AMBLYOPIA TREATMENT STUDY (ATS20)

Binocular Dig Rush Game Treatment for Amblyopia

PROTOCOL

Version 4.0
30 April 2018

PROTOCOL AMENDMENT #1
13 September 2017

This amendment provides for the following protocol change:

Protocol Change

Current Protocol
The original sample size of 84 study participants for the older cohort assumed a standard deviation of 5 letters after 4 weeks of treatment (see Section 5.2.2).

Although we believed that our estimate of 5 letters in the older cohort was reasonable, a sample size re-estimation was planned once approximately 50% of the pre-planned number of subjects have completed their 4-week outcome visit (see Section 5.3). In summary, a pooled estimate of variance without respect to treatment group will be calculated and used to re-estimate sample size. If the observed standard deviation of change is larger than the pre-study estimate of 5 letters, the sample size will be increased, up to a maximum limit corresponding to a standard deviation of change of 8 letters for the older cohort (206 subjects) with a 5% adjustment for loss to follow-up.

Proposed Change
The pooled standard deviation for 42 subjects completing their 4-week outcome visit was observed to be 6 letters (higher than the original estimate of 5 letters). The sample size for the older cohort will be increased to 116 study participants (58 per group) based on the results of the planned sample size re-estimation.

Other Change:
A typographical error has been corrected in section 2.5.

PROTOCOL AMENDMENT #2
12 February 2018

This amendment provides for the following protocol change:

Protocol Change

Current Protocol
The following criteria must be met for a child to be enrolled in the study:

- VA in the fellow eye 20/25 or better (ATS-HOTV) or ≥78 letters (E-ETDRS)

Proposed Change
Eligibility criteria with respect to fellow-eye visual acuity has been changed to depend upon the age of the subject. The following criteria must be met for a child to be enrolled in the study:

- Best-corrected fellow-eye VA meeting the following criteria:
  - If age 4, 20/40 or better by ATS-HOTV
  - If age 5 or 6, 20/32 or better by ATS-HOTV
  - If age 7 or older, 20/25 or better by E-ETDRS (≥78 letters)

The requirement for an interocular difference ≥3 logMAR lines (ATS-HOTV) or (≥15 letters (E-ETDRS) has not changed.

Rationale for Change
Normal visual acuity values depend upon the age of the subject. The eligibility values for the study have been changed to match the normal values based upon age cited in the following table:

Table: Normal Visual Acuity Values Based on Age

Age range	Subnormal if worse than
36-47 months	20/50
48-59 months	20/40
60-83 months	20/32
≥84 months	20/25

Normal values for children aged 30-72 months based on a study by Pan et al.[29] Normal values for children aged ≥72 months based on a study by Drover et al.[30]

PROTOCOL AMENDMENT #3
07 March 2018

This amendment provides for the following protocol change:

Protocol Change

References added to the protocol to support normal values for children aged 30-72 months based on a study by Pan et al and normal values for children aged >72 months based on a study by Drover et al added.

PROTOCOL AMENDMENT #4
30 April 2018

This amendment provides for the following protocol changes:

Protocol Change # 1

Current Protocol
The original sample size of 116 study participants for the younger cohort assumed a standard deviation (SD) of 1.2 logMAR lines after 4 weeks of treatment (see Section 5.2.1).

Although we believed that our estimate of 1.2 logMAR lines in the younger cohort was reasonable, as it was based on best available data, a sample size re-estimation was pre-specified once approximately 50% of the pre-planned number of participants reached their 4-week outcome visit (see Section 5.3): Once approximately 50% of the pre-planned number of subjects have completed the 4-week outcome 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. Within each age cohort, if the observed standard deviation of change is larger than the pre-study estimate, the sample size will be increased, up to a maximum limit corresponding to a standard deviation of change of 1.5 logMAR lines (182 subjects) for the younger cohort.

Proposed Change
The pooled SD for 51 subjects completing their 4-week outcome visit was 1.6 logMAR lines (95% confidence interval for SD = 1.2 to 2.0 logMAR). Since the observed SD is much higher than the 1.2 logMAR lines used to estimate sample size, the sample size for the younger cohort will be increased to 182 study participants (91 per group) based on the results of the planned sample size re-estimation.

Protocol Change # 2

Current Protocol
Due to the short duration of the primary outcome at 4 weeks and expected rapid recruitment, no interim monitoring will be conducted for either age cohort. This decision will be re-evaluated if the sample size is increased.

Proposed Change
Interim monitoring for futility will be conducted when approximately 50% of the revised sample size (n=91) has completed the 4-week primary outcome visit. Details of the interim analysis plan will be developed in consultation with the DSMC and documented in the statistical analysis plan.

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CHAPTER 1: BACKGROUND AND SUMMARY

This study is being conducted by the Pediatric Eye Disease Investigator Group (PEDIG) and is funded through a cooperative agreement from the National Eye Institute.

1.1 Background

Epidemiology & clinical characteristics

Amblyopia is the most common cause of reduced monocular VA (VA) 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 anisometropia and strabismus.

Treatment –current methods and outcomes

The current mainstay of amblyopia treatment is spectacle correction (when there is uncorrected refractive error) followed by part-time patching or atropine penalization of the fellow eye.[3-8] Although current treatments using part-time occlusion and atropine drops are effective in many younger children (3 to <7 years) [3-8] residual amblyopia (20/32 or worse) is still present in 54% of children at age 10 years[9] and 40% at age 15 years.[10] In older children, age 7 to 12 years, current treatments are less effective.[11] The majority of older children still have residual amblyopia after treatment; in 7- to 12-year-old children, 80% treated with atropine and 74% treated with patching had residual amblyopia of 20/32 or worse.[12]

One possible reason for failure of part-time patching treatment in some younger children and many older children is poor compliance with the prescribed treatment regimens.[13,14] Nevertheless, data from studies using an occlusion dose monitor[15,16] suggest that many children successfully comply with prescribed part-time patching treatment and yet fail to respond to treatment, supporting the assertion 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 compliance, and social stigma.[17,18]

Based on the prevalence of residual amblyopia with current part-time patching treatment and the challenges of compliance with patching, new treatments for amblyopia are needed, particularly those that can be visually unobtrusive and that do not overtly interfere with the vision of the fellow eye.

Binocular treatment

In 2010, Hess et al[19] reported a binocular approach to treating amblyopia, without patching, atropine drops or blurring filters, consisting of dichoptic stimuli presented to each eye. In laboratory-based sessions, dichoptic motion coherence thresholds were measured by adjusting contrast levels in the fellow eye to optimize combination of visual information from both eyes and overcome suppression of the amblyopic eye. In these adult subjects mean amblyopia eye VA and stereoacuity improved over several weeks.[19] This method of binocular treatment was then adapted to a “falling blocks” game, which was studied by Li et al,[20] reporting a mean amblyopic eye improvement of approximately 2 logMAR lines when treating adults in a supervised setting for 1 hour/day over 2 weeks.

