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3 **MYOPIA TREATMENT STUDY**
4 **MTS1**
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7 **Low-Dose Atropine for Treatment of Myopia**
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10 **STATISTICAL ANALYSIS PLAN**
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14 **Version 2.1**
15 **November 28, 2022**
16 **Based on Protocol Version v5.1 (April 06, 2020)**
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19 **Revision History**

VERSION NUMBER		AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION (INCLUDING SECTIONS REVISED)
SAP	Protocol				
1.0	V4.0 (Oct 4, 2019)	Rui Wu	Michele Melia	April 27, 2018	Initial version
2.0	V5.1 (April 6, 2020)	Rui Wu	Michele Melia	June 15, 2020	The following revisions from SAP Version 1.0 have been highlighted in this updated version. <u>Section 1</u> 1.It has been specified that if the fully-adjusted model failed to converge, or displayed evidence of estimate instability due to partial aliasing, the race/ethnicity and iris color variables would be combined as follows: East Asian (regardless of eye color), non-East Asian with brown eyes, non-East Asian with non-brown eyes. The number of East Asians with non-brown eye color would be tabulated and reported. 2.The maximum likelihood method is specified for the mixed model. 3.It is specified that the tipping point analysis will be conducted only if more than 10% of primary outcome data are missing. Details of implementing the tipping point analysis have been added.

VERSION NUMBER		AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION (INCLUDING SECTIONS REVISED)
SAP	Protocol				
					<p><u>Section 3</u> The overall false discovery rate (FDR) in the secondary analyses will be controlled at the 5% level using the two-stage step-up FDR procedure.</p> <p><u>Section 4</u> 1.It is specified that the subgroup analysis based on baseline age and baseline SER will be in 4 categories based on median split. 2.It is specified that an estimate of the treatment effect within each subgroup and a 95% confidence interval will be obtained for each of the times, 24 and 30 months, by adding a treatment by time by subgroup interaction into the primary analysis model and generating the appropriate contrasts. Each subgroup effect will be estimated separately, one subgroup per model. 3.The overall FDR in the subgroup analyses will be controlled at the 5% level using the two-stage step-up FDR procedure.</p> <p><u>Section 5</u> The overall FDR in the additional analyses will be controlled at the 5% level using the two-stage step-up FDR procedure.</p> <p><u>Section 6</u> 1.In the previous version, it was specified that the average of the item responses at the 6-month visit would be calculated and compared using a Wilcoxon Rank Sum test for difference between the treatment groups. In the current version, it has been updated to comparing the average of the item responses at 24-month visit using a t-test to be consistent with the protocol. 2.The adjustment of FDR has been removed given that there is only one average score for the questionnaire.</p> <p><u>Section 7</u> 1.Given that by the time sufficient data allowing for an analysis of efficacy or futility for the primary outcome would be possible, all or most subjects are expected to be within 1 year of completing the primary outcome, no formal statistical interim monitoring is proposed. 2.It has been added that the tabulated safety and efficacy data will be reviewed by the PEDIG Data and Safety Monitoring Committee at its biannual meetings,</p>

