AMBLYOPIA TREATMENT STUDY

(ATS22)

A Randomized Trial to Evaluate Sequential vs Simultaneous Spectacles plus Patching for Amblyopia in Children 3 to <13 Years Old

Protocol Identifying Number: ATS22

Version Number: 7.0

24 March 2023

PROTOCOL AMENDMENT #6
24 March 2023

This amendment provides for the following protocol changes:

Protocol Change #1:

Current Protocol
Section 2.3 (Participant Eligibility Criteria) of the current protocol states that spectacles cannot be worn for more than a total of 24 hours to be considered as having "no prior amblyopia treatment" and be eligible for the study, regardless of the amount of time has elapsed since spectacle wear was attempted.

Proposed Change
Section 2.3 was revised to extend the allowable duration of total spectacle wear time from 24 hours to 72 hours if spectacle wear was attempted more than 3 months prior to the Enrollment visit. If spectacle wear was attempted within 3 months of the Enrollment visit, the participant must not have worn spectacles for more than 24 hours.

Rationale for Change
Spectacle wear of 72 hours or less more than 3 months from the Enrollment visit is not likely to result in partial treatment of amblyopia, and so these potential participants would still meet the definition of "no prior treatment" and help to increase recruitment of eligible subjects.

Protocol Change #2:

Current Protocol
The current protocol section 2.3 (Participant Eligibility Criteria) criterion #4 indicates that a cycloplegic refraction must have been performed within 45 days of the Enrollment Visit *or* within 90 days of the Spectacle Baseline Visit.

Proposed Change
Section 2.3 (Participant Eligibility Criteria) criterion #4 has been revised to require a cycloplegic refraction within 45 days of the Enrollment Visit *and* within 90 days of the Spectacle Baseline Visit. This has been specified in section 3.1.1 (Lensometry).

Rationale for Change
The cycloplegic refraction measurement determines the spectacle correction, which is a treatment for this study. The cycloplegic refraction measurement can change over time. It is important that the cycloplegic refraction is current at the time of the Enrollment visit and the Spectacle Baseline/Randomization visit to ensure that a potential participant is wearing the most accurate spectacle correction for study testing.

Protocol Change #3:

Current Protocol
Section 4.2 (Simultaneous Spectacles and Patching) does not specify the treatment protocol for participants in the Simultaneous treatment group who meet definition of stable resolved.

Proposed Change
The following text was added to section 4.2: If non-improvement ("stable/worsening") is reached AND amblyopia is no longer present (less than or equal to 1 logMAR line) (meeting the definition for "stable resolved", *section 4.3.5*), participants will continue spectacle wear only (i.e. discontinue patching) and 8-weekly visits until 56 weeks.

Rationale for Change
Treatment protocol for participants in the Simultaneous treatment group who meet definition of stable resolved was not included as an oversight and the correction has been made to avoid any confusion.

Protocol Change #4:

Current Protocol
Section 4.4.1 (Testing Procedures at Study Visits), section 4.4.1.1.1 (At Visits Prior to the 56-week Study Visit), and section 4.4.1.1.2 (At the 56-week Study Visit) states that a test and retest of monocular distance visual acuity of both eyes must be performed after cycloplegic refraction at the time of the primary outcome visit if the refractive error has changed significantly and glasses are being updated.

Proposed Change
The proposed change is to remove the requirement for a test and retest of monocular distance visual acuity of both eyes after cycloplegic refraction at the time of primary outcome visit.

Rationale for Change
Participants have often undergone many hours of testing at the time of this test, and after data quality review, it was found that almost 50% of children in the younger age cohort are unable to complete this testing due to fatigue. To alleviate testing burden on participants and investigators, this requirement is being removed. It will still be possible to evaluate whether residual amblyopia is still present due to a change in refractive error without pursuing this additional testing.

Protocol Change #5:

Current Protocol
Contrast sensitivity is measured in both the right and left eyes using the SpotChecks™ Contrast Sensitivity Test at the Spectacle Baseline Visit and at every follow-up visit until the end of the study. Additionally, contrast sensitivity in the left eye is re-tested at the 8-week follow-up visit, for the purpose of evaluating test/retest reliability of the SpotChecks™ Contrast Sensitivity Test.

Proposed Change

The SpotChecks™ Contrast Sensitivity Test in the right and left eyes will now only be required at the Spectacle Baseline Visit and at the primary outcome visit. Retest of the contrast sensitivity test in the left eye at the 8-week follow-up visit will be removed from the protocol. Section 4.4.1 and the "Schedule of Study Visits and Procedures" table have been updated to reflect this change.

Rationale for Change

Recent quality review of contrast sensitivity data at the 8-week follow-up visit showed that only 37% (n=132) of younger cohort and 47% (n= 47) of older cohort yielded scoreable data. The most common reason for data that was not scoreable was due to poor participant tolerance of testing due to testing fatigue. Efforts to continue testing at every follow-up visit creates undue burden on participating sites. Reducing contrast sensitivity testing only to the Spectacle Baseline Visit and Primary Outcome Visit significantly decreases testing burden on participants and site personnel, but still allows for treatment group comparison and change from baseline analyses at the end of the study.

Re-testing contrast sensitivity in the left eye at the 8-week follow up visit is no longer necessary as the test/retest reliability analyses of the SpotChecks™ Contrast Sensitivity Test has been completed. Collecting test/re-test contrast sensitivity data from additional subjects will not significantly change the precision of the Bland-Altman analysis.

PROTOCOL AMENDMENT #5
19 April 2022

This amendment provides for the following protocol changes:

Protocol Change #1:

Current Protocol
The current protocol (Section 2.3 Eligibility Criteria) states that at the Enrollment Visit, VA will be tested with or without cycloplegia using the investigator's routine VA testing method, with the child wearing a trial frame or viewing through the phoropter with refractive correction meeting eligibility criteria based on a cycloplegic refraction that has been performed within 45 days.

If the amblyopic-eye VA criteria are not met (e.g., VA is too good in the amblyopic eye) at the Enrollment Visit when tested in trial frames, then the potential participant cannot be offered enrollment into the study because the VA would be expected to be too good to meet the criteria at the Spectacle Baseline / Randomization Visit. In these cases, spectacles will not be paid for by the study and the study will end for these participants.

Proposed Change
Section 2.3 Eligibility Criteria has been updated to state that VA testing at the Enrollment Visit is NOT required if spectacles meeting protocol criteria have already been prescribed prior to the study.

Rationale for Change
The purpose of the Enrollment visit is to determine whether participants qualify for study-provided glasses, by meeting visual acuity study criteria with trial frame or phoropter correction, assuming that spectacles are not yet available. Currently, some participants are being identified after they have already ordered glasses from an optical shop that meet protocol criteria and are available at the time of the Enrollment Visit. In these cases, the Enrollment Visit and Spectacle Baseline Visit can be done on the same day since the new glasses are available. Testing VA at the time of the Enrollment Visit with the child wearing a trial frame or viewing through a phoropter with the spectacle prescription is not required.

Protocol Change #2:

Current Protocol
The current protocol (Section 4.3.8 Recurrent Amblyopia and Section 6.8 Recurrence of Amblyopia) defines recurrent amblyopia as a decrease in amblyopic eye VA of 0.2 logMAR or more on a subsequent visit to the visit at which the definition of "stable resolved" was met.

Proposed Change
The definition for recurrent amblyopia has been modified to a decrease in amblyopic VA of 0.2 logMAR or more **and a 0.2 logMAR or more interocular difference of distance VA between the amblyopic eye and the non-amblyopic eye** on a subsequent visit to the visit at which the definition of "stable resolved" was met.

Rationale for Change

In the old definition of recurrent amblyopia, only a decrease in vision in the amblyopic eye was considered, without regard for the possibility of a concurrent decrease in fellow eye visual acuity, which could reasonably occur due to poor subject attention, cooperation, or motivation. This change in study definition for recurrent amblyopia is necessary since only a decrease in amblyopic eye VA accompanied by a significant IOD should be considered recurrent amblyopia.

Additional Protocol Changes

- The testing procedures during follow-up have been clarified to reflect that Masked Examiner Testing is required prior to **and at time of** primary outcome exam.

PROTOCOL AMENDMENT #4
24 January 2022

This amendment provides for the following protocol changes:

Protocol Change #1:

Current Protocol
The current protocol section 4.4.1 (Testing Procedures) did not specify that additional testing including a cycloplegic refraction and test and retest of monocular distance visual acuity in trial frames may be required as defined later in section 4.4.1.1 (Management of Refractive Error).

The current protocol section 4.4.1.1 (Management of Refractive Error) was also not clear with respect to when a cycloplegic refraction was required, when glasses needed to be changed, and what additional testing was required and at what visit relative to the end of the study.

Proposed Change
Both sections have been edited as follows:

- Section 4.4.1 (Testing Procedures) has been edited to reflect the possibility of additional testing including a cycloplegic refraction and test and retest of distance visual acuity in trial frames at the end of the visit that was already defined in section 4.4.1.1 (Management of Refractive Error).

- Section 4.4.1.1 has been split into two sections to make it clear when a cycloplegic refraction is required, when glasses need to be changed, and what additional testing is required.

    - One section has been added to clarify the protocol for visits prior to 56-weeks
    - Another section has been added to clarify the protocol for the 56-week visit.

Rationale for Change
These edits make the protocol consistent throughout the text and clearer.

Protocol Change #2:

Current Protocol
The current protocol section 4.4.1 (Testing Procedures) requires visual acuity test and re-test at all study follow-up visits to be performed by a masked certified examiner.

Proposed Change
Section 4.4.1 was edited to allow for visual acuity test and re-test at post-primary outcome follow-up visits to be performed by either a masked or unmasked certified examiner.

Rationale for Change
Once subjects meet primary outcome, treatment is at investigator discretion, so visual acuity testing does not need to be masked.

PROTOCOL AMENDMENT #3
26 August 2021

This amendment provides for the following protocol changes:

Protocol Change #1:

Current Protocol
Section 4.1 Sequential Spectacles (and Patching if Needed) incorrectly refers to "residual amblyopia" as including 1 logMAR line (0.1 logMAR) of interocular difference in distance visual acuity (VA); and incorrectly refers to resolved amblyopia as less than 1 logMAR line.

Proposed Change
The text in Section 4.1 has been edited to match the correct definitions of "residual amblyopia" (defined in Section 4.3.3 as more than 0.1 logMAR of interocular difference of distance VA) and resolved amblyopia (defined in Section 4.3.4 as 0.1 logMAR or less of interocular difference of distance VA).

Rationale for Change
These edits make the protocol definitions consistent throughout the text.

Protocol Change #2:

Current Protocol
Currently, a cycloplegic refraction is mandated at the time of the primary outcome exam for participants who demonstrate "stable resolved" or "stable residual" amblyopia, or at the 56-week study visit, if stable/worsening visual acuity status has not been declared by the 48-week visit.

Proposed Change
A cycloplegic refraction will be mandated at the time of the primary outcome visit if the previous amblyopic eye is still worse than the fellow eye (IOD > 0 and previously amblyopic eye worse) based on average of test and re-test); or at the 56-week study visit, if stable/worsening visual acuity status has not been declared by the 48-week visit.

This change has been made in Section 4.4.1.1. Management of Refractive Error.

Rationale for Change
The purpose of performing a cycloplegic refraction at the primary outcome exam is to determine if the non-improving and suboptimal visual acuity is because of uncorrected refractive error. The cycloplegic examination allows the investigator to determine if this is the case, so that the spectacle lenses can be changed. Participants whose previous amblyopic eye is no longer worse than the fellow eye at the primary outcome exam are more likely to truly have no residual amblyopia; thus, it is not necessary to conduct a cycloplegic refraction to look for uncorrected refractive error.

Additional Protocol Changes

- Within section **2.3 Participant Eligibility Criteria**, criterion #4 has been revised to indicate that a cycloplegic refraction must be performed within the last 45 days of the Enrollment visit, rather than 30 days, to be consistent with changes made in response to Protocol Amendment #2 (18 June 2021).

PROTOCOL AMENDMENT #2
18 June 2021

This amendment provides for the following protocol changes:

Protocol Change #1:

Current Protocol
Currently, the protocol specifies that a change in spectacle correction is allowable prior to randomization if the participant shows decreased visual acuity at the time of Spectacle Baseline / Randomization visit compared to the visual acuity measured at the Enrollment visit (Section 3.1.2.1). The current protocol does not specify if a spectacle change is allowed prior to randomization if there is no decrease in visual acuity from enrollment but the investigator believes that a change in spectacle correction is needed for optimal clinical management (e.g., new manifest strabismus).

Proposed Change
Section 3.1.2.2, titled "**Other Spectacle Change Allowances**" has been added with the following text:

> If an investigator identifies a new problem at the time of the Spectacle Baseline visit (e.g., new manifest strabismus) that could be appropriately managed by a change in spectacle correction, re-make of the glasses by the optical lab is permitted and will be paid for by the study, as long as the new spectacle prescription is within the refractive error requirements of the protocol, as specified in section 2.3 #4. The subject should return for another Spectacle Baseline / Randomization visit within 45 days.

Rationale for Change
This protocol change will allow investigators to modify the spectacle correction for the participant to allow for optimal clinical management prior to randomization.

Protocol Change #2:

Current Protocol
The current protocol specifies that cycloplegic refraction should be performed within 30 days of the Enrollment visit but it does not specify the allowable time period from the Spectacle Baseline / Randomization visit.

Proposed Change
Section 2.3 Patient Eligibility Criteria has been updated to specify that the cycloplegic refraction should be performed within 90 days of the Spectacle Baseline / Randomization visit.

Rationale for Change
To ensure that the refractive error has not changed significantly and that the participant is optimally corrected at the time of randomization.

Protocol Change #3:

Current Protocol
The current protocol requires the Spectacle Baseline / Randomization visit to be within 30 days of the Enrollment visit. If a re-make of the spectacle correction is necessary, another Spectacle Baseline / Randomization visit will need to be scheduled within 30 days.