Recently, binocular treatment using a "falling blocks" game has been adapted to an iPad® device, which uses red-green anaglyphic glasses. In children, non-randomized studies conducted by Birch's research group found an improvement in amblyopic-eye VA of approximately 1 logMAR line prescribing 4 hours/week of binocular treatment for 4 weeks in 4 to 12 year-olds,[21,22] and in 3- to 6-year-olds.[23] The studies[21-23] conducted by Birch's group in children included 4 different binocular games, one of which was the falling blocks game, and allowed concurrent patching at a different time of day at the eye care provider's discretion, although a sub-analysis of those only treated with binocular games yielded a similar magnitude of effect.

Knox et al[24] also found a comparable improvement (approximately 1 logMAR line) in children (mean age 8.5 years) treated with a similar game, using a head-mounted display in a supervised setting for 1 hour/day for 5 sessions over one week.

Based on these pilot studies, PEDIG performed a randomized clinical trial to compare amblyopic-eye VA improvement over 16 weeks in children age 5 to <13 years, with 20/40 to 20/200 amblyopic-eye VA, comparing a binocular iPad game (prescribed 1 hour per day) with patching of the fellow eye (prescribed 2 hours per day).

1.2 Results of PEDIG study of binocular treatment (ATS18)

In a recently completed PEDIG RCT,[25] 385 subjects 5 to <13 years of age (mean 8.5 years) with amblyopia (20/40 to 20/200, mean 20/63) resulting from strabismus, anisometropia, or both, were randomly assigned to either 16 weeks of a binocular iPad game, prescribed for 1 hour a day (n=190, binocular group), or patching of the fellow eye prescribed for 2 hours a day (n=195, patching group).

At 16 weeks, the mean amblyopic-eye VA improved 1.05 lines (2-sided 95% confidence interval (CI): 0.85 to 1.24 lines) in the binocular group and 1.35 lines (2-sided 95% CI: 1.17 to 1.54 lines) in the patching group, with an adjusted treatment group difference of 0.31 lines favoring patching (upper limit of the 1-sided 95% CI 0.53 lines). This upper limit exceeded the pre-specified non-inferiority limit of 0.5 lines. In a post hoc analysis, the two-sided 95% CI for the adjusted treatment group difference was 0.04 to 0.58 lines, favoring the patching group. Only 22% of subjects randomized to the binocular game performed >75% of the prescribed treatment (median 46%, interquartile range 20% to 72%). In younger subjects 5 to <7 years of age, without prior amblyopia treatment, amblyopic-eye VA improved 2.5 ± 1.5 lines in the binocular group and 2.8 ± 0.8 in the patching group. Adverse effects (diplopia, reduction of fellow-eye VA, new tropia) were uncommon and of similar frequency between groups.

We therefore concluded that in children 5 to <13 years of age, amblyopic-eye VA improved with both binocular game play and patching, particularly in younger children age 5 to <7 years without prior amblyopia treatment. However, based on a post hoc analysis, VA improvement with this particular binocular iPad treatment was not as good as with 2 hours of prescribed daily patching.

1.3 Rationale for Proposed Study Design

It is entirely possible that our failure to find non-inferiority of binocular treatment to part-time patching in our recent RCT was due to poor compliance with the binocular treatment. Only 22% of subjects randomized to the binocular game performed >75% of the prescribed treatment (median 46%, interquartile range 20% to 72%).

Recently, a new binocular game has been developed for children, called "Dig Rush," which is much more interesting than the falling blocks game because it involves more interesting tasks such as digging for gold and earning rewards. It has 42 levels, and therefore the child remains engaged for a much longer period of time than the falling blocks game.

The Birch group has studied the binocular Dig Rush game in children age 4 to <10 years and found that compliance with the game is excellent, with a mean amblyopic eye VA improvement of 1.5 lines at 2 weeks and 1.7 lines at 4 weeks.[26]

Based on several promising pilot studies of binocular treatment in children and in adults, a full RCT is warranted, to investigate whether binocular treatment is an effective treatment for amblyopia, and, analogous to the PEDIG RCT which investigated the effectiveness of patching,[27] the appropriate control group is continued spectacle treatment alone.

1.4 Study Objective

To compare the efficacy of 1 hour/day of binocular game play 5 days per week plus spectacle correction with spectacle correction only, for treatment of amblyopia in children 4 to <13 years of age.

1.5 Synopsis of Study Design

Major eligibility criteria: (*see section 2.2* for a complete listing)

- Age 4 to <13 years
- Amblyopia associated with anisometropia, strabismus (<5Δ at near measured by SPCT), or both
- No amblyopia treatment (atropine, patching, Bangerter, vision therapy, binocular therapy) in the past 2 weeks
- Spectacle 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 (ATS-HOTV) or 33 to 72 letters (E-ETDRS)
- Best-corrected fellow-eye VA meeting the following criteria:
  - If age 4, 20/40 or better by ATS-HOTV
  - If age 5 or 6, 20/32 or better by ATS-HOTV
  - If age 7 or older, 20/25 or better by E-ETDRS (≥78 letters)
- Interocular difference ≥3 logMAR lines (ATS-HOTV) or (≥15 letters (E-ETDRS)
- No myopia greater than -6.00D spherical equivalent in either eye
- Demonstrate in-office ability to play the Dig Rush game under binocular conditions (with red-green glasses) on at least level 3, including ability to see red "diggers" and blue "gold carts" at 20% contrast in the non-amblyopic eye

Treatment Groups

Subjects will be randomly assigned with equal probability to either:

- Binocular treatment group: binocular computer game play prescribed 1 hour per day 5 days a week (treatment time can be split into shorter sessions totaling 1 hour each day) with spectacles, if needed (*see section 3.1*)
- Continued spectacle correction, if needed (*see section 3.2*)

Sample Size – see details in Chapter 5

- Results will be analyzed separately in 2 age cohorts.
  - 182 children aged 4 to < 7 years (younger cohort)

- 116 children aged 7 to <13 years (older cohort) based on results of the sample size re-estimation
    i. A maximum of 20% of enrolled subjects in each age cohort may have had previous binocular therapy.

Visit Schedule

- Enrollment exam and randomization
- 1-week phone call (7 to 13 days from randomization) to inquire about issues with the binocular game (if applicable) and to encourage compliance with treatment for all groups (to be completed by site personnel)
- 4 weeks ± 1 week (primary outcome)
- 8 weeks ± 1 week (secondary outcome)
    - Binocular group final visit
    - Spectacle group switched to binocular treatment and followed for 8 weeks
- 9-week phone call (Spectacle group only: 7 to 13 days from 8-week exam) to inquire about issues with the binocular game (if applicable) and to encourage compliance with treatment for all groups (to be completed by site personnel)
- 16 weeks ± 1 week (final visit for group originally randomized to continued spectacle treatment who were switched to binocular treatment)

