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Targeted, Safe, and Efficient Gene Delivery to Human Hematopoietic Stem and Progenitor Cells *In Vivo* Using Engineered Avid Adenovirus Vector Platform

Jia Yao, PhD[1], *Svetlana Atasheva, PhD*[1], *Nicole Wagner, PhD*[2], *Nelson C Di Paolo, PhD*[1], *Phoebe L Stewart, PhD*[2], *Dmitry M. Shayakhmetov*[1]

[1] Emory University, Atlanta, GA
[2] Case Western Reserve University, Cleveland, OH

Precise editing of human genome using CRISPR-Cas nucleases, base editors, prime editors, or other technologies represents a promising approach for ameliorating or correcting many human genetic diseases. Although numerous pre-clinical and clinical proof-of-concept studies demonstrated feasibility of gene correction in the desired regions of the human genome *ex vivo*, targeted delivery to and cell-type-specific expression of gene editing proteins in various cell types *in vivo* represent major challenges for all viral and non-viral delivery platforms developed to date. Here, we describe the development and analysis of AVIDs, a novel adenovirus-based gene delivery platform, that allows for a highly targeted, safe, and efficient gene delivery to human hematopoietic stem and progenitor cells (HSPCs) *in vivo* after intravenous vector administration. The AVID vectors attach to human HSPCs via engineered fibers that bind to either CD46 or DSG2 receptors, which are highly expressed on human HSPCs. The efficient entry of AVIDs into these cells is mediated via a mutated penton that was engineered to interact with CD49f, α6 integrin class, whose expression is restricted to cells with long-term repopulating and stem cell capacity. To improve virus safety after intravenous administration, as well as to increase the efficacy of virus production for a cost-effective high-yield vector manufacturing, AVIDs further comprise mutations in hypervariable loops of the major capsid protein hexon. While *in vitro* infection with AVIDs leads to about 30% transduction of a pool of human CD34 $^{+}$ cells, the efficacy of transduction of CD34 $^{+}$CD38 $^{-}$CD45RA $^{-}$CD90 $^{+}$ subsets, highly enriched for hematopoietic stem cells (HSCs), ranged from 80% to 100% for individual donors. Single intravenous administration of AVIDs to humanized mice, grafted with human CD34 $^{+}$ cells, after mobilization led to up to 20% transduction of CD34 $^{+}$CD38 $^{-}$CD45RA- HSPC subsets in the bone marrow. Importantly, targeted *in vivo* transduction of CD34 $^{+}$CD38 $^{-}$CD45RA $^{-}$CD90 $^{-}$CD49f $^{+}$ subsets, highly enriched for human HSCs, reached up to 19%, which represented a 1900-fold selectivity in gene delivery to HSC-enriched over lineage committed CD34-negative cell populations in the bone marrow. Because the AVID platform allows for regulated, cell type-specific expression of gene editing technologies as well as for expression of immunomodulatory proteins to ensure persistence of corrected HSCs *in vivo*, the HSC-targeted AVID platform may enable development of novel curative therapies through safe and effective *in vivo* correction of disease-causing mutations in human HSCs after a single intravenous administration.

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