Luminopia One Amblyopia Vision Improvement Study (C-AM-2)

Protocol Identifying Number: C-AM-2

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Amblyopic Eye
ATS	Amblyopia Treatment Study
BCVA	Best Corrected (with current refractive correction) Visual Acuity
CFR	Code of Federal Regulations
CI	Confidence Interval
CRF	Case Report Form
D	Diopter
FDA	Food and Drug Administration
FE	Fellow Eye
GCP	Good Clinical Practice
ICH	International Council for Harmonization
IRB	Institutional Review Board
NIH	National Institutes of Health
PI	Principle Investigator
PEDIG	Pediatric Eye Disease Investigator Group
SE	Spherical equivalent
SPCT	Simultaneous Prism and Cover Test
VA	Visual Acuity

KEY ROLES

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1. BACKGROUND AND SUMMARY

1.1. Background

Epidemiology and Clinical Characteristics

Amblyopia is the most prevalent cause of reduced monocular visual acuity in children and young adults, with estimates of prevalence ranging from 1% to 5%.[1, 2] The most common associated amblyogenic risk factors are uncorrected anisometropia, strabismus, or a combination of these. In addition to reduced visual acuity, amblyopic participants may also have dysfunctions of accommodation, fixation, binocularity, vergence, reading fluency, depth perception, and contrast sensitivity.[3-10]

Current Treatments

The current standard of care for amblyopia treatment is refractive correction (when there is uncorrected refractive error) followed by part-time patching or atropine penalization of the fellow eye.[11-15] In younger children, aged 3 to 7 years, although current treatments with part-time occlusion and atropine drops are somewhat effective,[11-15] residual amblyopia (20/32 or worse) is still present in 54% of children at age 10 years[16] and 40% at age 15 years.[17]

One possible reason for failure of part-time patching treatment in some younger children and many older children is poor adherence with the prescribed treatment regimens.[21, 22] Nevertheless, data from studies using an occlusion dose monitor[23, 24] suggest that many children do successfully comply with prescribed part-time patching treatment and yet fail to respond to treatment, suggesting that part-time patching is ineffective for treating amblyopia in some children.

In addition, patching has negative psychosocial effects for many children, and children often resist wearing a patch. Some children and their parents rate patching poorly from the standpoint of adverse effects of treatment, treatment adherence, and social stigma.[25, 26] Based on the prevalence of residual amblyopia with current part-time patching treatment and the challenges of adherence with patching, new treatments for amblyopia are needed, particularly those that are engaging for patients and lead to improvements in vision.

Binocular Treatments

Several past studies have demonstrated the potential of "binocular treatments" for amblyopia, which rebalance visual input to the eyes using various modifications and encourage proper binocular vision. In 2010, Hess et al[27] measured dichoptic motion coherence thresholds in a series of sessions by adjusting fellow eye contrast levels to overcome suppression of the amblyopic eye. Nine adults (aged 24 to 49 years) achieved significantly improved amblyopic-eye visual acuity ($p < 0.008$) and stereoacuity ($p = 0.012$) after repeated measurements, despite 4 of 9 participants previously being treated with patching.

Knox et al[28] studied a similar paradigm with a binocular computer game using an in-office, head-mounted display over five 1-hour treatment sessions. Contrast was adjusted to equalize input from each eye. Fourteen children (aged 5 to 14 years) with prior patching treatment were included in the study, with initial amblyopic eye visual acuity ranging from 20/25 to 20/200. Following treatment, mean amblyopic eye visual acuity ($p = 0.0001$) and stereoacuity improved significantly ($p = 0.02$).

In another recent study published in 2015, Li et al[29] used dichoptic movies presented through a passive 3D display with a fellow eye contrast reduction to treat amblyopia in 8 amblyopic children. After only ten hours of treatment, amblyopic eye visual acuity improved on average by a clinically significant 0.2 logMAR. As a comparison, the average rate of improvement for patching is 0.1 logMAR per 120 hours of treatment[30].

In 2016, Kelly et al[31] compared the video game Dig Rush to patching for the treatment of amblyopia in 28 children. Dig Rush is played on an iPad with red-green anaglyphic glasses, and consists of a fellow eye contrast reduction and dichoptic presentation of game elements. The study employed a cross-in design, where half of the participants played the binocular game for 4 weeks and the other half underwent patching for 2 weeks before crossing into the binocular group for the remaining 2 weeks. After 2 weeks, the binocular game group improved an average of 0.15 logMAR compared to an average improvement of 0.07 logMAR in the patching group ($p = 0.02$). At the 4-week visit after the patching group had crossed into the binocular group, no difference was found in average visual acuity improvement between the groups.

In 2017, Bossi et al[32] studied a binocular treatment which used blurring modifications to balance visibility between the two eyes. Participants were asked to watch dichoptic movies for an hour/day for either 8 or 24 weeks, and the system applied a Gaussian blur to the fellow eye image based on the participant's initial interocular visual acuity difference. The mean improvement in visual acuity was 2.7 lines, a clinically significant gain.

1.2. Results of Luminopia Pilot Trials

The first version of the Luminopia One therapeutic was tested in a pilot trial for feasibility and safety at Boston Children's Hospital led by Dr. David Hunter and approved by the Boston Children's Hospital Institutional Review Board. The study enrolled participants aged 7+ with amblyopia who had failed prior treatment. All participants were prescribed a dosage of 1 hour per day and received either 4 or 8 weeks of treatment. Participants were able to understand the therapeutic well and use it at-home for treatment, and no instances of new heterotropias, diplopia, or other adverse events were reported.

The second version of the therapeutic was tested in an open-label, single-arm pilot trial at Houston Eye Associates led by Dr. Malcolm Mazow and Dr. Ann Stout, and approved by Alpha IRB. The mean (SD) age was 5.4 (1.2) years and the mean (SD) amblyopic eye BCVA at enrollment was 0.42 (0.12) logMAR (approximately 20/50) (unpublished data). All participants except one showed improvement in amblyopic eye BCVA, with 7 out of 10 participants gaining 0.2 logMAR (2 lines) or more and 6 out of 10 participants gaining 0.3 logMAR (3 lines) or more from baseline. The mean (SD) improvement in BCVA from baseline was 0.17 (0.09) logMAR (1.7 lines) after 4 weeks of treatment, 0.24 (0.16) logMAR (2.4 lines) after 8 weeks of treatment, and 0.29 (0.17) logMAR (2.9 lines) after 12 weeks of treatment. This is the largest known improvement in visual acuity from baseline with any binocular treatment for amblyopia to date. No adverse events were reported, and mean adherence to the prescribed dosage (1 hour per day, 7 days per week) was 77%.

The final version of Luminopia One is currently being tested in an open-label, single-arm, multicenter study enrolling children aged 4-12 at 9 centers to use Luminopia One at home for 1 hour/day, 6 days/week for 16 weeks. The study was reviewed and approved by Alpha IRB. Out of the 49 participants enrolled to date (mean age of 7.2 +/- 2.2 years), 21 participants have

completed 12 weeks of treatment with mean adherence 92% of the prescribed dose. Mean amblyopic eye BCVA improved 0.16 logMAR (1.6 lines; p=0.0014) after 12 weeks of treatment from baseline.

Although the latest study is ongoing, an analysis of the data collected across pilot trials to date demonstrates clinically and statistically significant improvements in visual acuity after 12 weeks of treatment with Luminopia One, as well as high adherence over the full treatment period.

1.3. Proposed Study Design

Study Design Overview

The proposed study is a multi-center randomized controlled clinical trial which compares mean change in amblyopic eye BCVA from baseline with Luminopia One ("therapeutic") to refractive correction ("control"). One-hundred and forty participants (n = 140) aged 4-7 years will be enrolled. Participants will be randomized 1:1 to the "therapeutic group", to use Luminopia One, or the "control group", to undergo continued refractive correction, for 12 weeks of treatment.

The objective of the study is to demonstrate the safety and efficacy of Luminopia One in amblyopia patients with amblyopia associated with anisometropia and/or with mild strabismus.

Proposed Treatment Dosage

The proposed study prescribes a treatment dosage of 1 hour per day, 6 days per week for the therapeutic group and full-time refractive correction for the control group. The selected dosage for the therapeutic group is based on pilot trials on the therapeutic and prior research with binocular treatments. Studies on binocular video games by Hess et al primarily used 1 hour per day[33], while studies on dichoptic movies and other video games used 5 hours per week[29, 34]. A treatment dosage of 1 hour per day, 6 days per week balances these two options, allowing participants to miss 1 day of the week while encouraging participants to undergo at least 5 hours of treatment per week. The selected dosage for the control group is consistent with guidelines recommended by the American Academy of Ophthalmology for treatment of amblyopia[35].

Refractive Correction Control Group

The control group of refractive correction is most appropriate for evaluating the therapeutic's safety and efficacy. Since refractive correction is the first step with all amblyopia treatments, refractive correction as a control group provides a robust baseline comparator for which any difference in effect between groups can be attributed to the therapeutic. The proposed design mimics the ATS5 RCT conducted by PEDIG, which established safety and efficacy of patching through a comparison to refractive correction[36]. In that study, the refractive correction group continued to improve, but more slowly than the patching group.