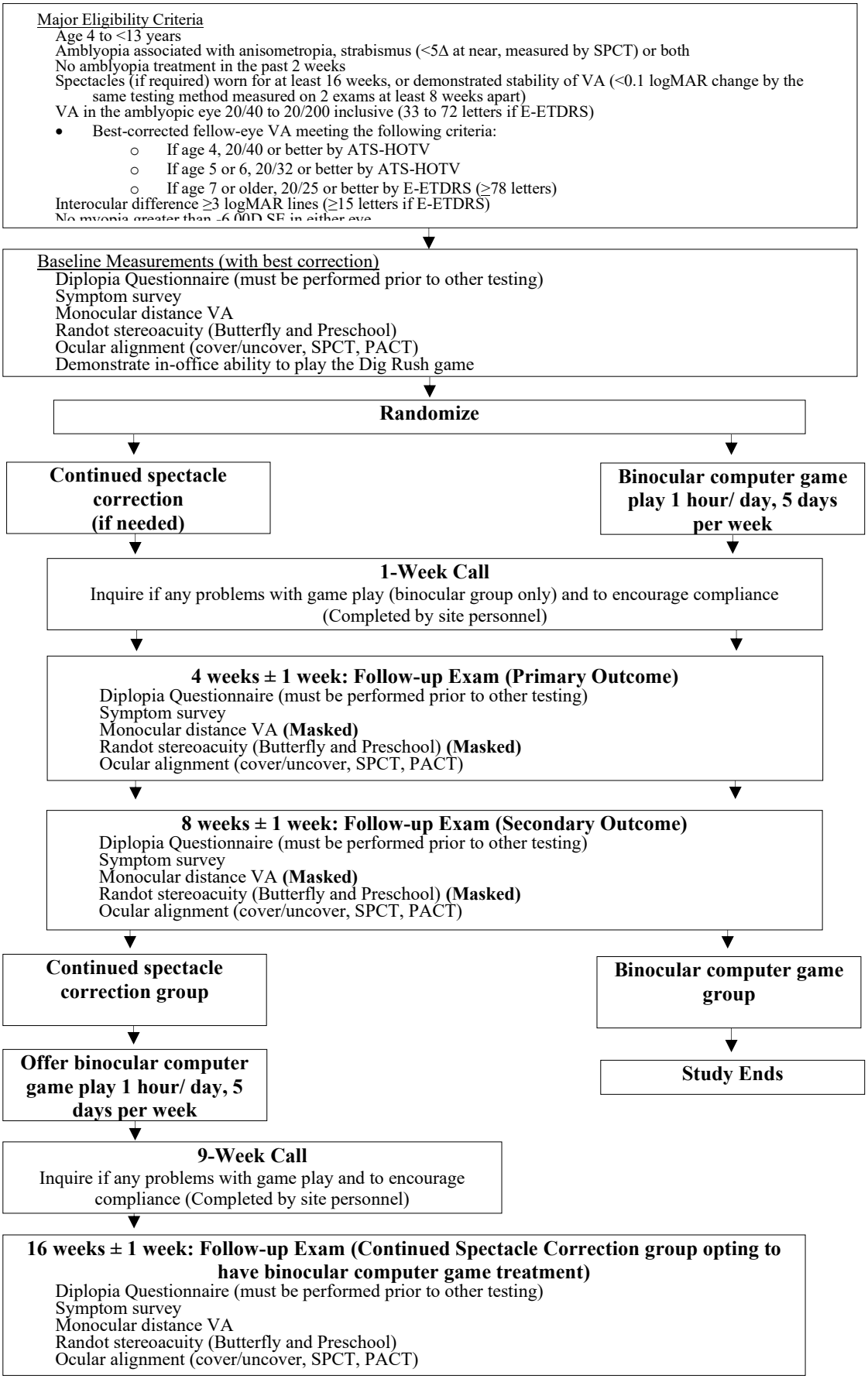
Testing Procedures

At each follow-up visit, distance VA will be measured in each eye using ATS-HOTV for children <7 years at enrollment and the E-ETDRS for children ≥7 years at enrollment (VA method used at enrollment will be used over the course of the trial). We will also assess near stereoacuity using the Randot Butterfly Stereoacuity test and Randot Preschool Stereoacuity test, history of diplopia, symptoms, and ocular alignment (distance and near) by cover test, simultaneous prism cover test (SPCT) (if manifest deviation present), and prism and alternate cover test (PACT) (for all subjects).

Analysis

The primary analysis will compare mean change in amblyopic-eye VA from enrollment to 4 weeks in the binocular computer treatment group with the continued spectacle treatment group.

Study Summary Flow Chart



CHAPTER 2: SUBJECT ENROLLMENT

2.1 Eligibility Assessment and Informed Consent/Assent

The study plans to enroll a minimum of 182 subjects aged 4 to < 7 years and 116 subjects aged 7 to <13 years (based on results of the sample size re-estimation). Up to 20% of enrolled subjects in each age cohort can have had previous binocular therapy. As the enrollment goal approaches, sites will be notified of the end date for recruitment. Subjects who have signed an informed consent form can be randomized until the end date, which means the expected recruitment might be exceeded.

A child 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 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 (depending on age and local IRB requirements) prior to performing any study-specific procedures that are not part of the child's routine care.

2.2 Eligibility and Exclusion Criteria

2.2.1 Eligibility Criteria

The following criteria must be met for a child to be enrolled in the study:

1. Age 4 to <13 years
2. Amblyopia associated with strabismus, anisometropia, or both (previously treated or untreated)
   a. 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, must be no more than 4pd by SPCT at near fixation (see #6 below)
      - Documented history of strabismus which is no longer present (which in the judgment of the investigator could have caused amblyopia)
   b. 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
   c. 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 amblyopia treatment other than optical correction in the past 2 weeks (patching, atropine, Bangerter, vision therapy, binocular treatment)
4. Requirements for required refractive error correction (*based on a cycloplegic refraction completed within the last 7 months*):
   - 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

   NOTE: Subjects with cycloplegic refractive errors that do not fall within the requirements above for spectacle correction may be given spectacles at investigator discretion but must follow the study-specified prescribing guidelines, as detailed below.

a. Spectacle prescribing instructions referenced to the cycloplegic refraction completed within the last 7 months:
    - SE must be within 0.50D of fully correcting the anisometropia.
    - SE must not be under corrected by more than 1.50D SE, and reduction in plus sphere must be symmetric in the two eyes.
    - Cylinder power in both eyes must be within 0.50D of fully correcting the astigmatism.
    - Axis must be within +/- 10 degrees if cylinder power is ≤1.00D, and within +/- 5 degrees if cylinder power is >1.00D.
    - Myopia must not be undercorrected by more than 0.25D or over corrected by more than 0.50D SE, and any change must be symmetrical in the two eyes.

b. Spectacle correction meeting the above criteria must be worn:
    - For at least 16 weeks ***OR*** until VA stability is documented (defined as <0.1 logMAR change by the same testing method measured on 2 consecutive exams at least 8 weeks apart).
        - For determining VA stability (non-improvement):
            - The first of two measurements may be made 1) in current spectacles, or 2) in trial frames with or without cycloplegia or 3) without correction (if new correction is prescribed),
            - The second measurement must be made without cycloplegia in the correct spectacles that have been worn for at least 8 weeks.
            - *Note: since this determination is a pre-study procedure, the method of measuring VA is not mandated.*

