Exploratory Safety Profile of EDIT-101, a First-in-Human *in vivo* CRISPR Gene Editing Therapy for *CEP290*-Related Retinal Degeneration

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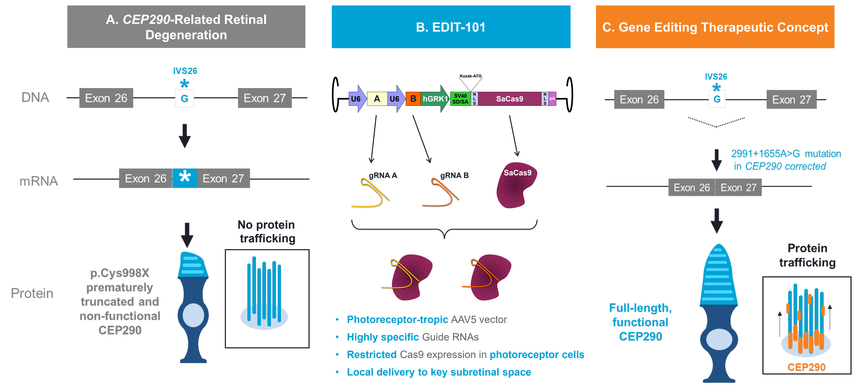
Introduction

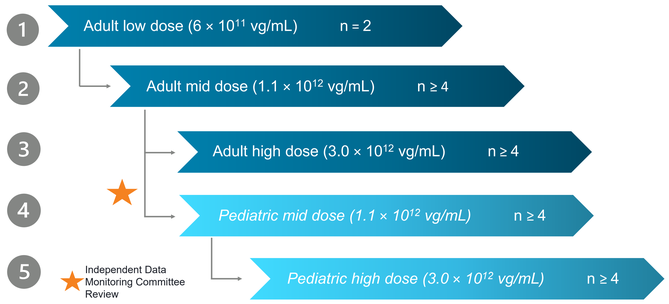
- CEP290 protein localizes to connecting cilium of photoreceptors and is essential for ciliary assembly and protein trafficking[1].
- *CEP290* mutations frequently cause Leber congenital amaurosis type 10 (LCA10), a retinal degenerative condition resulting in severe visual impairment[2].
- The most common *CEP290* mutation causing LCA10 is c.2991+1655A>G within intron 26 (CEP290-IVS26), which results in a nonfunctional CEP290 protein (Figure 1A)[2].
- Currently, there are no approved LCA10 treatments. To address this medical need, we developed EDIT-101, a Cas9-based gene editing medicine that specifically removes the CEP290-IVS26 mutation to restore photoreceptor function.
- The safety, tolerability, and efficacy of EDIT-101 are being assessed in the BRILLIANCE clinical trial (Figure 2). Viral shedding is a key exploratory safety end-point and here we present viral shedding data from EDIT-101 treated patients.

Figure 1. EDIT-101, a first-in-human *in vivo* CRISPR gene editing medicine for *CEP290*-related retinal degeneration



***CEP290*-Related Retinal Degeneration, EDIT-101, and Gene Editing Therapeutic Concept overview. A.** The most common *CEP290* mutation is c.2991+1655A>G within intron 26 (CEP290-IVS26), which generates a non-functional CEP290 and progressive retinal degeneration that results in LCA10[1,2]. **B.** EDIT-101 utilizes the photoreceptor-tropic adeno-associated virus type 5 (AAV5) to deliver DNA encoding *Staphylococcus aureus* Cas9 (SaCas9) expressed under the photoreceptor-specific *GRK1* promoter and two gRNAs under U6 promoters. Both gRNAs are highly specific to the CEP290 locus, with no off-targets identified[3]. **C.** The two gRNAs direct SaCas9-mediated DNA cleavage up- and downstream of the CEP290-IVS26 mutation, excising it, and restoring CEP290 splicing and function[3].

Figure 2. BRILLIANCE Phase 1/2 study overview (NCT03872479)



BRILLIANCE study overview: The BRILLIANCE study (NCT03872479) is a Phase 1/2, open-label, single ascending dose clinical trial designed to evaluate safety, tolerability, and efficacy of EDIT-101 in patients with *CEP290*-related retinal degeneration. Up to 34 patients will be enrolled in up to 5 cohorts to evaluate up to 3 dose levels of EDIT-101. Patients receive a single subretinal injection of EDIT-101 into their eye with the worst visual acuity. Study follow up occurs for 3 years, after which patients are asked to consent to participate in a long-term follow-up study for an additional 12 years.