Proposed Primary Endpoint Duration

The primary endpoint duration should be sufficient to demonstrate efficacy of Luminopia One while minimizing the amount of time for which study participants are unable to use alternative amblyopia treatments. Based on these tradeoffs, a 12-week primary endpoint duration was deemed most appropriate for both the therapeutic and control groups. For the therapeutic group, pilot trials on the therapeutic have demonstrated a significant effect after 12 weeks of treatment. This treatment duration is also supported by prior research on other binocular treatments[33], where 82% of the total effect was observed within 12 weeks of treatment. For the control group,

a 12-week treatment duration allows for comparison with the therapeutic at an equivalent time point while minimizing the delay in alternative treatment.

1.4. Study Endpoints

Primary Efficacy Endpoint:

- The mean change in amblyopic eye BCVA from baseline to 12 weeks of treatment with Luminopia One compared to mean change in amblyopic eye BCVA from baseline to 12 weeks with refractive correction alone.

Primary Safety Endpoint:

- The mean change in fellow eye BCVA from baseline to 12 weeks of treatment with Luminopia One compared to mean change in fellow eye BCVA from baseline to 12 weeks with refractive correction alone.
- Additionally, the primary safety endpoint will report the frequency and severity of all related Adverse Events (anticipated and unanticipated).

1.5. Synopsis of Study Design

Major Eligibility Criteria –see Section 2.2 for complete list

- Age 4 to 7 years (inclusive)
- Amblyopia associated with anisometropia, strabismus (≤ 5Δ at distance measured by SPCT in current refractive correction), or both
- Refractive correction (if required) worn for at least 16 weeks, or until stability of VA is demonstrated (≤ 0.1 logMAR change by the same testing method measured on 2 exams at least 8 weeks apart)
- VA in the amblyopic eye 20/40 to 20/200
- VA in the fellow eye 20/32 or better
- Interocular difference ≥ 3 logMAR lines
- No prior amblyopia treatment (other than refractive correction) for > 12 months in total

Treatment Groups – see Section 3.1 and 3.2 for more information

Participants will be randomly assigned 1:1 to either:

- **Therapeutic group**: Luminopia One prescribed 1 hour per day, 6 days per week with refractive correction
- **Control group**: Refractive correction alone full-time

Sample Size – see Section 6.1 for more information

- 140 participants

Visit Schedule – see Section 3.4 for more information

- **Visit 1:** Screening and Enrollment exam
- **Phone Call 1:** 1-week phone call ± 2 days
- **Visit 2:** 4-week visit ± 1 week
- **Phone Call 2:** 6-week phone call ± 1 week
- **Visit 3:** 8-week visit ± 1 week
- **Phone Call 3:** 10-week phone call ± 1 week
- **Visit 4:** 12-week visit ± 1 week (Primary endpoint)

- **Optional Phone Call 4**: 16-week phone call ± 1 week (for those randomized to control group)
- **Optional Visit 5:** 24-week visit ± 1 week (for those randomized to control group)

Primary Endpoint Testing Procedure – see Section 3.5 for more information
At each follow-up visit, best-corrected distance visual acuity will be measured in each eye using a standardized electronic ATS-HOTV system and protocol. The ATS-HOTV electronic visual acuity testing protocol inherently incorporates testing of the visual acuity limit twice in order to increase test-retest reliability[38]. All clinical sites will follow a standardized **Visual Acuity Protocol** for visual acuity testing.

Statistical Analysis – see Section 6.4 for more information
The sample size is based on a primary efficacy analysis that will compare mean change in amblyopic eye VA from baseline to 12 weeks in the therapeutic group with the control group. A group sequential design with a single interim analysis will be used to allow stopping for either early success or futility. The interim analysis will be conducted after 75% of subjects have completed their 12-week follow-up visit. The overall Type I error rate was set at 0.05.

The primary safety endpoint will test if the mean change in best-corrected fellow-eye visual acuity in the therapeutic group is noninferior to that in the control group from baseline to 12 weeks. Additionally, the primary safety endpoint will report the frequency and severity of all related Adverse Events (anticipated and unanticipated). The interim analysis will include an early test of the primary safety endpoint as well.

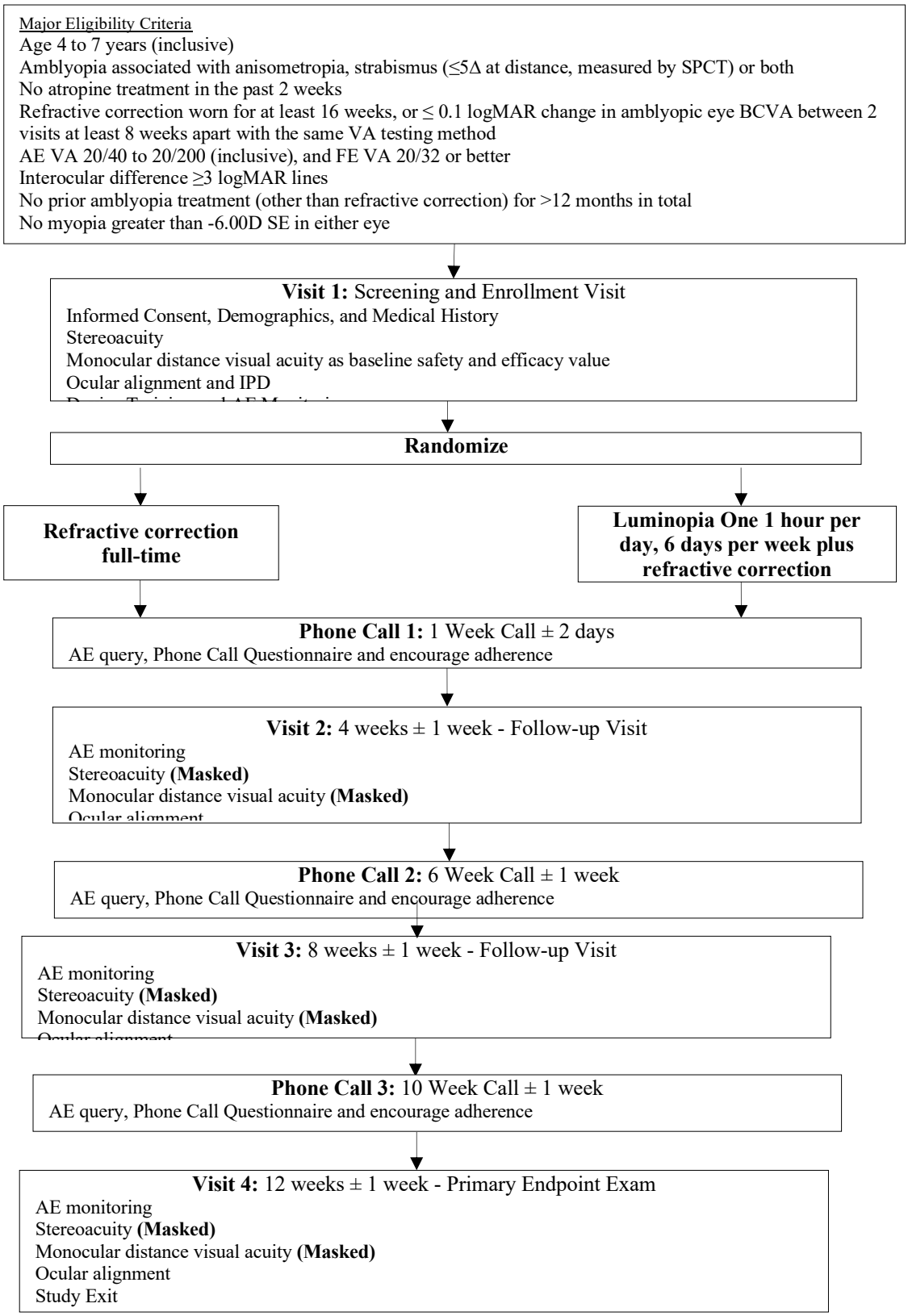
Secondary endpoints will evaluate the proportion of participants who improve 2 or more logMAR lines from baseline to 12 weeks, the mean change in stereoacuity from baseline to 4, 8, and12 weeks, the mean change in amblyopic-eye VA from baseline to 4 and 8 weeks, and the adherence from baseline to 4, 8, and 12 weeks. These endpoints will be summarized descriptively. No formal analyses are planned for secondary endpoints.

1.6. Study Administration and Oversight

Medical Monitor
Safety oversight will fall under the direction of a Medical Monitor with the appropriate medical and ophthalmological expertise. A **Safety Management Plan** will be developed prior to first participant enrollment in the trial. The **Safety Management Plan** will include expectations and the process for Medical Monitor oversight and frequency/timing of Medical Monitor review of safety events.

Contract Research Organization (CRO)
A contract research organization (CRO) will provide services surrounding data management and biostatistics for the study.

Study Summary Flow Chart (excluding Optional Visits and Calls)



2. PARTICIPANT ENROLLMENT

2.1. Informed Consent

The study plans to enroll a minimum of 140 participants.

A patient is considered for the study after undergoing a routine eye examination (by a study investigator as part of standard of care) that identifies amblyopia appearing to meet the eligibility criteria. The study will be discussed with the patient'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 must be obtained from a parent prior to any study-specific procedures that are not part of routine care being performed on the patient. Assent must be obtained from any study participant age 7 or older at time of consent.