5. VA, measured in each eye without cycloplegia in current spectacle correction (if applicable) within 7 days prior to randomization using the ATS-HOTV VA protocol for children < 7 years and the E-ETDRS VA protocol for children ≥ 7 years on a study-approved device displaying single surrounded optotypes, as follows:
    a. VA in the amblyopic eye 20/40 to 20/200 inclusive (ATS-HOTV) or 33 to 72 letters (E-ETDRS)
    b. Best-corrected fellow-eye VA meeting the following criteria:
        - If age 4, 20/40 or better by ATS-HOTV
        - If age 5 or 6, 20/32 or better by ATS-HOTV
        - If age 7 or older, 20/25 or better by E-ETDRS (≥78 letters)
    c. Interocular difference ≥ 3 logMAR lines (ATS-HOTV) or ≥ 15 letters (E-ETDRS)
6. Heterotropia with a near deviation of <5Δ (measured by SPCT) in habitual correction (Angles of ocular deviation >4Δ are not allowed because large magnitudes of the deviation would compromise successful playing of the game.)
7. Subject is able to play the Dig Rush game (at least level 3) on the study iPad under binocular conditions (with red-green glasses). Subject must be able to see both the red "diggers" and blue "gold carts" when contrast for the non-amblyopic eye is at 20%.
8. Investigator is willing to prescribe computer game play, or continued spectacle wear per protocol.
9. Parent understands the protocol and is willing to accept randomization.
10. Parent has phone (or access to phone) and is willing to be contacted by Jaeb Center staff or other study staff.
11. Relocation outside of area of an active PEDIG site for this study within the next 8 weeks is not anticipated.

2.2.2 Exclusion Criteria

A subject is excluded for any of the following reasons:

1. Prism in the spectacle correction at time of enrollment (eligible only if prism is discontinued 2 weeks prior to enrollment).
2. Myopia greater than -6.00D spherical equivalent in either eye.
3. Previous intraocular or refractive surgery.
4. Any treatment for amblyopia (patching, atropine, Bangerter filter, vision therapy or previous binocular treatment) during the past 2 weeks. Previous amblyopia therapy is allowed regardless of type, but must be discontinued at least 2 weeks prior to enrollment.
5. 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 subject if the above VA criteria are met*).
6. No Down syndrome or cerebral palsy
7. No severe developmental delay that would interfere with treatment or evaluation (in the opinion of the investigator). Subjects with mild speech delay or reading and/or learning disabilities are not excluded.
8. Subject has demonstrated previous low compliance with binocular treatment and/or spectacle treatment (as assessed informally by the investigator)

2.3 Historical Information

Historical information to be elicited will include the following: date of birth, sex, race, ethnicity, and history of prior eye-related treatment (including length of spectacle correction).

2.4 Procedures at the Enrollment Visit

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

All examination procedures at enrollment are performed in the subject's current spectacle correction, if required (testing in trial frames is not permitted), and without cycloplegia:

1. <u>ATS Diplopia Questionnaire</u>
   - The child and parent(s) will be specifically questioned regarding the presence and frequency of any diplopia within the last 2 weeks using a standardized diplopia assessment (*see ATS Miscellaneous Testing Procedures Manual*). The diplopia assessment must be performed prior to any other testing during the exam.

2. <u>Symptom Survey</u>
   - The child and parent(s) will complete a 5-item symptom survey regarding the presence of various ocular symptoms within the past 2 weeks (*see ATS Miscellaneous Testing Procedures Manual*). The symptom survey must be performed prior to any other testing during the exam.

3. <u>Distance VA Testing:</u> Monocular distance VA testing will be performed in current refractive correction (if required) 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*.
   - The same VA protocol used at enrollment will be used throughout the study regardless of age at follow-up.

4. Stereoacuity Testing:
    - Stereoacuity will be tested at near in current spectacle correction using the Randot Butterfly and Randot Preschool stereoacuity tests.

5. 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) as outlined in the *ATS Procedures Manual*.
        - See *section 2.2.1* for eligibility criteria related to ocular alignment.

6. Additional Clinical Testing:
    - Ocular examination as per investigator's clinical routine (if not performed within 7 months)

7. Demonstration of Game Understanding
    - The subject must be able to see both the red "diggers" and blue "gold carts" when contrast is at 20% for the non-amblyopic eye. Subjects must demonstrate that they understand the game by playing the game in the office on at least level 3. Subjects unable to play the game are not eligible for the study.

2.5 Randomization of Eligible Subjects

For each age cohort, the Jaeb Center will construct a Master Randomization List using a permutated block design stratified by visual acuity in the amblyopic eye as moderate 20/40 to 20/80 (53 to 67 letters) versus severe 20/100 to 20/400 (18 to 52 letters), which will specify the order of treatment group assignments.

All eligible subjects enrolled in the study will be followed for 8 weeks. Subjects will be randomly assigned in a 1:1 allocation to one of the following treatment groups for 8 weeks:

1. Binocular game play 1 hour per day, 5 days per week with spectacle correction (*see section 3.1*), if needed
2. Continued spectacle correction (*see section 3.2*), if needed

Once a child is assigned to treatment, he/she will be included in the analysis regardless of whether or not the assigned treatment is received. Thus, the investigator must not randomly assign a subject to treatment unless convinced that the parent will accept either of the treatments.

CHAPTER 3: TREATMENT AND FOLLOW-UP

3.1 Binocular Computer Game Treatment

Subjects assigned to the binocular treatment group will be prescribed the Dig Rush game to play for 1 hour per day, 5 days a week for 8 weeks. Parents of subjects will be instructed that the 1 hour of daily treatment should be completed in a single 60-minute session, but if this is not possible for whatever reason, the treatment may be divided into shorter sessions totaling 1 hour per day.

All subjects in the study will play the Dig Rush game presented on an iPad while wearing red/green (anaglyph) glasses (over current spectacle correction, if applicable) with the green filter placed over the amblyopic eye. The subject should be instructed to hold the iPad at his/her usual reading distance. Some game elements are only visible to the fellow eye viewing through the red lens, while other game elements are only visible to the amblyopic eye viewing through the green lens. Image contrast varies depending on depth of amblyopia to ensure stimulation of the amblyopic eye and binocular game play.

Contrast of the game elements in the amblyopic eye will be at 100% throughout the study. Contrast of game elements seen by the fellow eye will begin at 20% at the start of the study and will increase or decrease automatically in increments from the last contrast level (e.g., 20% to 22%) based on the subject's performance and duration of game play.

3.2 Continued Spectacle Correction Group

Subjects assigned to the continued spectacle wear group will continue wearing their appropriate spectacle correction (if required) for all waking hours, 7 days per week for 8 weeks. Subjects assigned to the continued spectacle wear group will be offered binocular treatment for 8 weeks after the initial 8 weeks of the study has been completed. Subjects in the continued spectacle correction group that choose to continue with binocular treatment after 8 weeks will return for a follow-up visit at 16 weeks.

3.3 Compliance

Parents will be asked to complete a compliance calendar by manually recording the number of minutes that the child played the game each day, and/or how long the child has worn the spectacle correction. The investigator will review the calendars at each follow-up visit. The amount of time the game is played will also be recorded automatically during game play by the iPad. These data will be downloaded at the Jaeb Center when the iPad is returned after the study.

