Characterization of Targeted Integration with Viral and Non-Viral DNA Donors

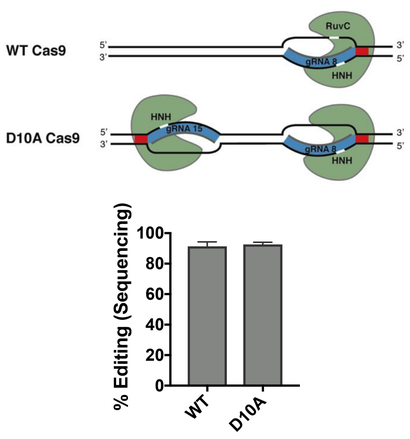
Carrie M. Margulies[1], Frank Buquicchio[1], Tanushree Phadke[1], John LaBella[1], Pietro Genovese[2], Giulia Schiroli[2,3], Valentina Vavassori[2,3], Charles Albright[1], Vic Myer[1], Luigi Naldini[2,3], Cecilia Cotta-Ramusino[1]

[1]Editas Medicine, 11 Hurley Street, Cambridge, MA 02141, [2]San Raffaele Telethon Institute for Gene Therapy, Milan, Italy, [3]Vita Salute San Raffaele University, Milan, Italy

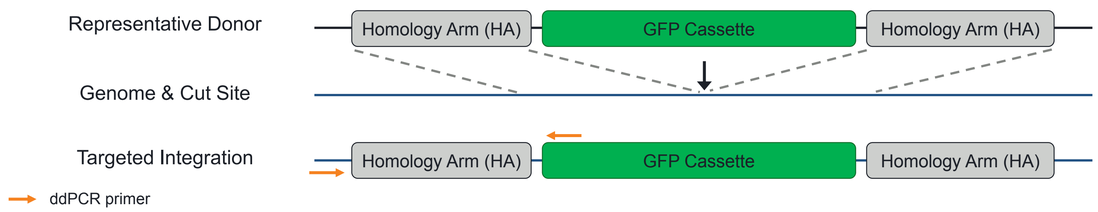
Introduction

- Targeted integration (TI) with high-efficiency and accuracy is a revolution in the field of genomic therapies
- One application of TI is the delivery of therapeutic transgene expression cargos to 'safe harbors', such as the *AAVS1* locus, allowing for uniform transgene expression while also lowering the potential risk associated with semi-random integration of viral vectors (e.g. lentivirus)
- When endogenous control of gene expression is required, TI of corrected genes/exons at the endogenous site is an attractive strategy
- To achieve the ideal level of targeted integration, we can choose from a selection of different CRISPR endonucleases and different types of donor templates