Proposed Change
The Spectacle Baseline / Randomization visit will be extended to be within 45 days of the Enrollment visit. If a re-make of the spectacle correction is necessary, another Spectacle Baseline / Randomization visit will need to be scheduled within 45 days.

Rationale for Change
During the COVID-19 pandemic, optical shops are having a longer turnaround time on making spectacles, and participants are requiring a longer amount of time to receive the new spectacles. The amount of time between the Enrollment visits and the Spectacle Baseline / Randomization visit has been extended from 30 to 45 days for a given prescription.

Protocol Change #4:

Current Protocol
Section 3.2.1.1. **"Visual Acuity Not Meeting Eligibility Criteria**" has two subsections, 3.1.2.1.1 "**Presumed Latent Hyperopia**" and 3.1.2.1.2 "**Other Cases of Reduced Visual Acuity**", describing the procedures that can be optionally performed at investigator discretion if latent hyperopia or a change in refractive error is suspected to be the cause for a reduction in visual acuity of ≥1 line when the mean of test and retest VA at the Spectacle Baseline / Randomization visit is compared to the Enrollment visit.

Proposed Change
The two subsections, 3.1.2.1.1 "**Presumed Latent Hyperopia**" and 3.1.2.1.2 "**Other Cases of Reduced Visual Acuity**" have been deleted and the title in section 3.1.2.1 has been changed from "**Visual Acuity Not Meeting Eligibility Criteria**" to **"Management of Reduced Visual Acuity"**, with the following clarifying text:

If the investigator suspects incomplete relaxation of accommodation in the current spectacles is causing reduction of the mean of test and retest VA at the Spectacle Baseline visit from the Enrollment visit in either eye (regardless of whether this initial VA measurement renders the participant eligible or ineligible), the investigator should consider (not mandatory) repeating test and retest VA with a -1.00 D lens in BOTH eyes, regardless of refractive error.

If the participant meets eligibility criteria based on mean of test and retest VA through the –1.00 D lens, the investigator has two options:

1. Randomize the participant in the current spectacles (the test and re-test VA using the -1.00 D lens will be used as the participant's baseline VA)

2. Change the spectacles by reducing the hyperopic power up to a symmetrical reduction of 1.50 D sphere, following guidelines in *section 2.3 (#4)*.

If the participant does not meet eligibility criteria based on mean of test and retest VA, the participant will be dropped from the study.

If the investigator suspects that a change in refractive error is causing reduction of the mean of test and retest VA at the Spectacle Baseline visit from the Enrollment visit in either eye (regardless of whether this initial VA measurement renders the participant eligible or ineligible), the investigator should consider (not mandatory) repeating cycloplegic refraction.

If the current spectacles **are not** within the lensometry tolerances specified in the protocol (section 3.1.1) based on the new cycloplegic refraction, the spectacles should be re-made and will be paid for by the study. An investigator may repeat visual acuity testing at their discretion to determine if a participant still meets the VA eligibility requirements to continue in the study, but this is not mandatory. The participant should not wear any glasses until they return for another Spectacle Baseline / Randomization visit within 45 days.

If the current spectacles **are** within the lensometry tolerances and the participant meets eligibility criteria, the participant may be randomized.

Rationale for Change
The current wording of the protocol is confusing for investigators, as it suggests that all participants with mean test and retest VA reduced ≥1 line at the Spectacle Baseline / Randomization visit from the Enrollment visit in one or both eyes should be re-tested with a -1.00D lens and should have repeated cycloplegic refraction.

This protocol change clarifies that test and re-test VA with a -1.00D lens and repeat cycloplegic refraction is not mandatory. It also provides detailed instruction on when these tests should be considered, and the appropriate actions based on the results of this additional testing.

Protocol Change #5:

Current Protocol
The current protocol does not specify whether all testing at the Spectacle Baseline / Randomization visit must be completed within a single visit.

Proposed Change
In section 3.1.3 “Testing in New Spectacles”, the following text has been added:

> If all components of the Spectacle Baseline / Randomization visit are not completed, the child may return within 7 days to complete the remaining study procedures or repeat all study procedures (at investigator discretion) and be randomized (NOT wearing the glasses in the meantime). If returning more than 7 days later, all testing must be repeated at a new Spectacle Baseline / Randomization visit (NOT wearing the glasses in the meantime).

Rationale for Change

Some participants may become tired or uncooperative during the Spectacle Baseline / Randomization visit, leading to incomplete or inaccurate baseline testing. This protocol change will make it possible to obtain a complete and accurate baseline dataset in these circumstances.

Additional Protocol Changes

- The **flow chart** has been updated to be consistent with the text already in section **4.3.8 Recurrent Amblyopia** that 'Participants meeting the definition for "Recurrent amblyopia" should be treated, and the method of treatment will be at investigator discretion.
- The age-normal fellow eye visual acuity criteria in section **2.3 Eligibility Criteria** have been corrected to reflect that smaller logMAR values equate to better visual acuity.

PROTOCOL AMENDMENT #1
27 August 2020

This amendment provides for the following protocol change:

Protocol Change

Current Protocol
At each follow-up visit post-randomization, monocular contrast sensitivity will be assessed in the right and left eyes using the SpotChecks™ Contrast Sensitivity Test.

Proposed Change
An additional monocular test of contrast sensitivity in the left eye will be performed at the 8-week post-randomization follow-up visit. In addition, the right eye will be tested first.

Rationale for Change
The test and retest data in the left eye collected at the 8-week post-randomization visit will allow us to evaluate the test/retest reliability of the SpotChecks™ Contrast Sensitivity Test in amblyopic and non-amblyopic eyes in children with amblyopia. This information will allow us to more accurately interpret any change in contrast sensitivity during the study.

Additional Protocol Changes

- The **protocol summary** has been updated to reflect the additional secondary objective to evaluate test/retest reliability of the SpotChecks™ Contrast Sensitivity Test.
- The **background** has been updated to reflect the rationale for evaluating the test/retest reliability of the SpotChecks™ Contrast Sensitivity Test.
- The **follow-up clinical testing** paragraph has been updated to reflect the retest in the left eye on the SpotChecks™ Contrast Sensitivity Test at the 8-week post-randomization visit only; and that the right eye should be tested first at all visits.
- The **statistical methods chapter** has been updated to reflect the analyses of the test/retest data for the SpotChecks™ Contrast Sensitivity Test.

Additional Protocol Changes
The **statistical methods chapter** has been updated to reflect adjustments to p-values and confidences intervals for secondary outcomes to maintain a false discovery rate of 5%.

Visual acuity logMAR equivalents have been updated in the **eligibility and spectacle baseline** chapters to reflect the mean of two measurements. The protocol with respect to testing visual acuity at enrollment versus spectacle baseline has been clarified to make it clearer as to what instruments are allowed and when.

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
ANCOVA	Analysis of covariance
ATS	Amblyopia Treatment Study
BCVA	Best corrected visual acuity
CI	Confidence interval
CFR	Code of Federal Regulations
CRF	Case report form
D	Diopter
DHHS	Department of Health and Human Services
DSMC	Data safety and monitoring committee
eCRF	Electronic case report form
FDA	Food and Drug Administration
GCP	Good clinical practice
HRQOL	Health related quality of life
ICH	International Council for Harmonisation
IOD	Interocular difference
IRB	Institutional Review Board
JCHR	Jaeb Center for Health Research
logMAR	Logarithm of the minimal angle of resolution
NIH	National Institutes of Health
ODM	Occlusion dose monitor
PACT	Prism and alternate cover test
PedEyeQ	Pediatric Eye Questionnaire
PEDIG	Pediatric Eye Disease Investigator Group
QA	Quality assurance
QC	Quality control
RBM	Risk based monitoring
RCT	Randomized clinical trial
SD	Standard deviation
SE	Spherical equivalent refractive error (Sphere + ½ Cylinder)
SPCT	Simultaneous prism and cover test
VA	Visual acuity

PROTOCOL SUMMARY

Title	A Randomized Trial to Evaluate Sequential vs Simultaneous Spectacles plus Patching for Amblyopia in Children 3 to <13 Years Old
Précis	Previous studies by the Pediatric Eye Disease Group (PEDIG) and others have found that treating anisometropic, strabismic, and combined-mechanism amblyopia with spectacle correction alone results in resolution of amblyopia in a proportion of previously untreated children, potentially eliminating the need for additional treatment modalities such as patching or atropine for those children, and potentially making subsequent patching easier in the remaining children. Other clinicians prefer to initiate spectacle correction and patching simultaneously, believing that improvement is more rapid and final visual acuity (VA) outcomes are superior. The present study is being conducted to determine whether simultaneous treatment with spectacles and patching has an equivalent VA outcome compared with sequential treatment, first with spectacles alone, followed by patching (if needed), for previously untreated amblyopia in children 3 to <13 years of age. If found to be equivalent, the study will also evaluate whether Child and Parent HRQOL outcomes are superior with sequential treatment versus simultaneous treatment.
Study Design	Multicenter, randomized clinical trial.
Number of Sites	The study is open to all clinical sites approved to participate in the PEDIG network.
Primary Objective	To determine whether simultaneous treatment with spectacles and patching has an equivalent VA outcome compared with sequential treatment, first with spectacles alone followed by patching (if needed), for previously untreated amblyopia in children 3 to <7 years of age (younger cohort) and 7 to <13 years of age (older cohort).
Primary Endpoint	The primary efficacy endpoint will be mean change in amblyopic eye logMAR distance VA between randomization and VA at completion of randomized treatment or at 56 weeks, whichever is earlier. The primary endpoint for each participant is defined as: 1. Amblyopic eye VA (calculated as mean of test and retest) at the last visit that was the basis for a "stable resolved" or "stable residual" determination (in the sequential group, stable residual amblyopia criteria can be reached only *after* patching has been instituted); or 2. Amblyopic-eye VA at 56 weeks (calculated as mean of test and retest) in those completing a 56 week visit without ever meeting criteria for "stable resolved" or "stable residual" amblyopia (if retest missing at 56 weeks, the single test value will be used).
Secondary Objectives/Endpoints	To determine if the following endpoints differ <u>between</u> simultaneous treatment and sequential treatment groups. **Efficacy:** • Child and parental health-related quality of life (HRQOL) domain scores as measured by PedEyeQ. • Proportion of participants with improvement of amblyopic eye distance VA of 2 or more logMAR lines (>=10 letters if E-ETDRS), using the primary endpoint as defined above. • Time to stable resolved amblyopia. • Amblyopic-eye distance VA (calculated as mean of test and retest) after 8 weeks. • Amblyopic-eye distance VA (calculated as mean of test and retest) at 56 weeks for all participants. • Binocularity by Randot Preschool Stereotest, Randot Butterfly Stereotest, and Worth 4 Shape Testing. • Monocular contrast sensitivity. **Safety:** • Worsening of best-corrected fellow-eye distance VA of 0.2 logMAR lines or more at any time during the study from baseline (based on mean of test and retest, or initial test if retest not done). • Proportion of participants who develop a new strabismus.

	• Proportion of participants who develop mild, moderate, and severe skin irritation from patching. To evaluate the following endpoint within simultaneous treatment and sequential treatment groups: • Recurrence of amblyopia in those who have resolved (defined as a 0.2 or more logMAR reduction and IOD of 0.2 logMAR or more from value at visit of resolution). To evaluate whether the following endpoints are associated with improvement in amblyopic eye distance VA within simultaneous treatment and sequential groups separately: • Percent of expected patching achieved (adherence) as measured by occlusion dose monitors (ODMs). • Percent of expected spectacle wear achieved (adherence) as measured by parental calendars. To evaluate the test/retest reliability of the SpotChecks™ Contrast Sensitivity Test.
Population	**Key Inclusion Criteria:** • Ages 3 to < 13 years • Amblyopia associated with anisometropia, strabismus, or both • Amblyopic-eye VA of 20/40 to 20/200 (0.26 to 1.04 logMAR inclusive, 33 to 72 letters inclusive) based on mean of test and retest • Fellow-eye VA normal for age (based on mean of test and retest) • Interocular difference in VA of 0.3 logMAR lines or more using the mean of test and retest VA in each eye **Key Exclusion Criteria:** • Previous spectacle wear (see section 2.3 #4) • Previous treatment for amblyopia
Sample Size	Results will be analyzed separately within 2 age cohorts. • 272 participants aged 3 to <7 years of age (younger cohort) • Up to 272 participants aged 7 to <13 years of age (older cohort) will be enrolled while recruitment is ongoing for the younger cohort
Phase	Phase III Randomized Clinical Trial
Treatment Groups	Random assignment within each age cohort (1:1) to: • Sequential treatment, first with spectacles alone followed by patching (if needed) 2 hours per day, 7 days per week (monitored by ODM) • Simultaneous treatment, with spectacles combined with patching 2 hours per day, 7 days per week (monitored with ODM)
Participant Duration	If randomized, participation in the study will last 56 weeks
Protocol Overview/Synopsis	Children will be prescribed spectacles and will then return for a spectacle baseline visit, where they will wear their new spectacles for the first time for at least 10 minutes (but no more than 24 hours) and will be tested in those new spectacles to confirm final eligibility prior to randomization. Participants not found to be eligible in their new spectacles will end study participation. Participants eligible for the study will be randomly allocated to one of two treatment groups: Sequential (spectacles alone) and then patching if needed (monitored by ODM), or Simultaneous (spectacles and patching monitored by ODM). After randomization, follow-up visits will occur at 8-week intervals through 56 weeks. At each visit on or after the 8-week visit, participants will be classified as either: stable/worsening or improving; those stable/worsening are then classified as having either resolved or residual amblyopia, provided that the current and most recent previous visit were completed at least 6-weeks apart (target 8 weeks) and provided the required test and retest VA testing was completed. Participants who are stable/worsening and have residual amblyopia in the sequential group will start patching (monitored by ODM) and continue to be followed every 8 weeks. Participants in the simultaneous group, or in the sequential group after having advanced to patching, who are stable/worsening but have residual amblyopia will be released to treatment at investigator discretion. All participants continue 8-weekly visits until 56 weeks when study participation ends.