VERSION NUMBER		AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION (INCLUDING SECTIONS REVISED)
SAP	Protocol				
					and if any unexpected safety or other issues arise, they can recommend to stop the study at any time.
2.1	V5.1 (April 6, 2020)	Rui Wu Rui Wu <small>Digitally signed by Rui Wu, DN: cn=Rui Wu, o=Novartis, email=Rui.Wu@Novartis.com, c=US, Date: 2022.12.20 17:57:05-0500</small>	Michele Melia Michele Melia I am digitally signing this document. 2022-12-20 17:57-05:00	12-19-2022	<p>The following revisions from SAP Version 2.0 have been highlighted in this updated version.</p> <p><u>Section 1</u></p> <p>Per discussion with M. Melia, the previous sensitivity analysis using multiple imputation has been replaced with an analysis of covariance (ANCOVA) model that compares change in SER between treatment groups at 24 months, while only adjusting for baseline factors as specified in the primary analysis. The rationale of this change was that the percentage of missing outcomes at the primary outcome visit (24 months) was low while, due to the virtual visits implemented for the intermediate visits (6, 12, and 18 months), the number of missing outcomes at those visits was higher. The imputation method previously specified would then primarily impute the outcomes at intermediate visits and would not be an effective check of the effect of missing data assumptions on the primary 24 months analysis. The new sensitivity analysis proposed will be used to assess if including the intermediate visits, which have the highest proportions of missing data, in the primary analysis longitudinal model affects the treatment group comparison of the primary outcome at 24 months.</p> <p><u>Section 2</u></p> <p>Based on the same rationale for the change in Section 1 above, the previous sensitivity analysis using multiple imputation has been replaced with an ANCOVA model that compares change in SER between treatment groups at 30 months, while only adjusting for baseline factors as specified in the primary analysis.</p> <p><u>Section 3</u></p> <p>1. Per discussion with the study leads, the proportion of participants with $\geq 0.5D$, 1D, and 2D progression in myopia at 12, 24, and 30 months will now be tabulated.</p> <p>2. Per discussion with M. Melia, the previous analysis calculating relative risk of $\geq 1D$ (or 2D) progression in myopia has been replaced with treatment group comparison of proportions at 12, 24, and 30 months using Barnard's test. The originally proposed method of analysis, an analysis of time to progression using the proportional hazards model, was not feasible due to a relatively large proportion of missing data at interim visits due to COVID. While missing data is still a</p>

VERSION NUMBER		AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION (INCLUDING SECTIONS REVISED)
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					<p>problem in the analyses of results of interim visits when using Barnard's test, the 24-month primary time point is largely unaffected, as the amount of missing data is minimal.</p> <p>3.Per discussion with the study leads, to evaluate biologic activity of the drops, and the potential effect of dilute atropine on accommodation, as a post hoc analysis, mean binocular near point of accommodation at 6 months was compared between treatment groups using an ANCOVA model, adjusting for baseline binocular near point of accommodation, age, iris color (brown vs. not brown), and race (East Asian vs. non-East Asian).</p>

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21 The primary objective of MTS1 is to determine the efficacy of daily low-dose atropine
22 (0.01%) for slowing myopia progression over a two-year treatment period in children
23 aged 5 to less than 13 years with myopia -1.00D to -6.00D at the time of enrollment.
24

25 Participants are randomly assigned 2:1 to the following two treatment groups:

- 26 • Atropine Group: 0.01% atropine eyedrops administered 1 drop to each eye daily for
27 24 months, followed by 6 months off atropine eyedrops
- 28 • Placebo Group: Placebo eyedrops administered 1 drop to each eye daily for 24
29 months, followed by 6 months off placebo eyedrops
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31 32 **1. Primary Analysis: Refractive Error at 24 Months (On-Treatment)**

33 The primary analysis will be a treatment group comparison of mean change from baseline
34 to 24 months in spherical equivalent refractive error (SER), as measured by a masked
35 examiner using cycloplegic autorefraction, using a longitudinal discrete time mixed
36 model. The population-averaged method will be used to model the repeated measures on
37 SER at 6, 12, 18, and 24-month visit. The time variable will be categorical to not impose
38 assumptions regarding trend of SER over time. The correlations between SER
39 measurements within person across visits will be estimated and a correlation structure
40 will be selected accordingly. (Given that the follow-up visits are approximately equally
41 spaced, an autoregressive covariance structure is considered likely. Other correlation
42 structures will also be fitted, and the information criteria will be used to select the most
43 appropriate covariance structure.) The model will include the interaction between time
44 and treatment group and adjust for baseline SER, age, iris color (brown vs. non-brown),
45 and race (East Asian vs. non-East Asian), to account for potential residual confounding,
46 and improve power for the treatment comparison. The web data entry system mandated
47 that baseline SER, age, and iris color could not be missing at enrollment. The value
48 ‘unknown’ will be used in the mixed model for missing race/ethnicity value.
49

