Development of an *in vitro* potency assay for AVB-101, a recombinant AAV9 gene therapy for the treatment of frontotemporal dementia with progranulin mutations

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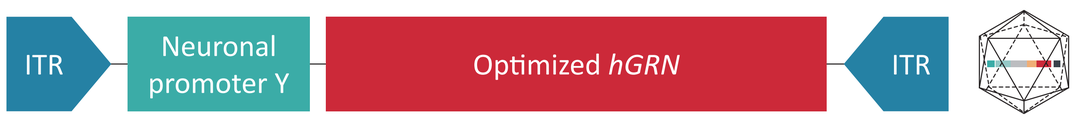
OBJECTIVE

To develop an *in vitro* potency assay which allows for accurate quantification of the progranulin (PGRN) protein to improve and replace an existing *in vivo*/*in vitro* qualitative potency assay.

INTRODUCTION

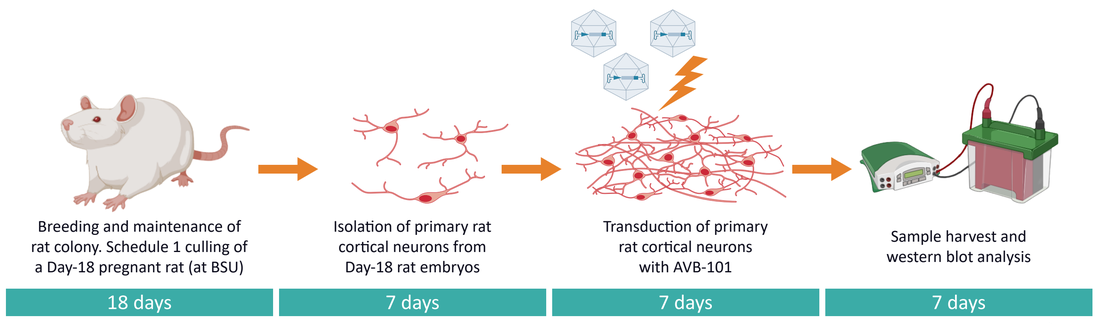
- AVB-101 (**Figure 1**) is a recombinant, adeno-associated virus serotype 9 (AAV9)-based gene therapy designed to deliver a functional copy of the human granulin (*hGRN*) gene to cortical neurons, restoring human PGRN to physiological levels and potentially preventing disease progression.[1,2]
- The AVB-101 therapeutic expression cassette is comprised of the codon optimized *hGRN* driven by a neuronal promoter, denoted as promoter Y and flanked by inverted terminal repeat (ITR) sequences (**Figure 1**).

Figure 1: AVB-101 key components



- Given the cell type specificity of promoter Y, development of a cell-based potency assay in ubiquitous cell lines, such as HEK293 and HeLa, can be challenging due to negligible expression.
- Whilst neuronal cell lines allow for transcriptional activity, variability arises due to low levels of promoter activity coupled with inconsistent cell growth. Additionally, these cell lines often exhibit a low permissivity to AAV9, resulting in low levels of transgene expression, making dose-dependent detection and quantitation challenging.
- The hybrid *in vivo/in vitro* rat cortical neuron assay (**Figure 2**) has significant drawbacks; requiring the use of animals and licensed operators, qualitative results, and inconsistencies in cell culture and operator performance. Thus, transfer of this assay to a good manufacturing practice (GMP) environment is difficult.
- Here, we describe the systematic engineering of HEK293 cells using gene editing tools to develop a quantitative *in vitro* potency assay.