STUDY SUMMARY FLOW CHART

SCHEDULE OF STUDY VISITS AND PROCEDURES

Visit	Consent	Distance VA in trial frames	Demographics / Medical History	Lensometry	Distance VA (test and retest) ***MASKED**	Binocularity	Ocular Alignment	Contrast sensitivity***	Quality of Life	Adherence Monitoring
Enrollment Visit	X	X**	X							
Spectacle Baseline / Randomization Visit				X	X **(unmasked)**	X	X	X	X	
8-Week Visit				X	X	X	X	X	X	X
16-Week Visit				X	X	X	X	X	X	X
24-Week Visit				X	X	X	X	X	X	X
32-Week Visit				X	X	X	X	X	X	X
40-Week Visit				X	X	X	X	X	X	X
48-Week Visit				X	X	X	X	X	X	X
56-Week Visit				X	X	X	X	X	X	X

Note: A cycloplegic refraction will be mandated at the end of the primary outcome visit if the previous amblyopic eye is still worse than the fellow eye (IOD > 0 and previously amblyopic eye worse) based on average of test and re-test); or at the 56-week study visit if primary outcome has not been declared by the 48-week visit AND the previous amblyopic eye is still worse than the fellow eye (IOD > 0 and previously amblyopic eye worse based on average of test and re-test).

After the cycloplegic refraction, monocular distance VA test and re-test in both eyes may be required with a trial frame correction as defined in section 4.4.1.1.

*Post-primary outcome follow-up visits vision test and retest may be performed by a masked or unmasked certified examiner as defined in section 4.4.1.1.

**Distance VA testing in trial frames required if spectacles not already prescribed.

*** The SpotChecks™ Contrast Sensitivity Test to be done at the Spectacle Baseline / Randomization Visit and at primary outcome only.

CHAPTER 1. Background Information

1.1 Epidemiology & Clinical Characteristics

Amblyopia is the most common cause of reduced monocular visual acuity (VA) in children and young adults, with estimates of prevalence ranging from 1% to 5%.[1,2] The most commonly associated risk factors are uncorrected anisometropia, strabismus, or a combination of these. In addition to reduced VA, amblyopia may also be associated with dysfunctions of accommodation, fixation, binocularity, vergence, reading fluency, and contrast sensitivity.[3-12]

1.2 Treatment – Current practice

"Sequential Treatment"

Based on amblyopia treatment studies over that last 2 decades by PEDIG and others, and American Academy of Ophthalmology preferred practice recommendations,[13] many pediatric eye care providers currently treat amblyopia first with optical correction alone[13,14] expecting at least 25-40% of cases of anisometropic, strabismic, or combined-mechanism amblyopia to resolve with no further treatment needed.[15-17] For those with residual amblyopia after such optical treatment, part-time patching or atropine is often prescribed, and this overall treatment approach could be termed "sequential." Nevertheless, even after sequential optical and patching treatment, approximately 50% of children with anisometropic, strabismic, or combined-mechanism amblyopia have residual or recurrent amblyopia.[18]

"Simultaneous Treatment"

Some clinicians prescribe spectacles and patching (or atropine) together at the initiation of treatment; this approach could be termed "simultaneous." In a survey of 74 pediatric ophthalmologists and fellows attending an ophthalmology forum at Wills Eye Hospital,[14] 24% reported that they would prescribe spectacles and patching or atropine simultaneously. In some areas of the USA (e.g., the Dallas, Texas metro area) the proportion of providers who adopt the "simultaneous" approach appears to be even higher (E. Birch personal communication, based on regular referral of children with amblyopia for research studies to the Retina Foundation of the Southwest).

1.3 Rationale for "Sequential" Treatment

There are several potential advantages to "sequential" treatment:

1. A proportion of children with amblyopia will never need patching (or atropine)[19-22]
2. Amblyopic-eye VA in children who do not completely resolve with spectacles alone is often better at the commencement of patching, and therefore subsequent patching is likely easier for the child and parents
3. The HRQOL of the child and/or the parents may be better due to #1 and #2

Previous studies of spectacle treatment alone are often limited by short durations between follow-up visits, e.g., every 4 or 5 weeks, which may not have allowed sufficient time for investigators to detect true improvement. In addition, criteria for "stability" have often not been strict, for example no improvement of 1 logMAR line over sequential 4-week visits. With short follow-up intervals, and less than strict criteria for stability, there is a risk of erroneously declaring stability (i.e., "no further improvement"), which may have underestimated the

effectiveness of treatment. It is entirely possible that the true proportion of children with amblyopia who improve and resolve with optical treatment alone is higher than reported in previously published studies.

The current study will address these previous study design limitations by extending the duration of follow-up and making the definition of "no further improvement" more stringent.

1.4 Rationale for "Simultaneous" Treatment

There are potential advantages to "simultaneous" treatment:

1. The overall VA outcomes may be superior because the child is younger at the commencement of patching
2. The rate of VA improvement may be faster
3. HRQOL of the child and/or the parents may be better due to #1 and #2

There are some data supporting better outcomes with earlier treatment. In a meta-analysis of 4 RCTs, treatment of amblyopia was found to be more effective (on average) when initiated at an earlier age, particularly for severe amblyopia,[23] therefore starting patching treatment earlier may lead to better overall outcomes. Time to reach a VA plateau with spectacle treatment alone may take up to 45 weeks,[15] thereby delaying patching treatment (when needed) in some children.

There are other data supporting a faster response with simultaneous treatment. Agervi et al[19] compared spectacles alone with spectacles plus a Bangerter filter for the treatment of anisometropic amblyopia, and reported mean time to resolution of amblyopia ($\leq$0.1 logMAR inter-ocular difference) in the Bangerter group was 2.2 +/- 1.9 months, which was significantly shorter than the spectacle-alone group (3.9 +/- 3.2 months), although mean final VAs were similar.

1.5 "Sequential" versus "Simultaneous"

In summary, the choice of a sequential approach versus a simultaneous approach to "optical treatment plus patching treatment" remains unresolved, with some existing data supporting one approach and some data supporting the other. There is a reasonable rationale for either approach. This unresolved controversy results in a dichotomy of current clinical practice, with some care providers favoring one approach and others favoring the opposite approach.

The currently proposed RCT will specifically address the following questions: 1) does simultaneous spectacle and patching treatment of amblyopia have equivalent VA outcomes compared with sequential treatment, first with spectacles-alone, and then patching only (if needed); and 2) if found to be equivalent, are Child and Parent HRQOL outcomes superior with sequential treatment or simultaneous treatment?

1.6 Measuring Adherence with Spectacles and Patching

In studies of amblyopia treatment effectiveness, interpretation of findings is often limited by lack of data on adherence to prescribed treatment. Uncertainty remains as to whether better treatment outcomes are related to better adherence with prescribed treatment and worse outcomes to poorer adherence. Some research groups have used occlusion dose monitors (ODMs) to record actual

patch wear time.[24-31] In general these previous studies concluded that adherence with therapy is a significant factor in determining treatment outcome, although there remains considerable individual variability.

Understanding the role of adherence with regard to treatment response is important for ongoing and future amblyopia studies, but lack of availability of the specific dose-monitoring devices has previously precluded implementation in PEDIG RCTs. Recently, a new method of monitoring patching adherence has become available. A thermosensor device (TheraMon,® originally developed to measure and store wearing time of dental retainers) has been piloted for adhesive patches in adults[32] and this technology appears to work well for monitoring adherence with amblyopia patching treatments in children. Ongoing pilot studies (accepted manuscript in press, Wang J et al, JAAPOS 2020) using the TheraMon® with eye patches in children, are confirming the feasibility of affixing the ODM to adhesive patches, and the feasibility of data collection.

We hypothesize that adherence with patching will be greater in the sequential group, towards the *start* of treatment, because that group will, on average, have better VA at the *start* of patching. But we also hypothesize that patching adherence will be worse in the sequential group towards the *end* of treatment, if that group indeed takes longer to reach amblyopia resolution or best VA. We further hypothesize that there will be a relationship between adherence with spectacles (measured by a parental calendar) and response to spectacles, and adherence to patching (measured with ODMs) and response to patching.

1.7 Health-Related Quality of Life

An area increasingly recognized as important in studies of treatment effectiveness is the assessment of health-related quality of life (HRQOL) in affected individuals and their families. We hypothesize that the HRQOL in children and parents will be better in the Sequential group compared with the Simultaneous group at 3 time points: 1) initially (at the first follow-up visit), 2) at the primary outcome visit, and 3) at the end of the study (56 weeks).

We propose to use the newly developed pediatric eye-related quality of life and functional vision instrument, the Pediatric Eye Questionnaire (PedEyeQ),[33] which has now been validated in children with bilateral visual impairment and children with strabismus.[34] The PedEyeQ is patient- and parent-derived and consists of age-appropriate Child and Proxy components as well as a Parent component (to assess the effect on the parent themselves). The PedEyeQ has the following domains:

Child PedEyeQ:

- Functional Vision
- Bothered by Eyes and Vision
- Social
- Frustration / Worry

Proxy PedEyeQ:

- Functional Vision
- Bothered by Eyes and Vision
- Social

- Frustration / Worry
- Eyecare

Parent PedEyeQ:

- Impact on Parent and Family
- Worry about Child's Eye Condition
- Worry about Self-perception and Interactions
- Worry about Functional Vision

Other pediatric HRQOL instruments are either not patient-derived,[35,36] are designed to assess the effects of specific treatments only,[37] or assess only general health-related quality of life (e.g. the Pediatric Quality of Life Inventory).[38,39] In addition, previously-developed instruments do not separately assess child and proxy components and/or the effects on the parents themselves. The PedEyeQ currently appears the most appropriate instrument to assess the functional vision and eye-related quality of life impact of treatment approaches in the present study.

1.8 Contrast Sensitivity

The SpotChecks™ Contrast Sensitivity Test will be used to measure monocular contrast sensitivity. The SpotChecks™ Contrast Sensitivity Test was designed as an inexpensive alternative for patients to self-monitor for changes in contrast sensitivity. It has been validated in adults against the near Pelli-Robson chart.[40,41] The test/retest reliability of this test in children has not yet been documented in the literature. As a part of this study, we will also evaluate the test-retest reliability of the SpotChecks™ Contrast Sensitivity Test.

1.9 Summary of Rationale for Present Study

Many clinicians currently treat anisometropic, strabismic, and combined-mechanism amblyopia first with optical treatment (spectacles) alone, and then only patch if needed ("sequential" treatment). Other clinicians start spectacle and patching treatments simultaneously. Our primary study hypothesis is that the VA outcomes are equivalent with both treatment strategies. If VA outcomes are equivalent, it is important to know whether HRQOL for the Child and/or the Parent is better with one treatment strategy. We hypothesize that Child and Parent HRQOL domain scores will be higher (better HRQOL) with sequential treatment than with simultaneous treatment when compared 1) initially (at the first follow-up visit), 2) at the primary outcome visit, and 3) at the end of the study (56 weeks). The currently proposed RCT will test each of these hypotheses.

Amblyopia treatment presents a significant healthcare burden and identifying whether "simultaneous" spectacle and patching treatment is equivalent to "sequential" treatment with an initial phase of spectacles alone, is important for efficient use of healthcare resources and for providing evidence for best clinical practice. On one hand, successful treatment using spectacle correction alone may reduce the number of children needing treatment with patching or atropine and may have less impact on the quality of life of children and parents, whereas on the other hand, potentially shorter treatment duration with simultaneous treatment may reduce overall healthcare burden, and may be reflected in better Child and Parent HRQOL.

1.10 General Considerations

The study is being conducted in compliance with the policies described in the Jaeb study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the study protocol described herein, and with the standards of Good Clinical Practice (GCP). When feasible, data will be directly collected in electronic case report forms, which will be considered the source data.

CHAPTER 2. **Enrollment Visit**

2.1 Purpose

The enrollment visit will occur either at the child's regular clinic visit, when first identified to have amblyopia, or at a separate research visit. Recall that potential study participants have never worn glasses or contact lenses in the past, and so reduced VA in the amblyopic eye could simply be from optical blur. The purpose of the enrollment visit is to determine whether the child meets eligibility criteria.

2.2 Participant Recruitment and Enrollment

The study plans to enroll a minimum of 272 participants into the younger cohort aged 3 to <7 years and up to 272 participants into the older cohort aged 7 to <13 years. As the enrollment goal approaches for the younger cohort, sites will be notified of the end date for recruitment. Participants who have signed an informed consent form can be randomized until the end date, which means the expected recruitment number might be exceeded.

Study participants will be recruited from clinical centers in the United States and Canada. All eligible participants will be included without regard to gender, race, or ethnicity. There is no restriction on the number of participants to be enrolled by each site toward the overall recruitment goal.

2.2.1 Informed Consent and Authorization Procedures

A child is considered for the study after undergoing a routine eye examination as part of standard of care) that identifies amblyopia appearing to meet the eligibility criteria. The study will be discussed with the child's parent(s) or guardian(s) (referred to subsequently as parent(s)). Parent(s) who express an interest in the study will be given a copy of the informed consent form to read. Written informed consent / assent must be obtained from a parent and child (the JCHR IRB requires assent for children 7 years of age and older) prior to performing any study-specific procedures that are not part of the child's routine care.

If the participant turns 7 years of age after initial consent, then assent must be obtained prior to performing any additional study-specific procedures, including data collection for the study. If assent cannot be obtained, then study-procedures, including data collection, cannot continue.

If a participant and/or legally authorized representative do not have English as their native language, then the consent and/or assent forms must be provided in the native language. Further, a qualified translator must be present for the consent process and must be present for all subsequent study-related interactions.