50 Given that at most a small number of participants of East Asian race are expected to have
51 non-brown iris, there is a possibility of partial aliasing when including both race and iris
52 color in the mixed model. If the fully-adjusted model fails to converge, or displays
53 evidence of estimate instability, such as very large standard error associated with a
54 partially-aliased covariate, the race and iris color variables will be combined as follows:
55 East Asian (regardless of eye color), non-East Asian with brown eyes, non-East Asian
56 with non-brown eyes. The number of East Asians with non-brown eye color will be
57 tabulated and reported.
58

59 At baseline and all follow-up visits, including the 24-month visit, the mean of the three
60 readings from autorefraction in each eye will be calculated and the mean of both eyes for
61 each participant will be used for the analysis. If fewer than 3 readings are available in
62 each eye, the mean of available readings will be used for each eye to obtain the mean of
63 both eyes for each participant. If data from only one eye is available, the mean of
64 readings on that eye will be used for analysis.
65

66 The mean change from baseline to 24 months in SER in each treatment group and the
67 treatment group difference (atropine – placebo), together with their corresponding 95%
68 confidence intervals, will be estimated using the mixed model with maximum likelihood
69 estimation. Maximum likelihood estimation gives unbiased estimates of treatment effect
70 in the presence of missing outcome data, as long as the missing data is missing at random
71 (MAR) conditional on the variables included in the analysis model. The 2-sided null
72 hypothesis of mean treatment difference equals zero (superiority hypothesis) will be
73 tested at an alpha level of 0.05.

74 75 **1.1.Principles to be Followed in Primary Analysis**

76 Model assumptions for the longitudinal discrete time mixed model will be assessed,
77 including linearity of the adjustment covariates (baseline SER and baseline age), and
78 normality and homoscedasticity of the outcome distribution across the treatment groups.
79 The linearity assumption of the baseline covariates of SER and age will be evaluated
80 using descriptive scatterplots and by categorizing each of the baseline factors in the
81 model to check for approximate linearity of the coefficients across ordered categories. A
82 baseline covariate will be included as a continuous variable in the model if the
83 assumptions for linearity are met for that covariate; otherwise it will be categorized. The
84 median split will be used for categorization.

85
86 The primary analysis will follow the intent-to-treat principle; all randomized participants
87 will be included in the analysis and analyzed according to their randomized treatment
88 group regardless of whether the assigned treatment was actually received. However, only
89 data from exams completed within a visit analysis window (± 3 months from the expected
90 visit date) will be included in the analysis. Given that a discrete time model will be used
91 and the time points will be grouped into 6-month intervals (i.e. 6, 12, 18, or 24 months
92 from randomization), if two consecutive visits are within 90 days from each other, only
93 one of the two visits will be included in the analysis. The following principles will be
94 used in choosing which visit to be included:

- 95 a. If a 24-month primary outcome visit is available, it will be included in the analysis.
- 96 b. Any other visit that is within 90 days of the 24-month visit will be excluded from the
97 analysis.
- 98 c. If any two visits (other than 24-month visit) are within 90 days of each other, the visit
99 closer to the expected visit date will be included in the analysis, and the other visit
100 will be excluded.

101
102 There will be no explicit imputation of outcome data for exams not completed or
103 completed outside the analysis window, as the mixed model will produce an unbiased
104 estimate of treatment effect as long as missing outcome data are missing at random
105 (MAR), and it is expected that including the baseline covariates and outcome data from
106 interim follow-up exams in the analysis model is likely to meet MAR requirements,
107 although this will not be verifiable. Hence, the sensitivity of results to the MAR
108 assumption will be explored in sensitivity analyses (Section 1.2).

