New gene therapy vehicle for the expression of multiple transgenes with distinct kinetics of expression

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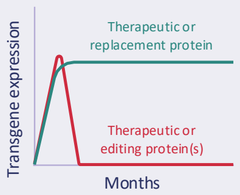
Introduction

Herpes simplex virus (HSV-1) is a non-integrative DNA virus that establishes a life-long latent infection in sensory neurons. In the nucleus of these cells, its double-stranded DNA genome is packed into chromatin and organized in two distinct epigenetic programs: heterochromatin marks repress all viral lytic genes, while euchromatin marks allow expression within a specific genomic region called the LAT region. We engineered unique, non-replicative HSV-1 (nrHSV-1) vectors harboring two transgenes positioned in distinct epigenetic regions of the viral genome, enabling dual expression kinetics from a single viral particle.



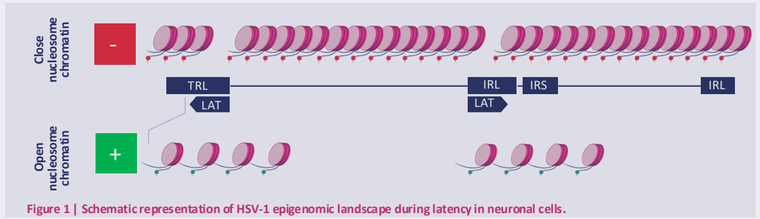


Figure 1 | Schematic representation of HSV-1 epigenomic landscape during latency in neuronal cells.

Our Objectives

Leveraging the epigenetic regulation system of the HSV-1 virus to develop a unique gene therapy vector enabling the expression of two transgenes, each with a distinct kinetic of expression.

1 Vector design

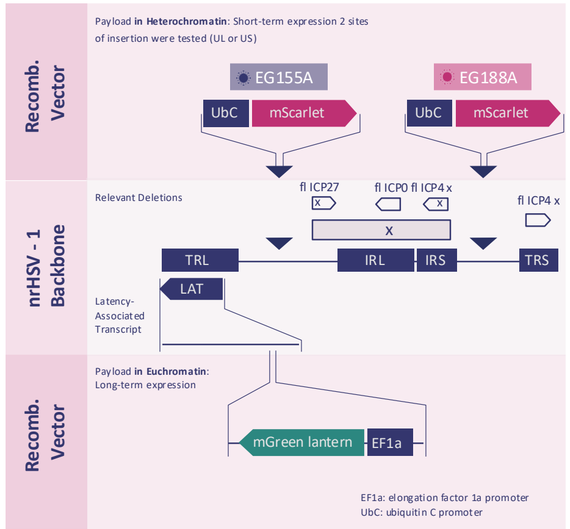


Figure 2 | Dual kinetics nrHSV-1 vectors design. The reporter mGreen Lantern gene is introduced into the LAT region, a region naturally protected from repression during latency in neuronal cells. A second reporter gene, the mScarlet is placed in heterochromatin-prone regions for transient expression, either in the Unique Long (UL) or Unique Short (US) region of nrHSV-1, namely EG155A and EG188A, respectively.

2 nrHSV-1 vectors drive transgenes expression with distinct kinetics *in vitro*

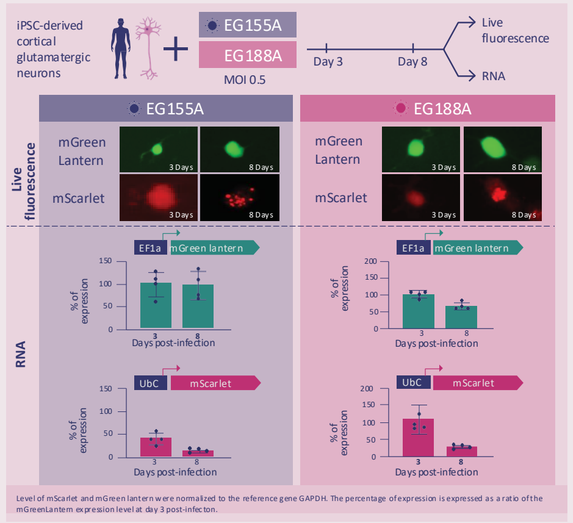


Figure 3 | Differential Expression Kinetics of Dual Transgenes in Human iPSC-Derived Neurons. Comparable levels of **mGreen Lantern** expression were observed between day 3 and 8 post-infection for both vectors, indicating sustained transgene activity in the euchromatic region. In contrast, **mScarlet** expression positioned in the heterochromatic region, declined significantly. These results suggest that repression of **mScarlet** is influenced by the specific site of insertion within the heterochromatinized domain of the HSV-1 genome.

3 nrHSV-1 vector enable differential kinetic of transgenes in mouse Dorsal Root Ganglia

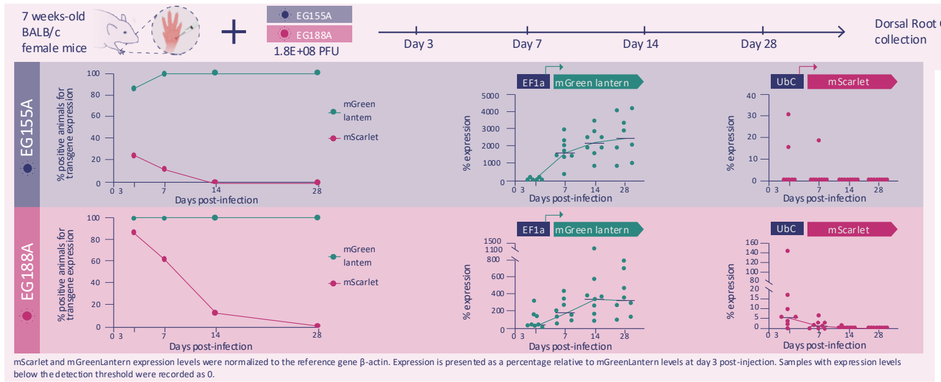


Figure 4 | Differential Transgene Persistence in Sensory Neurons Depends on Genomic Insertion Site. Expression of the **mScarlet** transgene inserted into the heterochromatic region of the HSV-1 genome is rapidly silenced in dorsal root ganglia (DRG) sensory neurons following footpad injection in BALB/c mice. Signal loss is observed by day 3 (EG155A) or day 7 (EG188A) post-injection, with only minimal expression detected in a few mice by day 14. In contrast, the **mGreen Lantern** transgene positioned within the euchromatic LAT region shows increased expression between days 3, 7 and 14, and stabilized expression by day 28, highlighting the critical role of genomic and epigenetic context in regulating transgene stability and persistence *in vivo*.

Conclusion

This innovative dual-payload non-replicative HSV-1 vector represents a key advancement in the field of gene therapy, enabling delivery of multiple transgenes with distinct kinetics in the same cell. Importantly, transient expression can be fine-tuned by selecting specific insertion sites. This level of control offers a versatile and powerful platform for next-generation therapies, particularly for *in vivo* gene editing and long-term correction of autosomal dominant diseases.