Supplementary Online Content

Repka MX, Weise KK, Chandler DL, et al; Pediatric Eye Disease Investigator Group. Low-dose 0.01% atropine eye drops vs placebo for myopia control: a randomized clinical trial. *JAMA Ophthalmol*. Published online July 13, 2023. doi:10.1001/jamaophthalmol.2023.2855

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods. The authors have provided this eMethods to give readers additional information about the work.

Additional Methods

Additional Information about Study Procedures

1. Autorefractors used in the study: Canon RK-F2Grand Seiko WR-5100K, Nidek ARK-1s, Nidek ARK-500A, Nidex ARK-530A, Nidek ARK-560A, Nidek 700A, Topcon KR-1, Topcon KR 800, Topcon, KR 8800, Zeiss Manufactured VISUREF 100. The same instrument as specified in the protocol was used for each participant throughout the course of the study.
2. Biometers used in the study: Zeiss IOLMaster 500, Zeiss IOLMaster 700, Haag-Streit Lenstar LS900. Site used the same biometer for each participant throughout the course of the trial.
3. Cycloplegia prior to autorefraction was obtained with 2 drops of 1% cyclopentolate administered 5 minutes apart 30 ± 5 minutes from the time the second drop of 1% cyclopentolate was administered.
4. One eyedrop of study drug (0.01% atropine or placebo) was to be administered nightly for 24 months. Parents were instructed to instill a second drop if the first did not go in, consistent with standard clinical practice.
5. At enrollment into the run-in phase, refractive correction was updated, if needed.
6. In addition to follow up visits, the clinical sites made phone calls to the participant at 2 weeks, 3, 9, 15, 21, and 27 months.
7. The virtual visits that were allowed at the 6-, 12-, and 18-month visits during the pandemic (if needed) were conducted by phone or video; only data on adherence with eye drops and eye glass wear, and adverse events were collected. Although participants did not attend in person, study medication was delivered to families after virtual visits.
8. At each follow up visit between 6 to 24 months, female participants who had experienced menarche completed a urine pregnancy test. No pregnancy tests were positive during the study in either treatment group; however, such an occurrence would have resulted in discontinuation of drug as a safety precaution.
9. During follow up, an optical correction update was required for refraction changes of ≥0.75 D sphere, ≥0.50D in spherical equivalent refraction (SER) of anisometropia, ≥0.75D of astigmatism, and/or ≥6 degrees in axis for astigmatism ≥1.00D.
10. Medical history since the previous study visit was obtained prior to administering the Eyedrop Symptom Questionnaire or performing any testing procedures. There was no formal script for querying participants about adverse events. Participants were asked about adverse events occurring since the previous visit 1) ocular adverse events (e.g., lid/conjunctival irritation, light sensitivity, near blur, and/or reading difficulty), and 2) systemic events (e.g., dry skin/mouth, tachycardia, fever, flushing, irritability, mental confusion, constipation, aggravation of asthma, or seizures). The Medical Dictionary for Regulatory Activities (MedDRA) preferred term was used to describe each event.
11. Unused ampules were to be returned to the study sites for disposal and as an incentive for adherence. Counts of unused ampules were performed when returned (eFigure 8).
12. Additional testing procedures:
    a. Cycloplegic outcomes were corneal radius, anterior chamber depth, and lens thickness (where available).
    b. Binocular near point of accommodation: Measured without cycloplegia with participant wearing their eyeglasses using a study-specified and provided near-point rule.). The target was a Gulden Ophthalmics (Elkins Park, PA) 20/30 single vertical line of letters and was pushed toward the participant's eyes until the participant reported first sustained blur. Measures were recorded in the nearest half centimeter from the forehead.
    c. Binocular near acuity was measured at the 6-month visit with the ATS4 Near Acuity Test (Precision Vision, Woodstock, IL).
13. An independent laboratory confirmed the accuracy of the atropine concentration from randomly selected samples of atropine and placebo at two time points.

Additional Statistical Methods

1. For the longitudinal discrete-time mixed model used for the primary analysis comparison of mean change in SER from baseline to 24 months (on treatment), the population-averaged method was used to model the repeated measures of SER at the 6-, 12-, 18-, 24, and 30-month visits. The time variable was categorical to not impose assumptions regarding the trend of SER over time.
2. Missing SER data were from the virtual visits at the 6-, 12-, and 18-month visits. The percentage of virtual visits was not expected to differ systematically between treatment groups as randomization was stratified and blocked by site and the study was masked in that the specific treatment group was unknown to 1) the participant and parent who administered the eyedrops, 2) the investigator and coordinator as caregivers, and 3) the examiners who assessed outcomes. The maximum likelihood method was used to provide unbiased estimates of treatment effect at 24 months in the presence of missing outcome data.
3. A sensitivity analysis was conducted to compare the mean change in SER from baseline to 24 months between treatment groups using the ANCOVA method, adjusting for baseline SER, age, iris color (brown vs. not brown), and race (East Asian vs. non-East Asian participants) and not considering SER at the intermediate 6-, 12-, or 18-month visit.
4. 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 analysis of covariance, adjusting for baseline binocular near point of accommodation, age, iris color (brown vs. not brown), and race (East Asian vs. non-East Asian participants).
5. The four-level response to each item in the Eyedrop Questionnaire was converted to numeric (never=0, sometimes=1, most of the time=2, always=3) and the mean and standard deviation of the converted numeric responses to each item at each visit were tabulated by treatment groups.

eResults.

The authors have provided these eResults to provide readers additional information about the work.