109 110 **1.2.Sensitivity Analysis**

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112 A sensitivity analysis will be conducted to compare the mean change in SER from
113 baseline to 24 months between the treatment groups using an analysis of covariance
114 (ANCOVA) model, adjusting for baseline SER, age, iris color (brown vs. non-brown),
115 and race (East Asian vs. non-East Asian). Possible partial aliasing of iris color and race
116 will be handled as specified for the primary analysis model.

117 118 119 **2. Secondary Objective: Efficacy off Atropine Treatment (30 Months)**

120 The secondary objective is to determine the efficacy of atropine treatment for slowing
121 progression of myopia after a period of 6 months off treatment. The same approach
122 defined in Section 1 (including the sensitivity analysis) will be used to obtain a treatment
123 group comparison of change from baseline to 30-months in SER, as measured by a
124 masked examiner using cycloplegic autorefraction. The mean treatment group difference
125 and the corresponding 95% confidence interval will be estimated at 30 months. However,
126 the statistical testing of significance will be performed only if a statistically significant
127 effect for treatment was found in the primary analysis at 24 months.

128
129 30-month visits will be included in the analysis as long as they are no earlier than 3
130 months and no later than 6 months from the expected visit date and same follow-up SER
131 measurements selected for the primary analysis (at 6, 12, 18, or 24 months from
132 randomization) using the principles specified in Section 1.1 will be included in the
133 analysis.

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135
136 A sensitivity analysis will be conducted to compare the mean change in SER from
137 baseline to 30 months between the treatment groups using an ANCOVA model, adjusting
138 for baseline SER, age, iris color (brown vs. non-brown), and race (East Asian vs. non-
139 East Asian).

140 141 142 **3. Secondary Outcomes**

143 The overall false discovery rate (FDR) in the secondary analyses specified in Section 3
144 below will be controlled at the 5% level using the two-stage step-up false discovery rate
145 procedure of Benjamini, Krieger, and Yekutieli.^{1,2} This involves first applying the false
146 discovery rate procedure of Benjamini and Hochberg³ at alpha level = (overall α)/(overall
147 $\alpha + 1$) to estimate the number of true null hypotheses, and then applying the adaptive
148 FDR adjustment⁴ conditional on the number of true null hypotheses. This method
149 generally has better power than the usual Benjamini-Hochberg FDR method.

150 151 152 **3.1. Proportion of Participants with Progression $\geq 0.5D$, $1D$, and $2D$**

153 The proportion of participants with progression $\geq 0.5D$, $\geq 1D$, and $\geq 2D$ from baseline
154 to 12, 24, and 30 months in each treatment group will be tabulated. The proportions will
155 be compared between treatment groups using Barnard's test.

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159

160 **3.2.Change in Axial Length at 12 and 24 Months (On Treatment)**

161 Axial length will be reported as the distributions of baseline length, 12-month length, 24-
162 month length, and change in axial length from baseline to 12 and 24 months. A treatment
163 group comparison of the change in axial length from baseline to 12 months and 24
164 months will be performed using a longitudinal discrete time mixed model with maximum
165 likelihood estimation, which allows for interaction between time and treatment group,
166 and adjusts for the same baseline covariates as the primary analysis. The same strategies
167 specified in the primary analysis (Section 1, excluding sensitivity analysis) will be used
168 to choose the appropriate covariance structure for the model.

169

170 At baseline and all follow-up visits, including the 12 and 24-month visits, the mean of the
171 axial length readings in both eyes for each participant will be used for the analysis. If data
172 from only one eye is available, the reading on that eye will be used for analysis. The
173 treatment group differences (atropine – placebo) and a 95% confidence interval will be
174 estimated using the mixed model.

175

176 **3.3.Change in Axial Length at 30 Months (Off Treatment)**

177 The same approach defined in Section 3.4 will be used to conduct a treatment group
178 comparison of the change in axial length from baseline to 30 months.