Methods

Table 1. Assay sensitivity and linear range

Assay sensitivity and linear range				
Matrix	**Linear range (copy number)**	**Inter-assay precision (% Ct CV)**	**E (%)**	**R^2**
Blood	25 to 10^7	0.8–1.8	94–104	≥0.998
Tears	25 to 10^7	0.6–2.0	92–103	≥0.998
Nasal mucosa	25 to 10^7	0.3–1.2	94–98	≥0.999

Sensitivity and linear range assessment: Assay sensitivity in all blood, tears, and nasal mucosa was determined over 5 runs using a linearized plasmid encoding SaCas9 (PLA323) as a surrogate for EDIT-101. Linear range is reported in copies of linearized PLA323 or double stranded EDIT-101 viral vector DNA. Limit of detection (LoD) and limit of quantification (LoQ) were determined to be 10 and 25 copies of either PLA323 or EDIT-101 DNA, respectively. Inter-assay precision indicates the coefficient of variation (CV) between different runs. PCR efficiency and goodness of fit are represented by the E and R^2 values, respectively.

Table 2. Matrix effect

Matrix effect of sample DNA on qPCR	
Matrix	**% of nominal concentration**
Blood	78–81
Tears	109–115
Nasal mucosa	110-113

Matrix effect assessment: Matrix effect in blood, tears, and nasal mucosa was determined by spiking PLA323 with matrix DNA and dividing the observed PLA323 copy number by the input copy number. Matrix DNA alone was not amplified above the LoD, indicating the assay is specific for the SaCas9 DNA sequence (data not shown). Nominal value is equal to $1.28e^{12}$ EDIT-101 viral genomes/mL for blood and tears and $5.49e^{11}$ EDIT-101 viral genomes/mL for nasal mucosa.

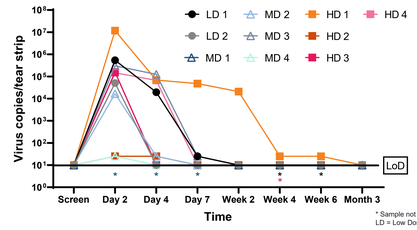
Table 3. Stability of EDIT-101 in matrices at different temperatures and timepoints

Stability of EDIT-101 in matrices (% of EDIT-101 at T = 0)												
Storage temperature (°C)	**18–25**		**2–8**		**−10 to −30**		**−60 to −90**					
Storage time	**4 hrs**		**24 hrs**		**1 m**		**Freeze/thaw (n=3)**		**1 m**		**6 m**	
Viral spike	Low	High	Low	High	Low	High	Low	High	Low	High	Low	High
Blood	101	102	93	91	90	93	89	82	76	77	95	92
Tears	103	93	110	101	121	104	121	110	87	90	91	84
Nasal mucosa	99	97	93	107	109	112	95	104	109	127	109	126

Stability of EDIT-101 viral vector in matrices mimicking anticipated clinical sample storage conditions: Stability was determined by spiking EDIT-101 into blood, tears, or nasal mucosa samples, subjecting the samples to conditions listed above, and then processing them for DNA extraction and subsequent qPCR analysis. Values within the table represent the observed value divided by the T = 0 value. Values at T = 0 were determined by spiking EDIT-101 into blood, tears, or nasal mucosa samples and immediately processing them for DNA extraction and qPCR analysis. High spike of EDIT-101 at T = 0 were $1.0e^5$, $1.0e^4$, and $5.0e^5$ for blood, tears, and nasal mucosa, respectively. Low spike of EDIT-101 at T = 0 were $1.0e^4$, $1.0e^3$, and $5.0e^4$ for blood, tears, and nasal mucosa, respectively.

Results

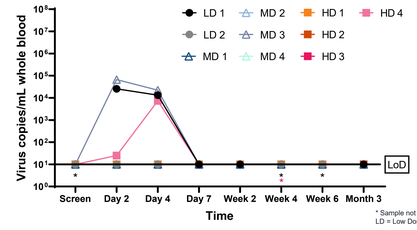
Figure 3. EDIT-101 viral shedding in tears from the treated eye



Tear samples were collected from inside the lower eyelid of both the treated and untreated eye. Tears collected 2-days post EDIT-101 administration contained the highest observed quantity of viral genomes. EDIT-101 was below the LoQ in most patient samples by day 7 and one patient by week 4. Only samples from the treated eye contained quantifiable levels of EDIT-101 viral genomes.