3.4 Phone Call

Site personnel will call all subjects at 1 week (7 to 13 days) to encourage compliance with treatment and to confirm that there are no technical problems playing the binocular game for those assigned to binocular treatment. Site personnel will call subjects in the continued spectacle treatment group who switch to binocular treatment at 9 weeks (7 to 13 days after the 8-week visit), again to encourage compliance with treatment and to confirm that there are no technical problems playing the binocular game for those assigned to binocular treatment.

3.5 Follow-up Visit Schedule

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

- 1-week phone call (7 to 13 days from randomization) to inquire about issues with the binocular game (if applicable) and to encourage compliance with treatment for all groups (to be completed by site personnel)
- 4 weeks ± 1 week (primary outcome)

- 8 weeks ± 1 week (secondary outcome)
  - Binocular final visit
  - Spectacle group switched to binocular treatment and followed for 8 weeks
- 9-week phone call (Spectacle group only: 7 to 13 days from 8-week exam) to inquire about issues with the binocular game (if applicable) and to encourage compliance with treatment for all groups (to be completed by site personnel)
- 16 weeks ± 1 week (final visit for group originally randomized to continued spectacle treatment who were switched to binocular treatment)

Additional non-study visits can be performed at the discretion of the investigator.

3.6 Resolution of Amblyopia

Subjects achieving amblyopic-eye VA equal to or better (0 lines or more lines better) than the better of the fellow-eye VA at baseline or 4-week visit, will be considered to have resolved and may discontinue binocular treatment, although these subjects will still return for all remaining follow-up exams.

3.7 Optional Post 8-week Treatment

For children originally randomized to binocular treatment, the study ends at 8 weeks.

Children who were originally assigned to continued spectacle treatment will be offered binocular treatment for 8 weeks following the 8-week visit. The study will end at the 8-week exam for children who choose not to receive binocular treatment. Children receiving binocular treatment at the 8 week exam will return at 16 weeks for a follow-up visit. The study will end for these children at the 16-week visit.

3.8 Follow-up Visit Testing Procedures

Subjects will be seen at follow-up visits as outlined in *section 3.5*. A Masked Examiner must complete distance VA and stereoacuity testing at these visits (*section 3.8.*1). All procedures will be performed with the subject's current spectacle correction. If a subject currently wears spectacles but is not wearing them at the follow-up examination for whatever reason, testing must be performed in trial frames.

Prior to the Masked Examiner entering the room, subjects and parents should be instructed not to discuss their treatment with the Masked Examiner.

The following study procedures are performed at each visit:

1. <u>ATS Diplopia Questionnaire</u>
   - The child and parent(s) will be specifically questioned regarding the presence and frequency of any diplopia within the last 2 weeks using a standardized diplopia assessment (*see ATS Miscellaneous Testing Procedures Manual*). The diplopia assessment must be performed prior to any other testing during the exam.

2. Symptom Survey
    - The child and parent(s) will complete a 5-item symptom survey regarding the presence of various ocular symptoms within the past 2 weeks (*see ATS Miscellaneous Testing Procedures Manual*). The symptom survey must be performed prior to any other testing during the exam.

3. Distance VA Testing (masked):
    - Monocular distance VA testing will be performed in the current spectacle correction in each eye using the same VA testing method that was used at enrollment, as described in the *ATS Testing Procedures Manual*.
        - The ATS HOTV testing protocol will always be used to test VA in the younger age cohort (4 to <7 years at enrollment) whereas the E-ETDRS protocol will always be used to test VA in the older cohort (7 to<13 years at enrollment).
        - Testing must be completed without cycloplegia.

4. Stereoacuity Testing (masked):
    - Near stereoacuity will be tested in habitual current refractive correction using the Randot Butterfly test and Randot Preschool Stereoacuity test at near (1/3 meter).

5. Ocular Alignment Testing:
    - Ocular alignment will be assessed in the current spectacle correction by the cover test, simultaneous prism and cover test (SPCT) (if strabismus is present on cover testing), and prism and alternate cover test (PACT) in primary gaze at distance (3 meters) and at near (1/3 meter) as outlined in the *ATS Procedures Manual*.

3.8.1 Masked Examiner

The Masked Examiner must be certified to test VA and stereoacuity. Because the Masked Examiner must be masked to the subject's treatment group, he/she must be someone other than the managing clinician (in many cases the managing clinician will be the investigator but this is not required).

3.9 16-Week visit

This visit is only for children randomized to continued spectacle treatment who opt to receive binocular treatment at the 8-week visit. At the 16-week visit, children will have the same testing as described in *section 3.8*; however, testing does not need to be completed by a masked examiner. Following this visit, the study will end for these children.

3.10 Non-Study Visits and Treatment

Investigators may schedule additional visits at their own discretion. Subjects 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.

Investigators must not start any additional treatment (other than that outlined in *section 3.1*) prior to the 8-week outcome visit.

CHAPTER 4: MISCELLANEOUS CONSIDERATIONS IN FOLLOW-UP

4.1 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 subjects. 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 subject and/or family and to help coordinate scheduling of the outcome examinations.

The site investigator or coordinator will contact the parents of each subject after the first week of the study to encourage compliance with treatment (spectacle or binocular) and to confirm that there are no technical problems playing the binocular game for those assigned to binocular treatment.

4.2 Subject Withdrawals

Parents may withdraw their child from the study at any time. This is expected to be a very infrequent occurrence in view of the study design's similarity to routine clinical practice and short duration. If the parents indicate that they want to withdraw their child from the study, the investigator personally should attempt to speak with them to determine the reason. If their interest is in transferring the child's care to another eye care provider, every effort should be made to comply with this and at the same time try to keep the child in the study under the new provider's care.

4.3 Management of Refractive Error

Because of the short duration of the study and the requirement to have a cycloplegic refraction within 7 months prior to enrollment, no cycloplegic refraction is mandated during the study. Nevertheless, whenever the investigator suspects that refractive error may not be corrected according to study guidelines, a cycloplegic refraction should be performed. Change in spectacle correction is at investigator discretion, but must be prescribed according to the guidelines described in *section 2.2.1*. and spectacles will be paid for by the study.

Contact lenses are not allowed during the study.

4.4 Management of Strabismus

Because of the short duration of the study and the age group being studied, strabismus surgery is not allowed prior to the end of the study. If surgery is performed, the date and type of surgery will be recorded in the comment section of the Follow-up Examination Form.

4.5 Risks

4.5.1 Development of Manifest Ocular Deviation or Diplopia

Diplopia is expected to be rare based on our experience during the previous ATS18 study comparing binocular treatment with patching. [25]

Data on frequency of diplopia will be collected from the child and parent(s) at each study visit.

If treatment precipitates the development of a manifest ocular deviation (e.g., esotropia) and/or diplopia, the parent will be advised to have the subject see the investigator as soon as possible. If a new manifest deviation is confirmed on examination, the decision as to whether to continue or discontinue therapy will be left to the investigator's and parent's decision. If the investigator determines that binocular diplopia is present, continuation of treatment is also at the discretion of the investigator and parent(s). If amblyopia treatment is to be discontinued during the study, a

Protocol Chair should be called to discuss the case. Subjects discontinuing treatment during the study will continue to be seen for the remaining regularly scheduled study visits.