Additional Results

1. Excellent adherence with eyeglasses wear was reported for 98% and 99% in the atropine and placebo groups, respectively.
2. The results from the sensitivity analysis were similar to the efficacy outcome in the primary analysis. Using ANCOVA and only adjusting for baseline factors in the primary analysis, at the 24-month visit, the adjusted mean change in SER from baseline was -0.81D in both treatment groups (treatment group difference: -0.005D; 95% CI: -0.18D to 0.17D).
3. The adjusted mean binocular near point of accommodation at 6 months was 6.03 cm and 5.34 cm in the atropine and placebo groups, respectively (adjusted treatment group difference with atropine minus placebo: 0.68 cm; CI: -0.29 to 1.65 cm).
4. The majority of mean responses to the Eyedrop Questionnaire were 1 or lower at each study visit and the responses were similar between the treatment groups.
5. Binocular near visual acuity at 6 months was reduced >1 logMAR line in 1 (1%) atropine participant and no placebo treated participant.
6. We estimated ampule use over 24 months by taking the number of ampules supplied to each participant completing the 24-month visit, subtracting the number of ampules returned, with the difference divided by the number of days between randomization and their 24-month visit, yielding estimated percent of prescribed drug utilized. All unreturned ampules were considered used. Participants who did not return any ampules were considered to have used the entire amount dispensed. Among the participants that completed the 24-month primary outcome, 65% (77 of 119) and 60% (35 of 58) in the atropine and placebo groups respectively were estimated to have used 90% or more of the expected amount of study medication (eFigure 8).

eTable 1. Eligibility Criteria

Inclusion Criteria for Enrollment into Run-In Phase (2 to 4 Weeks)

1. Age 5 years to 12 years at time of enrollment. Children within 4 weeks of their 13th birthday are not eligible.
2. Refractive error meeting the following by cycloplegic autorefraction:
   - Myopia -1.00D to -6.00D spherical equivalent (SER) in each eye
   - Astigmatism <=1.50D in each eye
   - Anisometropia <1.00D SER
3. Currently wearing refractive correction (single vision eyeglasses or contact lenses) for at least 4 weeks
4. Excellent compliance with refractive correction (more than 75% of all waking hours) for at least one month, based on investigator judgment after discussion with parent
5. Gestational age ≥ 32 weeks
6. Birth weight >1500g
7. Parent understands the protocol and is willing to accept randomization to atropine or placebo
8. Is willing to participate in a 2-to-4-week run-in phase using daily artificial tear eyedrops
9. Able to return in 2 to 4 weeks for possible randomization
10. Parent has a phone (or access to phone) and is willing to be contacted by Investigator's site staff
11. Relocation outside of the area of an active PEDIG site within next 32 months is not anticipated

Exclusion Criteria for Enrollment into Run-In Phase (2 to 4 Weeks)

1. Current or previous myopia treatment with atropine, pirenzepine or other anti-muscarinic agent
2. Current or previous use of bifocals, progressive-addition lenses, or multifocal contact lenses
3. Current or previous use of orthokeratology, rigid gas permeable, or other contact lenses being used to reduce myopia progression
4. Known atropine allergy
5. Abnormality of the cornea, lens, central retina, iris, or ciliary body
6. Current or prior history of manifest strabismus, amblyopia, or nystagmus
7. Prior eyelid, strabismus, intraocular, or refractive surgery
8. Down syndrome or cerebral palsy
9. Diseases known to affect accommodation, vergence, or ocular motility (e.g., multiple sclerosis, Grave's disease, myasthenia gravis, diabetes mellitus, Parkinson's disease)
10. Existing ocular conditions (e.g., retinal disease, cataracts, ptosis) or systemic/neurodevelopmental conditions (e.g., Down syndrome) which may influence refractive development
11. Any condition that in the judgement of the investigator could potentially influence refractive development
12. Existing conditions that may affect the long-term health of the eye or require regular pharmacologic treatment that may adversely interact with study medication (e.g., JIA, glaucoma, diabetes mellitus, pre-diabetes)
13. Inability to comprehend and/or perform any study-related clinical tests
14. A negative urine pregnancy test will be required for all females who have experienced menarche

Additional Inclusion Criteria for Randomization (Evaluated at Run-in Phase Follow Up Visit)

1. During the 2-4 weeks Run-in Phase:
   - Participants must have used artificial tear eyedrops in both eyes for at least 2 weeks and must have been at least 90% adherent with instilling the drops in both eyes (based on review of adherence calendar and count of unused ampules)
   - Participants must have excellent (76% to 100%) adherence with refractive correction
2. Best-corrected distance visual acuity in current correction meeting the following criteria:
   - 20/32 or better in each eye (>=76 letters by E-ETDRS testing)
   - Interocular difference <= 0.2 logarithm of the Minimal Angle of Resolution (<= 10 letters by E-ETDRS testing)
3. Refractive correction must meet the following criteria:

- Myopia (spherical equivalent) in both eyes must be corrected to within ±0.50 D of the investigator's cycloplegic measurement of refractive error.
- Cylinder power in both eyes must be within ±0.50 D of the investigator's standard refraction technique, which can be based on a cycloplegic or non-cycloplegic refraction.
- Cylinder axis for both eyes must be within ±5 degrees of the axis found on the investigator's refraction when cylinder power is >= 1.00 D or within ±15 degrees when the cylinder power is <1.00 D.

4. Refractive error meeting the following by cycloplegic autorefraction *(repeated only if Run-In Follow Up Visit occurred between 4-6 weeks after randomization (i.e., after the 2-4 weeks visit window)*
   - Myopia -1.00D to -6.00D spherical equivalent (SER) in each eye
   - Astigmatism <=1.50D in each eye
   - Anisometropia <1.00D SER

eTable 2. Treatment Modifications During 24 Months Follow Up

		Reason for stopping treatment	Initial time point it was reported	Type
Participant in whom study eyedrops were paused or stopped during the 24-month follow-up	Atropine Group (N=4)	Perceived lack of treatment effect	12-month visit	Permanent
		Burning in the eye	18-month visit	Permanent
		Sensitivity to light	15-month phone call	Permanent
		Sting in the eye upon study treatment administration	1 month from randomization	Temporary
	Placebo Group (N=6)	Sting in the eye upon study treatment administration	6-month visit	Permanent
		Eye injury due to hit in the head	20 months from randomization	Temporary
		Conjunctivitis, allergic	21-month phone call	Temporary
		Conjunctivitis, swollen and red eye	4 months from randomization	Temporary
		Conjunctivitis, allergic, swollen and red eye	4 months from randomization	Temporary
		Headache and vomiting	1 month from randomization	Temporary
		Type of treatment	**Initial time point it was reported**	
Additional treatment for myopia (besides study eyedrops) was used or prescribed during the 24-month follow-up	Atropine Group (N=2)	Multifocal soft contact lens wear and correction lens	18-month visit	--
		1% atropine	24-month visit	--
	Placebo Group (N=0)	--	--	--