A participant is considered enrolled when the informed consent and assent forms has been signed, as applicable.

2.3 Participant Eligibility Criteria

Participants must meet all the following inclusion criteria in order to be eligible to participate in the study. The VA criteria will need to be met at the subsequent Spectacle Baseline / Randomization Visit, at least 10 minutes after the new spectacles are first worn (Spectacles must

be based on a cycloplegic refraction performed within 90 days. The participant will be excluded, and randomization will not occur, if the new spectacles have been worn more than a total of 24 hours prior to the Spectacle Baseline / Randomization Visit – these participants would then be considered partially treated with spectacles).

Participants who previously attempted spectacle wear 3 months prior to the Enrollment Visit for 72 hours (3 days) or less of total wear time would meet the eligibility criteria of no previous spectacle wear. Participants who previously attempted spectacle wear less than 3 months prior the Enrollment visit for 24 hours or less of total wear time would meet the eligibility criteria of no previous spectacle.

For participants not already prescribed spectacles meeting eligibility criteria prior to enrollment, VA will be tested at the Enrollment Visit with or without cycloplegia using the investigator's routine VA testing method, with the child wearing a trial frame or viewing through the phoropter with refractive correction meeting eligibility criteria based on a cycloplegic refraction that has been performed within 45 days.

If the amblyopic-eye VA criteria are not met (e.g., VA is too good in the amblyopic eye) at the Enrollment Visit when tested in trial frames, then the potential participant cannot be offered enrollment into the study because the VA would be expected to be too good to meet the criteria at the Spectacle Baseline / Randomization Visit. In these cases, spectacles will not be paid for by the study and the study will end for these participants.

Eligibility Criteria:

1. Age 3 to <13 years at the time of randomization
2. Amblyopia associated with anisometropia, strabismus, or both
    - Criteria for strabismic amblyopia: At least one of the following must be met:
        - Presence of a heterotropia on examination at distance or near fixation (with or without optical correction)
        - Documented history of strabismus which is no longer present (which in the judgment of the investigator could have caused amblyopia)
    - Criteria for anisometropia: At least one of the following criteria must be met:
        - ≥1.00 D difference between eyes in spherical equivalent (SE)
        - ≥1.50 D difference in astigmatism between corresponding meridians in the two eyes
    - Criteria for combined-mechanism amblyopia: Both of the following criteria must be met:
        - Criteria for strabismus are met (see above)
        - ≥1.00 D difference between eyes in spherical equivalent OR ≥1.50 D difference in astigmatism between corresponding meridians in the two eyes
3. No previous treatment for amblyopia, including total spectacle wear as specified below:
    a. No more than 72 hours of spectacle wear if spectacle wear attempted more than 3 months prior to the Enrollment Visit
    b. No more than 24 hours of spectacle wear if spectacle wear attempted less than 3 months prior to the Enrollment Visit.

4. Investigator planning to initiate (or has already prescribed) spectacle correction of refractive error meeting the following criteria based on a cycloplegic refraction that has been performed within 45 days of the Enrollment visit **and** within 90 days of the Spectacle Baseline visit:
    a. Full correction of spherical and cylindrical anisometropia.
    b. Full correction of astigmatism with the same axis found by the cycloplegic refraction.
    c. Allow symmetric reduction of sphere up to 1.50, prescribing such that the most hyperopic meridian is not rendered myopic, in the non-amblyopic eye at baseline.
    d. Allow symmetric addition of only -0.25 sphere for participants with plano sphere, spherical myopia, simple or compound myopic astigmatism in the non-amblyopic eye at baseline.
5. For participants not already prescribed spectacles meeting eligibility criteria prior to enrollment, a single measurement of best-corrected VA in each eye, measured **using the investigator's preferred VA testing method,** must meet the criteria specified below. The refractive correction should correct for the full cycloplegic refractive error if the child is cyclopleged. If the child is not cyclopleged, the refractive correction should meet the refractive error prescribing criteria specified in Section 2.3 #4.
    - VA in the amblyopic eye approximately 20/40 to 20/200 (0.26 to 1.04 logMAR inclusive, 33 to 72 letters inclusive)
    - Age-normal VA in the fellow eye:[42,43]
        - 3 years: approximately 20/50 or better, ≤ 0.44 logMAR, ≥ 63 letters
        - 4 years: approximately 20/40 or better, ≤ 0.34 logMAR, ≥ 68 letters
        - 5-6 years: approximately 20/32 or better, ≤ 0.24 logMAR, ≥ 73 letters
        - 7-12 years: approximately 20/25 or better, ≤ 0.14 logMAR, ≥ 78 letters
    - Interocular difference ≥ 3 logMAR lines (0.3 logMAR) or ≥ 15 letters
    - When participants return for the Spectacle Baseline / Randomization Visit with their new spectacles, they will need to meet the same criteria as above **using the ATS-HOTV or E-ETDRS protocol** after wearing the new spectacles for at least 10 minutes (based upon the mean of a test and retest of VA in those new spectacles).
6. Investigator is willing to prescribe spectacle wear followed sequentially by patching or simultaneous spectacles and patching treatment per protocol.
7. Parent and participant willing to forego option of contact lens wear for the duration of the study.
8. Parent understands the protocol and is willing to accept randomization.
9. Parent has phone (or access to phone) and is willing to be contacted by Jaeb Center staff or other study staff.
10. Relocation outside of area of an active PEDIG site for this study within the next 56 weeks is not anticipated.

2.4 Participant Exclusion Criteria

Individuals meeting any of the following exclusion criteria at baseline will be excluded from study participation.

1. Myopia greater than -6.00D spherical equivalent in either eye.

2. Previous intraocular or refractive surgery.
3. Planned strabismus surgery in the next 56 weeks.
4. Any previous treatment for amblyopia (patching, atropine, Bangerter filter, vision therapy, or binocular treatment).
5. Previous spectacle or contact lens wear as specified below:
    - More than 72 hours of total spectacle wear time if attempted 3 months or more prior to the Enrollment Visit
    - More than 24 hours of total spectacle wear time if attempted less than 3 months prior to the Enrollment visit.
6. Parent and participant unwilling to forego option of contact lens wear for the duration of the study.
7. Ocular co-morbidity that may reduce VA determined by an ocular examination performed within the past 7 months (*Note: nystagmus per se does not exclude the participant if the above VA criteria are met*).
8. Severe developmental delay that would interfere with treatment or evaluation (in the opinion of the investigator). Participants with mild speech delay or reading and/or learning disabilities or ADHD are not excluded.
9. Known allergy to adhesive patches.
10. Known allergy to silicone.

2.5 Screening Examination Procedures

2.5.1 Historical Information

Historical information elicited will include the following: date of birth, sex, race, ethnicity, and prior amblyopia therapy (e.g., spectacles, contact lenses, patching, pharmacologic, filters).

2.5.2 Visual Acuity Testing for Participants Prescribed Spectacles at Time of Enrollment

For participants not already prescribed spectacles meeting eligibility criteria prior to enrollment, visual acuity will be tested once in each eye at the Enrollment Visit **using the investigator's preferred method.** VA will be tested with or without cycloplegia, using a trial frame or through the phoropter. The refractive correction should correct for the full cycloplegic refractive error if the child is cyclopleged. If the child is not cyclopleged, the refractive correction should meet the refractive error prescribing criteria specified in Section 2.3 #4. This VA test at the Enrollment visit will be done to determine whether the presenting VA deficit is likely to be primarily optical blur or primarily amblyopia. The final determination of VA eligibility will be made at the Spectacle Baseline Visit after the participant has worn his/her new spectacles for a minimum of 10 minutes (based upon the mean of a test and retest of VA in those new spectacles).

Potential participants who do not meet VA criteria in trial frames or phoropter (*section 2.3*) will not be eligible to participate in the study and will not return for the Spectacle Baseline / Randomization Visit.

Potential participants meeting the VA criteria with their prescription in trial frames or phoropter and who appear to meet other eligibility criteria may be offered participation in the study, in which case the current visit would become the Enrollment Visit. Alternatively, the child may return on a subsequent day for the Enrollment Visit.

All eligible participants will return for a Spectacle Baseline / Randomization Visit within 45 days (after collecting their spectacles from the optical shop but having been instructed not to wear the spectacles before the Spectacle Baseline / Randomization Visit).
Spectacles will be paid for by the study with the expectation that the majority of participants who appear eligible at the Enrollment Visit will still be eligible at the Spectacle Baseline / Randomization Visit.

2.5.3 Obtaining Spectacles

Study spectacles will be either be picked up by the participant prior to the Spectacle Baseline / Randomization Visit (with instructions not to wear) or sent directly to the Investigator. If picked up from the optical shop, the new spectacles should be carried to the office between pickup and the Spectacle Baseline / Randomization Visit and should not be worn for more than a total of 24 hours prior to the Spectacle Baseline / Randomization Visit.

CHAPTER 3. Spectacle Baseline Visit / Randomization

3.1 Spectacle Baseline / Randomization Visit

The purpose of the Spectacle Baseline / Randomization Visit is to confirm eligibility for the randomized trial with the participant wearing his or her new spectacle correction for the first time. The Spectacle Baseline / Randomization Visit should occur within 45 days of the Enrollment visit and is the visit at which eligible participants will be randomly assigned to either Sequential or Simultaneous treatment.

If the Spectacle Baseline / Randomization Visit does not occur within 45 days of the Enrollment visit, all Eligibility criteria (*section 2.3*) will need to be reconfirmed.

Prior to the visit, the office staff should verify that the spectacles prescribed at the Enrollment visit either have already been received at their office or that the participant will be picking their spectacles up by the day of the Spectacle Baseline / Randomization Visit.

Informed consent will have been previously obtained at the Enrollment Visit.

Participants who have worn their spectacles for more than a total of 24 hours prior to the Spectacle Baseline / Randomization Visit will not be eligible and study participation will end.

At the visit, the spectacle prescription should be verified with a lensometer. Sphere and cylinder power must meet the criteria in *section 2.3*.

3.1.1 Lensometry

When the participant returns with their new spectacles for the Spectacle Baseline / Randomization Visit the spectacles will need to meet the following criteria compared with the prescribed refractive correction:

- Sphere power must be within 0.25 D of the prescribed correction.
- Cylinder power must be within 0.25 D of the prescribed correction.
- Axis must be within +/- 10 degrees if cylinder power is ≤1.00D, and within +/- 5 degrees if cylinder power is >1.00D.

If these criteria are not met, the spectacles must be re-made by the optical lab and the participant will return within another 45 days for the Spectacle Baseline / Randomization Visit (see *section 2.3*). **Note:** The cycloplegic refraction meeting the Enrollment criteria (see *section 2.3 #4)* must have been performed within 90 days of the repeat Spectacle Baseline Visit. Otherwise, the participant will require repeat cycloplegic refraction to confirm that the spectacle prescription is still within study tolerances before the Spectacle Baseline visit can be conducted.

The PedEyeQ child questionnaire (or proxy questionnaire) and parent questionnaire should be completed before any clinical testing.

3.1.2 Visual Acuity Testing

Distance VA testing, with the participant wearing his/her new spectacle correction, will be performed in each eye by a certified examiner using the electronic ATS-HOTV VA protocol for

children <7 years and the E-ETDRS VA protocol for children ≥ 7 years on a study-certified acuity tester displaying single surrounded optotypes as described in the *ATS Testing Procedures Manual.*

Participants should wear the new spectacles for at least 10 minutes before visual acuity is tested. After an initial test of distance VA, distance VA will be retested, because study VA will be based on the mean of test and retest at each visit.

3.1.2.1 Management of Reduced Visual Acuity

If the investigator suspects <u>incomplete relaxation of accommodation</u> in the current spectacles is causing reduction of the mean of test and retest VA at the Spectacle Baseline visit from the Enrollment visit in either eye (regardless of whether this initial VA measurement renders the participant eligible or ineligible), the investigator should consider (not mandatory) repeating test and retest VA with a -1.00 D lens in BOTH eyes, regardless of refractive error.