* Sample not collected
LD = Low Dose Patient; MD = Mid Dose Patient; HD = High Dose Patient.

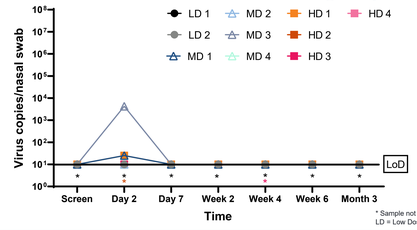
Figure 4. EDIT-101 viral shedding in blood samples



Blood samples from only three patients had quantifiable levels of EDIT-101 viral genomes 2- and 4-days post EDIT-101 administration. EDIT-101 viral clearance for all patients was observed by day 7.

* Sample not collected
LD = Low Dose Patient; MD = Mid Dose Patient; HD = High Dose Patient.

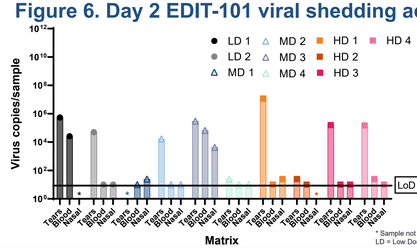
Figure 5. EDIT-101 viral shedding in ipsilateral nasal mucosa samples



Nasal mucosa samples were collected from both nostrils. Only one sample collected ipsilateral to the treated eye contained quantifiable levels of EDIT-101 viral genomes. EDIT-101 viral clearance for all patients was observed by day 7. Nasal mucosa samples collected contralateral to the treated eye did not contain quantifiable levels of EDIT-101 (data not shown).

* Sample not collected
LD = Low Dose Patient; MD = Mid Dose Patient; HD = High Dose Patient.

Figure 6. Day 2 EDIT-101 viral shedding across dose cohorts



The highest EDIT-101 viral shedding levels were observed in day 2 samples across most matrices and cohorts. For HD 4, their day 4 blood sample had the highest level of EDIT-101. Observed shedding levels are orders of magnitude lower than the administered dose. The HD 1 day 2 tear sample had the highest observed EDIT-101 level ($1.78e^7$ copies/tear strip). Compared to the administered dose of $9.15e^{11}$ EDIT-101 copies, the highest shed levels represent <1% of the administered dose. No correlation between doses and viral shedding was observed.

* Sample not collected
LD = Low Dose Patient; MD = Mid Dose Patient; HD = High Dose Patient.

Conclusions

- We developed a SaCas9-specific qPCR assay to evaluate EDIT-101 viral shedding in patient samples from the BRILLIANCE trial.
- Detected EDIT-101 viral genome levels in patient samples were significantly lower than administered doses (<1%). Viral genomes were detected more frequently, and at a higher concentration, in tear samples from the treated eye than any other matrix analyzed.
- EDIT-101 shedding was transient, with viral clearance below the LoQ observed for most patients by Day 7 and one HD patient by Week 4 in tears and Day 7 in blood and nasal mucosa, suggesting very little risk of systemic viral persistence following treatment.
- No correlation between EDIT-101 doses and viral shedding levels was observed. Taken together, these data suggest a favorable immunogenicity profile for EDIT-101.

Disclosures

MCJ, SE-H, BRD, AE, RM, MSS, KZ, & SM are employees of Editas Medicine. MEP is an employee of Casey Eye Institute. EP is an employee of the Ocular Genomics Institute. MEP and EP are PIs in the BRILLIANCE clinical trial and consult for Editas Medicine. MEP received funding from the Unrestricted Grant from Research to Prevent Blindness, New York, New York and P30 EY010572.

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References: [1]Drivas, T., Bennett, J. (2014). *Ret. Degen. Dis.* doi.org/10.1007/978-1-4614-3209-8_66; [2]den Hollander AI, et al. *Am. J. Hum. Genet.* (2006), doi.org/10.1086/507318 [3]Maeder ML, et al. *Nat. Med.* (2019) doi.org/10.1038/s41591-018-0327-9.