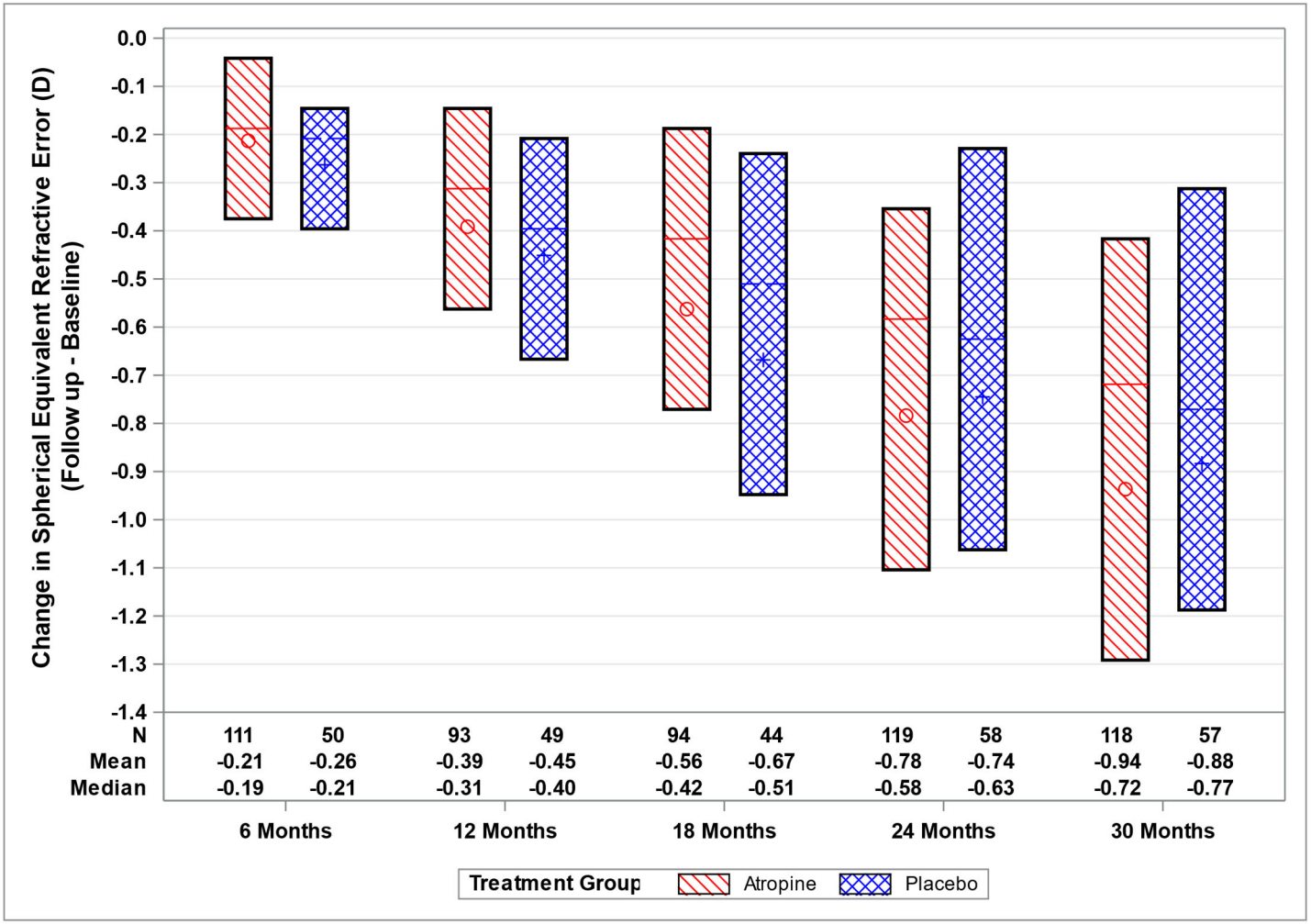
eTable 3. Responses to Eyedrop Symptom Questionnaire at Each Study Visit[a]