4.5.2 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 subject may receive cycloplegic/dilating eye drops.

4.5.3 Delay in Use of Traditional Amblyopia Treatment

The subjects in the either treatment group will not be able to perform any patching, atropine, Bangerter filter, or additional vision therapy treatment during the study.

4.6 Reporting of Adverse Events

No surgical procedures are part of the protocol. There are no expected long-term adverse events associated with playing the computer game on the iPad. Investigators will abide by local IRB reporting requirements.

4.6.1 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.

4.7 Discontinuation of Study

The study may be discontinued by the Steering Committee (with approval of the Data and Safety Monitoring Committee) prior to the preplanned completion of enrollment and follow-up for all subjects.

4.8 Travel Reimbursement

Parents of each subject will be compensated $40 per visit (by check or money-card) for completion of each protocol-specified visit, for a maximum of $160. If there are extenuating circumstances, and the subject is unable to complete study visits without additional funds for travel costs, additional funds may be provided.

4.9 Study Costs

The subject or his/her insurance provider will be responsible for the costs that are considered standard care.

Because the treatment used in the study is not standard of care, the enrollment, 4-, 8, and 16-week follow-up visits will be paid for by the study. The cost of the binocular game treatment related equipment will also be paid for by the study; however the iPad will need to be returned upon study completion.

Changes in spectacle correction if done (*see section 4.3*) will be paid for by the study.

4.10 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice.

Data will be directly collected in electronic CRFs, which will be considered the source data.

A risk-based monitoring approach will be followed, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).

CHAPTER 5: SAMPLE SIZE ESTIMATION AND STATISTICAL ANALYSIS

The approach to sample size estimation and the statistical analyses are summarized below. A detailed Statistical Analysis Plan document will be written and finalized prior to any tabulation or analysis of study outcome data.

5.1 Definition of Subject Cohorts

The study will enroll two cohorts of subjects with identical eligibility criteria, apart from age at time of randomization:

- Younger cohort: Children aged 4 to <7 years
- Older cohort: Children aged 7 to <13 years

Compliance may differ by age; therefore, we believe that there could be a differential treatment effect between age groups. If the binocular game is not as appealing to younger children with some not being able to play the game very well, it is possible that the treatment response with binocular therapy may be reduced in this age group due to poorer compliance with game play compared with the older cohort. The opposite may be true as well. Therefore, the current study will be powered for two separate age cohorts according to the criteria listed above.

5.2 Sample Size Estimation

Sample size estimates for each age cohort were based on data from previous PEDIG studies (ATS3[28], ATS5[27], and ATS18[25]), and data from a preliminary pilot study for subjects treated with the Dig Rush game on an iPad device (E. Birch Study[26]) limited to subjects meeting the eligibility criteria for the current protocol.

5.2.1 Younger Cohort (Children 4 to <7 years of age)

Control Group – Continued Spectacle Correction Alone

To estimate the treatment effect in our study for those randomized to continued spectacles alone after stability, data were reviewed from subjects aged 3 to <7 years who were randomly assigned to continue spectacle correction alone in a previous PEDIG study (ATS5, see Table 1). After adjusting for baseline VA, the mean change in VA at 5 weeks was 0.55 logMAR lines (95% confidence interval (CI): 0.19 to 0.90 logMAR lines) with standard deviation 1.28 logMAR lines (95% confidence interval (CI): 1.07 to 1.58 logMAR lines).

Given the more stringent criteria for assessing VA stability with spectacles and shorter outcome time in the current study as compared to the previous study, we anticipate that the magnitude and standard deviation of VA improvement after 4 weeks in the current study will be smaller than those in Table 1.

Binocular Treatment Group (Dig Rush Game):

Data from 2 studies were reviewed: (1) preliminary pilot data for subjects randomly assigned to 4 weeks of binocular treatment with the Dig Rush game on an iPad device (E. Birch Study) and (2) data from a previous PEDIG trial (ATS18) for subjects randomly assigned to binocular treatment with the Hess falling blocks game on an iPad device (Table 1). Based on the more conservative estimates from ATS18, a mean VA change at 4 weeks of 1.09 lines (95% CI: 0.63 to 1.55 lines), with standard deviation 1.32 logMAR lines (95% CI: 1.06 to 1.74 logMAR lines) after adjusting for baseline VA might be expected. However, we anticipate that the magnitude of VA improvement after 4 weeks in the current study will be somewhat larger than that in ATS18, due to better compliance.

Table 1: Previous Study Data from Subjects Aged 3 to <7 Years of Age

Cohort	N	Change in Amblyopic Eye VA at the 4 or 5 Week Visit (logMAR Lines) ‡	
		Mean Change (95% CI)	SD Change (95% CI)
Spectacle Correction Alone:			
ATS5: Age 3 to <7 years †	53	0.55 (0.19 to 0.90)	1.28 (1.07 to 1.58)
No prior amblyopia treatment	50	0.54 (0.17 to 0.91)	1.32 (1.10 to 1.64)
Prior amblyopia treatment	3	0.67 (-2.21 to 3.54)	0.39 (0.20 to 2.45)
Binocular Treatment:			
E. Birch Study (Age 4 to <7 years) ^	9	1.67 (0.73 to 2.61)	1.19 (0.80 to 2.28)
ATS18 (Age 5 to <7 years) §	34	1.09 (0.63 to 1.55)	1.32 (1.06 to 1.74)
No prior amblyopia treatment	19	1.47 (0.73 to 2.22)	1.54 (1.16 to 2.28)
Prior amblyopia treatment	15	0.60 (0.15 to 1.05)	0.81 (0.59 to 1.28)

‡ Positive values indicate improvement. Mean and SD adjusted for baseline visual acuity.
† ATS5 data were limited to randomized subjects 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 spectacle 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.
^ E. Birch study enrolled children aged 4 to <7 years of age. Change in VA after 4 weeks of binocular therapy was computed for subjects randomly assigned to receive binocular treatment using the Dig Rush game on an iPad device for 4 weeks (prescribed 1 hour per day, 5 days per week without patching). Subjects with >4pd magnitude of strabismus (measured by PACT) were excluded from the study.
§ ATS18 study subjects were prescribed binocular treatment for 1 hour per day, 7 days per week. The binocular game, Hess falling blocks, was played on an iPad device. The outcome data reported are from the 4-week masked outcome visit.

Summary:

Based on data from previous studies (Table 1), the treatment group difference for the current study for the mean change in VA at 4 weeks was estimated to be 0.75 logMAR lines with a pooled standard deviation of 1.2 logMAR lines. Although the expected group difference in the current study is larger than 0.75 logMAR lines (Table 1), there is limited data available on the Dig Rush binocular game and this estimate is consistent with the results of the previous ATS5 study comparing spectacle correction alone versus patching (treatment group difference of 0.7 line) after 5 weeks of treatment.

Sample Size Estimation:

Assuming a true difference in mean VA change between the two groups of 0.75 logMAR line after 4 weeks of treatment and a pooled standard deviation of 1.2 logMAR lines, a total sample size of 110 subjects (55 per group) has 90% power with a type I error rate of 5% to detect a treatment group difference between binocular treatment and spectacle correction alone (Table 2). Adjusting for 5% loss to follow-up, a total sample size of 182 (91 per group) is needed.