If the participant meets eligibility criteria based on mean of test and retest VA through the –1.00 D lens, the investigator has two options:

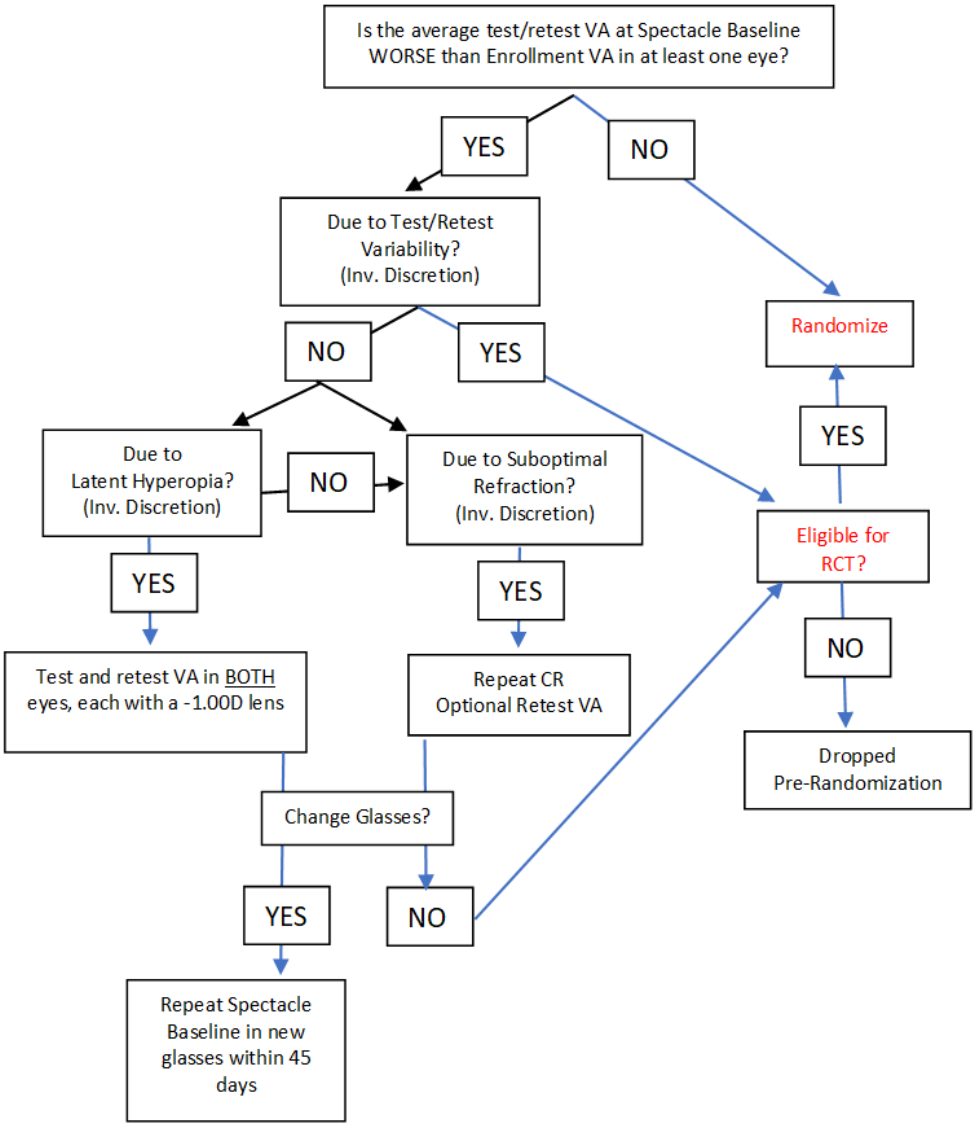
1. Randomize the participant in the current spectacles (the test and re-test VA using the -1.00 D lens will be used as the participant's baseline VA)
2. Change the spectacles by reducing the hyperopic power up to a symmetrical reduction of 1.50 D sphere, following guidelines in *section 2.3 (#4).*

If the participant does not meet eligibility criteria based on mean of test and retest VA, the participant will be dropped from the study.

If the investigator suspects that <u>a change in refractive error is causing reduction of the mean of test and retest VA at the Spectacle Baseline visit from the Enrollment visit in either eye (regardless of whether this initial VA measurement renders the participant eligible or ineligible)</u>, the investigator should consider (not mandatory) repeating cycloplegic refraction.

If the current spectacles **are not** within the lensometry tolerances specified in the protocol (section 3.1.1) based on the new cycloplegic refraction, the spectacles should be re-made and will be paid for by the study. An investigator may repeat visual acuity testing at their discretion to determine if a participant still meets the VA eligibility requirements to continue in the study, but this is not mandatory. The participant should not wear any glasses until they return for another Spectacle Baseline / Randomization visit within 45 days.

<u>If the current spectacles **are** within the lensometry tolerances and the participant meets eligibility criteria, the participant may be randomized.</u>



3.1.2.2 Other Spectacle Change Allowances

If the investigator identifies a new problem at the time of the Spectacle Baseline visit (e.g., new manifest strabismus) that could be appropriately managed by a change in spectacle correction, re-make of the glasses by the optical lab is permitted and will be paid for by the study, as long as the new spectacle prescription is within the refractive error requirements of the protocol, as specified in section 2.3 #4. The participant should return for another Spectacle Baseline / Randomization visit within 45 days.

3.1.3 Testing in New Spectacles

Participants will wear their prescribed spectacles for the first time for at least 10 minutes prior to testing. Participants who have worn their spectacles for more than a total of 24 hours prior to

this visit will end study participation. Participants who have worn their spectacles for less than 24 hours will have their VA measured twice while wearing their new spectacles.

If all components of the Spectacle Baseline / Randomization visit are not completed, the child may return within 7 days to complete the remaining study procedures or repeat all study procedures (at investigator discretion) and be randomized (NOT wearing the glasses in the meantime). If returning more than 7 days later, all testing must be repeated at a new Spectacle Baseline / Randomization visit (NOT wearing the glasses in the meantime).

The order of testing follows (additional details are described in the *ATS Testing Procedures Manual)*:

1. PedEyeQ:
    - The PedEyeQ is a patient-derived questionnaire designed to assess functional vision and eye-related quality of life. It consists of a child questionnaire for children ages 5 years and older, a proxy questionnaire completed by the parent regarding their child, and a parent questionnaire regarding the parent's own quality of life. The child questionnaire is administered by study personnel to children under the age of 8 years and is completed by the child for children 8 years of age or older (with the help of study personnel, if needed). Proxy and Parent questionnaires are completed by the parent. The questionnaires take about 10 minutes to complete.
2. Distance VA Testing:
    - Monocular distance VA testing will be performed in refractive correction in each eye by a certified examiner using the electronic ATS-HOTV VA protocol for children <7 years and the E-ETDRS VA protocol for children ≥ 7 years on a study-certified acuity tester displaying single surrounded optotypes.
    - The VA protocol used at enrollment will be used throughout the study regardless of age at follow-up.
2. Repeat Distance VA Testing: Monocular distance VA testing will be performed in current refractive spectacle correction in each eye by a certified examiner (as above).
3. Binocularity Testing:
    - Gross binocularity will be tested in current spectacle correction at near using the hand-held Worth 4-Shape test held at 40 cm.
    - Stereoacuity will be tested at near in current spectacle correction using both the Randot Butterfly and Randot Preschool stereoacuity tests.
4. Ocular Alignment Testing:
    - Ocular alignment will be assessed in current spectacle correction by the cover test, simultaneous prism and cover test (SPCT) (in cases of strabismus detected by cover test), and prism and alternate cover test (PACT) in primary gaze at distance (3 meters) and at near (1/3 meter).
5. Contrast Sensitivity Testing
    - Contrast sensitivity will be assessed in each eye (right eye first then left eye) using the SpotChecks™ Contrast Sensitivity Test. Contrast Sensitivity testing should be performed last prior to randomization.

3.2 Randomization

For each age cohort, the Jaeb Center will construct a Master Randomization List using a permutated block design stratified by VA in the amblyopic eye (calculated as a mean of the test and retest) as moderate 20/40 to 20/80 (53 to 72 letters; 0.26 to 0.64 logMAR) versus severe 20/100 to 20/200 (33 to 52.5 letters; 0.65 to 1.04 logMAR) which will specify the order of treatment group assignments.

All eligible participants enrolled in the study will be followed for up to 56 weeks. Participants eligible for randomization will be randomly assigned within each age cohort in a 1:1 allocation to one of the following treatment groups:

1. **Sequential treatment**: full-time spectacle correction first, with subsequent patching for 2 hours per day/7 days per week only if needed (no improvement (stable/worsening) and residual)
2. **Simultaneous treatment:** full-time spectacle correction and part-time patching for 2 hours per day/7 days per week

Once a participant is assigned to a treatment group, the participant will be included in the analysis regardless of whether or not the assigned treatment is received. Thus, the investigator must not enroll and randomize a child unless convinced that the parent will accept either treatment.

CHAPTER 4. Randomized Trial Procedures

4.1 Sequential Spectacles (and Patching if Needed)

Participants randomized to the sequential group will initially be treated with spectacle correction to be worn all waking hours (spectacles confirmed at the Spectacle Baseline / Randomization Visit in accordance with the criteria in *section 2.3*).

Adherence with spectacle wear will be monitored throughout the study by a parental calendar.

Participants will continue to wear their spectacles as their only treatment and return for scheduled follow-up visits until non-improvement of VA (i.e., "stable/worsening") is reached or participants reach the final 56-week visit (*section 4.3.3*). Once VA non-improvement ("stable/worsening") has been reached, if the participant has a residual interocular difference in distance VA of more than 1 logMAR line (meeting the definition for "stable residual," *section 4.3.6*), the participant will start patching the non-amblyopic eye 2 hours per day/7 days per week and continue to wear their spectacle correction full-time.

Adherence with patching will be monitored throughout the remainder of the study by an occlusion dose monitor (ODM) worn on the inside of the patch.

Once patching treatment has been initiated, participants in the sequential patching group will continue follow-up every 8 weeks until no further improvement ("stable/worsening") has been reached (see *section 4.3* below) or until 56 weeks.

If there has been no further improvement in VA, but the participant has residual amblyopia ("stable residual"), treatment will be at investigator discretion but participants will continue 8-weekly visits until 56 weeks.

If non-improvement ("stable/worsening") is reached AND amblyopia is no longer present (less than or equal to 1 logMAR line) (meeting the definition for "stable resolved", *section 4.3.5*), participants will continue spectacle wear and 8-weekly visits until 56 weeks.

In participants who meet the definition of stable resolved, but amblyopia recurs (defined as a 2 or more logMAR line decrease in VA from the level measured at the visit when the definition of "stable resolved" was last met) prior to the 56-week visit, they will be treated and the method of treatment will be at investigator discretion.

4.2 Simultaneous Spectacles and Patching

Participants randomized to the simultaneous group will be treated simultaneously with a spectacle correction worn all waking hours (spectacles confirmed at the Spectacle Baseline / Randomization Visit and in accordance with the criteria in *section 2.3*) and patching of the non-amblyopic eye for 2 hours per day / 7 days per week.

Adherence with spectacle wear will be monitored throughout the study by a parental calendar. Adherence with patching will be monitored throughout the study by an occlusion dose monitor (ODM) worn on the inside of the patch.

Participants will continue their treatment and return for scheduled follow-up visits until no further improvement or worsening ("stable/worsening") of VA is reached (see *section 4.3* below) or 56 weeks.

If no further improvement or worsening ("stable/worsening") of VA, but the participant has residual amblyopia ("stable residual"), treatment will be at investigator discretion but participants will continue 8-weekly visits until 56 weeks.

If non-improvement ("stable/worsening") is reached AND amblyopia is no longer present (less than or equal to 1 logMAR line) (meeting the definition for "stable resolved", *section 4.3.5*), participants will continue spectacle wear only (i.e. discontinue patching) and 8-weekly visits until 56 weeks.

In participants who meet the definition of stable resolved, but amblyopia recurs (defined as a 2 or more logMAR line decrease in VA from the level measured at the visit when the definition of "stable resolved" was last met) prior to the 56-week visit, they will be treated and the method of treatment will be at investigator discretion.

4.3 Amblyopia Status

At each follow up visit, the website will determine if a participant is no longer demonstrating improvement of visual acuity ("stable/worsening"). If a participant is no longer improving in visual acuity ("stable/worsening"), amblyopia status will then be defined as residual or resolved to direct the next step in randomized treatment, as described in 4.1 and 4.2. The subsections below provide the study definitions of VA improvement, VA non-improvement ("stable/worsening"), and the criteria for determining amblyopia status (residual, resolved, recurrent).

4.3.1 Improvement of Visual Acuity

Improvement of VA is defined as an improvement in distance VA of 0.05 logMAR or more, (VA is calculated as the mean of test and retest at each visit) over 2 sequential visits that are a minimum of 6 weeks (target of 8 weeks) apart. If visits are not 6 weeks apart, improvement/non-improvement will not be evaluated until this interval is met. If the amblyopic eye VA is not tested twice at a specific visit, improvement/non-improvement will not be evaluated until the next follow-up visit at which test and retest are completed.

4.3.2 Non-improvement of Visual Acuity ("Stable/Worsening VA")

Non-improvement of VA is defined as no improvement in distance VA of 0.05 logMAR or more (based on mean of test and retest) over 2 sequential visits, a minimum of 6 weeks (target of 8 weeks) apart. If the amblyopic eye VA is not tested twice at a specific study visit, improvement/non-improvement will not be evaluated until the next study follow-up visit at which a test and retest of VA are completed.

Non-improvement of VA ("stable/worsening") allows participants randomized to the sequential group to advance from spectacles alone (optical treatment) to added patching treatment.

Non-improvement of VA ("stable/worsening") allows participants who have been patching in either group to be released to treatment at Investigator discretion.

All participants will continue to return for follow-up visits every 8 weeks through 56 weeks. For participants who meet criteria of stable/worsening VA, amblyopia status is then determined in the second step in the classification (*see sections 4.3.3-7*).

4.3.3 Residual Amblyopia

Residual amblyopia is defined as more than 0.1 logMAR of interocular difference of distance VA between the amblyopic eye and the non-amblyopic eye (calculated for each eye as the mean of the test and retest VA measurements made at that visit).

4.3.4 Resolved Amblyopia

Resolved amblyopia is defined as 0.1 logMAR or less of interocular difference of distance VA between the amblyopic eye and the non-amblyopic eye (calculated for each eye as the mean of the test and retest VA measurements made at that visit).

4.3.5 Stable Resolved Amblyopia

Amblyopia is defined as "stable resolved" at the study visit where the participant's amblyopia both meets the definition of non-improved ("stable/worsening") VA (*section 4.3.2*) and "resolved" amblyopia (*section 4.3.4*). NOTE: Participants cannot be considered "stable resolved" if they meet the definition for "improving" (*section 4.3.1*).

4.3.6 Stable Residual Amblyopia

Amblyopia is defined as "stable residual" at the study visit where the participant's amblyopia meets the definition of non-improved ("stable/worsening") VA (*section 4.3.2*) and "residual" amblyopia (*section 4.3.3*). NOTE: Participants cannot be considered "stable residual" if they meet the definition for "improving" (*section 4.3.1*).

