2 SYNOPSIS

Name of Company: **Spark Therapeutics** 3737 Market Street, Suite 1300 Philadelphia, PA 19104 **Name of Finished Product:** AAV2-hRPE65v2 **Name of Active Ingredient(s):** AAV2-hRPE65v2	**Individual Study Table Referring to Part of the Dossier:** **Volume:** **Page:**	***(For National Authority Use Only)***

Title of Study: A Safety and Efficacy Study in Subjects with Leber Congenital Amaurosis (LCA) Using Adeno-Associated Viral Vector to Deliver the Gene for Human RPE65 to the Retinal Pigment Epithelium (RPE).

Number and Name of Study Center(s):
001: The Children's Hospital of Philadelphia, Department of Ophthalmology, 34th and Civic Center Blvd, Philadelphia PA 19104 [Administration site]

005: University of Iowa Hospitals and Clinics, Department of Ophthalmology and Visual Science, 200 Hawkins Dr, Iowa City IA 52242 [Administration site]

Publications (reference):
There are currently no publications based on the clinical study.

Study Period:	**Clinical Phase:**
First subject, first visit: 15-Nov-2012 Last subject, last visit: 06-Apr-2015 Data cutoff: 16-Jul-2015	Phase 3

Objectives:
The objectives of the study were to assess the safety, tolerability, and efficacy of sequential, bilateral, subretinal administration of AAV2-hRPE65v2 to subjects with Leber congenital amaurosis (LCA) due to *RPE65* mutations. In addition to monitoring for safety and tolerability in the stated population, efficacy was evaluated using a number of retinal and visual function tests.

The **primary objective** was to determine whether non-simultaneous, bilateral subretinal administration of AAV2-hRPE65v2 improves the ability to navigate (as measured by standardized mobility testing) in adults and children with LCA due to *RPE65* mutations, three years of age or older.

Endpoints:
Efficacy Endpoints
The primary efficacy endpoint was performance on the mobility test, as measured by a change score one year following vector administrations as compared to subjects' pre-administration mobility test performance. Secondary efficacy endpoints included full-field light sensitivity threshold (FST) testing and visual acuity (VA) testing. Additional efficacy endpoints included pupillary light reflex (PLR) testing, independent orientation and mobility

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assessments, and other evaluations/measurements of visual and retinal function including a visual function questionnaire, visual field testing (Humphrey and/or Goldmann), and contrast sensitivity.

Safety Assessments

Safety Assessments included adverse event recording, concomitant medications, physical examinations, ophthalmic exams (including direct and indirect ophthalmoscopy), clinical labs (including serum chemistries, hematology testing, and urinalysis), immunology studies (including anti-AAV2 antibodies and antigen-specific T cell reactivities on PBMCs), and vector shedding analyses (including peripheral blood and tear PCR) to detect vector spread.

Methodology:

This was a Phase 3 open label, randomized controlled trial of gene therapy intervention by subretinal administration of AAV2-hRPE65v2. At least twenty-seven subjects, three years of age or older, were to be recruited at either The Children's Hospital of Philadelphia or University of Iowa to provide for complete data in at least twenty-four subjects.

Subjects randomized to the Intervention group ($n \geq 16$) were to receive non-simultaneous injections of 1.5E11 vector genomes (vg) AAV2-hRPE65v2 to each eye; sequential subretinal injections were to occur within an eighteen-day period. Subjects randomized to the Control group ($n \geq 8$) were not to receive AAV2-hRPE65v2 for a period of at least one year from Baseline evaluation. Following retinal and visual function analysis, including mobility testing, at one year's time, subjects in the Control group were to receive non-simultaneous injections of 1.5E11 vg AAV2-hRPE65v2 to each eye (also within eighteen days), provided they still met all study eligibility criteria.

Number of Subjects (planned and analyzed):

Planned: ≥ 27 (Intervention ≥ 18; Control ≥ 9)

Analyzed: 31 (Intervention = 21; Control = 10)

Diagnosis and Criteria for Inclusion:

Inclusion Criteria:

1. Willingness to adhere to protocol and long-term follow-up (LTFU) as evidenced by written informed consent or parental permission and subject assent (where applicable).
2. Diagnosis of LCA due to *RPE65* mutations; molecular diagnosis is to be performed, or confirmed, by a CLIA-certified laboratory.
3. Age three years old or older.
4. Visual acuity worse than 20/60 (both eyes) and/or visual field less than 20^{o} in any

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meridian as measured by a III4e isopter or equivalent (both eyes).

