Statistical Analysis Plan

Trial ID: DEFOG

Effect of wearing peripheral focus-out glasses on Emmetropization in Chinese children aged 6–8 years

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Table of Contents

Scope of the statistical analysis plan 4

List of abbreviations 5

1 Introduction 6

1.1 Trial information 6

1.1.1 Background 6

1.1.2 Objective(s) 6

1.1.2.1 Primary objective 6

1.1.2.2 Secondary objectives 6

1.1.3 Endpoints 6

1.1.3.1 Primary endpoint 6

1.1.3.2 Secondary endpoints 7

1.1.4 Type of trial 7

2 Statistical considerations 7

2.1 Sample size determination 7

2.2 Definition of intercurrent events 8

2.3 Definition of analysis sets 9

2.4 Statistical analysis 9

2.4.1 Descriptive statistics 9

2.4.1.1 Baseline characteristics 9

2.4.1.2 Descriptive statistics of primary and secondary endpoints 10

2.4.2 Analysis for Primary endpoint 10

2.4.2.1 Primary analysis for primary endpoint 10

2.4.2.2 Secondary analysis for primary endpoint 11

2.4.3 Analysis for Secondary endpoints 12

2.4.3.1 Change in Spherical equivalent refraction (D) to month 6,12,18 12

2.4.3.2 Occurrence of myopia 13

2.4.3.3 Change in other clinical ophthalmological assessments 13

2.4.4 Safety and tolerability endpoints 14

2.4.4.1 Safety endpoints 14

2.4.4.2 Tolerability endpoints 14

2.4.5 Multiple testing 14

2.4.6 Statistical software 15

3 Change log 16

3.1 Version changes of SAP 16
3.2 Major changes to trial protocol 17
4 References 17

Scope of the statistical analysis plan

- This statistical analysis plan(SAP) covers a more technical and detailed elaboration of statistical analysis and detailed procedures for executing the statistical analysis in DEFOG trial, which is consistent with the principal features of the statistical methods described in the published study protocol[1].
- A change log describing any amendment in SAP is provided on section 3.
- This is a supportive document and replication with the protocol is minimized.
- The SAP will be finalized before database lock (i.e. completion of last visit of last participant).

List of abbreviations

AD Discontinue randomized treatment due to adverse events
AMP Amplitude of accommodation
ChT Choroidal thickness
CFP Continuous loss-to follow-up
DEFOG Direct Emmetropia with Focus-out Glasses
DS Discontinue randomized treatment due to other reasons
FAS The full analysis set
GLMM Generalized linear mixed model
IFP Intermittent loss-to follow-up
IQR Interquartile range
ITT Intention-to-treat
MAR Missing at Random
MI Multiple imputation
MMRM Mixed Model for Repeated Measures
MNAR Missing Not at Random
MP Myopia diagnosis
PPAS The per-protocol analysis set
PR Initiate other myopia-preventative measures
Q-Q plot Normal Quantile-Quantile plot
SAS The safety analysis set
UCVA Uncorrected visual acuity
WD Withdrawn from the trial due to objective reasons

1 Introduction

1.1 Trial information

1.1.1 Background

Myopia is one of the most common eye diseases causing visual impairment and blindness, and the high prevalence in adolescents remains a major public health concern. Based on clinical studies using optical defocus to regulate ocular growth and refractive changes through visual feedback, we hypothesize that early wearing of peripheral myopic defocusing spectacles in children at high risk of myopia may slow the process of emmetropization by inducing more peripheral myopic defocus.

1.1.2 Objective(s)

1.1.2.1 Primary objective

To assess the effect of wearing Direct Emmetropia with Focus-out Glasses (DEFOG) lenses versus blank control on cycloplegic objective refraction over 24 months in non-myopic children aged 6-8 years at high risk of myopia.

1.1.2.2 Secondary objectives

- To assess the effect of wearing Direct Emmetropia with Focus-out Glasses (DEFOG) lenses versus blank control over 24 months in non-myopic children aged 6-8 years at high risk of myopia on:
  - The occurrence of myopia
  - Other clinical ophthalmological assessments

To evaluate the safety and tolerability of DEFOG over 24 months in non-myopic children aged 6-8 years at high risk of myopia.