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

SD of Change (LogMAR lines)	Treatment Group Difference in Mean VA Change from Baseline at 4 weeks (LogMAR lines)				
	0.50	**0.75**	**1.00**	**1.25**	**1.50**
1.0	172	78	46	30	22
1.1	206	94	54	36	26
1.2	246	**110**	64	42	30
1.3	288	130	74	48	34
1.4	332	150	86	56	40
1.5	382	172	98	64	46

* Number in cells represents the total number of subjects required to detect a treatment group difference in amblyopic-eye VA change from baseline to 4 weeks using a t-test with a 2-sided alpha=0.05 and power 90% for a range of pooled SD of change in VA (logMAR lines).

5.2.2 Older Cohort (Children 7 to <13 years of age)

Control Group – Continued Spectacle Correction Alone

To estimate the treatment effect in our study for those randomized to continued spectacles alone after stability, data were reviewed from subjects aged 7 to <13 years who were randomly assigned to continued spectacle correction alone in a previous PEDIG study (ATS3, see Table 3). Similar to ATS18, the proportion of subjects with prior treatment in the current study is expected to be higher than the proportion observed in ATS3. Therefore, the proportions with and without prior treatment from ATS18 were used to weight ATS3 outcome data. After adjusting for baseline acuity, the weighted mean change in VA at 6 weeks was 1.3 letters (95% CI: 0.01 to 2.6 letters) with standard deviation of 5.3 letters (95% confidence interval (CI): 4.5 to 6.4 letters).

Given the more stringent criteria for assessing VA stability with spectacles and shorter outcome time than the previous study, we anticipate that the magnitude of VA change after 4 weeks will be close to zero, with standard deviation somewhat smaller than in Table 3.

Binocular Treatment Group (Dig Rush Game):

Data from 2 studies were reviewed: (1) preliminary pilot data[26] for subjects randomly assigned to 4 weeks of binocular treatment using the Dig Rush game on an iPad device and (2) data from a previous PEDIG trial[25] of subjects who were randomly assigned to binocular treatment using the Hess falling blocks game on an iPad device (Table 3).

A larger treatment effect at 4 weeks in the current study than in ATS18 is expected due to better compliance. Therefore, we used the 16-week data from ATS18[25] to estimate the expected mean and standard deviation of change in VA (4.1 letters, 95% CI: 3.0 to 5.1 letters) and (6.0 letters, 95% CI: 5.3 to 6.9 letters), respectively, adjusted for baseline acuity.

Table 3: Previous Study Data from Subjects Aged 7 to <13 Years of Age

		% of Enrolled Subgroup *		Change in Amblyopic Eye VA at the 4 or 6 Week Visit (Letters) ‡	
Cohort	**N**	**Actual**	**Expected**	**Mean Change (95% CI)**	**SD Change (95% CI)**
Spectacle Correction Alone:					
ATS3 (Age 7 to <13 years) †	67			1.3 (0.004 to 2.6)	5.3 (4.5 to 6.4)
No prior amblyopia treatment	27	40%	17%	3.4 (-0.1 to 6.8)	5.7 (4.5 to 7.8)
Prior amblyopia treatment	40	60%	83%	0.9 (-0.4 to 2.1)	4.6 (3.8 to 5.9)
Weighted Estimate**				**1.3 (0.01 to 2.6)**	**5.3 (4.5 to 6.4)**
Binocular Treatment:					
E. Birch Study[26] (Age 7 to <10 years)^	5			9.0 (5.5 to 12.6)	2.5 (1.5 to 7.2)
ATS18 (Age 7 to <13 years) † §	118			1.8 (0.9 to 2.8)	5.1 (4.5 to 5.8)
No prior amblyopia treatment	22			2.3 (-0.4 to 5.1)	6.2 (4.8 to 8.9)
Prior amblyopia treatment	96			1.7 (0.7 to 2.7)	4.8 (4.2 to 5.6)

* For the percentage of enrolled subjects within each subgroup of prior amblyopia treatment status, the actual percentage is based on the ATS3 enrollment characteristics while the expected percentage projects the characteristics of the cohort for the current study, which was based on ATS18 (younger cohort trial).

‡ Positive values indicate improvement. Mean and SD adjusted for baseline visual acuity.

† ATS3 data were limited to randomized subjects aged 7 to <13 years with an amblyopic-eye VA of 20/40 to 20/200 inclusive (33 to 72 letters if E-ETDRS), ≥ 3 lines of interocular difference (≥ 15 letters) and a fellow-eye VA of 20/25 or better (≥ 78 letters if E-ETDRS). The baseline magnitude of tropia at near (as measured by SPCT) was limited to <5pd. The outcome data reported were based on the 6-week visit.

** A weight was calculated for each subject in the ATS3 study based on prior amblyopia treatment status (no prior amblyopia treatment, prior amblyopia treatment) by computing the ratio of the expected to the actual percentage (Weight = Expected % / Actual %).
^ E. Birch study enrolled children aged 7 to <10 years of age and all of these study subjects had prior amblyopia treatment at enrollment. Change in VA after 4 weeks of binocular therapy was computed as logMAR lines for subjects randomly assigned to receive binocular treatment using the Dig Rush game on an iPad device for 4 weeks (prescribed 1 hour per day, 5 days per week without patching). Subjects with >4pd magnitude of strabismus (measured by PACT) were excluded from the study.
§ ATS18 study subjects were prescribed binocular treatment for 1 hour per day, 7 days per week. The binocular game, Hess falling blocks, was played on an iPad device. The outcome data reported are from the 4-week masked outcome visit.

Summary:
Due to the limited data available on the Dig Rush binocular game, ATS18 data were used to estimate a treatment group difference in mean change in VA at 4 weeks of 3.75 letters (0.75 logMAR line) with a pooled standard deviation of 5 letters (1.0 logMAR line) for the current study.

Sample Size Estimation:
Assuming a standard deviation of 5 letters, a total sample size of 78 subjects (39 per group) has 90% power with a type I error rate of 5% to detect a treatment group difference between binocular treatment and spectacle correction alone assuming the true difference in mean VA change between the two groups is 3.75 letters (0.75 logMAR line) after 4 weeks of treatment (Table 4). Adjusting for 5% loss to follow-up, a total sample size of 84 (42 per group) is needed.

Table 4. Total Sample Size Estimates for a 2-Arm Study *

SD of Change (Letters)	Treatment Group Difference in Mean VA Change from Baseline at 4 weeks (Letters)				
	2.50	**3.75**	**5.00**	**6.25**	**7.50**
5	172	**78**	46	30	22
6	246	110	64	42	30
7	332	150	86	56	40
8	434	194	110	72	50

* Number in cells represents the total number of subjects required to detect a treatment group difference in amblyopic-eye VA change from baseline to 4 weeks using a t-test with a 2-sided alpha=0.05 and power 90% for a range of pooled SD of change in VA (letters).