	Randomization Visit		6-Month Visit		12- Month Visit		18-Month Visit		24-Month Visit	
	Atropine (N=125)	Placebo (N=62)	Atropine (N=123)	Placebo (N=57)	Atropine (N=122)	Placebo (N=57)	Atropine (N=121)	Placebo (N=58)	Atropine (N=119)	Placebo (N=58)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Do you hate eyedrops?	0.6 (0.8)	0.5 (0.6)	0.7 (0.8)	0.8 (0.9)	0.6 (0.8)	0.7 (1.0)	0.7 (0.9)	0.7 (0.9)	0.7 (0.9)	0.8 (1.0)
Do your eyedrops hurt your eyes?	0.2 (0.6)	0.1 (0.5)	1.1 (1.0)	1.2 (1.0)	1.0 (1.0)	0.9 (0.8)	1.0 (1.0)	0.9 (0.9)	1.0 (0.9)	0.8 (0.9)
Do you have a hard time seeing?	0.2 (0.4)	0.1 (0.3)	0.1 (0.3)	0.2 (0.5)	0.2 (0.4)	0.1 (0.3)	0.1 (0.3)	0.2 (0.4)	0.3 (0.6)	0.1 (0.2)
Do you have trouble reading up close?	0.2 (0.5)	0.1 (0.2)	0.1 (0.4)	0.1 (0.3)	0.2 (0.5)	0.1 (0.5)	0.1 (0.3)	0.1 (0.5)	0.2 (0.6)	0.1 (0.4)
Does bright light make it hard for you to do things outside?	0.4 (0.7)	0.2 (0.5)	0.3 (0.5)	0.3 (0.6)	0.3 (0.5)	0.3 (0.6)	0.3 (0.5)	0.3 (0.7)	0.4 (0.7)	0.3 (0.6)
Are you bothered by how your eyedrops make your eyes look?	0.1 (0.2)	0.0 (0.0)	0.0 (0.1)	0.0 (0.1)	0.0 (0.2)	0.0 (0.0)	0.0 (0.2)	0.1 (0.4)	0.0 (0.2)	0.0 (0.0)
Does it bother you because your eyedrops hurt your eyes?	0.1 (0.3)	0.0 (0.2)	0.6 (0.9)	0.6 (0.9)	0.6 (0.9)	0.5 (0.8)	0.6 (0.9)	0.6 (0.9)	0.6 (0.9)	0.5 (0.9)
Does it bother you because you have a hard time seeing?	0.1 (0.5)	0.1 (0.4)	0.2 (0.5)	0.0 (0.3)	0.1 (0.3)	0.2 (0.5)	0.1 (0.4)	0.2 (0.6)	0.2 (0.5)	0.2 (0.6)
Does it bother you because you have trouble reading up close?	0.1 (0.5)	0.0 (0.2)	0.1 (0.4)	0.0 (0.2)	0.1 (0.3)	0.1 (0.4)	0.0 (0.1)	0.1 (0.5)	0.1 (0.5)	0.1 (0.4)
Does it bother you because bright light makes it hard to do things outside?	0.2 (0.6)	0.1 (0.3)	0.2 (0.5)	0.2 (0.5)	0.2 (0.4)	0.2 (0.6)	0.2 (0.5)	0.2 (0.6)	0.3 (0.6)	0.2 (0.6)

SD = standard deviation

[a]The response to each item had four levels which were converted to numeric (never=0, sometimes=1, most of the time=2, always=3). The mean and standard deviation (SD) of the numerical responses were provided.

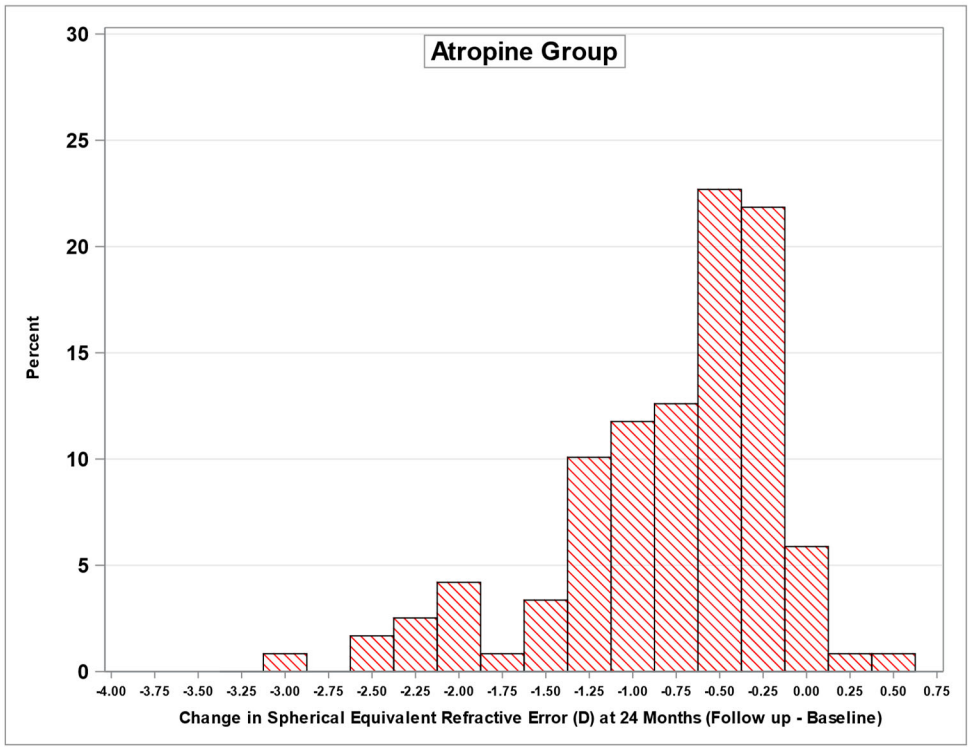
eFigure 1. Change in Spherical Equivalent Refractive Error (D) between Baseline and Outcome Visits (Outcome – Baseline) (Mean of OD and OS)