1.1.3 Endpoints

1.1.3.1 Primary endpoint

The primary endpoint addressing the primary objective:

- Change from baseline at month 0 to month 24 in spherical equivalent refraction (D)

1.1.3.2 Secondary endpoints

The secondary endpoints addressing the primary objective and secondary objectives:

Secondary endpoints:

- Change from baseline at month 0 to month 6, month 12, month 18 in spherical equivalent refraction (D)
- Occurrence of myopia
  - Time to myopia over 24 months
  - Fast myopia shift (spherical equivalent myopic shift of at least 1.00 D) at month 24
- Change from baseline at month 0 to month 6, month 12, month 18 and month 24 in :
  - Axial length (mm)
  - Amplitude of accommodation (AMP) (D)
  - Uncorrected visual acuity（UCVA）
  - Strabismus examination（△）
  - Choroidal thickness (ChT) (um)
  - Pupil size (mm)
  - Peripheral retinal refraction (D)
  - Corneal refractive (D)
  - Astigmatism (D)

- Safety and tolerability endpoints:
  - Number of adverse events from baseline at month 0 to month 24
  - Number of average level of adherence to DEFOG (high, moderate and low adherence) from baseline at month 0 to month 6, month 12, month 18 and month 24

1.1.4 Type of trial

This is a randomized, two-arm, parallel group, single-center clinical trial comparing wearing DEFOG lenses versus blank control on emmetropization in children aged 6-8 years at high risk of myopia.

2 Statistical considerations

2.1 Sample size determination

Below are the assumptions for the sample size calculation:

- The significance level is two-side 5%
- The power is 80%
- The randomization ratio is 1:1

- Drop-out rate of 20% at month 24
- Cohen's D of 0.5

There is a lack of reference information on the preventative effect on refraction changes which is relevant the study population; considering changes in refraction are proportional to changes in axial length, the sample size calculation is alternatively referenced from a previous large trial in China[2], by using average change in axial length for non-myopic children aged 6–8 years over 24 months. It is estimated that the axial length of the control group will increase by 0.6 mm ± 0.4 mm at the 24th month, and that of the experimental group will increase by 0.4 mm at the 24th month. Therefore, the value of Cohen's D for sample size calculation is referred as the estimated effect between groups divided by standard deviation in the control group, that is (0.6–0.4)/0.4 = 0.5, which will be subsequently converted into a suspected effect of change in refraction in this study. According to previous experience and the latest 2-year large trial in Shanghai, China[3], we conservatively assume an average change in refraction of − 1.00 D ± 1.00 D (i.e., 0.6-mm increase in axial length specified in the same study) over 2 years in children aged 6–8 years with a baseline refraction of 1.00 D ± 1.00 D in the control group. Thus, the estimated effect will be 0.5 × 1.00 + (− 1.00) = − 0.50 D for change in refraction in the experimental group. This means that the sample size is fulfilled for us to observe at least − 0.50 D change between groups to reject the null hypothesis of no difference.

Given the above-mentioned assumptions, the estimated sample size for each group is 80 participants (i.e., 160 participants in total). The calculation has been finalized before randomization.

2.2 Definition of intercurrent events

Intercurrent Events		Abbrev.		Definition
Myopia diagnosis		MP	Endpoint	At the visit of clinical diagnosis of myopia
Discontinue randomized treatment	Due to other reasons	DS	Non-adherence	
	Due to adverse events	AD	Endpoint	
Loss-to follow-up	Intermittent loss-to follow-up	IFP	Randomly missing data	Missed intermittent visits
	Continuous loss-to follow-up	CFP	Not-randomly missing & Non-adherence	Missed⩾3 consecutive visits
Withdrawn from the trial	Initiate other myopia-preventative measures	PR	Non-adherence	e.g. atropine ointment, low-dose atropine eye drops, Low-level red-light therapy, flippers, etc.
Withdrawn from the trial		WD	Randomly missing data	Due to objective reasons (e.g. moving, severe illness not related to the trial, etc.)

2.3 Definition of analysis sets

- The full analysis set (FAS):
  includes all randomized participants according to the intention-to-treat (ITT) principle, regardless of actual intervention participants receive.
- The per-protocol analysis set (PPAS):
  includes all randomized participants who receive, moderately or highly adhere to the randomized intervention during the whole intervention period (i.e. till 24 months). The adherence level is defined in 2.4.4.2.
- The safety analysis set (SAS):
  includes all randomized participants exposed to at least one day of randomized intervention.

  Any observation excluded from the analysis will be documented with the reason for exclusion provided.

2.4 Statistical analysis

Effect endpoints will be analyzed using the FAS and PPAS; safety endpoints will be analyzed using the SAS.

All analysis will be performed using the right eye only, except that a further inter-eye correlation analysis will be exclusively performed in sensitivity analysis (see 2.4.2.2).

Results from effect endpoints will be accompanied by two-sided 95% confidence intervals and corresponding two-sided p-values. Superiority will be claimed if p-values are less than 5% and the estimated intervention contrasts favors the DEFOG lenses.