2.2. Inclusion and Exclusion Criteria

2.2.1. Inclusion Criteria

A participant may be enrolled in the study if all of the following criteria are met:

1. Signed and dated informed consent form.
2. Parent and participant understand and are willing to comply with study procedures, and will be available for the duration of the study.
3. Age 4 to 7 years at the time of consent (inclusive).
4. Amblyopia associated with anisometropia and/or with mild strabismus, previously treated or untreated.
   - Criteria for mild strabismus: At least one of the following must be met:
     - Presence of a heterotropia on examination at distance or near fixation
     - Documented history of strabismus or strabismus surgery 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
     - ≥ 1.50 D difference in astigmatism between corresponding meridians in the two eyes
5. Must have refractive error correction (based on a cycloplegic refraction completed within the last 7 months) if any of the following are true:
   - Hypermetropia of 2.50 D or more by spherical equivalent (SE)
   - Myopia of amblyopic eye of 0.50D or more SE
   - Astigmatism of 1.00D or more
   - Anisometropia of more than 0.50D SE
6. Current refractive correction must be worn:
   - For at least 16 weeks **OR**
   - Until 2 consecutive BCVA measurements at least 8 weeks apart do not change by more than 0.1 logMAR lines

Note: Since this determination is a pre-study procedure, the method of measuring VA is not mandated.

7. Visual acuity (VA), measured in each eye without cycloplegia in current refractive correction (if required) within 7 days prior to randomization using the electronic ATS-HOTV visual acuity protocol, as follows:
   - VA in the amblyopic eye 20/40 to 20/200 inclusive
   - VA in the fellow eye 20/32 or better
   - Interocular difference ≥ 3 logMAR lines
8. Heterotropia with a distance deviation of ≤ 5Δ in current refractive correction measured by SPCT.
9. Interpupillary distance ≥ 52mm.
10. Participant understands how to use Luminopia One and wear refractive correction and has access to wireless internet at home which is able to support the therapeutic – See Section 2.5.

2.2.2. Exclusion Criteria

A participant will be excluded from the study if any of the following criteria are met:

1. Atropine treatment in the past 2 weeks.
2. Prior amblyopia treatment (other than refractive correction) for > 12 months in total.
3. Myopia greater than -6.00D spherical equivalent in either eye.
4. Previous intraocular or refractive surgery.
5. Any other condition which could be a potential cause for reduced BCVA according to the Investigator.
   - *Note: Nystagmus does not exclude the participant if the above VA criteria are met.*
6. Severe developmental or cognitive delay that would interfere with treatment or evaluation according to the Investigator.
   - *Note: Participants with mild speech delay or reading and/or learning disabilities are not excluded.*
7. History of low adherence with amblyopia treatment as assessed informally by the investigator.
8. History of light-induced seizures.

2.3. Testing Procedures at the Enrollment Visit

All examination procedures must be conducted within 7 days prior to the date of enrollment, except the cycloplegic refraction and the ocular examination, which may be performed within 7 months prior to enrollment.

All examination procedures at enrollment should be performed in the participant's current refractive correction (testing in trial frames is not permitted), and without cycloplegia:

1. Demographics and Medical History
   - The study personnel will collect demographic information from the participant and/or parent including: date of birth, sex, ethnicity, and race.
   - The study personnel will collect medical history information from the participant and/or parent including: ocular/non-ocular medical history, length of refractive correction wear, amblyopia treatment history, strabismus surgery history, and concomitant medications.

2. Stereoacuity – see Section 8.2
   - Stereoacuity testing will be performed in current refractive correction by an examiner using the Titmus Fly and Randot Preschool tests.

3. Distance VA Testing – see Section 8.3
   - Monocular distance VA testing will be performed in current refractive correction in each eye by an examiner using the electronic ATS-HOTV visual acuity protocol.

4. Ocular Alignment Testing – see Section 8.4
   - Ocular alignment will be assessed in current refractive correction by the simultaneous prism and cover test (SPCT) at distance.

5. Additional Clinical Testing
   - Ocular examination as per investigator's clinical routine (if not performed within 7 months).
   - Interpupillary distance – see Section 8.5.

6. Demonstration of Ability to Use Interventions
   - The participant must be able to use Luminopia One and be able to wear refractive correction properly.

7. AE Monitoring – see Section 5.1
   - The study personnel will query the participant and parent(s) to assess the occurrence of known and unknown adverse events during the screening visit.

2.4. Randomization of Enrolled Participants

Participants will be randomly assigned in a 1:1 allocation to one of the following treatment groups for 12 weeks:

1. **Therapeutic group**: Luminopia One therapeutic for 1 hour per day, 6 days per week with refractive correction
2. **Control group**: Refractive correction alone full-time

2.5. Training with Study Interventions

During the enrollment visit, but prior to enrollment and randomization, study personnel will train all participants on using the therapeutic. Training will consist of the participant and parent(s) watching an IRB-approved explainer video. In addition, study personnel will ask all participants to use the therapeutic which will be in "demonstration mode" and ensure that the participant understands how to complete the following activities:

1. Select a video to begin the therapeutic modifications

2. Pause/play the video
3. Change the volume of the video, and
4. Return to the menu to select a different video,

The participant will also be instructed how to put on their refractive correction. Once the participant and parent have watched the explainer video, and the participant has shown the ability to complete the aforementioned tasks within the therapeutic and to wear refractive correction properly, the participant is eligible for randomization and enrollment. As per the Inclusion Criteria (see Section 2.2.1), the participant must understand how to use **both** interventions to be eligible for randomization and enrollment.

In addition, participants must have access to wireless internet at home which is able to support the therapeutic to be eligible for the study. The participant will be withdrawn from the study if they find that videos buffer and don't play, and will be asked to return for a final follow-up visit. The participant will not count towards the study's enrollment target (see Section 6.1) and will not be included in the primary analysis population (see Section 6.5).

Study personnel will have access to a Study Coordinator Portal, which allows for enrollment of new participants. The Study Coordinator Portal takes in the age and amblyopic eye and produces a unique access code for each new participant. When the participant enters the access code into a device, the software application will apply the participant's prescription to their therapeutic.

2.6. Control Participants Optional Study Extension

Prior studies have indicated that parents may not be interested in being part of a trial if not all study participants will have the opportunity to use the investigational therapeutic. This creates a risk that parents may withdraw their child from the study after randomization if they are randomized to the control group, creating unbalanced randomization, or may decide not to participate at all. In order to mitigate this risk, and in line with studies published by PEDIG with other binocular treatments for amblyopia, the control group participants will be offered the opportunity to use the Luminopia One therapeutic for 12 weeks after the primary endpoint visit (Visit 4). Study measurements will be recorded at the end of the additional 12 weeks (Visit 5); however, only safety analysis will be conducted on data collected during this optional visit for control group participants only. Participants randomized to Luminopia One will exit the study after Visit 4 and will discuss options for continued treatment of amblyopia with their clinician.

3. TREATMENT GROUPS AND FOLLOW-UP

3.1. Therapeutic Group

Participants assigned to the therapeutic group will be prescribed Luminopia One for 1 hour per day, 6 days per week for 12 weeks. Parents will be instructed that the 1 hour of daily treatment should be completed in a single 60-minute session. Participants will be provided with the **Luminopia One Directions For Use** and **Luminopia One Quick Start Guide**, which contain instructions on how to use the therapeutic.

3.2. Control Group

Participants assigned to the control group will wear their current refractive correction full-time for 12 weeks.

3.3. Phone Calls

Unmasked study staff will call all participants at 1, 6 and 10 (and an optional call at 16) weeks to encourage adherence with the interventions, administer the phone call questionnaire and to query for Adverse Events in accordance with the **Phone Call Adverse Event Query** – see Section 8.7.

3.4. Follow-up Visit Schedule

The follow-up schedule is timed from randomization as follows:

- **Phone Call 1:** 1-week phone call ± 2 days
- **Visit 2:** 4-week visit ± 1 week
- **Phone Call 2:** 6-week phone call ± 1 week
- **Visit 3:** 8-week visit ± 1 week
- **Phone Call 3:** 10-week phone call ± 1 week
- **Visit 4:** 12-week visit ± 1 week (Primary endpoint)
  - Control group participants offered therapeutic
- **Optional Phone Call 4:** 16-week phone call ± 1 week (for control group participants only)
- **Optional Visit 5:** 24-week visit ± 1 week (for control group participants only)

3.5. Follow-up Visit Testing Procedures

Participants will be seen at follow-up visits as outlined in Section 3.4. All procedures will be performed with the participant's current refractive correction. **Visit 1 and Visit 4 visual acuity must ONLY be performed in current refractive correction glasses (not trial frames).**

The following procedures should be performed at each visit:

1. Medical History Update
   - The study personnel will inquire about any updates to medical history information from the participant and/or parent(s) at Visit 2, 3, and 4.

2. AE Monitoring – see Section 5.1
   - The study personnel will query the participant and parent(s) to assess the occurrence of known and unknown adverse events, in accordance with the **In Person Adverse Event Query** – see Section 8.7.

- The study personnel will also follow-up with any adverse events identified at previous study visits.

3. Stereoacuity – see Section 8.2
   - Stereoacuity testing will be performed in current refractive correction by a Masked Examiner using the Titmus Fly and Randot Preschool tests.

4. Distance VA Testing – see Section 8.3
   - Monocular distance VA testing will be performed in current refractive correction in each eye by a Masked Examiner using the electronic ATS-HOTV visual acuity protocol.

5. Ocular Alignment Testing – see Section 8.4
   - Ocular alignment will be assessed in current refractive correction by the simultaneous prism and cover test (SPCT) at distance.

