# Development of cell-based expression and functional potency assays for OPGx-BEST1 gene therapy

3617 - 0710

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## Purpose

- Bestrophinopathies are inherited retinal dystrophies caused by pathogenic variants in the *BEST1* gene.[1]
- OPGx-BEST1 is an AAV-based gene therapy designed to deliver *BEST1* transgene to the retinal pigment epithelium (RPE) cells to restore BEST1 expression and function.
- Analytical methods were developed to determine the relative potency of OPGx-BEST1 using RT-dPCR, western blot, and ELISA.
- The feasibility of quantitating the functional activity of OPGx-BEST1 was evaluated.

## Methods

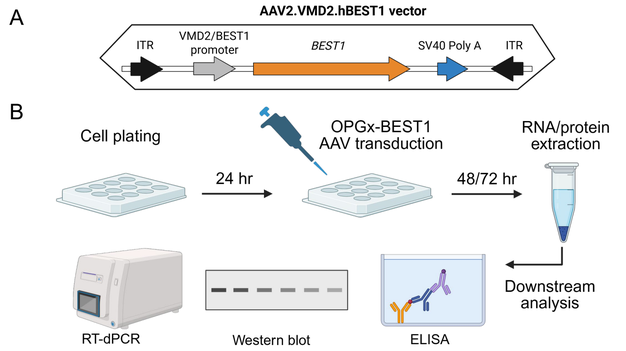



**Figure 1. In vitro cell-based potency assay workflow for OPGx-BEST1. (A) Schematic of OPGx-BEST1 AAV vector. (B) Schematic of assay workflow.**

- ARPE19, ARPE19-AAVR (engineered to express AAV receptor), and HEK293 cells were screened to establish potency assays.
- Cells were transduced with OPGx-BEST1 and RNA and/or protein extracts were prepared at 48- and 72-hour time points. RNA extracts were subjected to RT reaction and QIAcuity digital PCR system was used for thermocycling and detection.
- The linear range of the RT-dPCR method was established by testing 5E2 to 6E3 vg/cell in ARPE19 cells. Method accuracy was evaluated by comparing the nominal % potency and determined % potency. Method precision and specificity were evaluated by using biological replicates (n=4) and diluent treatment. Additionally, 3 lots of OPGx-BEST1 drug substance and drug product material were tested in the method.
- BEST1 protein expression was assessed by western blot (E6-6 monoclonal antibody) and by a commercially available ELISA kit.
- A halide sensitive-YFP quenching assay was developed to assess functional potency.[2] HEK293 cells stably expressing YFP (H148Q/I152L) were transiently transfected with plasmids expressing WT or mutant BEST1, then treated with 75 mM NaI with or without calcium Ionophore A23187 (5 µM). YFP fluorescence intensities were monitored in 20 sec intervals.

## Results

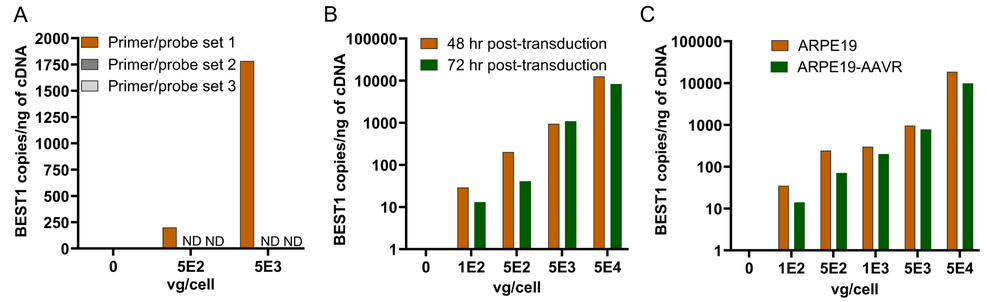
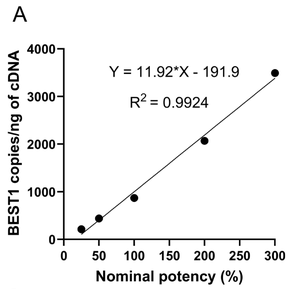



**Figure 2. Development of an RT-dPCR method for the quantification of OPGx-BEST1 gene expression. (A) Evaluation of Primer/Probe variants for BEST1 detection in ARPE19 cells. (B) BEST1 expression at different time points following OPGx-BEST1 transduction in ARPE19 cells. (C) Comparative evaluation of OPGx-BEST1 across multiple cell models.**




B

| vg/cell | BEST1 copies/ng of cDNA | Avg. BEST1 copies/ng of cDNA | % RSD | Nominal % Potency | Determined % Potency | % Accuracy |
|---|---|---|---|---|---|---|
| 5E2 | 241.3 | 212.4 | 19% | 25% | 28% | 98% |
| | 183.5 | | | | 21% | |
| 1E3 | 467.2 | 440.4 | 9% | 50% | 54% | 101% |
| | 413.7 | | | | 48% | |
| 2E3 | 850.6 | 869.2 | 6% | 100% | 98% | 100% |
| | 845.8 | | | | 97% | |
| | 941.4 | | | | 108% | |
| | 839.1 | | | | 97% | |
| 4E3 | 2385.2 | 2069.6 | 22% | 200% | 274% | 119% |
| | 1754.0 | | | | 202% | |
| 6E3 | 3308.3 | 3493.6 | 8% | 300% | 381% | 134% |
| | 3678.9 | | | | 423% | |