4.3.7 Amblyopia Status Examples

The following are examples of amblyopia status:

	Visit 1 VA Test		Visit 1 VA Retest		Visit 2 VA Test		Visit 2 VA Retest		Mean Amb Eye logMAR		IOD logMAR Visit 2	
Ex	**Amb**	**Fellow**	**Amb**	**Fellow**	**Amb**	**Fellow**	**Amb**	**Fellow**	**Vis 1**	**Vis 2**		**Status**
1	20/50	20/20	20/50	20/20	20/50	20/20	20/40	20/20	0.40	0.35	0.35	Improving
2	20/60	20/20	20/60	20/25	20/50	20/20	20/50	20/20	0.50	0.40	0.40	Improving
3	20/60	20/20	20/50	20/20	20/60	20/25	20/40	20/20	0.45	0.40	0.35	Improving
4	20/25	20/20	20/25	20/25	20/20	20/20	20/25	20/20	0.10	0.05	0.05	Improving
5	20/32	20/20	20/32	20/20	20/20	20/20	20/20	20/20	0.20	0.00	0.00	Improving
6	20/50	20/20	20/60	20/20	20/60	20/20	20/60	20/20	0.45	0.50	0.50	Stable residual (not improving)
7	20/50	20/20	20/60	20/20	20/50	20/20	20/60	20/20	0.45	0.45	0.45	Stable residual
8	20/25	20/20	20/20	20/20	20/25	20/20	20/20	20/20	0.05	0.05	0.05	Stable resolved

4.3.8 Recurrent Amblyopia

Recurrent amblyopia is defined as a decrease in amblyopic eye VA of 0.2 logMAR or more and a 0.2 logMAR or more interocular difference of distance VA between the amblyopic eye and the non-amblyopic eye on a subsequent visit to the visit at which the definition of "stable resolved" was met. VA is calculated for each eye as the mean of the test and retest VA measurements

made at the visit. If VA is not tested twice at a specific visit, then the change in VA will not be evaluated until the next study follow-up visit.

Participants meeting the definition for "Recurrent amblyopia" should be treated, and the method of treatment will be at investigator discretion.

4.4 Study Visits

Study visits following randomization will occur at:

Visit	Target Day Post-Randomization	Target Window Post-Randomization*	Allowable Window Post-Randomization**
8 weeks	56 days	42 to 70 days	28 to 83 days
16 weeks	112 days	98 to 126 days	84 to 139 days
24 weeks	168 days	154 to 182 days	140 to 195 days
32 weeks	224 days	210 to 238 days	196 to 251 days
40 weeks	280 days	266 to 294 days	252 to 307 days
48 weeks	336 days	322 to 350 days	308 to 363 days
56 weeks	392 days	378 to 406 days	364 to 504 days

* Target window is target day $\pm$ 2 weeks.
* Allowable window is (target day – 4 weeks) to (target day + 4 weeks minus 1 day); except for 56-week visit which is (target day – 4 weeks) to (target day + 16 weeks).

A text message will be sent by the Coordinating Center to remind the participant and family of the next study visit for participants who consent to receive text messages during the study.

Parents will be asked to complete a daily compliance calendar by recording how long the patch was worn each day (if applicable) and/or how long the child wore his/her spectacles each day.

If visits are not at least 6 weeks apart, improvement/non-improvement will not be evaluated until the participant has 2 visits at least 6 weeks apart. If VA is not tested twice at a specific study visit, the change in VA will not be evaluated until the next study follow-up visit.

Participants who (because of unforeseen circumstances) are unable or unwilling to return for all study follow-up visits will be permitted to return for the 56-week final visit *only* as an alternative to withdrawal from the study.

Additional office visits may occur as needed, however VA results from these non-study office visits will not be used for determination of VA stability.

4.4.1 Testing Procedures at Study Visits

The following procedures will be performed in the following order in **current spectacle correction** in both groups at each visit, unless otherwise specified. A Masked Examiner must complete distance VA testing at visits prior to and at time of primary outcome (*section 4.4.1)*. If a participant currently wears spectacles but is not wearing them at the follow-up examination for whatever reason, testing must be performed in trial frames using the last spectacle prescription. Prior to the Masked Examiner entering the room, participants and parents should be instructed not to discuss their assigned treatment with the Masked Examiner. Prior to performing any testing, the participant's spectacle correction will be verified using a lensometer. Spectacles should meet the following tolerances compared with the last cycloplegic refraction:

- Sphere power must be within 0.25 D of the prescribed correction.
- Cylinder power must be within 0.25 D of the prescribed correction.
- Axis must be within +/- 10 degrees if cylinder power is ≤1.00D, and within +/- 5 degrees if cylinder power is >1.00D.

If spectacles do not meet these tolerances, the participant should be tested with trial frames using the last spectacle prescription.

The PedEyeQ child questionnaire (or proxy questionnaire) and parent questionnaire should be completed before any clinical testing.

For more details, see the *ATS Procedures Manual*:

1. <u>PedEyeQ:</u>
    - The PedEyeQ is a patient-derived questionnaire designed to assess functional vision and eye-related quality of life. It consists of a child questionnaire for children ages 5 years and older, a proxy questionnaire completed by the parent regarding their child, and a parent questionnaire regarding the parent's own quality of life. The child questionnaire is administered by study personnel to children under the age of 8 years and is completed by the child for children 8 years of age or older (with the help of study personnel, if needed). Proxy and Parent questionnaires are completed by the parent. The questionnaires take about 10 minutes to complete.
2. <u>Distance VA Testing (performed by a **masked** examiner prior to and at time of Primary Outcome):</u>
    - Monocular distance VA testing will be performed in the participant's current spectacle correction in each eye by a masked certified examiner using the electronic ATS-HOTV VA protocol for children <7 years and the E-ETDRS VA protocol for children ≥ 7 years on a study-certified acuity tester displaying single surrounded optotypes.
    - The same VA protocol used at enrollment will be used throughout the study regardless of age at follow-up

3. Repeat Distance VA Testing (performed by a **masked** examiner prior to and at time of Primary Outcome): Monocular distance VA testing will be performed in current refractive correction in each eye by a masked certified examiner (as above).
4. Binocularity Testing (may be performed by an unmasked examiner)
    - Gross binocularity will be tested in current spectacle correction at near using the hand-held Worth 4-Shape test held at 40 cm.
    - Stereoacuity will be tested at near in current spectacle correction using both the Randot Butterfly and Randot Preschool stereoacuity tests.
5. Ocular Alignment Testing (may be performed by an unmasked examiner)
    - Ocular alignment will be assessed in the current spectacle correction by the cover test, simultaneous prism and cover test (SPCT) (in cases of strabismus detected by cover test), and prism and alternate cover test (PACT) in primary gaze at distance (3 meters) and at near (1/3 meter).
6. Contrast Sensitivity Testing (only at the time of the Primary Outcome visit; May be performed by an unmasked examiner)
    - Contrast sensitivity will be assessed in the current spectacle correction in each eye (right eye first then left eye) using the SpotChecks™ Contrast Sensitivity Test. Testing will be repeated in the left eye at the 8-week post-randomization visit, until there is sufficient sample size to perform test-retest reliability analysis.
7. Adherence Monitoring: Adherence data from the ODM will be downloaded and compliance calendars will be reviewed.

The following additional testing is performed at the end of the visit when required as defined below in section 4.4.1.1.:

8. Cycloplegic Refraction (at the time of the Primary Outcome visit, if required by 4.4.1.1)

4.4.1.1 Management of Refractive Error

A cycloplegic refraction is mandated at the time of the primary outcome visit for participants if the previous amblyopic eye is still worse than the fellow eye (IOD > 0 and previously amblyopic eye worse based on average of test and re-test); or at the 56-week study visit if primary outcome has not been declared by the 48-week visit AND the previous amblyopic eye is still worse than the fellow eye (IOD > 0 and previously amblyopic eye worse based on average of test and re-test).

4.4.1.1.1 At Visits Prior to the 56-week Study Visit

If the new cycloplegic refraction compared to the old cycloplegic refraction differs by ≥0.75 D sphere or ≥0.75 D cylinder or ≥0.75 D in SE anisometropia or axis change of 6 degrees or more when cylinder is 1.00 D or more; then a change in spectacles is required. Whether to update the spectacles for smaller changes in refraction is at investigator discretion.

When new spectacles are prescribed, the refractive correction prescribed must meet the requirements as described in section 2.3#4. The updated spectacles will be paid for by the study.

All participants will continue study follow-up regardless of whether spectacles are changed or not.

4.4.1.1.2 At the 56-week Study Visit

Since the 56-week study marks the end of the study, whether to update the spectacles is at investigator discretion. Any change in spectacles will NOT be paid for by the study.

4.4.1.2. Masked Examiner

The Masked Examiner must be certified to test visual acuity. Because the Masked Examiner must be masked to the participant's treatment group before primary outcome, he/she must be someone other than the managing investigator.

4.4.2 Unscheduled Visits

Investigators may schedule additional visits at their own discretion. Participants will continue to follow the study-specified follow-up schedule regardless of any non-study visits. No data will be collected at non-study visits for the purpose of the study. Data from non-study visits will not be used to determine VA non-improvement ("stable/worsening") or whether the amblyopia is resolved or residual.

Investigators must not start any additional treatment (other than that outlined in *section 4.1 and 4.2*) prior to the 56-week outcome visit.

CHAPTER 5. Miscellaneous Considerations

5.1 Contacts by the Jaeb Center for Health Research and Sites

The Jaeb Center serves as the PEDIG Coordinating Center. The Jaeb Center will be provided with the parent's contact information. The Jaeb Center may contact the parents of the participants. Permission for such contacts will be included in the Informed Consent Form. The principal purpose of the contacts will be to develop and maintain rapport with the participant and family and to help coordinate scheduling of the outcome examinations.

5.2 Spectacles

Participants who have been screened and meet eligibility criteria while wearing trial frames with the cycloplegic refraction at the screening exam will be provided spectacles paid for by the study.

Any change of spectacles will be paid for by the study.

Contact lenses are not allowed during the study.

5.3 Management of Strabismus

Strabismus surgery may be performed after the participant has been released to non-study treatment. If surgery is performed, the date and type of surgery will be recorded in the comment section of the Follow-up Examination Form. Note that participants should not be enrolled in the study if strabismus surgery is planned or desired by families in the next 56 weeks (see exclusion criteria *section 2.4*).

5.4 Risks

5.4.1 Risks of Examination Procedures

The procedures in this study are part of daily eye care practice in the United States and pose no known risks. As part of a routine usual-care exam, the participant may receive cycloplegic/dilating eye drops.

5.4.2 Risks of Patching

If skin irritation occurs, the parent will be advised to put an emollient on the skin and discontinue use of the patch for a day.

Patching could potentially decrease the VA in the sound eye, although this is almost always reversible and extremely unlikely when the sound eye has several hours without occlusion each day. The diagnosis and management of reverse amblyopia is left to the Investigator's judgment.

Patching could precipitate the development of an ocular deviation (strabismus), although this has been found to be very rare in our previous studies and indistinguishable from the natural history of amblyopia. If treatment precipitates the development of an ocular deviation (e.g., esotropia in participants with hyperopia), the parent will be advised to have the child see the investigator as soon as possible.

5.5 Reporting of Adverse Events

No surgical procedures are part of the protocol. There are no expected long-term adverse events associated with spectacles or patching. Investigators will abide by local IRB reporting requirements.

5.5.1 Risk Assessment

It is the investigators' opinion that the protocol's level of risk falls under DHHS 46.404 which is research not involving greater than minimal risk.

5.6 Participant Compensation

Participant compensation will be specified in the informed consent form.

5.7 Participant Withdrawal

Participation in the study is voluntary, and a participant may withdraw at any time. For participants who withdraw, their data will be used up until the time of withdrawal.

5.8 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. De-identified participant information may also be provided to research sites involved in the study.

CHAPTER 6. Statistical Considerations

6.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below.

6.2 Definition of Participant Cohorts

Two cohorts of participants with identical eligibility criteria, apart from age at time of randomization will be enrolled:

- Children aged 3 to <7 years at randomization referred to as the 'younger cohort'
- Children aged 7 to <13 years at randomization referred to as the 'older cohort'

Overall response to amblyopia treatment has been found to be somewhat reduced in children 7 years and older[23] and it is likely that there are differences in adherence with spectacle correction and with prescribed patching between children <7 years and children 7 years and older; therefore, we believe that there could be a differential treatment effect between the two age cohorts. Recruitment potential is also expected to differ between the two age cohorts given the requirement for no prior treatment for amblyopia. Therefore, statistical considerations are different for the two age cohorts and all analyses described below will be conducted separately within each age cohort.

6.3 Primary Objective

The primary objective is to evaluate whether or not VA outcomes are equivalent after completion of up to 56 weeks of "sequential treatment" (spectacles first, with patching if needed for 2 hours per day/7 days per week) versus completion of up to 56 weeks of "simultaneous treatment" (spectacles together with patching 2 hours per day/7 days per week) in children newly diagnosed with amblyopia caused by anisometropia, strabismus, or both.

6.4 Primary Efficacy Outcome – Distance Visual Acuity in Amblyopic Eye

The primary efficacy outcome will be mean change in amblyopic eye logMAR distance VA between randomization and completion of randomized treatment or at 56 weeks, whichever is earlier.

The outcome VA for each participant is defined as either of the following:

1. Amblyopic eye VA (calculated as mean of test and retest) at the last visit that was the basis for a "stable resolved" or "stable residual" determination (in the sequential group, stable residual amblyopia criteria can be reached only *after* patching has been instituted); or

2. 56-week visit amblyopic-eye VA (calculated as mean of test and retest) in those completing a 56-week visit without ever meeting criteria for "stable resolved" or "stable residual" amblyopia (if retest missing at 56 weeks, the single test value will be used).

The outcome VA for participants who never meet criteria for "stable resolved" or "stable residual" amblyopia and do not complete the 56-week visit, or receive non-randomized treatment for amblyopia prior to meeting criteria for "stable resolved" or "stable residual" amblyopia, will be imputed using multiple imputation based on amblyopic eye VA scores while on randomized treatment (using the mean of test and retest if done).