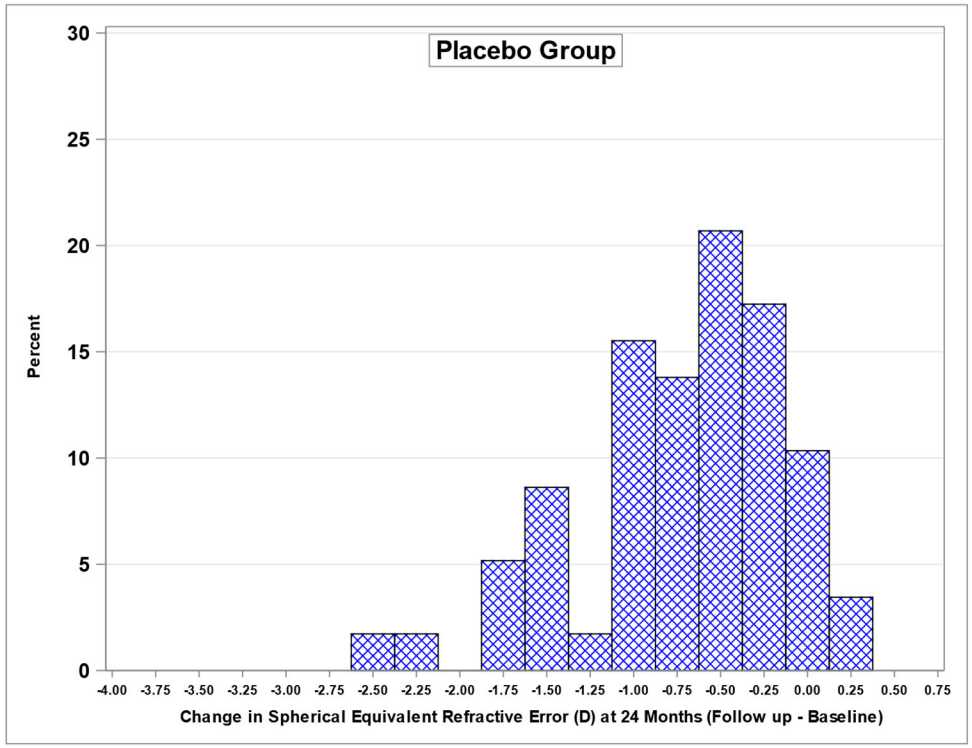


Elements of the Box Plot:

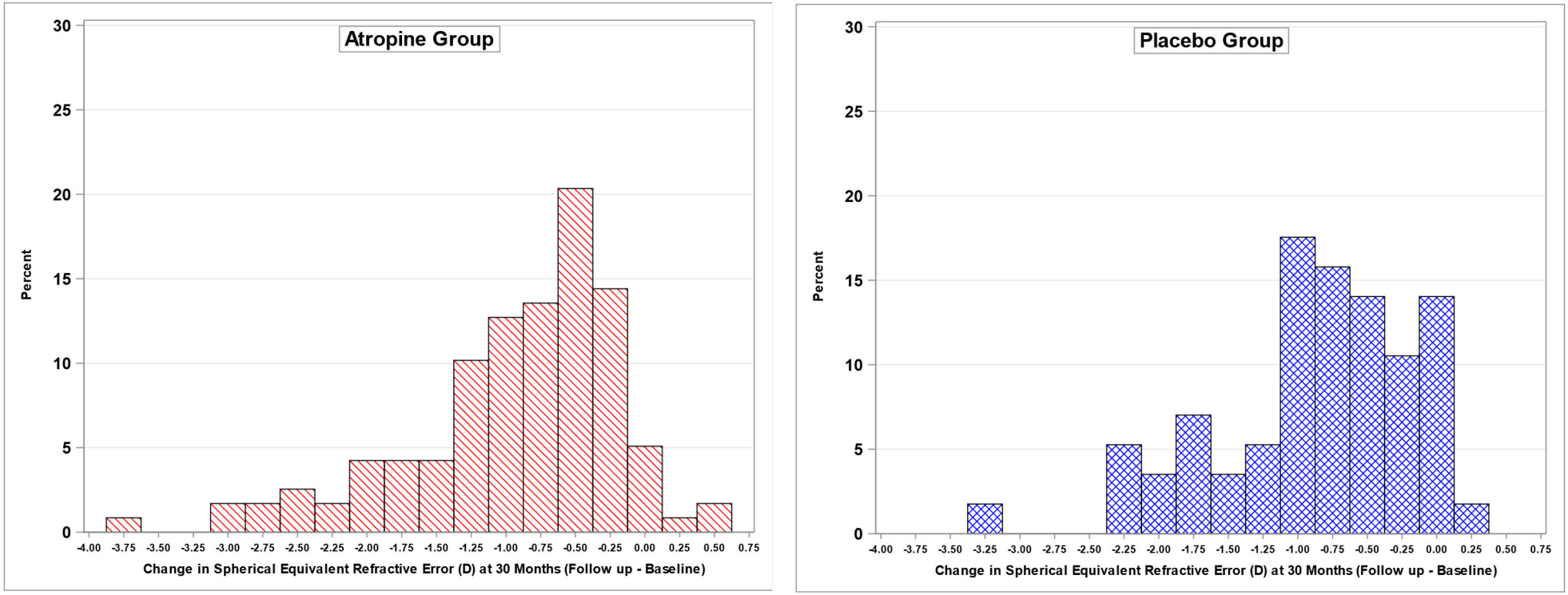
- Marker in the box: mean.
- Horizontal line in the box: median.
- Upper and lower edges of the box: 75th and 25th percentiles respectively.

eFigure 2. Change in Spherical Equivalent Refractive Error (D) between Baseline and Outcome Visits (Outcome – Baseline) at 24 Months (Mean of OD and OS)