5.3 Interim Analysis and Sample Size Re-estimation

The sample size estimates for both the younger and older age cohorts are based on previous studies of spectacle correction and binocular treatment. Although we believe that our estimates of variation are reasonable for both cohorts, a sample size re-estimation will be performed once approximately 50% of the pre-planned number of subjects have completed the 4-week outcome 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.[28] Within each age cohort, if the observed standard deviation of change is larger than the pre-study estimate, the sample size will be increased, up to a maximum limit corresponding to a standard deviation of change of 1.5 logMAR lines (182 subjects) for the younger cohort and 8 letters for the older cohort (206 subjects) with a 5% adjustment for loss to follow-up.

Due to the short duration of the primary outcome at 4 weeks and expected rapid recruitment, no interim monitoring will be conducted for either age cohort. This decision will be re-evaluated if the sample size is increased.

5.4 Analyses

All analyses described below will be conducted separately for both of the age cohorts.

5.4.1 Primary Analysis

5.4.1.1 Mean Amblyopic Eye VA at 4 Weeks

For both age cohorts, the primary objective is to compare the efficacy of 4 weeks of treatment with 1 hour/day of binocular game play 5 days per week plus spectacle correction to treatment with spectacle correction alone (subsequently referred to as "control" treatment).

An analysis of covariance (ANCOVA) will be performed to compute the 4-week mean change in amblyopic-eye VA for the binocular and control treatments, adjusted for baseline acuity, and a 95% confidence interval will be constructed on the treatment group difference.

The primary analysis will follow a modified intent-to-treat principle. Data will be included only from subjects who complete the 4-week exam within the pre-defined analysis window. There will be no imputation of data for subjects who are lost to follow-up or withdraw from the study prior to the 4-week exam. Multiple imputation for missing data will be performed as a secondary approach, and results of the analysis with imputation of missing data assessed for consistency with the primary analysis.

Additional approaches to the primary analysis include the following:

- Limit the analysis to subjects whose 4-week outcome exams were performed within the protocol window (3 to 5 weeks post-randomization)
- Include subjects who completed the 4-week exam outside of the pre-defined analysis window

5.4.2 Secondary Analyses

5.4.2.1 VA Improvement at 4 Weeks Defined as a Binary Outcome

A secondary analysis will estimate the proportion of subjects with amblyopic-eye VA improvement of ≥ 2 logMAR lines (≥ 10 letters if E-ETDRS) at 4 weeks after baseline.

The proportion of subjects who achieve this outcome will be tabulated by treatment group and an exact 95% confidence interval will be computed on the group proportion. A p-value for the treatment group comparison will be computed using binomial regression with adjustment for baseline VA. If the binomial regression model does not converge, Poisson regression with robust variance estimation or an exact method (without baseline adjustment) will be used to derive a p-value for the treatment group comparison.

5.4.2.2 Stereoacuity

Stereoacuity will be tabulated at baseline and 4 weeks according to treatment group with computation of descriptive statistics. The change in stereoacuity from baseline to 4 weeks will be tabulated for each group and compared between treatment groups using the exact Wilcoxon rank-sum test.

5.4.2.3 Treatment Compliance with Binocular Therapy

Data from the automated iPad log files will be used to provide an objective measure of compliance with binocular treatment. The total amount of game play will be computed for the initial 4 weeks of treatment for the binocular treatment group. Secondary analyses will evaluate the relationship between the total amount of game play with (1) change in VA and (2) change in stereoacuity after the first 4 weeks of binocular treatment.

5.4.2.4 Fellow-eye Contrast with Binocular Therapy

Data from the automated iPad log files will be used to assess game performance as measured by the fellow-eye contrast. The level and change in fellow-eye contrast will be computed for the initial 4 weeks of treatment for the binocular treatment group. Secondary analyses will evaluate the relationship between the change in fellow-eye contrast with (1) change in VA and (2) change in stereoacuity after the first 4 weeks of binocular treatment.

5.4.3 Safety

5.4.3.1 VA in Fellow Eye

The mean change in fellow-eye VA from baseline to 4 weeks will be calculated and compared between treatment groups using ANCOVA with adjustment for baseline VA. The proportion of subjects with loss of 2 or more logMAR lines (10 or more letters) of VA in the fellow eye from baseline to the 4-week exam will be reported for each treatment group and compared using Barnard's exact test.

5.4.3.2 Ocular Alignment

The proportion of subjects with development of new strabismus (no heterotropia at baseline and the presence of near and/or distance heterotropia at 4 weeks) or an increase from baseline ≥10Δ in a pre-existing strabismus at 4 weeks will be reported by treatment group and compared using Barnard's exact test.

5.4.3.3 Diplopia

The proportion of subjects with each level of diplopia frequency will be reported by treatment group at 4 weeks. Data will also be tabulated based on the maximum frequency of diplopia reported by treatment group. The change in diplopia frequency level from baseline to 4 weeks will be compared between treatment groups using the exact Wilcoxon rank-sum test.

5.4.3.4 Adverse Symptoms

The child and parent(s) will complete a 5-item symptom survey regarding the presence of various ocular symptoms within the past 2 weeks at enrollment and at each visit. The distribution of scores on each symptom survey item will be described for the enrollment exam and the 4-week exam for each treatment group. The distribution of change in scores on each symptom survey item will also be described for each treatment group.

5.4.4 Outcomes at 8 Weeks

As secondary analyses, all analyses described above will be repeated using data obtained from the 8-week visit.

5.4.5 Exploratory Analyses

5.4.5.1 Subgroup Analysis at 4 Weeks

The treatment effect after 4 weeks in subgroups based on baseline factors will be assessed in exploratory analyses and used to suggest hypotheses for further investigation in future studies. The following baseline factors are of interest: amblyopic-eye VA, stereoacuity, the presence of a tropia at near, and prior amblyopia treatment (other than spectacle correction). In accordance with NIH guidelines, subgroup analyses of treatment effect according to gender and race/ethnicity will be conducted. However, based on results from previous studies, a differential treatment effect by these variables is not expected.

The general approach for these exploratory analyses will be to conduct an analysis of covariance similar to the primary analysis adding an interaction for treatment and the subgroup covariate of interest.

The subgroup definitions for the planned subgroup analyses are as follows:

1. Amblyopic-eye VA at baseline (20/40, 20/50, 20/63, 20/80 or worse)
2. Age group (specified for each age cohort separately in the Statistical Analysis Plan)

3. Stereoacuity (nil versus better than nil)
4. Presence of a near heterotropia at baseline (yes/no)
5. Prior amblyopia treatment (yes/no)

5.4.5.2 Effect of Binocular Treatment in Children Randomized to Continued Spectacles

The following exploratory analyses will evaluate the effect of binocular treatment in children randomized to continued spectacles alone who are prescribed binocular treatment at the 8-week visit.

- A point estimate and 95% confidence interval will be calculated for the mean change in amblyopic eye VA between the 8-week and 16-week visits while on binocular treatment.
- The total amount of game play will be computed for the duration of binocular treatment as an estimate of compliance.
- The distribution of scores on each symptom survey item will be described for the 8-week exam prior to starting binocular treatment and the 16-week exam after a period of binocular treatment. The distribution of change in scores between 8 and 16 weeks on each symptom survey item will also be described

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