Figure 2: Workflow for hybrid *in vivo/in vitro* AVB-101 potency assay

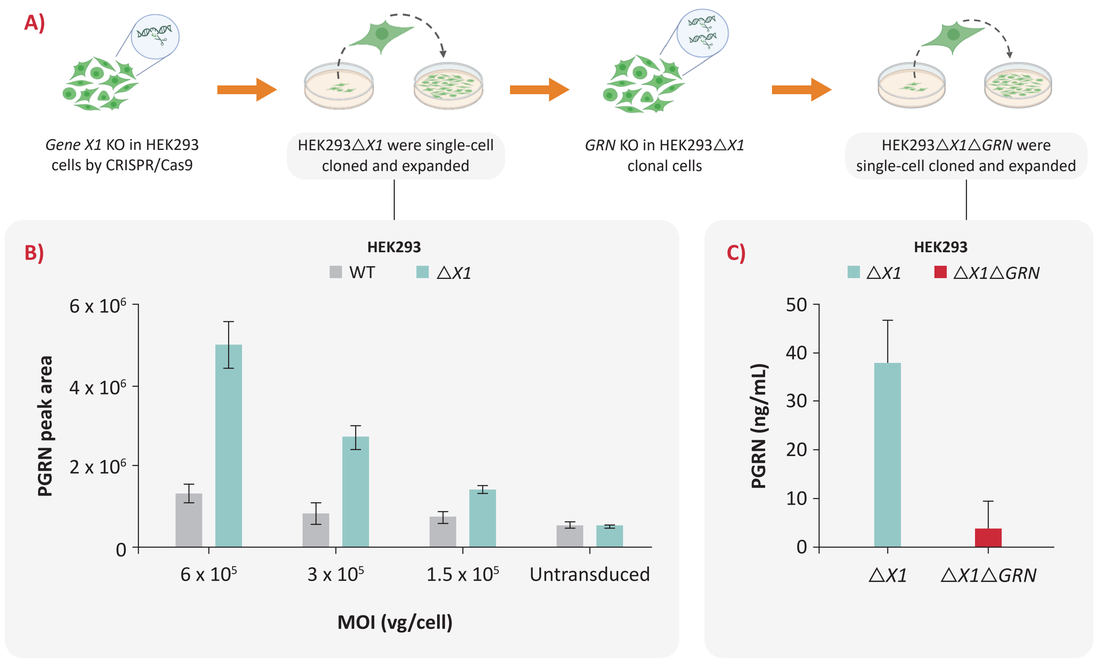


METHODS

Cell line development

- To increase AAV9 transduction, a gene encoding for a critical protein linked to AAV9 binding and cellular uptake (Protein X1) was inactivated by means of CRISPR/Cas9-mediated gene editing (**Figure 3A**). Several clones were isolated, and a clonal line (HEK293△*X1*) was generated and assessed for AAV9 transduction (**Figure 3B**). △X1 cells showed improved transduction efficiency (**Figure 3B**).
- Next, the endogenous *GRN* gene was disrupted in the clonal knockout (KO) cell line (HEK293△*X1*) to abrogate expression of endogenous PGRN from the cell line, increasing assay sensitivity (**Figure 3C**). Once again, the modified pool was cloned, and reduced expression of *GRN* in the resulting clone confirmed.

Figure 3: Generation of HEK293△*X1*△*GRN* cells



A) Cell line development workflow. **B)** Peak area of PGRN (identified by automated western blot system [JESS Simple Western™]) is directly related to PGRN concentration. **C)** In cells where the *GRN* gene was disrupted, endogenous PGRN expression was reduced ~10 fold. Error bars represent standard deviation.

CRISPRa – sgRNA guide screen

- Finally, the double-KO, clonal cell line (HEK293△*X1*△*GRN*) was further modified to stably express a deactivated Cas9 (dCas9) fused to the hybrid VP64-p65-Rta tripartite activator (VPR), as well as a guide RNA (gRNA) targeting the promoter Y sequence (**Figure 4**). Binding of the gRNA to the latter sequence elicited transcriptional activation, and thus *hGRN* gene expression from AVB-101.
- Four gRNA sequences targeting promoter Y were screened to determine the single gRNA that brought about the greatest degree of transcriptional activation.
- Guide performance was determined by expression of endogenous *gene Z*, known to be downstream of promoter Y.
- All four gRNA sequences showed an increase in expression of *gene Z*, compared to cells transduced with the negative, non-targeting control (NTC) (**Figure 5**).
- Single-guide RNA (sgRNA) 2 showed the greatest increase in transcriptional activation, with a 484-fold increase in *gene Z* expression. These cells were selected to develop the AVB-101 potency assay with and referred to as HEK293△*X1*△*GRN-sgRNA2*.

