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Supplemental Information

AAV6 Is Superior to Clade F AAVs in Stimulating Homologous Recombination-Based Genome Editing in Human HSPCs

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Methods:

Plasmids:

pKanAAV2/6.2 and pKanAAV2/9 was a gift from L.H. Vandenberghe. Site-directed mutagenesis kit (Agilent #200524) was used to generate pKanAAV2/6, and pKanAAV2/9 variants. pAdΔF6 was a gift from James M. Wilson (Addgene plasmid #112867). HBB donor construct has been described previously.[26]

Molecular modeling:

The AAV9 60-mer was assembled in Mac pymol from the AAV9 crystal structure[28] PDB: 3UX1 and is represented as a surface rendering with heat map demonstrating the B-factor and individual VP3 monomer chains within the capsid trimer highlighted white, grey, or black. The location of the mutations generated by site-directed mutagenesis of the AAV9 capsid are highlighted magenta.

CD34+ HSPC isolation and cell culture:

CD34+ HSPCs were isolated from fresh cord blood using human CD34 UltraPure MicroBeads (Miltenyi 130-100-453) provided by the Binns Family program for Cord Blood Research. Cells were cultured in StemSpan SFEMII (Stemcell technologies) supplemented with stem cell factor (SCF) (100 ng/mL), thrombopoietin (TPO) (100 ng/mL), FLT3-ligand (100 ng/mL), IL-6 (100 ng/mL), 20mg/mL penicillin, and 20mg/mL streptomycin at 37°C, 5% CO_2 and 5% O_2.

AAV production and titration:

12 million 293T cells per dish in 5 15-cm dishes were plated 1 day prior to three-plasmid transfection with 26 μg pAdΔF6, 13 μg pKanAAV2/(cap), and 13 μg HBB donor transgene per plate using PEI. 48-72 hours post-transfection cell-associated vector was harvested from cell pellets using the AAVpro Purification Kit (Takara #6666). Genomic DNA from purified vector was extracted using QuickExtract DNA Extraction Solution (Lucigen #QE09050) followed by titration by ddPCR using AAV2 ITR-specific primer/probe.[41]

AAV transduction, targeting, and analysis:

CD34+ cell editing and AAV transduction was done as previously described.[15, 24, 42] Briefly, RNP complex was generated using sgRNA target sequence for *HBB*: 5′ - CTTGCCCCACAGGGCAGTAA-3′ and WT Cas9 by incubation at room temperature for 15-20 minutes and electroporated into CD34+ cells using Lonza 4D Nucleofector (program DZ100). AAV was added at 5,000 VG/cell immediately after electroporation and then cells were diluted and cultured at 100,000 cells/mL. Editing and transduction was done after 2-3 days in culture with addition of fresh cytokine-containing media every 48 hours. GFP positive cells were analyzed on a FACSAria II SORP (BD Biosciences) 12-14 days after targeting.

References

41. Aurnhammer, C., Haase, M., Muether, N., Hausl, M., Rauschhuber, C., Huber, I., Nitschko, H., Busch, U., Sing, A., Ehrhardt, A., and Baiker, A. (2012). Universal real-time PCR for the detection and quantification of adeno-associated virus serotype 2-derived inverted terminal repeat sequences. Hum. Gene Ther. Methods *23*, 18–28.

42. Bak, R.O., Dever, D.P., Reinisch, A., Cruz Hernandez, D., Majeti, R., and Porteus, M.H. (2017). Multiplexed genetic engineering of human hematopoietic stem and progenitor cells using CRISPR/Cas9 and AAV6. eLife *6*, 6.