179

180 **3.4.Refractive Error at 12 Months (On Treatment)**

181 The primary analysis specified in Section 1 (excluding the sensitivity analysis) will be
182 used to obtain a treatment group comparison of change from baseline to 12-months in
183 SER, as measured by a masked examiner using cycloplegic autorefraction.

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185 **3.5.Near Accommodation at 6 Months**

186 As a post hoc analysis, mean binocular near point of accommodation at 6 months will be
187 compared between treatment groups using an ANCOVA model, adjusting for baseline
188 binocular near point of accommodation, age, iris color (brown vs. not brown), and race
189 (East Asian vs. non-East Asian).

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192 **4. Subgroup Analysis**

193 The treatment group difference for change in SER from baseline to 24 and 30 months
194 within the following subgroups will be explored:

195

- East Asian vs. non-East Asian race

196

- Brown iris versus non-brown iris

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- Baseline younger versus older age (based on median split)

198

- Baseline lower versus higher myopia level by SER (based on median split)

199

- Baseline age and baseline SER (4 categories based on median splits)

200

201 An estimate of the treatment effect within each subgroup and a 95% confidence interval
202 will be obtained for each of the times, 24 and 30 months, by adding a treatment by time

203 by subgroup interaction into the primary analysis model and generating the appropriate
204 contrasts. Each subgroup effect will be estimated separately, one subgroup per model.

205

206 Previous studies have suggested that race and/or eye color may interact with the
207 treatment effect of atropine. Several studies have consistently found atropine to be
208 effective in East Asian populations and a meta-analysis has suggested that atropine might
209 be more effective in Asian populations than in white children, although this conclusion is
210 limited by the lack of studies in non-Asian populations. Other research has shown that
211 atropine is rapidly taken up by melanocytes and released over time, leading to a longer
212 time to achieve mydriasis and a prolonged mydriatic effect in heavily-pigmented eyes as
213 atropine is released over time, potentially leading to an increased treatment effect.

214 Conversely, if the mechanism by which atropine slows myopic progression is through
215 local retinal effects, one might speculate that higher melanocyte density might prevent
216 the atropine from reaching the retina, which might result in brown eyes having less
217 treatment effect than non-brown eyes.

218

219 Atropine might be expected to be more effective in children with lower amounts of
220 myopia given more potential for suppression of myopia progression. Likewise, atropine
221 might be hypothesized to have a greater treatment effect in younger than in older children
222 given they are earlier in the course of myopic progression and have more room for
223 potential suppression.

224

225 For each time point, the planned subgroup analyses also will be conducted using a
226 continuous time longitudinal model, if linearity assumptions with time are met, to obtain
227 p-values for the subgroup effect. Even if change in SER is not precisely linear with time,
228 if it is monotonically decreasing, the continuous model is expected to have higher power
229 than the discrete time primary analysis model, and will be favored over the discrete time
230 model for obtaining p-values. The baseline factor and the baseline factor by treatment
231 interaction will be included as terms in the model, and the 3-way subgroup, time, and
232 treatment interaction will be used to determine whether there is a significant subgroup
233 effect. The false discovery rate for the subgroup analyses will be controlled using the
234 two-stage step up FDR procedure to control the overall FDR at 5%. Subgroup analyses
235 will be interpreted with caution, particularly if the corresponding overall analysis does
236 not demonstrate a significant treatment group difference.

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238

239 **5. Additional Analyses**

240 The overall false discovery rate for the additional analyses specified in Section 5.1 and
241 5.2 below will be controlled at the 5% level using the two-stage step up false discovery
242 rate (FDR) procedure.

243

244 **5.1. Treatment Effect Over First Year of Treatment**

245 The treatment effect on change in SER from baseline through the first year will be
246 compared with the treatment effect on change in SER from end of first year through the
247 second year, by constructing the appropriate contrasts in the primary analysis model.

