additional outcomes.

6.2.2 Proportion of patients that received appropriate antimicrobial therapy

The analysis of the proportion of patients that receive appropriate antimicrobial therapy will proceed identically to the analysis of patients with clinical improvement.

6.2.3 Provider certainty of belief that the patient has an infection

The outcome Y_{ij} is a continuous outcome that indicates the certainty of belief at the final visit for provider i , given a patient in treatment level j . We will estimate the difference in means of provider certainty of belief across treatment groups and 95% CI while adjusting for certainty of belief at randomization.

$$Y_{ij} = \tilde{\mu} + \alpha_j + \beta x_{ij} + e_{ij}$$

The P -value for differences between groups will be an F test (Type II test) if the difference between groups is not equal to 0 $\alpha_j \neq 0$.

If the assumptions for the ANCOVA model above are not met, a multiple linear regression model may be used instead. The final model will be chosen based on model diagnostic metrics, such as the coefficient of determination and the significance of predictors included in the model.

6.2.4 Patient quality of life

Patient composite and/or sub-scale quality of life as measured by the NEI-VFQ instrument will be measured in concert with each patient enrolled at the time of randomization and again at 4 weeks after randomization. The outcome Y_i is continuous outcome of quality of life measured for patient i 4 weeks after randomization $Y_i(0)$ is their corresponding measure at baseline. We will estimate the difference between groups in quality of life and its 95% confidence interval using linear regression that includes an indicator for group assignment, A_i , and the patient's baseline quality of life measure:

$$E(Y_i|A_i, S_i, Y_i(0)) = \alpha + \beta A_i + \sum_k \gamma_k I(S_i = k) + \delta Y_i(0)$$

The P -value for differences between groups will be the test that the difference in means, adjusted for baseline quality of life, is different from zero $\hat{\beta} \neq 0$.

6.2.5 Performance of MDS compared to conventional diagnostics

For Aim 3, testing will be divided into 3 categories: (1) MDS, (2) traditional diagnostics (HSV-, VZV-, CMV-, *Toxoplasma gondii*-PCR, 16S PCR, 23- and ITS-PCR, cultures, stains, and serology), and (3) clinical diagnosis at presentation (any combination of testing or clinical evaluation that can be done at the physician's clinic that help forms the clinician's belief).

The 3 diagnostic categories will be evaluated for their performances on identifying whether the patient has (a) an infectious vs non-infectious etiology, (b) a bacterial vs fungal vs viral vs parasitic infection, and (c) has a specific infection such as *P. acnes*, VZV, etc...

The sensitivity and specificity of these diagnostics and the latent gold standard prevalence will be estimated using latent class analysis (LCA). Uncertainty will be estimated using bootstraps percentile 95% confidence intervals (1,000 simulations). If estimates do not vary from unity under resampling, then the Poisson approximation will be reported for the lower confidence bound. For the analysis of class and specific organism, only diagnoses with more than 10 patients will be considered. We propose to use the R package `poLCA` v.1.4 or higher [5].

6.2.6 Host and non-host genomic markers

The analysis of host and non-host genomic markers is strictly exploratory and hypothesis-generating and will be identified as such in all reporting.

In brief, we propose to systematically compare the relative abundances of individual transcripts between groups at the species levels by negative binomial Wald test using a Benjamini-Hochberg False Discovery Rate (FDR) correction for multiple comparisons. The transcript count data will be pre-filtered, so that only genes that were represented in 25% or greater in the dataset will be used. Differentially abundant transcripts with $FDR < 0.05$ and a 2-fold change will be considered as notable.

6.3. Missing data

Missing Data. The primary analysis will be a complete case analysis, under the assumption that outcomes are missing completely at random. All efforts will be made to prevent loss to follow-up during the trial. We will assess whether missing outcomes are differential by arm. If we find differential loss to follow-up, or if primary outcomes are missing in >15% of patients, we will conduct a sensitivity analysis that uses inverse probability of censoring weights to correct for potential bias due to potentially informative censoring, following best practice guidelines for the prevention and treatment of missing outcomes in clinical trials [6–8]. Inverse probability weights will be estimated using logistic regression and baseline characteristics that predict loss to follow-up, such as baseline outcome measurements, age, sex, country, and any prognostic baseline clinical measurements.