6. Exit Questionnaire – see Section 8.6
   - Participants will be asked a questionnaire about their experience with the Luminopia One therapeutic at Visit 4 only, and at optional Visit 5 if applicable.

3.5.1. Masked Examiner

Stereoacuity and distance visual acuity testing must be completed by a Masked Examiner at follow-up visits. Prior to the Masked Examiner entering the room, participants and parents will be instructed not to discuss their treatment with the Masked Examiner. Masked Examiners will not have access to the treatment groups of participants. Whenever possible, stereoacuity and distance visual acuity testing should be completed by the **same Masked Examiner** at each visit for a given participant.

Masked Examiners will be certified during the site initiation process and will be trained on the **Visual Acuity Protocol** and **Masking Plan** which ensure that these individuals remain properly masked for the duration of the study.

3.6. Adverse Event Monitoring

All adverse events which occur during the study will be reported, regardless of whether or not they are considered to be related to treatment. The following adverse events will be specifically monitored in both the therapeutic and control groups:

1. Risks relating to amblyopia treatment
   - Diplopia
   - New/worsening heterotropia of greater than 10 prism diopters
   - Worsening of BCVA in either eye of 2 logMAR lines or greater from baseline
2. Risks relating to technological usage
   - Headaches
   - Nausea
   - Eye strain
3. New risks

See **Section 5: Adverse Events and Risks** for details relating to safety monitoring.
1.

3.7. Non-Study Visits and Treatments

Investigators may schedule additional non-study visits at their own discretion. Participants will continue to follow the study-specified follow-up schedule regardless of any non-study visits. All concomitant medications taken during the study will be recorded into the appropriate Case Report Form. Participants must not undergo any additional amblyopia treatment other than the assigned study treatment prior to study completion (either Visit 4 or Visit 5 if applicable). Refractive correction may not be changed during the study. Because of the short duration of the study, strabismus surgery is not allowed prior to the end of the study. If surgery is performed, the occurrence will be recorded in the appropriate Case Report Form and the participant will be discontinued from the trial.

4. MISCELLANEOUS CONSIDERATIONS

4.1. Study Sites

The study will include at least 10 clinical sites located throughout the US. Each clinical site will enroll at maximum 20% of the entire enrolled population. The randomization of participants at each site will be 1:1.

4.2. Participant Withdrawals

Parents or participants may withdraw from the study at any time. If the parents indicate that they want to withdraw their child from the study, the participant should be asked to come in for a study exit visit and to return the therapeutic (if applicable).

In addition, the Investigator may decide to discontinue a participant from the study for safety reasons or when it is in the best interest of the subject. Reasons for participant withdrawal may include but are not limited to the following:

- At the participant's request for any reason.
- At the Investigator's request due to safety concerns, AEs, or other medical reasons.
- Due to non-compliance (e.g., failure to follow application instructions, missing visits) or other protocol violation.
- When a participant is lost to follow-up.
- When a participant is erroneously enrolled into the study or does not meet eligibility criteria.

Participants who do not complete the entire study should be fully evaluated when possible. If, for any reason, a participant is discontinued before completing the primary endpoint visit, the subject should return and perform all the assessments scheduled for the primary endpoint visit. Notification of a participant discontinuation and the reason for discontinuation will be clearly documented on the participant's CRF.

4.3. Participant Compensation

Parents/participants will be compensated $25 by Amazon gift card at each visit for Visits 1 through 4, up to a total of $100.

4.4. Study Costs

The participant or their insurance provider will be responsible for costs incurred during the study that are considered standard of care, whereas procedures and visits not considered standard of care will be paid for by the study. The cost of the Luminopia One therapeutic and associated equipment will be paid for by the study; however, the therapeutic will need to be returned upon study completion. The cost of refractive correction will not be paid for by the study as refractive correction is standard care.

4.5. General Considerations

The study will be conducted in compliance with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, with ICH E6 and the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and with the standards of Good Clinical Practice.

5. ADVERSE EVENTS AND RISKS

The following section outlines the Adverse Events and Risks for the study. For more information, please refer to the **Safety Management Plan.**

5.1. Definition

An AE is any untoward medical occurrence, unfavorable and unintended sign (including an abnormal lab finding), symptom, or disease in a clinical investigation associated with the use of an investigational product, whether or not considered related to the investigational product.

The following specific adverse events will be monitored throughout the study:

1. Risks relating to amblyopia treatment
    - Diplopia
    - New/worsening heterotropia
        - New heterotropia: any new ocular deviation in a participant without a tropia at baseline
        - Worsening heterotropia: an increase in ocular deviation ≥ 10Δ in a participant with a tropia at baseline
    - Worsening of BCVA in either eye
        - A decrease ≥ 2 logMAR lines from baseline
2. Risks relating to technological usage
    - Headaches
    - Nausea
    - Eye strain

5.2. Recording of Adverse Events

Throughout the course of the study, all efforts will be made to remain alert to possible adverse events. The first concern will be the safety of the study participants, and appropriate medical intervention will be made accordingly.

All reportable adverse events, whether discovered by study personnel during AE monitoring, through physical examination, laboratory tests, or other means will be recorded on an Adverse Event Case Report Form.

Study personnel will also capture the following information for each adverse event:

- Eye (OD, OS, OU, or non-ocular)
- Relatedness to study interventions
- Intensity
- Onset Date
- Serious or Non-Serious
- Action Taken
- Resolution Date

<u>Relatedness</u>

The study investigator will assess the relationship of any adverse event to treatment to determine if there is a reasonable possibility that the adverse event may have been caused by treatment. To ensure consistency of adverse event assessments, investigators should apply the following general guidelines when evaluating whether an adverse event is related to treatment:

Definite: A clear cut causal relationship with the study device and no other possible cause;
Probable: A causal relationship with the study device is likely although alternate etiologies are also possible;
Possible: A causal relationship with the study device is not definite, and alternate etiologies are also possible;
Not related: The AE has no causal relationship to the study device and/or there is evidence of alternative etiology such as concurrent medication or illness;
Not applicable: The participant has not been exposed to the study device.

Intensity
The maximum intensity that occurred since the onset of an adverse event will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe, categorized as follows:

Mild: Symptom(s) barely noticeable to participant or does not make participant uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s).
Moderate: Symptom(s) of sufficient severity to make participant uncomfortable; performance of daily activity is influenced; participant is able to continue in study; treatment for symptom(s) may be needed.
Severe: Symptom(s) cause severe discomfort; severity may cause cessation of treatment with study medication or device; treatment for symptom(s) may be given and/or participant hospitalized

It is emphasized that the term severe is a measure of intensity: thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

Serious or Non-Serious
A serious adverse event (SAE) is any untoward occurrence that:

- Results in death.
- Is life-threatening (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (sight-threatening).
- Is considered a significant medical event by the investigator based on medical judgment (ex. may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above).

A non-serious adverse event is any adverse event which is not categorized as a serious adverse event.

An unanticipated adverse device event (UADE) is defined as a serious adverse event caused by, or associated with, a device, if that effect was not previously identified in nature, severity, or degree of incidence.

Resolution Date

Adverse events that occur during the study will be followed until resolution. Adverse events which continue after the participant's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

5.3. SAE and UADE Reporting Requirements

Study investigators are required to report any instance of an SAE or UADE to the study sponsor, reviewing IRBs, and Medical Monitor no later than 10 working days after the Investigator first learns of the effect. The study sponsor is responsible for conducting an evaluation of the UADE and will report the results of the evaluation to FDA, the Medical Monitor, all reviewing IRBs, and participating Investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effect as FDA requests.

5.4. Risks

Standard of care treatment for amblyopia comes with the accepted risks of developing a new/worsening heterotropia or diplopia. However, the risk of developing new/worsening heterotropias or developing diplopia from the Luminopia One therapeutic is expected to be no greater than it would be with standard of care. In a 2016 study comparing binocular treatment to patching, the rate of new heterotropias was 8.8% in the binocular group and 5.9% in the patching group, a statistically and clinically insignificant difference[33]. The same study showed that diplopia was rare after both treatments[33].

The procedures to be conducted in the study during visits are part of daily eye-care practice and pose no known risks. As part of a routine eye-care exam, the participant may receive cycloplegic eye drops.

The participants in both groups will be unable to perform any amblyopia treatment other than the intervention they are prescribed for the duration the study. Given the relatively short study duration of 12 weeks, we believe the risk of delaying standard of care treatment is minimal and justified.

Extended screen time with technological products in general are associated with the risks of nausea, headaches, and eye-strain. Prevalence of these symptoms will be monitored throughout the study, and the **Luminopia One Directions For Use** provide additional warnings for these symptoms. More information can be found in the **Clinical Risk Benefit Analysis** (CRBA) and the **Investigator's Brochure** for Luminopia One.

5.5. 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. It is also believed that the Luminopia One is a non-significant risk (NSR) device as per FDA 21 CFR 812.66 because:

- by design it is NOT intended as an implant and does NOT presents a potential for serious risk to the health, safety, or welfare of a subject;

- based on the device's Indication For Use it is NOT purported or represented to be for use supporting or sustaining human life and does NOT present a potential for serious risk to the health, safety, or welfare of a subject;

- based on the device's Indication For Use it is NOT for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject;

- by design it does NOT present a potential for serious risk to the health, safety, or welfare of a subject.

Please refer to the **Study Risk Determination** and the **Clinical Risk Benefit Analysis** for additional justification.