The primary analysis will follow the intent-to-treat (ITT) principle whereby all participants randomized will be included in the analysis according to the treatment group assigned at randomization. An analysis of covariance (ANCOVA) will be performed to compute the difference in mean change in amblyopic-eye distance VA (in logMAR lines) between the sequential and simultaneous treatment groups, adjusted for baseline amblyopic eye distance VA, and a 2-sided 95% confidence interval will be constructed on the treatment group difference. Equivalence of treatment groups will be declared if the upper and lower 95% CI limits are smaller (in absolute value) than the equivalence margin of 0.75 logMAR lines. Superiority of one treatment over the other will be declared if the upper and lower ends of the 95% CI exclude 0 difference.

The ANCOVA model assumptions of normality and homoscedasticity (equal variance in treatment groups) will be checked, and if violated, an alternative method of analysis that is robust to the assumption(s) violated will be performed to check the sensitivity of the primary analysis model to the violation of assumptions. The methods to be considered include, but are not limited to, outcome VA transformation and the non-parametric randomization-based method of Koch et al.[44] If results differ, the robust method will be used for trial conclusions and formulation of resulting recommendations.

Additional approaches to the primary analysis include the following:

- ITT with no imputation - Including only participants who meet the criteria for "stable resolved" or "stable residual" (only *after* patching has been instituted in the sequential group) or complete the 56-week visit (not including those for whom an outcome VA is imputed).

The multiple imputation ITT analysis is considered primary. If the ITT with no imputation and primary analyses give inconsistent results, exploratory analyses will be performed to evaluate possible factors contributing to the difference.

6.5 Secondary Efficacy Outcomes

All secondary outcomes involving amblyopic eye distance VA will use the ITT dataset with amblyopic eye distance VA imputed when missing. Secondary outcomes not involving amblyopic eye distance VA will use observed data, with no imputation for missing data.

For secondary outcome comparisons that are based on a parametric statistical model, all model assumptions will be checked, and if violated, data transformation or a non-parametric alternative will be used instead.

The distribution of all secondary outcomes will be tabulated at randomization and the outcome visit for each randomized treatment group and described using descriptive statistics.

Treatment group comparisons on secondary clinical outcomes will be controlled using the false discovery rate (FDR) method at 5% and to adjust p-values and confidence intervals for multiplicity.

6.5.1 Pediatric Eye Questionnaire (PedEyeQ)

Rasch scores for each questionnaire item will be obtained from published look-up tables available at www.pedig.net, and used to calculate a score for each participant and a separate treatment group mean for each of the PedEyeQ domains of the Child, Proxy, and Parent PedEyeQ at randomization and at each visit. Scores will also be converted to a 0-100 scale to aid in interpretation.

- Child PedEyeQ:
  - Functional Vision
  - Bothered by Eyes and Vision
  - Social
  - Frustration / Worry
- Proxy PedEyeQ:
  - Functional Vision
  - Bothered by Eyes and Vision
  - Social
  - Frustration / Worry
  - Eye care
- Parent PedEyeQ:
  - Impact on Parent and Family
  - Worry about Child's Eye Condition
  - Worry about Self-perception and Interactions
  - Worry about Functional Vision

Comparisons of interest, following the hypotheses detailed in the Background section are as follows:

- Comparison of domain scores between treatment groups at 8 weeks (the first follow-up visit, before the sequential group has the opportunity to start patching and is still in spectacle correction).
- Comparison of domain scores between treatment groups at the primary outcome visit defined as in *section 6.4*.
- Comparison of domain scores between treatment groups at the conclusion of the study (56 weeks).

Mean Rasch scores for each domain will be compared between randomized treatment groups using linear models, adjusting for baseline domain score and other baseline factors known to be associated with health-related quality of life (HRQOL).

All analyses will test the null hypothesis of no difference between treatment groups. The false discovery rate (FDR) method will be used to control the FDR at 5% and to adjust p-values and confidence intervals for multiplicity.

6.5.2 Proportion Achieving Stable Resolved Outcome with Spectacles Alone

The proportion of participants in the Sequential Spectacles group who achieve "stable resolved" outcome status with spectacles alone will be calculated, along with a 95% confidence interval.

6.5.3 Binary Outcomes – Distance Visual Acuity

The proportion of participants who achieve the following binary outcomes will be tabulated by treatment group to aid in the interpretation of the primary outcome:

- The proportion of participants with outcome amblyopic-eye distance VA improvement of ≥ 2 logMAR lines (≥ 10 letters if E-ETDRS) from baseline.
- The proportion of participants with stable resolved VA (as defined in *section 4.3.5*).

Poisson regression with the log link will be used to estimate the relative risk of each outcome for the sequential versus simultaneous and an FDR-adjusted confidence interval. The Poisson models will include an adjustment for baseline amblyopic eye VA.

In the event the number of outcomes is too small for reliable estimation with Poisson regression,[45] a treatment group difference and 95% confidence interval will be estimated using the Farrington-Manning Score test or other exact method with no adjustment for baseline VA.

6.5.4 Time to Stable Resolved Amblyopia

For those participants who are classified as "stable resolved," the time from baseline to the time meeting that classification will be compared between treatment groups, using a Kaplan-Meier analysis with the logrank test.

6.5.5 Distance Visual Acuity after 8 Weeks and 56 Weeks on Randomized Treatment

An analysis of covariance (ANCOVA) will be performed to compute the difference in mean change in amblyopic-eye distance logMAR VA after 8 weeks and 56 weeks between the sequential and simultaneous treatment groups, adjusted for baseline amblyopic eye distance VA, and a 2-sided p-value and an FDR-adjusted confidence interval will be constructed on the treatment group difference for each time point.

6.5.6 Binocularity

Binocularity will be assessed on an ordered scale combining the results of the Randot Preschool Test, Randot butterfly, and Worth 4-Shape at near. Results of each individual test also will be tabulated at baseline and at the final study visit according to treatment group.

The possible levels of binocularity will be 40, 60, 100, 200, 400, 800 seconds of arc (Randot Preschool test), 2000 seconds of arc (Randot butterfly), binocular perception by W4D (4 or 5 lights), or no binocular perception by W4D (2 or 3 lights). This yields an ordered binocularity scale with 9 ordered levels. The change in binocularity levels for each test will be tabulated and compared between treatment groups using the exact Wilcoxon rank-sum test. The proportion of

participants in each treatment group unable to perform testing will be tabulated but these participants will not be included in the analysis of change.

6.5.7 Contrast Sensitivity

Contrast sensitivity scores range from unable (<0.90), and then from 0.90 to 2.05 (by 0.05 log contrast sensitivity units). An analysis of covariance (ANCOVA) will be performed to compute the difference in mean change in amblyopic-eye log contrast sensitivity units between the sequential and simultaneous treatment groups, adjusted for baseline amblyopic eye contrast sensitivity, and a 2-sided p-value and FDR-adjusted confidence interval will be constructed.

Test-retest reliability will be evaluated at the 8-week post-randomization visit. The difference between the initial and repeat contrast sensitivity score of the left eye will be calculated and evaluated across the age distribution to see if it is appropriate to combine data from both age cohorts, or if the data should be analyzed within subgroups defined by age. Analyses (overall or by age) will then evaluate the test/retest differences in contrast sensitivity as a function of the mean value for the initial and repeat test (Bland-Altman type analyses). Confidence intervals for the upper and lower limits of agreement will be calculated either overall or within subgroups defined by the mean test/retest contrast sensitivity score if the data suggest there is a difference across the distribution of scores.

6.6 Safety

The PEDIG DSMC will review safety data tabulated by treatment group at each of its semi-annual meetings and can request formal statistical comparison of any safety outcome at any time if they have cause for concern. As type II error is more of a concern than type I error in safety analyses, we will use $p<0.05$ without adjustment for multiplicity to define statistical significance in all safety analyses.

6.6.1 Distance VA in Fellow Eye

The mean change in fellow-eye distance VA from randomization to the last visit while on randomized treatment (using the mean of test and retest if done or initial test if retest not done at each visit) will be calculated and compared between treatment groups using ANCOVA with adjustment for baseline VA, and a 2-sided p-value and 95% confidence interval will be constructed on the estimated treatment group difference.

The proportion of participants with loss of 2 or more logMAR lines (10 or more letters) of VA in the fellow eye from randomization to their last visit (using the mean of test and retest if done or initial test if retest not done at each visit) while on randomized treatment will be reported for each treatment group and compared using Barnard's exact test.

6.6.2 Ocular Alignment

The proportion of participants with development of new strabismus by cover testing at distance or near (no heterotropia at baseline and the presence of near and/or distance heterotropia at a subsequent visit) or an increase from baseline ≥10Δ by PACT in a pre-existing strabismus at any time on randomized treatment during the study will be reported by treatment group and compared using Barnard's exact test.

6.6.3 Skin Irritation from Patching

The proportion of participants with skin irritation from patching reported as "severe" at any time during the study, using the severe, moderate, or mild scale, will be tabulated for each randomized treatment group.

6.7 Adherence with Randomized Treatment

Adherence with Spectacles

Data from the parental calendar of spectacle wear will be used to calculate a subjective measure of adherence. The average % of spectacle treatment completed across visits will be defined by the investigator as poor (<25%), fair (25% to <50%), good (50% to <75%), or excellent (>=75%), based on investigator impression of the total hours of spectacle wear since the last study visit.

Adherence with Patching

Data from the occlusion dose monitor (ODM) will be used to calculate an objective measure of adherence. The proportion of patching completed will be defined as the total hours of patching divided by the total hours of prescribed patching over a specified period of time.

6.7.1 Adherence within the Sequential Treatment Group

6.7.1.1 While Wearing Spectacles Alone

The adherence with spectacles between randomization and the visit at which VA stability or resolution with spectacles alone was achieved will be calculated as described in *section 6.7* for each participant and the distribution within those randomized to sequential treatment will be summarized.

Average adherence with spectacle wear over the total number of study visits will be dichotomized based on having excellent (an average of 3 or higher) adherence, and the mean improvement in amblyopic eye VA will be compared between the 2 groups (average adherence of ≥3 or higher versus average of <3) thus defined using ANCOVA, with adjustment for baseline age and amblyopic eye VA.

6.7.1.2 While Wearing Spectacles and Patching After Stable VA on Spectacles Alone

For participants who initiate patching after reaching stable amblyopic eye VA with spectacles alone, their adherence with patching between the visit at which patching was initiated and the visit at which a "stable resolved" or "stable residual" determination was made while on spectacles and patching will be calculated as described in s*ection 6.7* for each participant, and the distribution within those randomized to sequential treatment will be summarized.

A Spearman correlation coefficient and 95% CI for the correlation between adherence with patching and improvement in amblyopic eye VA while wearing the patch, adjusted for age and baseline amblyopic eye VA, will be calculated to assess the strength of the relationship.

Adherence with patching will be dichotomized based on having 80% or higher adherence. The mean improvement in amblyopic eye VA will be compared between the 2 groups (≥80% versus

<80% adherence with patching) using ANCOVA, with adjustment for baseline age and amblyopic eye VA.

6.7.2 Adherence within the Simultaneous Treatment Group

6.7.2.1 While Wearing Spectacles and Patching

For participants who are randomized to simultaneous patching, the adherence with patching between randomization and the visit at which a "stable resolved" or "stable residual" determination is made while on spectacles and patching (or 56 weeks if a determination was never made and the 56-week visit was completed) will be calculated as described in *section 6.7* and the distribution within those randomized to sequential treatment will be summarized.

A Spearman correlation coefficient and 95% CI for the correlation between the adherence with patching and improvement in amblyopic eye VA, adjusted for age and baseline amblyopic eye VA, will be calculated to assess the strength of the relationship.

Adherence with patching will be dichotomized based on having 80% or higher adherence. The mean improvement in amblyopic eye VA will be compared between the 2 groups (≥80% versus <80% adherence with patching) using ANCOVA, with adjustment for baseline age and amblyopic eye VA.

6.8 Recurrence of Amblyopia

The proportion of participants who meet criteria for stable resolved and then have a recurrence of amblyopia will be tabulated within each treatment group and an exact 2-sided 95% confidence interval will be computed on the proportion within each treatment group.

Recurrent amblyopia is defined as a decrease in amblyopic eye VA of 0.2 logMAR or more and a 0.2 logMAR or more interocular difference of distance VA between the amblyopic eye and the non-amblyopic eye on a subsequent visit compared to the visit at which the definition of "stable resolved" was met. VA is calculated for each eye as the mean of the test and retest VA measurements made at the visit.

6.9 Protocol Adherence and Retention

A flow-chart will summarize visit completion by randomized treatment group.

The proportion of participants within each randomized treatment group receiving non-randomized treatment at any time during the study will be calculated and will include the following:

- The proportion of participants within the sequential patching treatment group who initiate patching prior to meeting stable residual amblyopia criteria (as defined in *section 4.3*).
- The proportion of participants within either group who receive a non-randomized treatment other than patching.
- The proportion of participants within the simultaneous treatment group who are non-compliant with patching, defined as never wearing a patch.

6.10 Baseline Descriptive Statistics

Baseline demographic and clinical characteristics will be tabulated by randomized treatment group.

6.11 Subgroup Analyses

An analysis to evaluate potential effect modification (interaction) between baseline amblyopic-eye distance VA and randomized treatment will be conducted. It is possible that participants with worse baseline amblyopic eye VA may have different improvement by treatment assignment compared with participants with less severe amblyopia. The more severe amblyopes may have additional benefit from sequential treatment due to improved adherence with patching because of better VA after initial improvement with spectacles. On the other hand, the more severe amblyopes may benefit more from simultaneous treatment because patching is starting sooner. We will construct a 95% confidence interval for the baseline amblyopic eye VA by treatment group interaction term to test the hypothesis that there is a non-zero interaction between baseline amblyopic eye VA and treatment assignment. In addition, the treatment effect and 95% confidence interval for treatment effect will be calculated within subgroups defined by baseline amblyopic eye VA as moderate (VA 20/40 to 20/80, 68 to 53 letters) versus severe (20/100 to 20/200 (52 to 33 letters).