5. Sufficient viable retinal cells as determined by non-invasive means, such as optical coherence tomography (OCT) and/or ophthalmoscopy. Must have either: *1)* an area of retina within the posterior pole of >100 µm thickness shown on OCT; *2)* ≥ 3 disc areas of retina without atrophy or pigmentary degeneration within the posterior pole; or *3)* remaining visual field within 30^{o} of fixation as measured by a III4e isopter or equivalent.[1]
6. Subjects must be evaluable on mobility testing (the primary efficacy endpoint) to be eligible for the study. Evaluable is defined as:
   a. The ability to perform mobility testing within the luminance range evaluated in the study. Individuals must receive an accuracy score of ≤ 1 during Screening mobility testing at 400 lux or less to be eligible; individuals with an accuracy score of > 1 on all Screening mobility test runs at 400 lux, or those who refuse to perform mobility testing at Screening, will be excluded.
   b. The inability to pass mobility testing at 1 lux. Individuals must fail Screening mobility testing at 1 lux to be eligible; individuals that pass one or more Screening mobility test runs at 1 lux will be excluded.

Exclusion Criteria

1. Unable or unwilling to meet requirements of the study, including receiving bilateral subretinal vector administrations.
2. Any prior participation in a study in which a gene therapy vector was administered.
3. Participation in a clinical study with an investigational drug in the past six months.
4. Use of retinoid compounds or precursors that could potentially interact with the biochemical activity of the RPE65 enzyme; individuals who discontinue use of these compounds for 18 months may become eligible.
5. Prior intraocular surgery within six months.
6. Known sensitivity to medications planned for use in the peri-operative period.
7. Pre-existing eye conditions or complicating systemic diseases that would preclude the planned surgery or interfere with the interpretation of study. Complicating systemic diseases would include those in which the disease itself, or the treatment for the disease, can alter ocular function. Examples are malignancies whose treatment could affect central nervous system function (for example: radiation treatment of the orbit; leukemia with

[1] Equivalent test objects used in other, similar VF testing protocols

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central nervous system (CNS)/optic nerve involvement). Subjects with diabetes or sickle cell disease would be excluded if they had any manifestation of advanced retinopathy (*e.g.*, macular edema or proliferative changes). Also excluded would be subjects with immunodeficiency (acquired or congenital) as there could be susceptibility to opportunistic infection (such as CMV retinitis).

8. Individuals of childbearing potential who are pregnant or unwilling to use effective contraception for four months following vector administration.
9. Individuals incapable of performing mobility testing (the primary efficacy endpoint) for reason other than poor vision, including physical or attentional limitations.
10. Any other condition that would not allow the potential subject to complete follow-up examinations during the course of the study or, in the opinion of the investigator, makes the potential subject unsuitable for the study.

Subjects were not to be excluded based on their gender, race, or ethnicity.

Test Product, Dose, Mode of Administration and Lot Number:
AAV2/2.CMV.CβA.hRPE65: Adeno-associated viral type 2 vector with cytomegalovirus (CMV) enhancer, chicken beta actin (CβA) promoter driving expression of human retinal pigment epithelium 65 kDa protein (*RPE65*) gene with an optimized Kozak sequence.

AAV2-hRPE65v2 was formulated in 180 mM Sodium Chloride, 10 mM Sodium Phosphate, 0.001% Lutrol® F68 (Pluronic F68/Poloxamer 188), pH 7.3

The AAV2-hRPE65v2 used was Lot 142-07001 (DOM: 03-Jan-2007).

Duration of Treatment:
This study involved bilateral, subretinal injections of the test article (one-time per eye) with at least 1 year of post-injection follow-up.

Reference Therapy, Dose and Mode of Administration, Lot Number:
Control group subjects who did not receive AAV2-hRPE65v2 for a period of at least one year from Baseline evaluation.

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Criteria for Evaluation:

Efficacy Assessments

Efficacy assessments included:

- Mobility testing
- Full-field light sensitivity threshold testing
- Visual acuity
- Pupillary light reflex testing
- Visual field testing – Humphrey and/or Goldmann
- Contrast sensitivity
- Visual function questionnaire
- Orientation and mobility assessment

Safety Assessments

Safety assessments included:

- Adverse event recording
- Concomitant medications
- Physical examinations
- Ophthalmic exams, including direct and indirect ophthalmoscopy
- Clinical labs: serum chemistries including liver and renal function panels, hematology tests, CBC with differential, urinalysis
- Immunology studies: anti-AAV antibodies and antigen-specific T cell reactivities on PBMCs
- Vector shedding: peripheral blood and tear PCR to detect vector spread

Statistical Methods:
Full statistical methodology was developed in a formal statistical analysis plan.

