

Consideration of Endpoints for the First Interventional Gene Therapy Clinical Study in Inherited Retinal Diseases Caused by BEST1 Mutations

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ABSTRACT

Purpose: The first interventional clinical study to assess the safety and efficacy of a novel gene therapy in the treatment of patients with autosomal dominant and recessive inherited retinal diseases caused by pathogenic BEST1 variants (BEST1-IRD) has been designed. Studies in patients were reviewed to select appropriate endpoints for a future clinical trial.

Methods: Nine canines with a BEST1 mutation were bred to the appearance of a vitelliform lesion at week 27. The canines were treated with either subretinal balanced salt solution (BSS) or subretinal AAV therapy with human transgene (1.4E9 vg/eye or 4.5E9 vg/eye). Safety and efficacy were evaluated to 103 weeks following treatment. Results of the canine model were integrated with prior natural history studies of BEST1-IRD patients to consider endpoints for the proposed clinical study.

Results: All canines tolerated the procedure and therapy well. Restoration of the RPE-photoreceptor interface was demonstrated, including resolution of the retinal microdetachments. Three natural history studies were identified with long-term follow up of multi-model imaging and functional analysis in BEST1 patients. While best corrected visual acuity is not a highly sensitive biomarker, rod and cone sensitivity measures are correlated with anatomical features of disease.

Conclusions: The results of canine interventional and human natural history studies in BEST1-IRD suggest a human interventional study may show anatomical resolution of the retinal changes with functional improvement in localized photoreceptor function and dark adaptation kinetics.

METHODS

Canine Interventional Study¹: Nine cBEST canines were injected with AAV-hBEST1 (1.4E9 vg/eye or 4.5E9 vg/eye) or BSS at 27 weeks. Subjects were injected unilaterally with AAV-hBEST1, leaving the fellow eye uninjected, or with AAV-hBEST1 in one eye and control (BSS) in the contralateral eye. Eyes were monitored clinically and by in vivo imaging.

Human Natural History Studies^{2,4}: Three natural history studies have evaluated retinal function and structure in patients with BEST1-IRD:

(1) 17 autosomal dominant BEST1 patients (ages 6-59) were evaluated using dark- and light-adapted chromatic perimetry, near infrared excited reduced illuminance autofluorescence imaging (NIR-RAFI), and optical coherence tomography (OCT). 6 patients were followed long-term (14-22 years) and dark adaptation kinetics was measured in 5 patients.²

(2) 4 patients with the autosomal recessive bestrophinopathy (ARB) phenotype (ages 22-39) were evaluated using dark- and light-adapted chromatic perimetry, dark-adaptation kinetics, short-wavelength excited reduced-illuminance autofluorescence imaging (SW-RAFI), and OCT.³

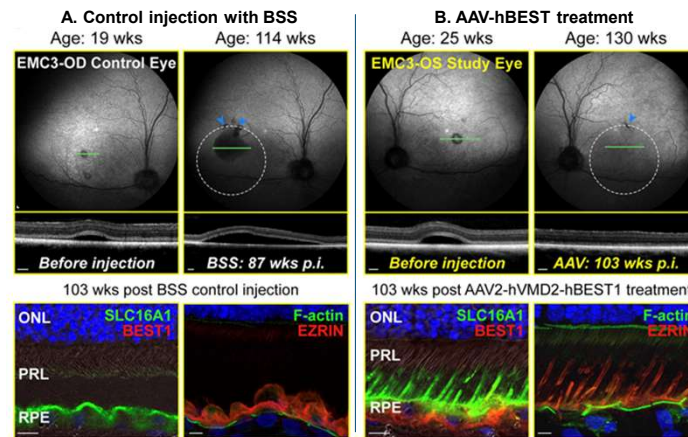
(3) Multimodal adaptive optics (AO) imaging was performed in 3 subjects with BEST1 mutations.⁴

RESULTS

Canine Interventional Study¹: In a representative result from EMC3 eyes exhibiting early vitelliform lesions at ages 19 and 25 weeks, the lesion in the study eye (EMC3-OS) disappeared after injection with AAV-hBEST1 treatment (Figure 1B, upper right), while the lesion in the control eye (EMC3-OD) injected with BSS continued to enlarge. (Figure 1A, upper left).

In all cases, transient retinal detachment associated with injection resolved within 24 to 48 hours, however, in control eyes, lesions reappeared as early as 1 week and progressed, while lesions in the study eyes fully resolved within 6 weeks and remained disease free. Furthermore, there was pronounced restoration of the RPE-PR interface in the study eye (Figure 1B, lower right) compared to the control eye (Figure 1A, lower left). No adverse effects on the retina were observed up to 207 weeks after treatment.