Figure 5: Fold change in expression of *gene Z* normalized to a housekeeping gene (*RPP30*) and non-targeting control cells

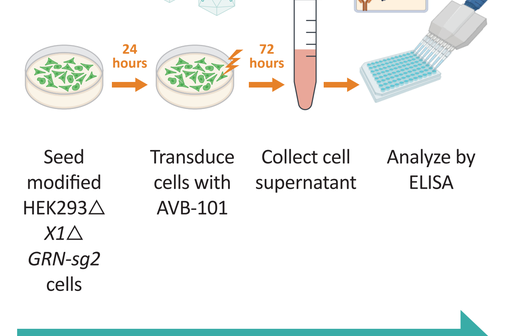
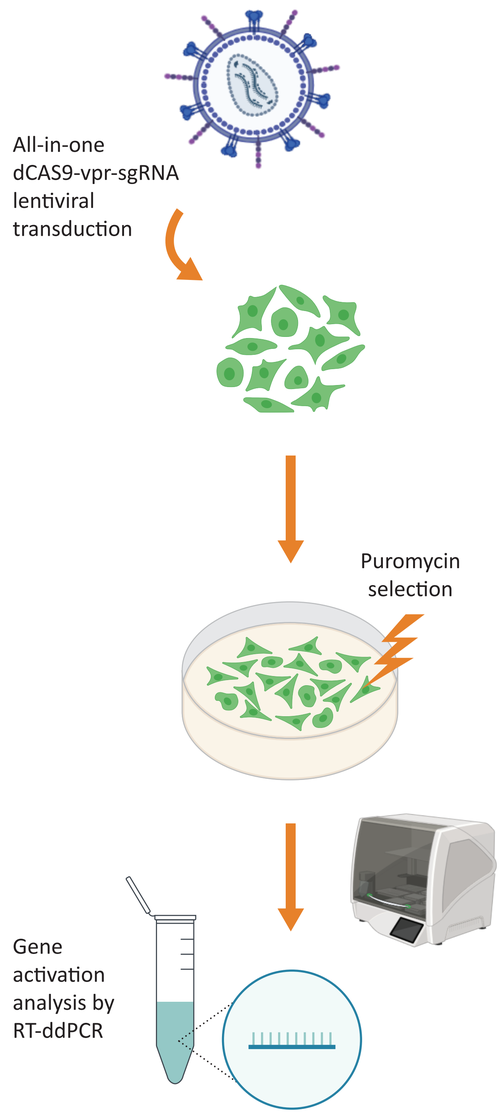


Figure 4: Generation of dCAS9-vpr-sgRNA lentiviral stable cell lines



Positive control: sgRNA targeting the low/no-expression gene titin (*TTN*) used to optimize transduction conditions (data not shown). Negative Control (NTC): sgRNA designed to not target any human gene, to establish experimental baselines and differentiate specific biological effects.

RESULTS

- HEK293△*X1*△*GRN-sgRNA2* demonstrated a dose-dependent response in relation to multiplicity of infection (MOI) after transduction with AVB-101 (**Figures 6–7**).
- No PGRN expression was observed in the NTC cells transduced with AVB-101 (**Figure 7**).

Figure 6: Workflow for *in vitro* AVB-101 potency assay

Figure 7: Transduction of HEK293△*X1*△*GRN-sgRNA2* and NTC cells with AVB-101

An AAV9 vector with a constative promoter (AAV9-CMV-PGRN) was used as a positive transduction control. Error bars show standard deviation. Each MOI was transduced in three separate wells.

CONCLUSIONS

- **We have successfully generated an immortalized engineered cell line with improved AAV9 permissivity, increased sensitivity by reduction of endogenous PGRN expression, and tissue-specific promoter activation.**
- **The engineered cell line has enabled the development of an *in vitro* potency assay for AVB-101, replacing the previous *in vivo/in vitro* hybrid method.**
- **The cell line engineering strategy employed provides an elegant solution for gene therapy potency assay development where tissue-specific promoters are utilized.**

REFERENCES: [1]Lee YB, et al. Oral presentation at ESGCT Annual Congress 2022; [2]Miranda CJ, et al. Poster presented at ESGCT Annual Congress 2023.

ABBREVIATIONS: **AAV9:** adeno-associated virus serotype 9; **BSU:** biological services and biobanking; **Cas9:** CRISPR-associated protein 9; **CRISPR:** clustered regularly interspaced short palindromic repeats; **dCas9:** deactivated Cas9; **ELISA:** enzyme-linked immunosorbent assay; **GMP:** good manufacturing practice; ***GRN:*** granulin; **gRNA:** guide RNA; **HEK:** human embryonic kidney; ***hGRN*:** human granulin; **ITR:** inverted terminal repeat; **KO:** knockout; **MOI:** multiplicity of infection; **NTC:** non-targeting control; **PGRN:** progranulin; **RT-ddPCR:** reverse-transcriptase droplet-digital polymerase chain reaction; **sgRNA:** single-guide RNA; **TTN:** titin; **VPR:** VP64-p65-Rta tripartite activator; **WT:** wild type.

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