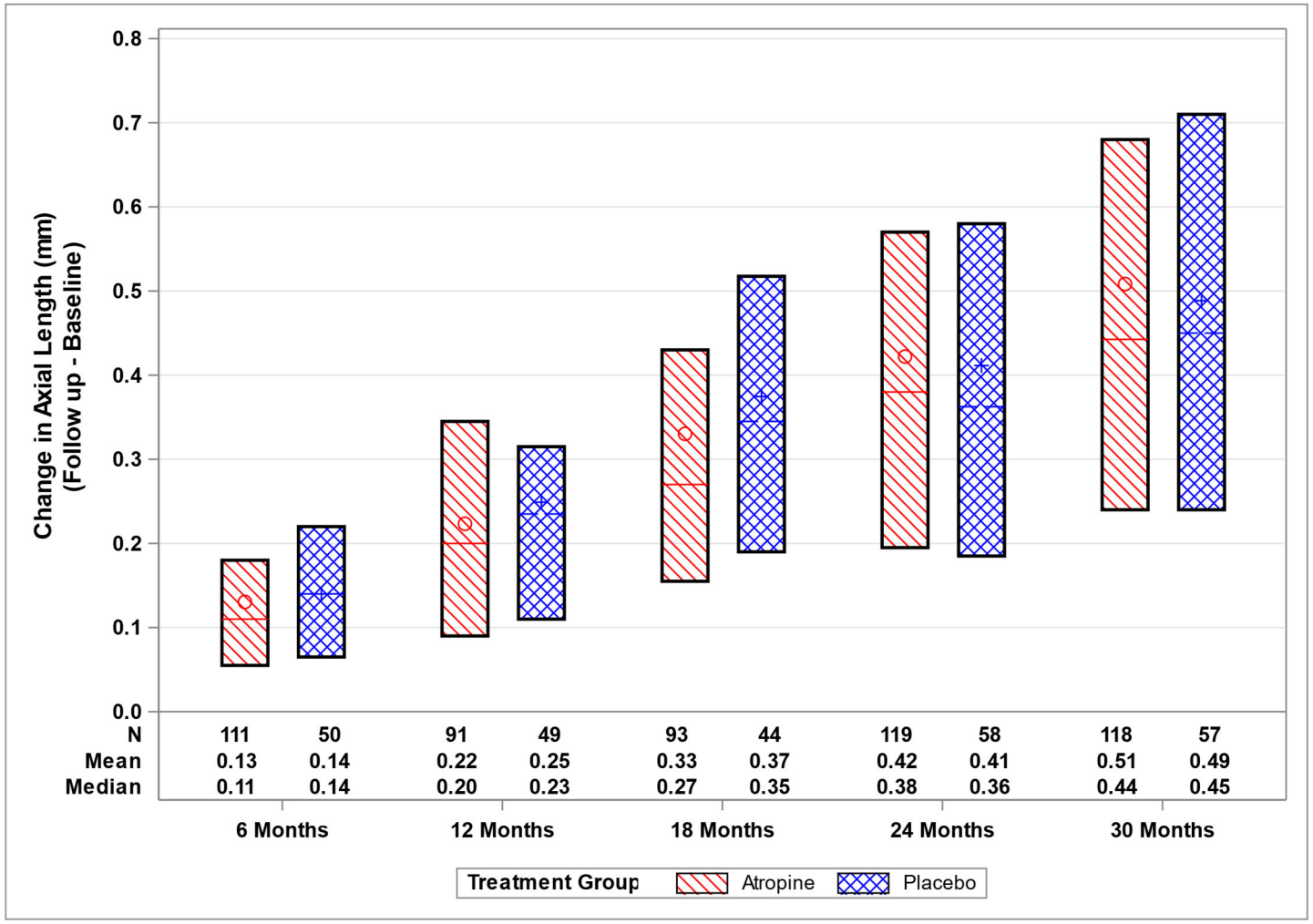




eFigure 3. Change in Spherical Equivalent Refractive Error (D) between Baseline and Outcome Visits (Outcome – Baseline) at 30 Months (Mean of OD and OS)



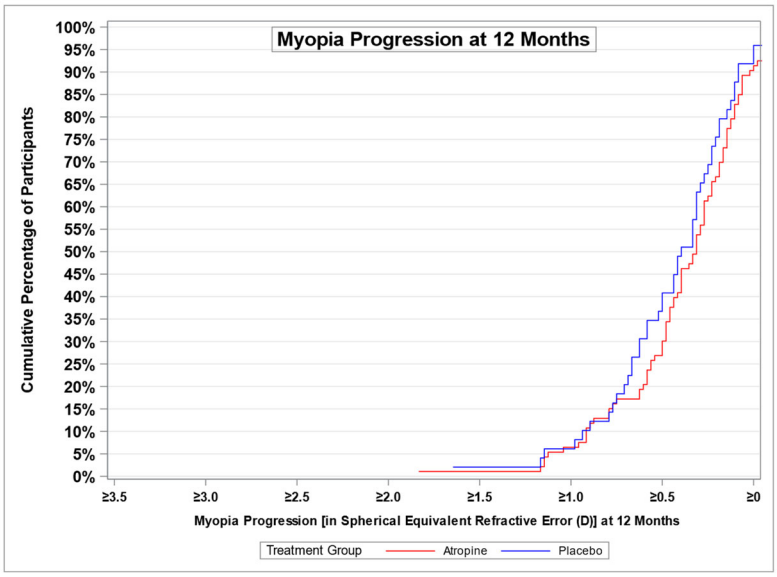
eFigure 4. Change in Axial Length (mm) between Baseline and Outcome Visits (Outcome – Baseline) (Mean of OD and OS)

