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In Vivo Transduction of Murine Hematopoietic Stem Cells After Intravenous Injection of AAVHSC15 AND AAVHSC17

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AAVHSCs are a group of Clade F AAVs originally isolated from human hematopoietic stem cells (HSCs). Understanding the transduction properties of recombinant versions of AAVHSCs in HSCs, particularly *in vivo*, will support their development for HSC-directed gene therapies. It has been established that AAV transduction of HSCs can be transient or result in very low levels of gene expression, which is difficult to detect with conventional methods. To more sensitively investigate AAVHSC transduction efficiency of HSCs *in vivo*, we utilized a loxP-STOP-loxP-tdTomato (Ai14) transgenic reporter murine model. In Ai14 murine cells, Cre expression induces tdTomato reporter expression. Here, we intravenously infused AAVHSC15 and AAVHSC17 expressing Cre:eGFP for detection of transient (tdTomato+) and stable (eGFP+) expression in HSCs from Ai14 mice. AAVHSC15.Cre:eGFP and AAVHSC17.Cre:eGFP vectors were injected retro-orbitally at a dose of 6E11 or 1.2E12 vector genomes (vgs) per mouse. At two weeks post-injection, mice were euthanized and bone marrow was isolated by standard procedures. Isolated bone marrow was depleted of red blood cells, filtered and the resulting cells stained for Lin, Sca-1 and c-Kit (LSK, markers of progenitor cells). AAVHSC trafficking to the hematopoietic niche was evident by the distinct tdTomato-positive population in the isolated femoral bone marrow, specifically, 6 to 8% of bone marrow LSK (Lin-Sca-1+c-Kit+) cells. Higher levels of vector genomes were observed in the tdTomato-positive LSK cells compared to tdTomato-negative LSK cells. Subsequently, peripheral blood (red blood cell depleted) from treated Ai14 mice and non-injected controls was evaluated at 2, 6, 10, 14, 18 and 21 weeks (terminal) post-injection. tdTomato-positive cells were detected in the peripheral blood of treated animals at all time points tested, with up to 21% at 21 weeks. The identification of tdTomato-positive cells in the peripheral blood long-term is indicative of LSK cell transduction, followed by differentiation and daughter cell proliferation. tdTomato-positive cells were identified in B-cell, T-cell and erythroid lineages evident by expression of cell-specific markers CD19, CD3e, and TER119, respectively. Thus, AAVHSC15 and AAVHSC17-transduced and -modified cells were capable of hematopoiesis. Furthermore, the percentage of tdTomato-positive LSKs at 21 weeks post-injection in the bone marrow was 7-13%, indicating a population of active

transduced progenitor cells that are capable of self-renewal and long-term stability. In summary, AAVHSC15 and -17 productively transduced mouse hematopoietic stem cells after retro-orbital I.V. injection, providing an opportunity for HSC-directed gene therapies for long-term expression and disease treatments.