2.4.1 Descriptive statistics

2.4.1.1 Baseline characteristics

Baseline characteristics of participants is defined as baseline values of the first available and eligible observation at or after randomization covering all participants, including:

- Continuous:
  - Age (years)
  - Body Mass Index
  - Baseline refraction (D)
  - Baseline axis length (mm)
  - Daily class hours (h)
- Categorical:

- Sex (1,Boy;2,Girl)
- Maternal myopic status (0,No;1, Yes) ; If yes:
  - Maternal myopic refraction (1,≥-3.00D；2,-3.00~-6.00D；3,≤-6.00D)
- Paternal myopic status (0,No;1, Yes) ; If yes:
  - Paternal myopic refraction (1,≥-3.00D；2,-3.00~-6.00D；3,≤-6.00D)
- Average outdoor activity time per day (1,<30min; 2,30-60min;3,1-2hours;4,>2hours)
- Average homework time per day (1,<30min; 2,30-60min;3,1-2hours;4,>2hours)
- Daily sleep hours (1,>10hours; 2,8-10hours;3,6-8hours;4,<6hours)
- Daily usage time of electronic products (1,<2hours;2,2-4hours;4-6hours;>6hours)
- Eye care knowledge (1,rich;2,enough;3,lack;4,not at all)
- Reading while lying down (0,no;1,sometime;2,frequently)
- Holding the pen too low or having fingers block one eye's vision (0,No;1, Yes)
- Writing with body or head slouched or lopsided (0,No;1, Yes)
- Distance from eye to paper (1, 10cm; 2, 15cm; 3, 20cm; 4, 25cm)
- Reading while walking or travelling (0,No;1, Yes)
- Light type for study (1, LED+daylight bulbs; 2, lamp+ daylight bulbs; 3, daylight bulbs only; 4, LED only; 5, lamp only)
- Study stress (1,heavy;2,moderate;3,light;4, not at all)

Baseline characteristics of participants will be presented as mean and standard deviation for continuous variables with normal or approximately normal distribution; otherwise, median values and interquartile range (IQR) will instead be calculated. Categorical variables will be described as numbers with proportions(number of participants within category ÷ all participants×100%).

2.4.1.2 Descriptive statistics of primary and secondary endpoints

Mean and standard deviation of continuous endpoints at each visit, and changes of mean and standard deviation from baseline to each visit will be presented. For myopia occurrence, numbers and proportion of participants with myopia at each visit and over 24 months, in total and in each group will be presented.

2.4.2 Analysis for Primary endpoint

The primary endpoint is defined as change in cycloplegic objective refraction from baseline till month 24.

2.4.2.1 Primary analysis for primary endpoint

Because repeated measures are completed at baseline, month 6, month 12, month 18 and month 24, Mixed Model for Repeated Measures (MMRM), preferably generalized linear mixed model (GLMM) will be performed using unimputed data in FAS, with cycloplegic objective refraction in

all visits included. Prior to the primary analysis, the normality of residuals will be evaluated graphically using Normal Quantile-Quantile plot (Q-Q plot).

This analysis investigates if there are group differences over time. The maximum likelihood method will be used to estimate the mean difference between the two groups and their 95% confidence interval, with Gaussian distribution, identity link function and random intercept with unstructured covariance; baseline refraction, group (group), time point (visit) and group × visit interaction as fixed effects. The time point(visit) is treated as categorical variable in the models.

For imputed data of primary endpoints, different imputation methods will be considered for handling missing outcomes. More details are described in 2.4.2.2 of complete data analysis.

2.4.2.2 Secondary analysis for primary endpoint

（1） Covariate-adjusted analysis

Covariates are baseline values (missing imputed) including:

- Age (years)
- Sex (1,Boy;2,Girl)
- Maternal myopic status (0,No;1, Yes)
- Paternal myopic status (0,No;1, Yes)
- Average outdoor activity time per day (1,<30min; 2,30-60min;3,1-2hours;4,>2hours)
- Daily sleep hours (1,>10hours; 2,8-10hours;3,6-8hours;4,<6hours)

Imputation of missing baseline covariates data:

- Continuous: If no assessments are available, the mean of baseline values within the same sex and age(±1year) is used as the baseline value.
- Categorical: If no assessments are available, a new category coded as 999 is used as the baseline value

（2） Complete data analysis

Handling of missing data for spherical equivalent refraction

Missing data will be handled based on the nature and pattern of missingness, which is pre-specified as follows:

Conditions	Assumption	Handling method	Statistical analysis
Missing due to IFP & WD	Missing at Random (MAR)	Multiple imputation(MI)	Regression (chained equation of arbitrary missing pattern with 10 imputations) on missing data, dependent on baseline age, sex, maternal and paternal myopia status, average outdoor activity time per day, daily sleep hours, baseline and follow-up available scores.
MP	Structural	No imputation	

	missing		
Missing due to CFP &DS	DEFOG group: Missing Not at Random (MNAR)	Incremental imputation	All subsequent missing data will be imputed based on last observed value, as the previous observed value plus the corresponding mean change observed in the control group over the same interval.
	Control group: MAR	MI	Regression (chained equation of arbitrary missing pattern with 10 imputations) on missing data, dependent on baseline age, sex, maternal and paternal myopia status, average outdoor activity time per day, daily sleep hours, baseline and follow-up available scores.

（3） Per-protocol analysis for primary endpoint

Using unimputed data of primary endpoints in PPAS, the statistical method is the same as primary analysis for primary endpoint.

（4） Analysis accounting for Inter-eye correlation

To assess the robustness of results from primary analysis for primary endpoint, within-participant inter-eye correlations, by utilizing all available data from both eyes, will be considered. A random intercept for different eye side nested within participant, with an unstructured covariance structure will be included in the same MMRM as primary analysis for primary endpoint.

（5） Subgroup analysis

The treatment effect will be explored, stratified by the following pre-specified subgroups in models used in primary analysis:

- Age (years)
- Sex (1,Boy;2,Girl)
- Maternal myopic status (0,No;1, Yes)
- Paternal myopic status (0,No;1, Yes)
- Average outdoor activity time per day (1,<30min; 2,30-60min;3,1-2hours;4,>2hours)
- Daily sleep hours (1,>10hours; 2,8-10hours;3,6-8hours;4,<6hours)

The results will be presented graphically as forest plots, using the same estimates as primary analysis for endpoints.

2.4.3 Analysis for Secondary endpoints

2.4.3.1 Change in Spherical equivalent refraction (D) to month 6,12,18

Using unimputed data in FAS, with the same MMRM as primary endpoints (i.e. the same scale in different time points will be included simultaneously), preferably GLMM for repeated measures analysis. The statistical method is the same as primary endpoints.

2.4.3.2 Occurrence of myopia

- **Time to myopia over 24 months**
  Using unimputed data in PPAS, the Kaplan-Meier method will be used to visualize the survival curves (i.e., the cumulative incidence of myopia over time) for each treatment group, and the Log-rank test will be used to compare the curves. Cumulative incidence at month 24 for each group will be reported as point estimates and their 95% confidence intervals. The risk difference, and risk ratio between groups and its 95% confidence interval will be calculated and reported as a key measure of clinical benefit. Participants with intercurrent events except myopia will be censored at the time of any intercurrent event that precludes the observation of the endpoint or at the end of the study.
- **Fast myopia shift (spherical equivalent myopic shift of at least 1.00 D) at month 24**
  A Fisher's Exact Test will be used to compare the proportion of participants with fast myopia shift between treatment groups at month 24., with the endpoint categorized as:
  - 0：spherical equivalent myopic shift < 1.00 D
  - 1：spherical equivalent myopic shift ≥1.00 D

2.4.3.3 Change in other clinical ophthalmological assessments

Using unimputed data in PPAS, MMRM, preferably GLMM will be performed. This analysis investigates if there are group differences over time (from baseline at month 0 to month 6, month 12, month 18 and month 24). The statistical method is the same as primary endpoint for all continuous secondary endpoints of clinical ophthalmological assessments.

Gaussian distribution and identity link function will be preferred for the endpoints:

- **Axial length (mm)**
- **Amplitude of accommodation (AMP) (D)**
- **Uncorrected visual acuity（UCVA）**
  - The decimal UCVA will be logMAR transformed before MMRM. The formula is defined as: logMAR UCVA= $-\log_{10}$(decimalUCVA)
- **Strabismus examination（△）**
- **Choroidal thickness (ChT) (um)**
- **Pupil size (mm)**
- **Peripheral retinal refraction (D)**
- **Corneal refractive (D)**
- **Astigmatism (D)**

If the number is very low at the completion of trial (e.g., <5 in each group) or failure to achieve normality after log-transformation, a Fisher's Exact Test will be used to compare the proportion of participants between treatment groups at month 24.