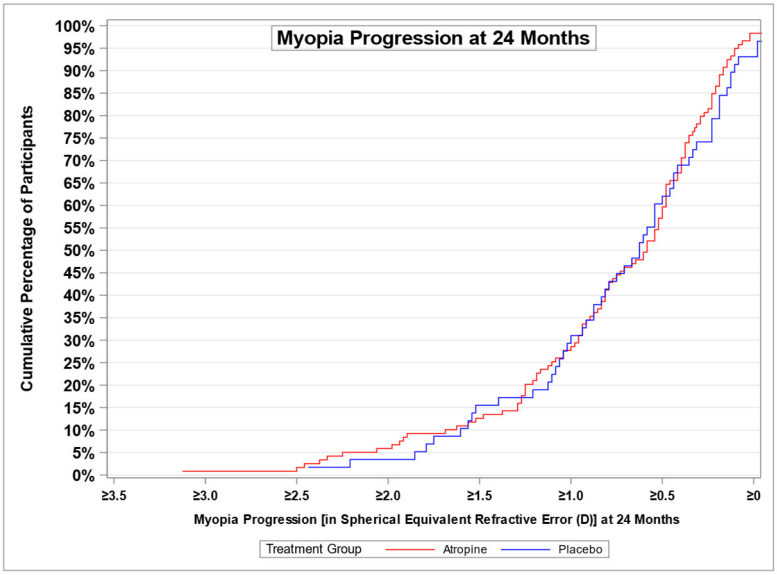


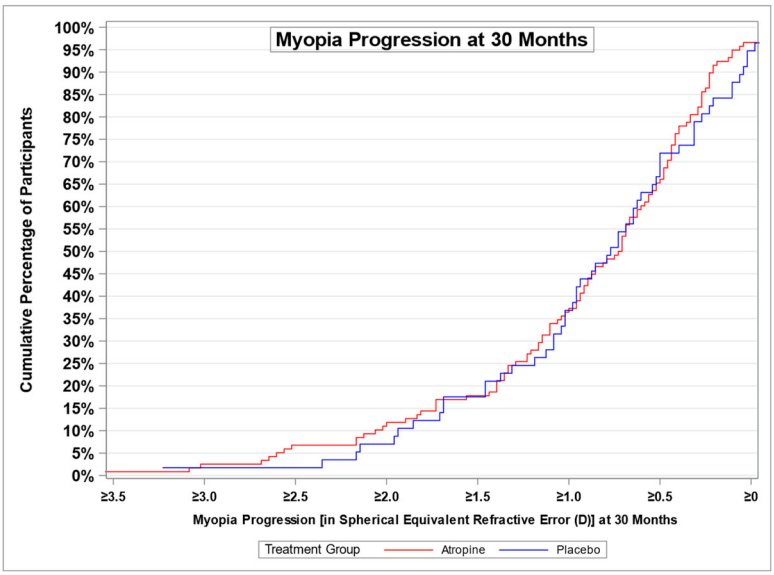
Elements of the Box Plot:

- Marker in the box: mean.
- Horizontal line in the box: median.
- Upper and lower edges of the box: 75th and 25th percentiles respectively.

eFigure 5. Myopia Progression at the Follow-up Visits

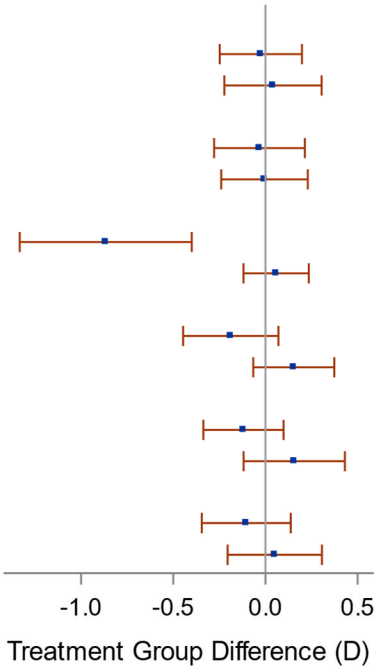






eFigure 6. Mean Change in Spherical Equivalent Refractive Error (SER) from Baseline at 24 Months in Subgroups

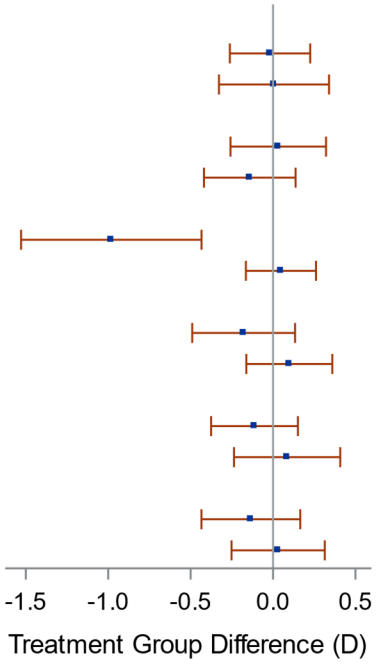
Subgroup	Atropine: # of Participants at 24 Months	Atropine: Adjusted Mean Change (D) in SER from Baseline at 24 Months	Placebo: # of Participants at 24 Months	Placebo: Adjusted Mean Change (D) in SER from Baseline at 24 Months	Treatment Group Difference (Atropine - Placebo) and 95% Confidence Interval of Adjusted Mean Change (D) in SER from Baseline at 24 Months
Age[a]					
Older age group	57	-0.59	33	-0.57	-0.03 (-0.25,0.20)
Younger age group	62	-1.02	25	-1.06	0.04 (-0.22,0.30)
Sex					
Female	63	-0.82	33	-0.79	-0.03 (-0.28,0.21)
Male	56	-0.82	25	-0.82	-0.01 (-0.24,0.23)
Race/Ethnicity: East Asian vs. Non East Asian					
East Asian[b]	15	-1.09	4	-0.23	-0.87 (-1.33,-0.40)
Non East Asian	104	-0.72	54	-0.78	0.06 (-0.12,0.24)
Race/Ethnicity: White vs. Non White					
Non White	62	-0.88	30	-0.69	-0.19 (-0.45,0.07)
White (Not Hispanic or Latino)	57	-0.66	28	-0.81	0.15 (-0.07,0.37)
Eye Color					
Brown	78	-0.87	37	-0.75	-0.12 (-0.34,0.10)
Not brown	41	-0.75	21	-0.90	0.16 (-0.12,0.43)
SER at Baseline[a]					
Higher baseline SER level	58	-0.92	31	-0.81	-0.10 (-0.35,0.14)
Lower baseline SER level	61	-0.72	27	-0.78	0.05 (-0.21,0.31)



[a]The age and baseline SER groups were based on the median split (median age = 10.2 years, median baseline SER = -2.73D).
[b]Myopic progression was greater in the East Asian participants in the atropine group (N=15) versus those in the placebo group (N=4). This finding was likely due to chance given the small sample sizes.