C

| Sample | BEST1 copies/ng of cDNA |
|---|---|
| Diluent prep1 | 0 |
| Diluent prep2 | 0 |
| 5E2 vg/cell prep1 | 241.3 |
| 5E2 vg/cell prep2 | 183.5 |

D

| Sample | % Relative Potency |
|---|---|
| OPGx-BEST1 standard | 100 |
| OPGx-BEST1 lot 1 | 112 |
| OPGx-BEST1 lot 2 | 110 |

**Figure 3. RT-dPCR potency assay performance in ARPE19 for fit-for-purpose prequalification. (A) Linearity analysis. (B) Precision across replicates and accuracy of BEST1 quantification. (C) Assay specificity for BEST1 detection. (D) Relative potency comparison of OPGx-BEST1 lots.**

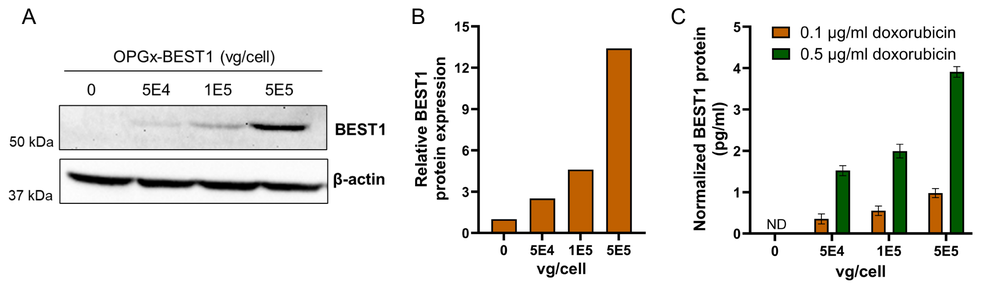



**Figure 4. Detection of OPGx-BEST1 protein expression in ARPE19 cells. (A) Western blot detection of BEST1 in cells 7 days post-transduction with OPGx-BEST1 and treatment with 0.1 µg/ml doxorubicin. (B) Quantification of relative BEST1 protein expression from western blot analysis. (C) BEST1 protein expression quantified by ELISA in cells 7 days post-transduction with OPGx-BEST1 and treatment with 0.1 or 0.5 µg/ml doxorubicin.**

## Results

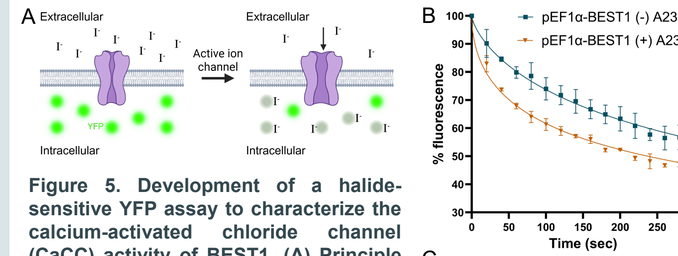



**Figure 5. Development of a halide-sensitive YFP assay to characterize the calcium-activated chloride channel (CaCC) activity of BEST1. (A) Principle of the assay: Iodide redistribution upon chloride channel activation and quenching of YFP fluorescence. (B) BEST1-dependent chloride channel activity in HEK293 cells stably expressing YFP (H148Q/I152L) with or without calcium ionophore A23187. (C) Screening of known BEST1 mutants using the assay. ARB: Autosomal Recessive Bestrophinopathy, LOF: loss-of-function, GOF: gain-of-function**

## Conclusions

- The RT-dPCR potency assay for OPGx-BEST1 demonstrates strong linearity, high precision, accurate quantification, and robust specificity in ARPE19 cells, supporting its use in fit-for-purpose prequalification.
- Different lots of OPGx-BEST1 showed a relative potency within the expected proposed range of 50-150%. This is in accordance with regulatory expectations for potency assurance during early-stage clinical development of OPGx-BEST1.
- Preliminary western blot and ELISA results confirmed BEST1 protein expression in transduced cells.
- Preliminary feasibility studies indicate that the developed halide-sensitive YFP assay can quantify functional activity of BEST1-overexpressing plasmids, and testing of OPGx-BEST1 within this system is currently underway.

**The developed RT-dPCR, western blot, ELISA, and halide-sensitive YFP assays establish a robust framework for evaluating OPGx-BEST1 expression and potency.**

## References

1. Singh Grewal S, Smith JJ, Carr AF. Bestrophinopathies. *Ther Adv Ophthalmol*. 2021;13:2515841421997191.
2. Galietta LJ, Haggie PM, Verkman AS. GFP-based halide indicators with improved $Cl^-/I^-$ affinities. *FEBS Lett*. 2001;499:220-224

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