Subjects were randomized to either the Intervention or the Control group stratified by Screening age category (≥ 10 years or < 10 years) and Screening mobility testing passing level (≥ 125 lux or < 125 lux) in a 2:1 ratio of Intervention to Control. Within each age and mobility testing stratum, randomized blocks governed the allocation to treatment group.

Descriptive statistics were to be tabulated for the study population. Subject disposition, demographic and baseline characteristics, extent of exposure, and information on study

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termination and withdrawal were to be summarized and presented by the means, standard deviation (SD) or standard error (SE), and ranges for continuous variables, as well as by counts and percentages for categorical variables. The efficacy analyses included all randomized subjects (intent to treat, or ITT, population). The safety analyses (adverse event data and labs) included all subjects who received injection for the Intervention group, and all Control subjects who did not withdraw prior to Baseline.

Primary efficacy endpoint analyses: The primary efficacy endpoint was the mobility test change score. Specifically, the study was to measure the ability of vector administration to increase visual function, as evidenced by an increase relative to controls in mean mobility test change score at one year after Baseline. The analysis was to use a non-parametric permutation test based on a Wilcoxon Rank-Sum as the observed test statistic and an exact method for the corresponding *p*-value. Additional analyses were performed with the modified ITT (mITT) and per protocol (PP) populations. Additional sensitivity analyses were performed to determine the robustness of the results of the primary analysis.

Second efficacy endpoint analyses: The secondary efficacy endpoints of FST and visual acuity were to be analyzed based on longitudinal repeated measures models that provided estimates of the difference between Baseline and Year 1 between the two treatment groups. For the monocular mobility testing, analyses were to use models analogous to the model described for the primary outcome. The secondary outcomes were only to be formally tested statistically if the primary outcome was statistically significant; testing of the secondary outcomes was to be performed using a hierarchical ordering.

Summary and Conclusions:

<u>Efficacy Results</u>:

Efficacy results at Year 1 are as follows:

- For the primary endpoint, analysis of the bilateral MT change score for the ITT population indicated a statistically significant treatment effect, with a mean (95% CI) group difference (Intervention – Control) of 1.6 (0.72, 2.41; $p = 0.001$). Similar results were observed for the mITT (1.6 [0.76, 2.50]; $p = 0.004$) and PP (1.7 [0.79, 2.56]; $p = 0.004$) analysis populations.
- For the mITT population, 65% of subjects in the Intervention group passed the mobility testing at the lowest light level evaluated (1 lux; score = 6), representing the maximal improvement measurable while no Control subject passed at this low light level.
- Supportive of the primary endpoint, analysis of the MT change sum score for the ITT

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population showed a statistically significant treatment effect, with a mean (95% CI) group difference (Intervention – Control) of 5.3 (3.11, 7.42; $p < 0.001$). The mITT and PP analysis populations showed similar results (5.5 [3.35, 7.64]; $p < 0.001$ for mITT; 5.5 [3.29, 7.70]; $p = 0.001$ for PP).

- 45% of subjects in the Intervention group demonstrated the maximum possible MT sum score of 18 (*i.e.*, passed the mobility testing at 1 lux for each individual eye and both eyes together), while no Control subject demonstrated this degree of low light performance.
- For the secondary endpoint of full-field light sensitivity threshold testing, which reflects underlying physiological function by measuring light sensitivity of the entire visual field, the mean (SE) change across both eyes from Baseline was -2.08 (0.29) log10(cd.s/m^2) for the Intervention group and 0.04 (0.44) log10(cd.s/m^2) for the Control group, for a statistically significant ($p < 0.001$) between-group mean (95% CI) treatment difference of -2.11 (-3.19, -1.04) log10(cd.s/m^2).
- For the secondary endpoint of the monocular MT change score for the first eye, the mean (SD) change from Baseline was 1.9 (1.2) for the Intervention group and 0.2 (0.6) for the Control group, resulting in a statistically significant ($p = 0.001$) mean (95% CI) treatment effect difference of 1.7 (0.89, 2.52).
- For the secondary endpoint of visual acuity, which measures changes in central vision by assessing the ability of the subject to read a standard eye chart, the mean (SE) change across both eyes from Baseline was -0.16 (0.07) LogMAR for the Intervention group and 0.01 (0.10) LogMAR for the Control group. The observed LogMAR changes reflect a mean 8-letter improvement on the eye chart for Intervention subjects vs. a mean 0.5-letter loss for Control subjects. The resulting mean (95% CI) treatment difference of -0.16 (-0.41, 0.08) LogMAR (an 8-letter improvement) was not statistically significant ($p = 0.17$).
- If the Lange scale is used for off-chart VA results, the mean (SE) change across both eyes from Baseline was -0.18 (0.04) LogMAR (a 9-letter improvement) for the Intervention group and -0.03 (0.06) LogMAR (a 1.5-letter improvement) for the Control group, resulting in a statistically significant (nominal $p = 0.047$) mean (95% CI) treatment difference of -0.15 (-0.29, -0.00) LogMAR (a 7.5 letter improvement).
- Analysis of visual function by subject- and parent-completed questionnaires indicated a statistically significant reduction in the perceived difficulty of daily living activities for subjects in the Intervention group, while no such improvement was noted for subjects in the Control group. Mean (95% CI) differences in changes from Baseline between the two