5.6. Clinical Monitoring

The sponsor is responsible for ensuring that adequate monitoring at each site is completed to guarantee protection of the rights and safety of participants and the quality and integrity of the data collected and submitted. Monitors must be appropriately trained and qualified to check for adherence to the investigational plan and the signed Investigator agreement, compliance to the IRB conditions and guidelines, and compliance to applicable regulations. Any non-compliance with these items that is not adequately addressed by the Investigator and/or study personnel is cause for the sponsor to put the site on hold or withdraw the Investigator site or study personnel from participation in the study.

During a monitoring visit, the monitor may review source documents, CRFs, and informed consent forms for a representative number of participants. Frequency of monitoring visits will be based upon enrollment, study duration, compliance and any suspected inconsistency in data that requires investigation. Both on-site monitoring and remote monitoring will be conducted.

A separate detailed **Monitoring Plan** will be prepared prior to the start of the study and maintained in the Trial Master File with the sponsor and CRO.

6. STATISTICAL CONSIDERATIONS

The approach to sample size and statistical analyses are summarized below. A detailed **Statistical Analysis Plan** will be written and finalized without knowledge of study data. The analysis plan synopsis in this chapter contains the framework of the anticipated final analysis plan.

6.1. Sample Size Estimation

Sample size estimates were based on data from previous PEDIG studies (ATS3[20], ATS5[36], ATS18[33] and ATS20) and data from participants in Luminopia One pilot trials who would meet the eligibility criteria for the current protocol.

Control Group –Refractive Correction
To estimate the mean change in amblyopic eye visual acuity for the primary efficacy endpoint, data were reviewed from participants aged 4 to 6 years who were randomly assigned to continue refractive correction alone in a previous PEDIG study (ATS5, see Table 1). The mean change in VA at 5 weeks was 0.55 logMAR lines (95% CI: 0.19 to 0.90 logMAR lines) with standard deviation 1.28 logMAR lines (95% CI: 1.07 to 1.58 logMAR lines).

Given the more stringent eligibility criteria in the proposed study as compared to the previous study, we anticipate that the magnitude and standard deviation of mean change in visual acuity after 12 weeks with refractive correction in the current study will be smaller than those in Table 1.

To estimate the mean change in fellow eye visual acuity for the primary safety endpoint, data were reviewed from participants who were randomly assigned to continue refractive correction alone in a previous PEDIG study (ATS5, see Table 2). The mean change in VA at 5 weeks was 0.02 logMAR lines (95% CI: 0.00 to 0.04 logMAR lines) with standard deviation 0.10 logMAR lines (95% CI: 0.08 to 0.12 logMAR lines).

Therapeutic Group – Luminopia One
To estimate the mean change in amblyopic eye visual acuity for the primary efficacy endpoint, data from 3 studies were reviewed: (1) data for participants randomly assigned to a similar treatment of "Dig Rush" on an iPad device (E. Birch Study); (2) data from a previous PEDIG trial (ATS18) for participants randomly assigned to a similar treatment of "Hess falling blocks" on an iPad device; and (3) data from Luminopia One pilot trials (Table 1).

To estimate the mean change in fellow eye visual acuity for the primary safety endpoint, data were reviewed from participants aged 4 to 7 years enrolled in Luminopia One pilot trials. The mean change in VA at 12 weeks was 0.05 logMAR lines (95% CI: 0.02 to 0.08 logMAR lines) with standard deviation 0.10 logMAR lines (95% CI: 0.08 to 0.13 logMAR lines).

Table 1: Previous Study Data (Primary Efficacy Endpoint Reference)

Cohort	N	Change in Amblyopic Eye VA at the 4 or 5 Week Visit (logMAR Lines)		N	Change in Amblyopic Eye VA at 8-12 Week Visit (logMAR Lines) ‡	
		Mean Change (95% CI)	SD Change (95% CI)		Mean Change (95% CI)	SD Change (95% CI)
Refractive Correction Alone:						
ATS5: Age 3 to 6 years [1]	53	0.55 (0.20 to 0.89)	1.28 (1.07 to 1.58)		n/a	n/a
Binocular Therapeutic:						
E. Birch Study (Age 4 to 6 years) [2]	9	1.67 (0.89 to 2.45)	1.19 (0.80 to 2.28)		n/a	n/a
Luminopia Pilot Trials (Age 4 to 7 years) [3]	34	1.36 (0.96 to 1.76)	1.18 (0.95 to 1.55)	35	1.64 (1.09 to 2.19)	1.66 (1.34 to 2.17)
ATS18 (Age 5 to 6 years) [4]	39	1.15 (0.73 to 1.57)	1.35 (1.10 to 1.74)	39	1.23 (0.79 to 1.67)	1.39 (1.14 to 1.79)
Patching Therapeutic:						
ATS18 (Age 5 to 6 years) [4]	50	0.90 (0.50 to 1.30)	1.45 (1.21 to 1.81)	48	1.43 (0.98 to 1.88)	1.62 (1.35 to 2.02)

[1] ATS5 data were limited to randomized participants with an amblyopic-eye VA of 20/40 to 20/200 inclusive with ≥ 3 lines of interocular difference and a fellow-eye VA of 20/25 or better who had stabilized with refractive correction prior to randomization. The baseline magnitude of tropia at near (as measured by SPCT) was limited to <5pd. The outcome data reported were based on the 5-week primary masked outcome visit.
[2] E. Birch study enrolled children aged 4 to <7 years of age. Change in VA after 4 weeks of binocular therapy was computed for participants randomly assigned to receive binocular the Dig Rush game on an iPad device for 4 weeks (prescribed 1 hour per day, 5 days per week without patching). Participants with >4pd magnitude of strabismus (measured by PACT) were excluded from the study
[3] Data from Luminopia pilot trials are limited to participants 4 to 7 years old with ocular alignment ≤5pd.
[4] ATS18 study participants were prescribed binocular therapy for 1 hour per day, 7 days per week vs 2-hours per day of patching. The binocular game, Hess falling blocks, was played on an iPad device. The outcome data reported are from the 4-week and 8-week masked outcome visits.

Table 2: Previous Study Data (Primary Safety Endpoint Reference)

Cohort	N	Change in Fellow Eye VA at the 4 or 5 Week Visit (logMAR Lines)		N	Change in Fellow Eye VA at 12 Week Visit (logMAR Lines) ‡	
		Mean Change (95% CI)	SD Change (95% CI)		Mean Change (95% CI)	SD Change (95% CI)
Refractive Correction Alone:						
ATS5: Age 3 to 6 years [1]	68	0.02 (0.00 to 0.04)	0.10 (0.08 to 0.12)			
Binocular :						
Luminopia Pilot Trials (Age 4 to 7 years) [3]	36	0.00 (-0.02 to 0.02)	0.05 (0.04 to 0.06)	35	0.05 (0.02 to 0.08)	0.10 (0.08 to 0.13)

Effect Size Estimates

Efficacy:

Based on Luminopia One pilot trials (Table 1), the estimate for mean change in amblyopic eye visual acuity for the primary efficacy endpoint is 1.64 logMAR lines in the therapeutic group and 0.45 logMAR lines in the control group, with a pooled standard deviation of 1.40 logMAR lines.

The true difference between the therapeutic and refractive correction might be as large as 1.19 logMAR lines; however, a conservative approach was taken to detect a true difference as small

as 0.75 logMAR, and therefore a group difference of 0.75 logMAR will be used to estimate sample size.

Safety:
Safety will be measured as change of visual acuity in the fellow eye. This is a noninferiority analysis to demonstrate that change of VA in the fellow eye when using Luminopia One is not worse than that observed in the control group.

Based on previous studies (Table 2), the estimate for mean change in fellow eye visual acuity for the primary safety endpoint is 0.05 logMAR lines in the therapeutic group and 0.02 logMAR lines in the control group, with a pooled standard deviation of 0.10 logMAR lines.

A noninferiority margin of 1.0 logMAR lines was determined to be most appropriate, given that 1.0 logMAR line is the minimum detectable difference in clinical practice with a standard eye chart for this age group[38]. Consequently, 1.0 logMAR line would be the smallest unacceptable difference in fellow eye visual acuity change between the therapeutic and control groups, and therefore the most appropriate noninferiority margin.

Additionally, the primary safety endpoint will report the frequency and severity of all related Adverse Events (anticipated and unanticipated).

Group Sequential Design:
The sample size for this trial will be based on a group sequential design with a single interim analysis to allow for stopping due to either early success (rejecting the null hypothesis) or futility. The goal of the group sequential design is to conserve precious time and resources and prevent unnecessary enrollment of participants if there is no observed difference in effect between groups **or** if there is a significant effect in favor of Luminopia One. It is important to allow for early stopping for success since there is a real possibility the therapeutic could perform significantly better than expected.

Boundaries for both alpha and beta will be calculated using a power cumulative error spending function. The mathematical form of this spending function is:

$$E(t,\rho) = \begin{cases} 1 \; if \; t \geq 1 \\ t^{\rho} \; if \; 0 < t < 1 \\ 0 \; otherwise \end{cases}$$

where ρ is the power parameter and t is the information fraction.

For beta spending, rho was set to 1.0 which results in a spending function much like the Pocock method (i.e. equal spending between the interim and final analyses). For alpha spending, rho was set to 2.0 resulting a spending function somewhere between that found for the O'Brien-Fleming method and the Pocock method.