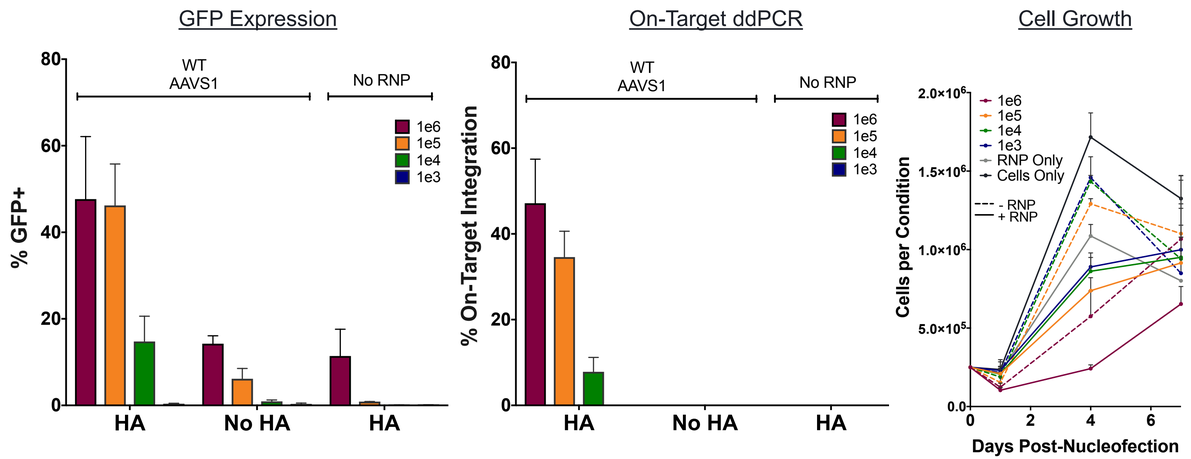
HBB Locus



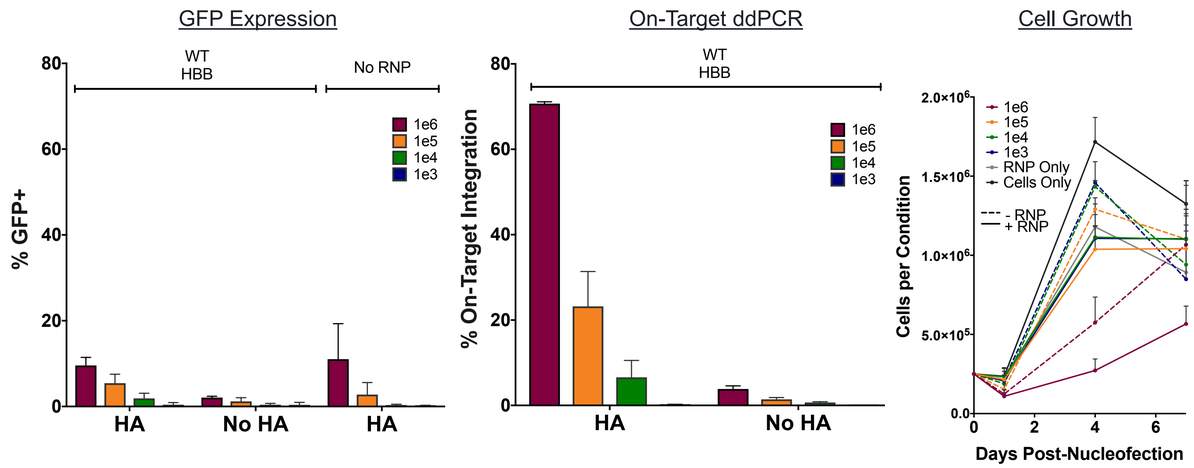
Targeted Integration and Viability in Response to Increasing AAV6 MOI



AAVS1 Locus

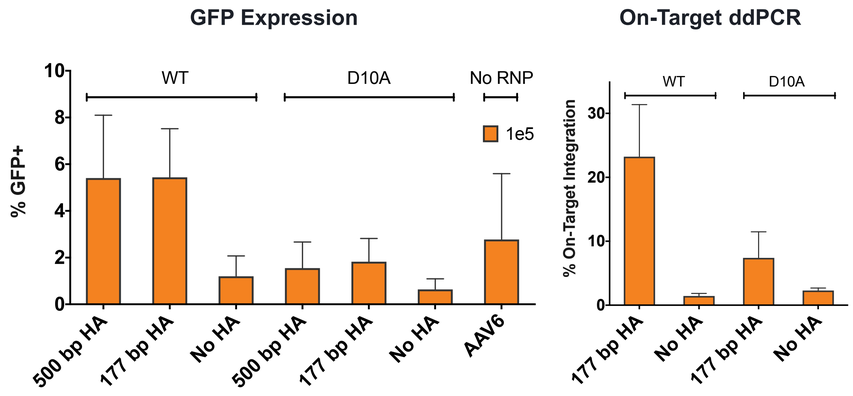


HBB Locus

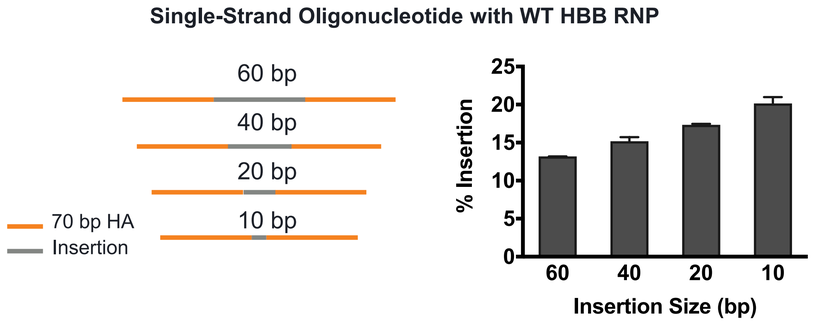


No homology arm (No HA) donor is a GFP cassette flanked by 500 bases that are non-homologous to the genome.

Impact of Cas9 Variants and Homology Arm Length on Targeted Integration by AAV6



Targeted Integration with Non-Viral DNA Donors at HBB



Conclusions

- TI at AAVS1 and HBB is comparable; we detected up to 60% on-target integration by ddPCR at either locus
- At HBB, we detect high levels of on-target integration despite very low GFP expression
- No HA donors delivered with RNP exhibit background GFP levels, suggesting integration of the donor is dependent on homology-directed repair
- WT outperformed D10A for TI with RNP at the HBB locus
- Shorter homology arms perform as well as longer homology arms
- Integration with ssODN donor does not exceed 20% at HBB locus