The following subgroup analyses, will be considered exploratory:

- Sex (male vs female)
- Race/ethnicity (white non-Hispanic vs non-white or Hispanic)
- Type of amblyopia (strabismic, anisometropic, or both)
- Binocularity as measured by the Randot Preschool, Randot Butterfly, and Worth 4-Shape tests

The approach for these analyses will be to estimate the treatment effect and 95% confidence interval within each subgroup (male versus female, white non-Hispanic versus non-white or Hispanic, type of amblyopia); using the same analytic methods as for the primary analysis. The interaction between treatment assignment and binocularity will also be explored.

6.12 Sample Size

6.12.1 Younger Cohort 3 to <7 Years of Age

The study will have 90% power to reject two one-sided null hypotheses that one treatment group is >=0.75 logMAR lines better than the other in favor of an alternative hypothesis that the simultaneous and sequential treatment groups are equivalent with respect to change in VA, where “equivalent” is the 2 groups having mean change in amblyopic eye VA within <0.75 logMAR lines, as determined by statistical tests of:

$\mathbf{H_{Null\ 1}}$ = $(\text{Simultaneous}_{\text{mean change}} - \text{Sequential}_{\text{mean change}}) \geq$ 0.75logMAR lines VA

$\mathbf{H_{Null\ 2}}$ = $(\text{Sequential}_{\text{mean change}} - \text{Simultaneous}_{\text{mean change}}) \geq$ 0.75logMAR lines VA

$H_{Alternative\ 1}$ = 0 ≤ ($Simultaneous_{mean\ change}$ - $Sequential_{mean\ change}$) < 0.75logMAR lines VA

$H_{Alternative\ 2}$ = 0 ≤ ($Sequential_{mean\ change}$ - $Simultaneous_{mean\ change}$) < 0.75logMAR lines VA

The primary analysis will follow the intent-to-treat principle whereby all participants randomized will be included in the analysis.

6.12.1.1 Sequential Treatment

To estimate the treatment effect for those randomized to sequential treatment, data from a previous PEDIG study, ATS5, [17,46] were used; the data used were limited to ATS5 participants who met the eligibility criteria for the current study (see Table 1).

One-hundred thirty-nine (139) participants aged 3 to 6 years with amblyopia due to anisometropia, strabismus, or both were treated with spectacles alone in a spectacles-only phase of the ATS5 study. Participants had visits every 5 weeks until amblyopia resolved (amblyopic eye VA same or better than best fellow-eye VA during study) or VA was stable (stable defined as <1 line improvement in logMAR VA between 2 subsequent 5-week visits). Upon completion of the spectacles-only phase, those with residual amblyopia were randomized to patching 2 hours per day/7 days per week or continued spectacles alone with visits every 5 weeks until VA was stable or amblyopia resolved.

The mean change in logMAR VA from the spectacle phase baseline visit to the visit of maximum VA in the ATS5 study is summarized in Table 1 below.

Table 1 – Visual Acuity Response in the ATS5 Study[17,46]

	N	Change in logMAR lines from Spectacle Phase Baseline Visit to Visit of Maximum Visual Acuity	
		Mean (95% CI)	SD (95% CI)
Enrolled into Spectacle Phase*	**139**		
Dropped prior to stability or resolution with spectacles	10	1.9 (1.1 to 2.7)	1.1 (0.8 to 1.9)
Resolved in spectacle phase	28	4.3 (3.7 to 5.0)	1.7 (1.4 to 2.3)
Stable residual amblyopia; Randomized to Patching	46		
Completed RCT phase	45	3.8 (3.4 to 4.3)	1.5 (1.3 to 1.9)
Dropped prior to stability or resolution in RCT phase	1	2.0	-
Stable residual amblyopia; Randomized to Continued Spectacles	55		
Completed RCT phase	52	3.2 (2.8 to 3.7)	1.6 (1.4 to 2.0)
Dropped prior to stability or resolution in RCT phase	3	2.7 (1.2 to 4.1)	0.6 (0.3 to 2.2)
Overall – Including those Randomized to Continued Spectacles****	**139**	**3.5 (3.2 to 3.8) Median (Range)**	**1.7 (1.5 to 1.9)**

		3 (0 to 8)	
Overall – Excluding those Randomized to Continued Spectacles*****	**84**	**3.7 (3.4 to 4.1)** **Median (Range)** **3 (0 to 8)**	**1.7 (1.5 to 2.0)**

* Meeting criteria for current study: age 3 to <7, 20/40 to 20/200 amblyopic eye visual acuity, age normal fellow-eye visual acuity, IOD >=3 lines, no prior amblyopia treatment including spectacles. SD=standard deviation.

After a median of 20 weeks of treatment with spectacles alone followed by patching (if needed), the mean change in VA at the study visit of maximum acuity was 3.7 logMAR lines (95% CI = 3.4 to 4.1 logMAR lines) with a standard deviation of 1.7 logMAR lines (95% CI = 1.5 to 1.9 logMAR lines).

Given the more stringent criteria for VA stability in the present study (3 visits at least 8 weeks apart with no improvement) compared to the previous study (2 visits at least 5 weeks apart with no improvement), we anticipate that the magnitude of VA improvement in the current study will be larger.

6.12.1.2 Simultaneous Treatment

There are few data in the literature to help us estimate the treatment effect in our study for those randomized to simultaneous treatment with spectacles and patching. Agervi et al[19] compared treatment with simultaneous spectacles plus Bangerter filters with spectacle correction alone to treat anisometropic children with previously untreated amblyopia.

Sixty-six participants were randomized to treatment with spectacles alone (n=33) or spectacles plus Bangerter filters (n=33) and completed a visit 1-year post-randomization. Age ranged from 4 to 5 years of age. The median logMAR BCVA in the amblyopic eye in each group at baseline was 0.4 logMAR (20/50) with a median IOD of 3 lines in each group; 3 (9%) and 4 (12%) had severe amblyopia of 20/125 or worse. Table 2 summarizes the response to treatment after 1 year.

Table 2 – Visual Acuity Response after Spectacles Alone or Spectacles Plus Bangerter

		Lines Change in Amblyopic Eye at 1 Year	**Time to Resolution**
	N	**Median (range)**	**Mean (range)**
Spectacles Alone	33	4 lines (2 to 9)	3.9 months (0.5 to 13.3 months)
Spectacles Plus Bangerter	33	4 lines (2 to 8)	2.2 months (0.4 to 9.7 months)

The median and range of amblyopic eye improvement in the Agervi et al study[19] is similar to that seen in the previous ATS5 study (4 logMAR lines vs 3.7 logMAR lines, respectively). Given that a lower proportion of participants in the Agervi study had severe amblyopia 20/125 or worse, compared with ATS5 (11% vs 19%), it is therefore reasonable to expect the two treatment regimens (sequential and simultaneous) are equivalent with respect to VA outcomes; hence, an equivalence design is proposed.

The standard deviation for improvement in the spectacles plus simultaneous patching treatment group is expected to be the same as seen in ATS5 (1.7 logMAR lines) given the similar ranges of improvement in the two studies.

6.12.3 Choice of Equivalence Margin

The equivalence margin was determined by the Protocol Leads and the PEDIG Operations Committee, using clinical judgment, based on simulated distributions of VA improvement corresponding to a mean difference between 2 populations of 0.25, 0.50, 0.75 and 1.0 logMAR lines. We presented side-by-side histograms of the distributions illustrating the shift in the distribution for each mean, along with corresponding differences for clinically meaningful cut points, e.g., difference in percent improving 2 or more logMAR lines, or percent with final VA of 20/32 or better. The consensus was that if the difference in mean improvement between treatments was less than 0.75 logMAR lines, then the treatments would be considered equivalent.

6.12.4 Sample Size Estimation

Based on previous data from ATS5 on sequential treatment (mean improvement of 3.7 logMAR lines) and data from Agervi et al (median improvement of 4 logMAR lines), we propose to design the present study to test whether the two treatments are equivalent, where equivalent is defined as a difference in mean improvement of less than 0.75 logMAR lines.

Assuming that the true difference in mean VA change between the two groups is 0 and assuming a pooled standard deviation of 1.7 logMAR lines, a total sample size of 272 participants (136 per group) has 90% power with a type I error rate of 2.5% to reject the null hypothesis of non-equivalence in favor of the alterative that simultaneous treatment is equivalent to sequential treatment (Table 3).

The percentage of participants lost to follow-up or withdrawn is expected to be small overall (10%) and equal between the two treatment groups. No adjustment to sample size has been made since outcome VA will be imputed for these participants (see *section 6.4*).

Table 3. Total Sample Size Estimates for a 2-Arm Study *

SD of Change (LogMAR lines)	Treatment Group Difference in Mean VA Change from Baseline (logMAR lines) Equivalence Margin	
	<0.50	**<0.75**
1.6	538	242
1.7	606	**272**
1.8	678	304
1.9	756	338

* The number of participants in cells represents the total number of participants required to reject a 2-sided null hypothesis that simultaneous treatment is not equivalent to sequential treatment by >=0.75 logMAR lines in favor of the alternative that simultaneous treatment is equivalent to sequential, with an equivalence margin of <0.75 logMAR lines; from 2 one sided T-tests with an alpha=0.025 and power 90% for a range of pooled SD of change in VA (logMAR lines).

A sample size of 272 participants for an equivalence study will have 90% power to reject a null hypothesis of no difference in favor of an alternative superiority hypothesis that there is a

difference between groups, if the true difference between groups is as small as 0.67 logMAR lines of VA, with a type 1 error rate of 5%.

6.12.5 Interim Analysis and Sample Size Re-estimation

An interim monitoring plan will be developed in conjunction with the DSMC and will be detailed in the statistical analysis plan.

6.12.6 Secondary HRQOL Outcomes

We will test the null hypothesis of no difference between groups in each HRQOL domain with an alternative hypothesis that the treatment groups differ. The false discovery rate (FDR) method will be used to control the FDR at 5% and to adjust p-values for multiplicity.

6.13 Older Cohort 7 to <13 Years of Age

Given the eligibility requirement of no prior amblyopia treatment, we expect to recruit fewer participants for the older cohort. The study will not be powered for a specific hypothesis test within the older cohort; rather an analysis of covariance (ANCOVA) will be performed to compute the mean logMAR change in amblyopic-eye VA for the sequential and simultaneous treatment groups, adjusted for baseline amblyopic eye VA, and a 95% confidence interval will be constructed on the treatment group difference.

The primary analysis will follow the intent-to-treat principle with outcome VA defined as in *section 6.4*.

Table 4 summarizes the half-width of a 95% confidence interval for a treatment group difference for various pooled standard deviations and sample sizes.

Table 4. Half-width 95% Confidence Interval for Treatment Group Difference in Older Cohort for Various Sample Sizes

SD of Change (logMAR lines)	N=50	N=75	N=125	N=150
1.6	± 0.90*	± 0.73	± 0.56	± 0.51
1.7	± 0.95	± 0.78	± 0.60	± 0.55
1.8	± 1.01	± 0.82	± 0.63	± 0.58

* Number in cells represents the half-width 95% confidence interval for logMAR treatment group difference.

CHAPTER 7. Data Collection and Monitoring

7.1 Case Report Forms and Other Data Collection

The main study data are collected on electronic case report forms (CRFs). When data are directly collected in electronic case report forms, this will be considered the source data. For any data points for which the eCRF is not considered source (e.g., lab results that are transcribed from a printed report into the eCRF), the original source documentation must be maintained in the participant's study chart or medical record. This source must be readily verifiable against the values entered into eCRF. Even where all study data are directly entered into the eCRFs at office visits, evidence of interaction with a live participant must be recorded (e.g., office note, visit record, etc.)

Electronic device data files are obtained from the study software and individual hardware components. These electronic device files are considered the primary source documentation. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

7.2 Study Records Retention

Study documents should be retained for a minimum of 3 years after completion of the final grant reporting. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

7.3 Quality Assurance and Monitoring

Designated personnel from the Coordinating Center will be responsible for maintaining quality assurance (QA) and quality control (QC) systems to ensure that the clinical portion of the trial is conducted and data are generated, documented and reported in compliance with the protocol adhering to Good Clinical Practice (GCP) and the applicable regulatory requirements, as well as to ensure that the rights and wellbeing of trial participants are protected and that the reported trial data are accurate, complete, and verifiable. Adverse events will be prioritized for monitoring.

A risk-based monitoring (RBM) plan will be developed and revised as needed during the course of the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013). This plan describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

The data of most importance for monitoring at the site are participant eligibility and adverse events. Therefore, the RBM plan will focus on these areas. As much as possible, remote monitoring will be performed in real-time with on-site monitoring performed to evaluate the verity and completeness of the key site data.

Elements of the RBM may include:

- Qualification assessment, training, and certification for sites and site personnel

- Oversight of Institutional Review Board (IRB) coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits/audit trail, protocol review of entered data and edits, statistical monitoring, study closeout
- On-site monitoring (site visits): source data verification, site visit report
- Agent/Device accountability
- Communications with site staff
- Patient retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- Adverse event reporting and monitoring

Coordinating Center representatives or their designees may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and progress of the study. The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

7.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The site PI/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the monitoring plan.

CHAPTER 8. Ethics/Protection of Human Participants

8.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

8.2 Institutional Review Boards

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

8.3 Informed Consent Process

8.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing.

The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

8.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the sponsor, representatives of the IRB, regulatory agencies or company supplying study product may inspect all documents and records

required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Jaeb Center for Health Research. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by Jaeb Center for Health Research staff will be secured and password protected.

At the end of the study, all study databases will be de-identified and archived at the Jaeb Center for Health Research.

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

8.3.3 Future Use of Data

Data collected for this study will be analyzed and stored at the Jaeb Center for Health Research. After the study is completed, the de-identified, archived data will be made available to the public.

CHAPTER 9. **References**

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