The planned interim analysis will also involve an analysis of the primary safety endpoint, including both the test of noninferiority in mean fellow eye BCVA change between groups and a report of the frequency and severity of all related Adverse Events (anticipated and unanticipated).

Sample Size Estimation

The sample size was calculated using the SEQDESIGN procedure in SAS version 9.3. The alternative reference was set to 0.75. As part of the design statement, the number of analyses was set to 2 where the first analysis was planned after 75% of the expected participants complete their 12-week primary outcome visit and the second after all participants complete their 12-week primary outcome visit. The method of alpha spending was set to "errfuncpow(rho=2.0)" and the method of beta spending was set to "errfuncpow(rho=1.0)." The "alt" parameter was set to "upper" to indicate a one-sided test, alpha was set to 0.05, and beta was set to 0.10 in order to achieve 90% power. The "stop" parameter was set to "both" in order to have boundaries for both success and futility.

This specification results in an effective sample size of 132 participants (66 per arm) providing 90% power to detect a 0.75 logMAR line difference between the therapeutic and control groups using a one-sided test (primary **efficacy** endpoint).

With an equal beta-spending function, if there truly is no treatment effect favoring Luminopia One, the chance of stopping the study early for futility at the interim analysis is approximately 89%.

The primary **safety** endpoint will test noninferiority in fellow eye visual acuity change from baseline to 12 weeks endpoint. The sample size required to achieve 90% power, for a non-inferiority window of 1.0 logMAR line with Type I error rate of 0.05, is fewer than 132 participants. Therefore, the primary efficacy endpoint will be used to drive sample size.

Adjusting the effective sample size for a 5% loss to follow-up results in a target enrollment of **140 subjects**.

6.2. Masked Sample Size Re-estimation

The sample size estimates are based on previous studies of refractive correction and binocular treatments. Although we believe that our estimates of variance are reasonable, a sample size re-estimation will be performed once 50% of the pre-planned number of participants have completed the primary endpoint visit. A pooled estimate of variance without respect to treatment group will be calculated and used to re-estimate sample size using a procedure that maintains masking and has a negligible effect on the Type I error rate, as the data of treatment effect will remain masked. If the observed standard deviation is larger than the pre-study estimate, the sample size will be increased to maintain adequate power. The masked sample size re-estimation will be conducted separately from the interim analysis.

6.3. Bias and Error Rates

Minimizing Bias

The interim analysis will be designed to optimize usage of time and resources while controlling the Type I and II error rates and minimizing operational bias, in accordance with FDA's guidance on Adaptive Designs for Medical Devices.

Type I and II Error Rates

Analyzing interim data during the study can lead to Type I error inflation and increase the chance of a false positive conclusion. The proposed group sequential design controls for Type I error inflation by using the well-established statistical method of alpha-spending, so the Type I error for the overall study is controlled at an acceptable level. A similar approach of a beta-spending function is used to keep the Type II error for the overall study at an acceptable level.

Operational Bias

The details of the group sequential design will be included in the full **Statistical Analysis Plan** and approved prior to collection of study data. To avoid any change in behavior between study personnel and participants before and after the interim analysis, the interim analysis will be conducted by the CRO and Sponsor without the involvement of participating investigators. The Medical Monitor will be informed of the interim analysis findings.

In the event that the study is stopped early for success or futility, randomization will be halted and the Medical Monitor will be asked to make a recommendation as to whether follow-up exams for existing participants should continue.

6.4. Analyses

6.4.1. Primary Efficacy Analysis

The primary analysis of efficacy will compare the mean change in amblyopic eye BCVA from baseline after 12 weeks (Visit 4) of treatment with Luminopia One compared to continued refractive correction. The 12-week visit in the therapeutic and control groups will determine success and failure of this endpoint. An analysis of variance will be performed to compare the therapeutic and control groups at 12 weeks. The mean change in amblyopic-eye VA for the therapeutic and control will be used as the dependent variable. The independent predictors will be the randomized treatment group (therapeutic/control). A standard F-test will be used to determine if significant differences exist in mean change from baseline between groups. A 95% confidence interval on the difference between groups will also be provided. If the mean change from baseline in VA is significantly larger for the therapeutic group over the control group then the primary efficacy endpoint will have been successfully passed.

The linear model used for this analysis is as follows:

$$\mu_{ijk} = \alpha + \beta_i * Group + \varepsilon_{ijk}$$

where α is the intercept, ßi is the coefficient associated with treatment group, coded 1 for the therapeutic group and 0 for the control group.

The formal hypothesis being tested is:

$$Ho: \beta_i \leq 0$$
$$Ha: \beta_i > 0$$

Testing will be done at the 0.05 level of significance.

6.4.2. Primary Safety Analysis

The coprimary analysis of safety will test if the mean change in fellow eye visual acuity of the therapeutic group from baseline to 12 weeks is noninferior to the mean change in fellow eye visual acuity of control group within a margin of 1.0 logMAR lines. The formal hypotheses being tested are:

$$Ho: \mu_{control} - \mu_{therapeutic} \leq \delta$$
$$Ha; \mu_{control} - \mu_{therapeutic} > \delta$$

where μ is the change from baseline for both the control and therapeutic subjects and δ is the non-inferiority window. The statistical test is a two-sample T-test which will be done at the 0.05 level of significance. In addition to the formal test on the mean change in fellow eye visual acuity, the primary safety endpoint will report the frequency and severity of all related Adverse Events (anticipated and unanticipated).

6.4.3. Subpopulation Analysis

The primary efficacy analysis will be redone on various subpopulations. Those subpopulations are type of amblyopia (anisometropia without vs with strabismus) , age (≤5, >5), and baseline VA where the range of baseline VA is split into two groups (better than 20/100, 20/100 or worse). The proportion of amblyopic eyes demonstrating each number of lines of improvement in BCVA at each study visit will also be reported. These analyses are not powered and are only supportive in nature.

6.4.4. Study Success Definition

The overall trial will be deemed a success if both the primary efficacy and primary safety endpoints are passed.

6.4.5. Secondary Analyses

6.4.5.1. VA Improvement at 12 Weeks of 2.0 or more logMAR Defined as a Binary Outcome

A secondary analysis will estimate the proportion of participants with amblyopic eye BCVA improvement of ≥ 2 logMAR lines at 12 weeks (Visit 4) after baseline. An exact 95% confidence interval will also be given along with the estimate.

6.4.5.2.

6.4.5.3. Stereoacuity Improvement at 4, 8 and 12 Weeks

A secondary analysis will report the mean change in stereoacuity from baseline to 4, 8 and 12 weeks along with 95% confidence intervals about the mean change.

6.4.5.4. VA Improvement at 4 and 8 Weeks

A secondary analysis will repeat the primary analysis except for the 4 and 8 week visit instead. Ninety-five percent confidence intervals will also be given.

6.4.5.5. Adherence at 4, 8, and 12 Weeks

A secondary analysis will report the mean adherence from baseline to 4, 8, and 12 weeks along with 95% confidence intervals about the mean adherence in the therapeutic group.

6.4.6. Safety Reporting

6.4.6.1. Adverse Events

The participant and parent(s) will be asked about known and unknown adverse events at each visit and phone call. The frequency of known and unknown adverse events will be summarized for all subjects.

6.5. Analysis Populations

The intent to treat (ITT) population will be those participants enrolled and randomized. For the purposes of analysis of the primary endpoints, subjects will be analyzed in the group to which they were assigned regardless of the treatment actually received. The ITT population will not include participants who do not meet eligibility criteria and are ultimately deemed ineligible for the study, including participants who are withdrawn from the study due to insufficient internet.

The as treated population (AT) will be those participants enrolled and randomized; however, treatment group assignment for the purposes of analysis will be based on the actual treatment type received.

The per protocol population (PP) is defined as those participants enrolled, randomized, who have adherence > 25%, and who have no major protocol deviations that could affect the endpoints of interest. The 25% adherence threshold for the PP population was determined from the first pilot trial on Luminopia One, in which all enrolled participants with > 25% adherence and no major protocol deviations showed improvements from using the therapeutic. The adherence data will be captured during follow-up visits and calls for the control group and with electronic tracking in the therapeutic group. Adherence will be captured while maintain masking of the study in accordance with the **Masking Plan**.

The ITT population will be the population used for analysis and interpretation of the primary and secondary efficacy endpoints. The analysis for the primary and secondary efficacy endpoints will also be conducted on the PP population and the AT population and these analyses will be considered supportive in nature. The AT population will be used to assess and characterize all safety endpoints.

Additional approaches to the primary analysis will also include participants who completed the primary endpoint visit outside of the pre-defined visit window. These will be considered supportive in nature.

6.6. Missing Data

Multiple imputation for missing data will be performed as a secondary approach with the ITT population, and results of the analysis with imputation of missing data will be considered supportive in nature and assessed for consistency with the primary analysis. Imputation will be based on regression methods using baseline covariates as independent predictors. If no significant predictors can be found, the following method will be used. Those subjects completing the study will be categorized by age, sex, and treatment received. For a subject with missing data, the imputed value will be a random draw from those subjects with data that have the same age, sex, and treatment.

6.7. Site Poolability

Data from this trial will be pooled across study site for the purposes of analysis. To demonstrate poolability of the primary endpoint data, the change in amblyopic eye BCVA between baseline and 12 weeks will be calculated for each subject. This value will be used as the dependent variable in a linear model. The independent predictors will be study site, treatment group, and a study site by treatment group interaction. If the interaction term is considered non-significant then the data will be pooled across site for the purposes of analysis. If the interaction term is considered significant then the analysis of the primary endpoint will have to be adjusted for study site. A significance level of 0.15 will be used for this analysis.

