Submitter Name: Xinxu Yuan
Submitted Email: Xinxu.yuan@vcuhealth.org
PI Name: Wenhui Hu
PI Email: wenhui.hu@vcuhealth.org

All-in-one AAV delivery of multiplexed hypercompact OsCas12f/sgRNAs to eradicate HIV

Xinxu Yuan[1], Araba Abaidoo-Myles[2], Abdul Rasheed Baloch[1], Dabbu Jaijyan[1], Fang Li[1], Qingsheng Li[4], Binhua Ling[3], and Wenhui Hu[1,2,]

[1]Department of Anatomy and Neurobiology; [2]Department of Microbiology and Immunology, Virginia Commonwealth University School of Medicine, Richmond, VA 23098; [3]Texas Biomedical Research Institute, San Antonio, TX 78227; [4]Nebraska Center for Virology, School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68583

Numerous studies, including ours, have highlighted the potential of CRISPR/Cas gene editing to eliminate latent viruses and cure HIV, as evidenced by an ongoing phase 1/2 clinical trial approved by the FDA. However, the targeted delivery of HIV eradicators to cellular reservoirs remains a significant challenge. While AAV delivery shows promise, the size limitations necessitate exploration of compact CRISPR/Cas editors, offering advantages in both viral packaging and targeting efficiency. AsCas12f from *Acidibacillus sulfuroxidans* (422 aa) and OsCas12f from *Oscillibacter Sp.* (433 aa) represent the smallest editors to date, exhibiting notable editing efficiency in mammalian cells both *in vitro* and *in vivo*. In this study, we initially validated the editing efficiency of enhanced OsCas12f (enOsCas12f) in mammalian cells using the LoxP-STOP-LoxP-tdTomato (LSL-tdT) reporter system, both *in vitro* and *in vivo*. Subsequently, we developed an "all-in-one" AAV vector containing enOsCas12f and multiplexed sgRNAs targeting two sites of HIV LTR and one site of Gag, utilizing multiple promoters (U6, H1, 7SK). Multiple cuts maximize HIV eradication and avoid escape. Utilizing the EcoHIV-Luciferase reporter virus, both AAV-DJ8 and AAV-PhP-eB delivery in mouse neural stem cells significantly reduced luciferase. HIV proviral excision was confirmed via PCR genotyping and next-generation sequencing. Similar eradication outcomes were observed in EcoHIV-Luciferase HEK293T cell line and neural stem cells from HIV Tg26 transgenic mice. These findings suggest that AAV delivery of multiplexed enOsCas12f/sgRNAs achieves high efficiency in HIV eradication. Evaluation of AAV-mediated enOsCas12f delivery and HIV eradication *in vivo* is ongoing.