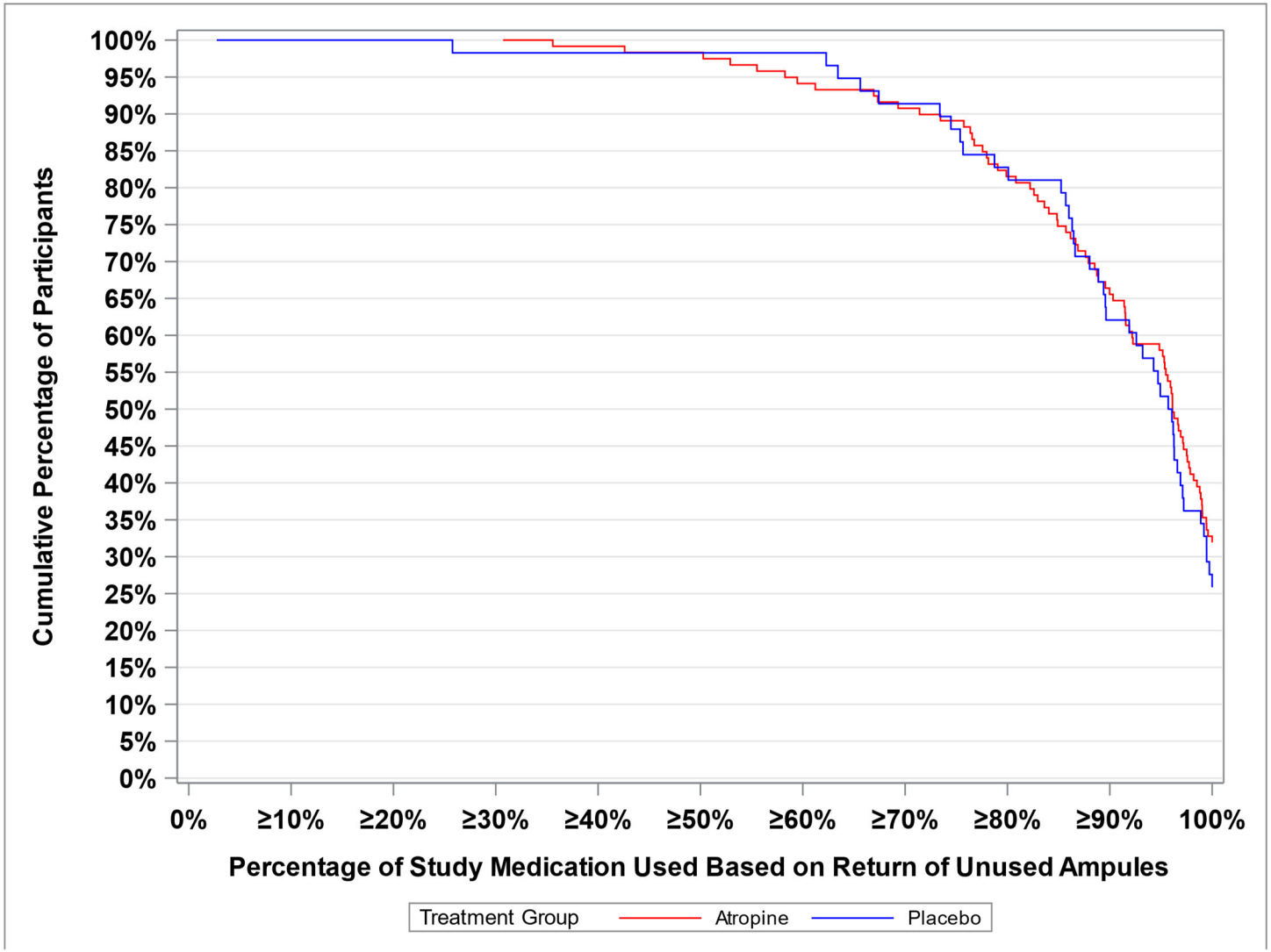
eFigure 7. Mean Change in Spherical Equivalent Refractive Error (SER) from Baseline at 30 Months in Subgroups

Subgroup	Atropine: # of Participants at 30 Months	Atropine: Adjusted Mean Change (D) in SER from Baseline at 30 Months	Placebo: # of Participants at 30 Months	Placebo: Adjusted Mean Change (D) in SER from Baseline at 30 Months	Treatment Group Difference (Atropine - Placebo) and 95% Confidence Interval of Adjusted Mean Change (D) in SER from Baseline at 30 Months
Age [a]					
Older age group	57	-0.68	32	-0.66	-0.02 (-0.26,0.23)
Younger age group	61	-1.24	25	-1.24	0.01 (-0.33,0.34)
Sex					
Female	63	-0.96	33	-0.99	0.03 (-0.26,0.32)
Male	55	-0.99	24	-0.85	-0.14 (-0.42,0.14)
Race/Ethnicity: East Asian vs. Non East Asian					
East Asian [b]	14	-1.32	4	-0.34	-0.98 (-1.53,-0.43)
Non East Asian	104	-0.87	53	-0.92	0.05 (-0.16,0.26)
Race/Ethnicity: White vs. Non White					
Non White	61	-1.05	29	-0.87	-0.18 (-0.49,0.13)
White (Not Hispanic or Latino)	57	-0.80	28	-0.90	0.10 (-0.16,0.36)
Eye Color					
Brown	77	-1.02	36	-0.91	-0.11 (-0.38,0.15)
Not brown	41	-0.90	21	-0.99	0.09 (-0.24,0.41)
SER at Baseline [a]					
Higher baseline SER level	58	-1.11	31	-0.98	-0.13 (-0.43,0.17)
Lower baseline SER level	60	-0.84	26	-0.87	0.03 (-0.25,0.31)



[a]The age and baseline SER groups were based on the median split (median age = 10.2 years, median baseline SER = -2.73D).
[b]Myopic progression was greater in the East Asian participants in the atropine group (N=14) versus those in the placebo group (N=4). This finding was likely due to chance given the small sample sizes.

eFigure 8. Estimated Prescribed Medication Used Based on Return of Unused Ampules



The medication used for each participant that completed the 24-month primary outcome visit was calculated as the total number of ampules dispensed during the study minus the number of unused ampules returned during the study. That difference was divided by the number of ampules that should have been used during the study. The expected number of ampules used during the study was equal to the number of days between randomization and each participant's 24-month visit.