6.8. Data Handling and Record Keeping

Source documentation is defined as the first place where data is recorded. This includes, but is not limited to, medical records (paper and electronic), test results and reports, and study worksheets. Original source documentation must be maintained at each site to substantiate data entered on case report forms. Source data must be made available by the Investigator to facilitate monitoring and audits. Monitors must have access to medical records sufficient to ensure the completeness and accuracy of reported data.

Investigators are required to maintain complete and accurate records pertaining to the study. Relevant records include:

1. IRB approvals, renewals, and withdrawals;
2. Records of interventions used in the study, including traceability to the participant that received the intervention;
3. Records of any intervention returned to the sponsor;
4. Signed informed consent form(s) for each enrolled participant;
5. Completed case report forms;
6. Records of all adverse events and supporting documentation;
7. Copies of the current approved version of the protocol.

Case Report Forms should be submitted within 10 business days after the corresponding visit. However, adverse events should be reported according to the timelines in Section 5.3. Investigators are responsible for notifying the sponsor of any changes to previously reported data.

Records should be maintained for at least 2 years following the completion or termination of the investigation or the time dictated by national guidelines if longer.

6.9. Publication Policy

This study is designed to gather and report clinically relevant information for the advancement of patient care. It is intended that peer-reviewed publications/abstracts will be generated to report the primary study results. The study's investigators and sponsor will have priority to author the primary manuscript(s) and initial abstract(s) related to overall study results. At the discretion of the sponsor, contributors to the writing of these pieces may be sought from Investigators who have contributed most significantly to the study. Contribution considerations include but are not limited to protocol compliance, data accuracy/completeness, number of participants enrolled, and willingness to adhere to established guidelines outlined in the International Committee of Medical Journal Editors (ICMJE).

Sites shall be prohibited from individually publishing any reports, abstracts, articles or data compilations concerning the study until its termination or a notification from the sponsor that the main study manuscript and/or abstract(s) have been accepted for publication/presentation. Additionally, the sponsor shall be provided a period of time for prior written notice to review and comment on any publications by a site concerning the study data. If the sponsor, in its reasonable judgment, needs additional time to seek patent protection for the information or to remove confidential information, the sponsor can have the submission or publication deferred for an agreed upon period of time. The sponsor alone reserves ownership of all data collected during this study and publication rights regarding any endpoints or hypotheses not specifically outlined in this investigational plan.

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8. APPENDICES

8.1. Appendix 1: Schedule of Events Table

Procedures	**Visit 1**: Screening and Enrollment	**Phone Call 1**: Week 1 Call	**Visit 2**: Week 4 Visit	**Phone Call 2**: Week 6 Call	**Visit 3**: Week 8 Visit	**Phone Call 3**: Week 10 Call	**Visit 4**: Week 12 Visit	**Optional Phone Call 4:** Week 16 (Control participants only)	**Optional Visit 5:** Week 24 (Control participants only)
Informed consent	X								
Demographics & Medical history	X								
Randomization	X								
Therapeutic administration and training	X								
Refraction (if applicable)	X								
Phone call questionnaire		X		X		X		X	
Concomitant Tx	X		X		X		X		X
AE monitoring	X	X	X	X	X	X	X	X	X
Medical history update			X		X		X		X
Adherence			X		X		X		X
Distance visual acuity	X		X		X		X		X
Stereoacuity	X		X		X		X		X
Ocular alignment	X		X		X		X		X
Interpupillary distance	X								
Exit questionnaire							X*		X
Compensation	X		X		X		X		
Recollect therapeutic							X*		X

*Luminopia One active group only

8.2. Appendix 2: Stereoacuity Testing

Titmus Fly

The Titmus Fly test is to be conducted with current refractive correction. The testing distance is 40 cm. If the participant identifies the fly correctly, their stereoacuity is presumed to be 3000 seconds of arc or better. If the participant does not identify the fly correctly their stereoacuity is presumed to be 10000 seconds of arc or worse.

Procedure

- Place Polaroid glasses on subject (over prescription glasses or contact lenses, if worn) before opening the test booklet.
- Display the fly. Ask the subject to pinch the tips of the wings.

Randot Preschool

The Randot Preschool test measures random dot stereoacuity from 800 to 40 arc seconds (800, 400, 200, 100, 60, 40). The Randot Preschool test consists of 3 booklets each with 2 sets of 4 random dot shapes, which can be matched to non-stereo shapes on the opposite side of the booklets. There are six levels (seconds of arc) in the test with two levels in each book. Each level has 4 rectangles that contain 3 shapes and one blank. Testing order is Book 3, Book 1, Book 2.

Procedure

1. As a pretest, use Book 3.
2. Point to the top 4 panels on the non-stereo side (black on white shapes) and ask, “Can you point to the duck?” If the child does not correctly identify the duck, do not proceed with the rest of the test.
3. Starting with Book 3, turn to the Randot side of the test booklet starting with the top level, point to one of the boxes containing a Randot shape, and ask the subject what shape is in the box. The child should be encouraged to match one of the black and white shapes to the Randot shape.
4. Continue by pointing to another shape at the same level. For each shape, indicate whether the patient identified correctly or incorrectly by tapping the image.
5. If 2 shapes are identified correctly at a level, testing will proceed to the next level.
6. If 2 shapes are identified incorrectly at a level, testing will stop at the current level.
7. The final score will be calculated as the lowest level of seconds of arc measured at which 2 shapes were correctly identified.

The final stereoacuity measurement used for analysis for each visit is the higher measurement between the Titmus Fly and the Randot Preschool tests.

8.3. Appendix 3: Distance VA Testing

Visual acuity testing is to be performed using the ATS-HOTV testing protocol with single surrounded optotypes on an electronic visual acuity (EVA) system supported by the EMMES Corporation or obtained from M&S Technologies. The **Visual Acuity Protocol** contains further details on the conduct of this procedure.

The examiner will test the participant's amblyopic eye visual acuity first, followed by the participant's fellow eye visual acuity.

The ATS-HOTV electronic visual acuity testing protocol inherently incorporates testing of the visual acuity limit twice in order to increase test-retest reliability[38]. The ATS-HOTV programs on the EVA system automatically execute the following Visual Acuity Testing Protocol:

Single letters with surround bars are presented in four phases:

Screening Phase I Reinforcement Phase II

- In Phase I and II, up to 4 single letters are sequentially presented at each logMAR level that is tested.
- LogMAR levels are: 20/800, 20/640, 20/500, 20/400, 20/320, 20/250, 20/200, 20/160, 20/125, 20/100, 20/80, 20/63, 20/50, 20/40, 20/32, 20/25, 20/20, 20/16, 20/12
- A level is "passed" if 3 of 3 or 3 of 4 letters are correct and "failed" if 2 letters are missed.
- Testing of a level stops as soon as criteria are met for either "pass" or "fail".

Screening Phase

1. Tester selects 20/100 or 20/400 size letter to present as a starting point, based on prior knowledge about the participant's visual acuity.
2. If response is correct, letter at next smallest logMAR level is presented and testing continues sequentially with one letter per logMAR level towards 20/20 until there is an incorrect response.
3. If starting point was 20/100 and response is incorrect at either 20/100 or 20/80, screening is restarted at 20/400.
4. If screening starts at 20/400 and response is incorrect, testing continues sequentially with one letter per logMAR level towards 20/800 until there is a correct response.
5. If 20/800 is missed, 20/800 becomes the starting level for phase I.

Phase I

1. Up to 4 single letters are sequentially presented 2 logMAR levels above the level missed in screening.
    a. Exception: If 20/20 was correct in Screening, phase I starts at 20/30.
    b. Exception: If 20/800 or 20/640 was missed in Screening, phase I starts at 20/800.
2. If the first level tested is failed, testing continues at sequentially larger logMAR levels until a level is passed.
    a. If 20/800 is failed, phase I ends, the Reinforcement phase is skipped, and 20/800 is retested in phase II.
3. If the first level tested is passed, testing continues at sequentially smaller logMAR levels until a level is failed.

Reinforcement Phase

1. Starting 3 levels larger than the level missed in phase I, 3 successively smaller single letters are presented.
    a. Exception: If the level failed in phase I is 20/500 or 20/640, 3 20/800 letters are shown for reinforcement.
    b. Note: The reinforcement phase responses do not contribute to the visual acuity score and even if the responses are incorrect, the test proceeds to phase II.

Phase II

1. Up to 4 single letters are sequentially presented at the last level missed in phase I.
2. If the level is failed, testing stops.
3. If the level is passed, testing continues at sequentially smaller logMAR levels until a level is failed.

Final Visual Acuity Score

The visual acuity score is the smallest logMAR level passed in Phase I or Phase II.

8.4. Appendix 4: Ocular Alignment (Simultaneous Prism Cover Test)

The Simultaneous Prism Cover Test (SPCT) is to be conducted with current refractive correction. The SPCT is used to measure a tropia. The SPCT is performed at distance (3m) fixation using an accommodative target (never a fixation light).

