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Efficient genome editing using AsCas12a-VLPs produced with Pol II-transcribed crRNA

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HIV- and MMLV-based virus-like particles (VLPs) are a promising delivery vehicle for CRISPR/Cas gene editing tools in the form of ribonucleoprotein (RNP) complexes of Cas nuclease and guide RNA (gRNA). VLPs combine the advantages of viral transduction and gene editing with RNP, can target specific cell populations due to pseudotyping and are potentially applicable *in vivo*. In most VLP systems RNP complexes are incorporated via the fusion or induced interaction of nucleases with Gag, whereas gRNA is packaged only as a complex with the nuclease. VLPs assemble in the cytoplasm, but gRNA is mostly expressed from the RNA polymerase III (Pol III)-transcribed U6 promoter and enriched in the nucleus, which hinders its packaging and reduces VLP editing efficiency.

As an approach to overcome this problem, we replaced SpCas9, which had been used in all previous VLP systems, by AsCas12a which can excise mature crRNA from a pre-crRNA transcript. Using the nuclease packaging mechanism of NanoMEDIC VLPs, we produced VLPs with AsCas12a and crRNA expressed from the Pol II-dependent CMV promoter. crRNA was directed into the cytoplasm as part of a Pol II-driven transcript and excised by AsCas12a followed by RNP formation and packaging into VLPs. The CMV-driven crRNA increased the knockout levels of transgenes in 293 cells from 30% to 50–90% and raised the level of endogenous *CXCR4* knockout in Jurkat T cells from 1% to 20%. Replacing a single crRNA with an array of three or six identical crRNAs improved *CXCR4* knockout rates by up to 60–70%. The RT-qPCR method that we developed for the quantification of crRNA confirmed increased crRNA levels in VLP samples with six crRNA copies. Pseudotyping AsCas12a-VLPs with a combination of VSVG and BaEVRless further improved editing efficiency by enhancing transduction levels. As a result of all modifications, *CXCR4* knockout levels in Jurkat T cells increased from the background levels to 80%. This effect was observed not only for NanoMEDIC VLPs but also for AsCas12a-containing eVLPs and Cas-VLPs based on another packaging mechanism. Regardless of promoter usage, the editing efficiencies of AsCas12a-VLPs were always higher compared to SpCas9-VLPs. AsCas12a-VLPs mediated the knock-in of the HIV fusion inhibitor MT-C34 into the *CXCR4* locus, achieving 60% knock-in levels with an AAV6 donor. Collectively, we proposed a method to improve VLP editing efficiency using AsCas12a with its pre-crRNA processing activity and Pol II-driven crRNA.

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