248

249 **5.2.Exploratory Analyses of Additional Ocular Biometric Parameters**

250 As exploratory analyses at 24 and 30 months, change in flat corneal radius, anterior
251 chamber depth, and lens thickness from baseline will each be compared between
252 treatment groups using a longitudinal discrete time mixed model with maximum
253 likelihood estimation, including the interaction between time and treatment group, and
254 adjusting for the baseline covariates from the primary analysis model. The same
255 strategies specified in the primary analysis (Section 1, excluding sensitivity analysis) will
256 be used to choose the appropriate covariance structure for the model.

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258 At baseline and all follow-up visits, including the 12 and 24-month visits, the mean of the
259 readings in both eyes for each participant will be used for the analysis. If data from only
260 one eye is available, the reading on that eye will be used for analysis. The treatment
261 group differences (atropine – placebo) and a 95% confidence interval will be estimated
262 using the mixed model.

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264 **5.3.Data Tabulations**

265 The following tabulations will be performed both according to treatment group and
266 among overall sample:

- 267 • Baseline demographics and clinical characteristics
- 268 • A flow chart accounting for all participants for all visits and phone calls
- 269 • Visit and phone contact completion rates for each follow-up visit
- 270 • Protocol deviations
- 271 • Proportion of participants needing bifocals by 24 months (i.e. during treatment)

272
273 **5.4.Compliance**

274 Compliance with study medication will be assessed at the 6-month, 12-month, 18-month,
275 and 24-month outcome exams. For each of these exams, the proportion of calendar days
276 that study medication was reported used and the proportion of unused study medication
277 ampules will be tabulated in each of the two treatment groups.

278
279 Compliance with refractive correction will be assessed at the 6-month, 12-month, 18-
280 month, and 24-month outcome exams. After discussion with the parent and child, study
281 personnel will classify the proportion of time refractive error was worn will be described
282 as excellent (76% to 100%), good (51% to 75%), fair (26% to 50%), or poor ($\leq 25\%$).
283 The distribution of refractive correction compliance will be tabulated in each of the two
284 treatment groups.

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287 **6. Safety Analyses**

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289 **6.1.Adverse Events of Eye Drops**

290 An eye drops questionnaire will be administered at randomization and at each follow-up
291 visit. The distribution of scores on each survey item will be summarized by treatment
292 group at the time of randomization and at each follow-up exam up until and including the
293 24-month visit. The average of the item responses at the 24-month visit will be calculated
294 and compared with a t-test for difference in distributions between treatment groups.

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6.2. Visual Acuity

The proportion of participants with loss of best corrected distance vision >1 logMAR line at 30 months in either eye will be compared between treatment groups using Barnard’s test. The proportion of participants with loss of best corrected near binocular vision >1 logMAR line at 6 months will be tabulated by treatment groups and compared using Barnard’s test. The Bonferroni correction will be used to account for multiplicity and control the type I error rate.

7. Interim Monitoring

By the time sufficient data allowing for an analysis of efficacy or futility for the primary outcome would be possible, all or most subjects are expected to be within 1 year of completing the primary outcome; hence, no formal statistical interim monitoring is proposed.

Tabulated safety and efficacy data will be reviewed by the PEDIG Data and Safety Monitoring Committee (DSMC) at its biannual meetings, and if any unexpected safety or other issues arise, they can recommend stopping the study at any time.

During the DSMC meeting in October 2019, the DSMC approved the proposal of NOT performing interim monitoring analysis for futility based on the rationale that by the time 50% of the 24-month data are available (May 2021), the recruitment will have been finished and the remaining participants will have between <1 to 7 months of remaining time on treatment before all participants have 24-month data (December 2021). The DSMC also approved the proposal of NOT performing interim monitoring analysis for efficacy based on the rationale that even if efficacy was found in an interim analysis for the 24-month on-treatment primary outcome analysis, the 30-month off-treatment secondary analysis is needed to understand whether the benefit persists after treatment is discontinued.

328 **References**

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