Procedure

1. Determine the fixating eye by inspection and/or a cover test.
2. Rapidly and simultaneously, position a cover before the fixating eye while placing a prism before the deviating eye.
3. Watch for movement of the nonfixating eye. The cover and prism are quickly removed and the binocular state reestablished.
4. Repeat steps #2 and #3, increasing the power of the prism until a reversal of the movement of the deviating eye is seen, where the prism is overcorrecting the deviation. Record the magnitude of prism that either neutralized the deviation or was closest to neutralizing the deviation.

8.5. Appendix 5: Interpupillary Distance

Interpupillary Distance (IPD) can either be measured by a ruler or by a pupilometer. The procedure for measuring IPD with a ruler is as follows:

Procedure

1. Examiner stands at 40 cm (16 inches).
2. Examiner closes right eye, participant fixes on examiner's left eye.
3. Examiner lines up the ruler zero point on the participant's right pupil, left pupillary border, or left limbus.
4. Examiner closes left eye, participant fixes on examiner's right eye.
5. Examiner reads scale directly in line with participant's life pupil center, left pupillary border, or left limbus.
6. Examiner closes right eye, participant fixes on examiner's left eye.
7. Examiner checks to make sure that zero point is still correct.

Common Difficulties and Solutions

8. If participant cannot close one eye, the eye can be occluded with a free hand.
9. If participant has strabismus, cover the participant's eye which is not being observed.
10. If participant is uncooperative, take a canthus-to-canthus measurement.

The procedure for measuring IPD with a pupilometer is as follows:

Pupilometer

1. Rest the instrument lightly on the nose with the child looking into the instrument. Move the slide so that the vertical line bisects the pupil and read the value from the instrument.

8.6. Appendix 6: Exit Questionnaire

For parent to complete during primary endpoint visit (Visit 4) or Visit 5 (if applicable).

1. On a scale of 0 (not likely at all) to 10 (extremely likely), how likely is it that you would recommend the Luminopia therapeutic to someone else with lazy eye?

0	1	2	3	4	5	6	7	8	9	10

2. How likely would you be to choose the Luminopia therapeutic over eye-patching to treat your child's amblyopia in the future?

Very unlikely	Unlikely	Neutral	Likely	Very likely

3. Do you have any suggestions on how to make the therapeutic more comfortable or more captivating for your child?
4. How did Luminopia One fit into your daily routine?

Very disruptive	Disruptive	Neutral	Easily	Very easily

5. What was the most enjoyable part of you and/or your child's experience with Luminopia One?
6. What was the most frustrating part of you and/or your child's experience with Luminopia One?
7. How valuable did you find the ability to review your child's usage data on the Patient Portal?

Not valuable at all	Not valuable	Neutral	Valuable	Very valuable

8. How valuable did you find the ability to curate content for your child on the Patient Portal?

Not valuable at all	Not valuable	Neutral	Valuable	Very valuable

9. Do you have any suggestions on how to make the Patient Portal more useful?
10. The average cost of braces for children's teeth is just under $5000. Given that, if your child continued to need an amblyopia therapeutic, how likely would you be to purchase Luminopia One at $1500?

Very unlikely	Unlikely	Neutral	Likely	Very likely

11. If your child continued to need an amblyopia therapeutic, how likely would you be to purchase Luminopia One at $950?

Very unlikely	Unlikely	Neutral	Likely	Very likely

8.7. Appendix 7: Phone Call Questionnaire and Adverse Event Queries

Phone Call Questionnaire
For study personnel to ask parent/guardian during phone calls.

1. What are your general thoughts about you and/or your child's experience using Luminopia One so far?
2. Have you and/or your child encountered any issues with the usability of Luminopia One?
3. How long does your child use Luminopia One for each day? (If less than an hour) What stops your child from using Luminopia One for an hour each day?
4. What are your child's favorite shows in Luminopia One?
5. What shows does your child wish were available in Luminopia One?

Phone Call Adverse Event Query
For study personnel to ask parent/guardian during phone calls.

1. Have you noticed any change in your child's health since the last visit?

O No O Yes → Explain and record AE

2. Has your child reported Double Vision (Diplopia)?

O No O Yes → Explain and record AE

3. Has your child reported or have you noticed any worsened Eye Turn (Ocular Misalignment)?

O No O Yes → Explain and record AE

4. Has your child reported Headaches?

O No O Yes → Explain and record AE

5. Has your child reported Nausea?

O No O Yes → Explain and record AE

6. Has your child reported Eye Strain?

O No O Yes → Explain and record AE

In Person Adverse Event Query
For study personnel to ask parent/guardian and participant during study visits.

1. Have you noticed any change in your child's health since the last visit?

O No O Yes → Explain and record AE

2. Has your child reported Double Vision (Diplopia)?

O No O Yes → Explain and record AE

3. *(To the child)* Have you seen "two Moms or two Dads" at any time?

O No O Yes → Explain and record AE

4. Has your child reported or have you noticed any worsened Eye Turn (Ocular Misalignment)?

O No O Yes → Explain and record AE

5. Has your child reported Headaches?

O No O Yes → Explain and record AE

6. *(To the Child)* Has your head hurt at all?

O No O Yes → Explain and record AE

7. Has your child reported Nausea?

O No O Yes → Explain and record AE

8. Has your child reported Eye Strain?

O No O Yes → Explain and record AE

9. *(To the Child)* Have your eyes hurt or felt blurry at all?

O No O Yes → Explain and record AE

8.8. Appendix 8: Summary of Amendment Changes

Page 1-3

- Background and Summary Current Treatments
    - Paragraph beginning with "In teenagers…" since that information is irrelevant to study population age of 4-7 years.

Page 1-4

- Background and Summary Results of Luminopia One Pilot Trials
    - Results from pilot trials updated

Page 1-5

- Proposed Study Design
    - Change of N from 130 to 140 following statistics evaluation, change of age group from 4-6 to 4-7 years old and change of study duration to 12 weeks, in paragraph 1
    - Added exclusion for > 12 months of prior amblyopia treatment

Page 1-6

- Study Objective
    - Study objectives section revised to specify study endpoints – specifically, primary efficacy and primary safety endpoint
- Synopsis of Study Design Major Eligibility Criteria –see Section 2.2 for complete list
    - Upper age changed limit changed from 6 to 7 years of age
- Treatment Groups – see Section 3.1 and 3.2 for more information
    - Therapeutic group prescription refined
    - Control group prescription refined
- Sample Size
    - Study N changed from 130 to 140 and age upper bound changed from 6 to 7 years
- Visit Schedule – see Section 3.4 for more information
    - Visit 4 added as primary endpoint visit after 12 weeks.
    - Call 3 added

Page 1-7

- "Testing Procedure" paragraph changed to "Primary Endpoint Treatment Procedure" to further describe the VA procedure
- "Statistical Analysis" section updated and expanded after statistician review
- Steering committee section eliminated

Page 1-9

- Visit 4 information added to flow chart
- Flow Chart updated
- Call 3 added

Page 2-1

- Informed Consent updated to include Assent procedures of 7 year old participants and updating study N
- Inclusion 3 updated from 6 to 7 years
- Inclusion 4a refined
- Inclusion 5, cycloplegic refraction changed from 9 to 7 months, and the "Note" eliminated as it caused covered by inclusion/exclusion

Page 2-2

- Inclusion 5a and 5b refined
- Inclusion 5b of Fellow Eye requirement eliminated
- Inclusion 9, refined

- Exclusion 1 eliminated as a discontinuation of prism would require stability in new refraction and is contradictory with other criteria
- Added exclusion for > 6 months of prior amblyopia treatment
- Exclusion 4 refined

Page 2-4

- Update of study duration from 8 to 12 weeks
- Therapeutic and Control prescription refined
- Section 2.4.1 on Luminopia One Therapeutic Administration refined and expanded to describe use of both Therapy and Controls
- Section of Optional Crossover for participants in control group placed from page 3-3 in protocol

Page 3-1

- Therapeutic group restricted to use of 60 continuous minutes
- Study duration updated to 12 weeks and usage updated from 7 days to 6 days.
- Study calls refined
- Change in Follow-up visit Testing Procedure refining requirement of corrective glasses in VA testing
- Inclusion of Phone Call Questionnaire

Page 3-2

- Clarification that all comparator group will have refraction
- Restriction of Masked Examiner access to treatment information and training.
- Inclusion of Exit Questionnaire

Page 3-3

- Section of Optional Crossover for participants in control group removed from page 3-3 in protocol and moved to page 2-4
- Corrective Refraction restriction added.

Page 4-1

- Participant Compensation updated
- Study Costs Refined
- Inclusion of study sites and enrollment limitation
- Elimination of Adherence procedures to prevent bias between active and control groups
- Elimination of Management of Refractive Error section, refraction will be kept the same during the study.
- Elimination of Discontinuation of Study, as there is no steering committee

Page 5-5

- Risk assessment expanded

Page 6-1

- Statistics section – Extensive Rewrite

Page 8-3

- Schedule of Events Table updated

Page 8-5

- Distance VA Testing procedure updated

Page 8-9

- Inclusion of Appendix 6

Page 8-10

- Inclusion of Appendix 7

8.9. Appendix 9: Investigators Signature

Protocol Title: Luminopia One Amblyopia Vision Improvement Study

Protocol Number: C-AM-2 Amendment 1

I agree to implement and conduct the study diligently and in strict compliance with the protocol, conditions of the IRB, ICH Guidelines for Good Clinical Practice (GCP) and all applicable laws and regulations, to supervise all usage of the device involving human subjects, and to ensure that the requirements for obtaining informed consent are met.

Printed Name:_____

Signed:_____ Date:_____