6.4. Additional analyses

We plan to carry out descriptive analyses and summaries of provider quality of life as measured by the WHOQOL-BREF instrument at the time of randomization and again at 4 weeks after randomization. We will use the measurements to inform the design of future studies that focus on the effect of incorporating MDS diagnostics into treatment on provider quality of life, such as the SD of provider quality of life scores and the within-provider correlation between scores over the study period (key parameters to inform future trials with this as an outcome).

6.5. Harms

Section 10 of the trial protocol includes details of adverse event monitoring and reporting. All serious adverse events including blindness and deaths will be recorded for the period between the date of enrollment and the last clinic visit that accesses the primary outcome.

6.6. Statistical software

Analyses will be conducted in R version 4.1.2 or later. Packages used in specific analyses have been listed above.

7. References

1. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B Methodol. 1995;57: 289–300. doi:10.1111/j.2517-6161.1995.tb02031.x
2. Kahan BC, Morris TP. Reporting and analysis of trials using stratified randomisation in leading medical journals: review and reanalysis. BMJ. 2012;345: e5840. doi:10.1136/bmj.e5840
3. Kahan BC, Morris TP. Improper analysis of trials randomised using stratified blocks or minimisation. Stat Med. 2012;31: 328–340. doi:10.1002/sim.4431
4. Sun CQ, Prajna NV, Krishnan T, Mascarenhas J, Rajaraman R, Srinivasan M, et al. Expert Prior Elicitation and Bayesian Analysis of the Mycotic Ulcer Treatment Trial I. Investig Ophthalmology Vis Sci. 2013;54: 4167. doi:10.1167/iovs.13-11716
5. Linzer DA, Lewis JB. polCA: An R Package for Polytomous Variable Latent Class Analysis. J Stat Softw. 2011;42. doi:10.18637/jss.v042.i10
6. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. Epidemiology. 2004;15: 615–625.
7. Little RJ, D’Agostino R, Cohen ML, Dickersin K, Emerson SS, Farrar JT, et al. The prevention and treatment of missing data in clinical trials. N Engl J Med. 2012;367: 1355–1360. doi:10.1056/NEJMSr1203730
8. National Research Council (U.S.), National Research Council (U.S.), National Academies Press (U.S.), editors. The prevention and treatment of missing data in clinical trials. Washington, D.C: National Academies Press; 2010.

8. Revision history

Version	Date	Summary of Changes, Justification, and Timing vis-à-vis key trial events (enrollment completion, interim analyses, unmasking, etc)
1	2022-03-02	First version
2	2024-05-31	Updated analysis 6.2.3. Changed analysis from computing Bayesian credible intervals over time to ANCOVA because we did not conduct a pre-test survey. <i>The outcome Y_{ij} is a continuous outcome that indicates the certainty of belief at the final visit for provider i, given a patient in treatment level j. We will estimate the difference in means of provider certainty of belief across treatment groups and 95% CI while adjusting for certainty of belief at randomization.</i>

		$Y_{ij} = \mu + \alpha_j + \beta x_{ij} + e_{ij}$ <i>The P-value for differences between groups will be an F test (Type II test) if the difference between groups is not equal to 0 $\alpha_j \neq 0$.</i> <i>If the assumptions for the ANCOVA model above are not met, a multiple linear regression model may be used instead. The final model will be chosen based on model diagnostic metrics, such as the coefficient of determination and the significance of predictors included in the model.</i>
2	2024-08-02	- Updated analysis 6.2.1. Added clarification on model adjustments for relevant covariates. <i>The model will also be adjusted for age and sex. Subsequent models will be adjusted for covariates individually, and the simplest, relevant model will be chosen.</i> <i>Unrestricted Model: $P(Y_i A_i, S_i) = \alpha + \beta_1 A_i + \beta_2 Sex + \beta_3 Age + \sum_k \gamma_k I(S_i = k)$.</i> - Updated analysis 6.2.4. Clarify composite and sub-scale NEI-VFQ scores.
2	2024-08-05	Updated analysis 6.2.1. Added clarification on model adjustments for relevant covariates. <i>We will also fit an adjusted model that includes covariates age and sex, which may increase power if age and sex are strongly associated with the outcome and/or control for any chance imbalance between groups by age or sex despite randomization.</i> <i>$P(Y_i A_i, S_i) = \alpha + \beta_1 A_i + \beta_2 Sex + \beta_3 Age + \sum_k \gamma_k I(S_i = k)$.</i>