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treatment groups were 2.4 (1.0, 3.8), nominal $p = 0.001$ for subjects and 4.0 (2.1, 6.0), nominal $p = 0.002$ for parents.

- Analysis of visual fields via Goldmann perimetry showed increased mean sum total degrees for subjects in the Intervention group as compared to no change or reduced mean values for subjects in the Control group, indicating improvements in peripheral vision following vector administration; these improvements reached the level of significance for the III4e test stimulus (mean [95% CI] treatment difference: 378.7 [145.5, 612.0] sum total degrees; nominal $p = 0.006$) but not the V4e test stimulus (mean [95% CI] treatment difference: 86.0 [-186.1, 358.1] sum total degrees; nominal $p = 0.67$).
- Humphrey computerized testing showed statistically significant increased mean macula threshold values for the Intervention group with no meaningful change in the Control group (Year 1 treatment difference [95% CI]: 7.9 [3.5, 12.2] decibels [dB]; nominal $p < 0.001$). No statistically significant changes were noted for foveal threshold levels (Year 1 treatment difference [95% CI]: 0.04 [-7.1, 7.2] dB; nominal $p = 0.18$).
- Pupillometry testing showed a statistically significant (nominal $p = 0.007$) overall mean (95% CI) difference of 11.37 (3.13, 19.61) for low mesopic light levels, with increased geometric mean (SE) values for the Intervention group (7.43 [2.30]) as compared to reduced values for the Control group (-3.94 [3.45]). No statistically significant changes were noted for scotopic or high mesopic light levels, in fact the right and left flash test group trends differed for the scotopic testing condition. This likely reflects inherent variability of PLR testing as performed rather than an actual treatment effect difference.

Safety Results:

- Bilateral, subretinal administration of AAV2-hRPE65v2 was generally well tolerated, both locally and systemically.
- No deaths or related serious adverse events were reported. Two subjects in the Intervention group experienced three SAEs at time points distant from vector administration. Each of these SAEs were considered unlikely to be related to study drug or study drug administration procedure and the events were recovered/resolved with no sequelae.
- No TEAEs were considered related to the study drug; 13 (65%) subjects in the Intervention group experienced TEAEs considered related to the study drug administration procedure. The most common TEAEs that were considered related to the administration procedure were cataract and increased intraocular pressure, which were both reported in three (15%) subjects. Most TEAEs were mild in severity and recovered

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or resolved with no sequelae.

- One subject (IA-33) developed an asymptomatic full thickness macular hole that was noted 10 days after Day 0A; this event improved to mild macular degeneration (thinning) that was noted 37 days after Day 0A and recovered or resolved with no sequelae by the Day 90B visit. These adverse events did not prevent improvement in retinal/visual function, including MT performance.
- Based on an estimated area of retina without confluent atrophy measure, all subjects, in both the Intervention and the Control groups, exhibited stable fundus examinations between Baseline and Year 1 visits.
- Of the four different OCT evaluations, only Heidelberg OCT foveal thickness for the first eye showed a statistically significant difference (95% CI) of -14.2 (-26.9, -1.5) microns (nominal $p = 0.017$) between the Intervention and the Control groups. Variability from differing anatomic locations and fixation challenges may limit comparisons of Baseline and follow-up OCT evaluations.
- Occasional subjects have presented with areas of fundus depigmentation, including outside the area of the injection blebs. Subjects remain asymptomatic, with no functional correlate reported.
- No clinically significant changes in laboratory tests or physical examinations were observed at any time point; 14 (70%) subjects in the Intervention group had isolated increases or decreases in blood pressure or heart rate, with no apparent safety signals associated with these events and no adverse events linked to changes in vital signs.

CONCLUSIONS:

Subretinal injection of AAV2-hRPE65v2 was safe and well-tolerated. The retinal and visual function changes observed through one year following bilateral administration of AAV2-hRPE65v2 suggest durable improvements in visual performance. These improvements contrast with the progressive nature of inherited retinal degenerative conditions in which subjects face an inexorable deterioration of retinal and visual function, which progresses until no useful vision remains.

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