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Zinc finger nuclease gene editing for functional cure in a nonhuman primate model of HIV/AIDS

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Background: Nuclease-mediated gene editing in hematopoietic stem cells (HSCs) holds great promise in the cure of HIV infection, but the feasibility and translatability of this approach to patients is unclear. To better evaluate the function of HSCs following gene editing, we have engineered cells with disrupted CCR5 alleles and assessed engraftment following autologous transplant in a clinically relevant large animal model, the pigtailed macaque (*M. nemestrina*). Disrupted CCR5 alleles in this model should directly protect against infection with simian/human immunodeficiency virus (SHIV). We are evaluating the extent to which CCR5-disrupted cell progeny engraft, and testing whether these cells impede infection by SHIV.

Methods: Zinc Finger Nucleases (ZFNs) are used to target the CCR5 locus in macaque HSCs. Engraftment and persistence of these stem cells, and stem-cell derived lymphoid and myeloid cells, are measured *ex vivo* and *in vivo*. Animals are challenged with SHIV containing an HIV envelope and suppressed by three-drug combination antiretroviral therapy (cART) following viral set point. Animals reach undetectable plasma viral loads prior to autologous transplant with gene-edited cells.

Results: CCR5 targeting experiments yield up to 60% gene disruption in CD34+ cells *ex vivo*, translating to approximately 5% steady state bulk disruption *in vivo*. Gene-disrupted cells demonstrate long-term, biallelic, multilineage engraftment in macaques. We have recently shown that this approach is equally feasible in SHIV-naïve and in SHIV-infected, cART-suppressed animals. Off-target analyses show overwhelming preference of ZFNs for the on-target CCR5 locus. In early experiments utilizing adeno-associated virus (AAV) to knock in a chemoselection marker at the ZFN-disrupted CCR5 locus in HSCs, we observe up to 30% efficiency of targeted integration.

Conclusions: This is the first demonstration of successful long-term multilineage engraftment of ZFN-edited, CCR5-deleted HSCs in SHIV-naïve and SHIV-infected, cART-suppressed macaques. Our strategy results in robust levels of target gene disruption without impairing HSC engraftment or differentiation. Although CCR5-deleted cells can undergo SHIV-dependent positive selection, we are using gene-targeted *in vivo* selection to enrich for CCR5-edited cells without the need for ongoing viral replication. These results have important implications not only for HIV, but also other genetic diseases that can be treated by gene-editing of HSCs.

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