2.4.4 Safety and tolerability endpoints

2.4.4.1 Safety endpoints

Counts with proportion (%) of adverse events in DEFOG group will be summarized.
The types of adverse events include:

- Dizziness after DEFOG wearing (1,Yes;0,No)
- Eye discomfort after DEFOG wearing (1,Yes;0,No)
- Blurred vision after DEFOG wearing (1,Yes;0,No)

2.4.4.2 Tolerability endpoints

Average level of adherence to DEFOG from baseline at month 0 to month 6, month 12, month 18 and month 24 will be collected at each visit in DEFOG group only. The adherence will be quantified based on the following wearing frequencies:

- Daily Hours
  - ⩾8 hours/day
  - 6-8 hours/day
  - 3-5 hours/day
  - ⩽2 hours/day
- Weekly Days
  - ⩾5 days/week
  - 3-5 days/week
  - 1-2 days/week
  - <1 day/week

Responses will be converted to numerical values representing the midpoint of each range. A Composite Adherence Score will be calculated for each participant at each visit as: (Daily Hours) × (Weekly Days), resulting in a continuous measure of average hours per week.

According to time-weighted average Composite Adherence Score over the 24-month study period, participants in DEFOG group will be categorized as:

- High adherence: average of ⩾5days×8hours=40 hours per week
- Moderate adherence: average of 18-40 hours per week
- Low adherence: average of ⩽3days×6hours=18 hours per week

Numbers and proportions of participants with high, moderate and low adherence at each visit will be summarized.

2.4.5 Multiple testing

Multiple testing on P-values is not considered in this trial, as there is only one primary endpoint.

Secondary endpoints are not pre-specified as formal hypothesis tests for claiming efficacy, but rather to assess the consistency of findings and generate hypotheses for future research. Thus, the results will be therefore interpreted with caution, with emphasis placed on the effect sizes rather than on statistical significance alone.

2.4.6 Statistical software

STATA® (version 16.0, Stata Corp, Texas, USA) will perform all data analyses and generate most data displays. R4.1.2 (Vienna, Austria) may also be used for some data analyses and generating statistical graphs.

3 Change log

3.1 Version changes of SAP

Version	Date	Major Changes
0.0	28 Feb 2023	Clinical Trial registration
0.5	22 Nov 2023	Study protocol published
0.75	2 Jan 2024	Secondary endpoints updated: 1. Corneal refractive 2. Fast myopia shift 3. Astigmatism
1.0	21 Nov 2025	SAP initialized
2.0	5 Dec 2025	SAP revised; 1. Adherence level updated 2. Analysis for time to myopia updated
2.5	16 Dec 2025	SAP revised; secondary endpoints updated: 1. Removed absolute value of AL and cycloplegic objective refraction at month 24
3.0	13 Feb 2026	SAP revised: 1. Analysis for secondary endpoints revised
3.5	20 Jun 2026	More details clarified: 1. PPAS clarified 2. Analysis for myopia revised 3. Methods of missing data imputation revised 4. Other TYPO corrected

3.2 Major changes to trial protocol

Section	Date	Major Changes	Reason
Secondary outcomes	Jan 2024	1. Outcomes added: corneal refractive, fast myopia shift and astigmatism	Consideration on clinical benefits and importance
	Dec 2025	2. Outcomes removed: absolute value of axial length and cycloplegic objective refraction	Consideration on clinical benefits and importance
	Jun 2026	3. The definition of myopia revised: the cycloplegic SER≦ -0.50D in either eye	Consideration on clinical practice
Statistical methods	Feb 2026	1. Adjusted covariates for primary outcome updated: age, sex, parental myopia, daily outdoor activity time and daily sleep hours	Consideration on clinical benefits and importance
	Jun 2026	2. Analysis for time-to-myopia revised: from rate difference to cumulative incidence	more clinically interpretable
	Jun 2026	3. Post-hoc analysis for time-to-myopia in full analysis set	more clinically interpretable

4 References

1. Shen L, He W, Yang W, Yan W and Yang C. Effect of wearing peripheral focus-out glasses on emmetropization in Chinese children aged 6–8 years: study protocol for a 2-year randomized controlled intervention trial. *Trials*. 2023;24:746.

2. Jiang Y, Zhu Z, Tan X, Kong X, Zhong H, Zhang J, Xiong R, Yuan Y, Zeng J, Morgan IG and He M. Effect of Repeated Low-Level Red-Light Therapy for Myopia Control in Children: A Multicenter Randomized Controlled Trial. *Ophthalmology*. 2022;129:509-519.

3. He X, Sankaridurg P, Wang J, Chen J, Naduvilath T, He M, Zhu Z, Li W, Morgan IG, Xiong S, Zhu J, Zou H, Rose KA, Zhang B, Weng R, Resnikoff S and Xu X. Time Outdoors in Reducing Myopia: A School-Based Cluster Randomized Trial with Objective Monitoring of Outdoor Time and Light Intensity. *Ophthalmology*. 2022;129:1245-1254.