Figure 1. Reversal of lesions and restoration of RPE-photoreceptor interface structure post-AAV-BEST1 treatment in the cBest (R25*/R25*) model compared to control



Top row: Bleb boundaries marked by dashed circles; the locations of corresponding OCT scans cut through the subretinal lesions before injection or through the matching locations mapped p.i. are marked by green horizontal lines; retinotomy sites are indicated by blue arrowheads. Scale bars, 200 µm. Bottom row: Scale bars, 10 µm.

Human Natural History Studies^{2,4}:

(1) The majority of BEST1 eyes had relatively preserved best-corrected visual acuity (BCVA) (0.2 logMAR; 20/30 Snellen equivalent) at baseline despite the presence of large vitelliform lesions.² Over long-term follow-up (up to ~15 years), BCVA declined slowly at a rate of approximately one ETDRS letter per year. Rod sensitivity was significantly reduced in lesion areas, whereas cone function remained relatively intact, even in eyes with large serous retinal detachments. Slowed dark-adaptation kinetics further indicated a defect in chromophore recycling, likely involving rod photoreceptors.

Structural abnormalities were observed including large intraretinal cysts, serous subretinal detachments, and hyperthickening of the outer nuclear layer (ONL), extending from the fovea to mid-peripheral retina. Notably, widening of the subretinal space in extrasilesional areas was associated with slowed rod function and later formation of satellite lesions. A retina-wide ONL thickening was also observed, which progressed over time in many patients.

RESULTS, CONT.

(2) ARB patients exhibited significant retinal changes including large intraretinal cysts in the inner nuclear layer, shallow serous retinal detachments, disruptions from the external limiting membrane (ELM) to RPE, and unusual prominence in the ELM peak. Despite this, some patients maintained preserved VA and cone sensitivity within central lesions. Rod-mediated function was severely compromised, with substantial loss of dark-adapted rod sensitivity and slowed dark-adaptation kinetics, suggesting a chromophore recycling defect. Over long-term follow-up, some eyes developed new satellite lesions in retinal areas that previously showed subretinal widening.

(3) RPE cells were significantly more affected than cones in patients with BEST1. Significant differences in RPE density were observed within 1mm of lesion edges. Even in contralateral eyes without visible lesions, similar patterns of cone and RPE disruption were detected, suggesting subclinical changes. Areas that developed new lesions over time showed a decrease in both cone and RPE densities, with RPE cells being more affected. After lesion resorption, further reductions in cell densities were observed.

CONCLUSIONS

- Subretinal injection of AAV gene therapy for BEST1 mutations reversed lesions and microdetachments and restored cytoarchitecture of the RPE-PR interface in a canine BEST1 interventional study, establishing proof-of-concept of AAV gene therapy for the treatment of BEST1-IRD.¹
- Many BEST1 patients retain VA despite significant lesions and rod dysfunction; Low-luminance visual acuity (LLVA) has been used in AMD studies and several central serous retinopathy patients have shown large LLVA reductions despite retaining BCVA; LLVA could be evaluated as a viable outcome measure, in addition to standard BCVA.²
- Rod sensitivity was consistently more affected than cone function, even in areas without overt lesions; Standard microperimetry has several limitations and requires fixation, which is difficult with BEST1 patients; A method of microperimetry that allows for early and accurate results in terms of function of the underlying photoreceptor system would be important in future studies.²
- NIR-RAFI can assess RPE structure and integrity with less light hazard compared to SW-AF and shorter exam time; NIR-RAFI could be more sensitive to early signs of disease and new lesions, making it a viable option for monitoring disease activity and progression.^{2,3}
- OCT cross-sectional imaging helps measure the size of lesions and detect subtle changes like thickening of the ONL and increased spacing between photoreceptor layers and the RPE. These subtle changes may be the only early signs of disease in nearby retina and can serve as useful markers to evaluate progression and treatment effects.
- Multimodal AO imaging allows for simultaneous quantification of the cone photoreceptor and RPE, as well monitoring the RPE outside the lesion area and/or in contralateral eyes, which is particularly important in BEST1 patients.⁴
- The results of natural history studies in humans underscore the importance of selecting and utilizing outcome measures to assess meaningful changes in retinal structure and visual function in BEST1 patients.²⁻⁴

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