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EX VIVO AND IN VIVO EDITING OF THE SIV GENOME IN NONHUMAN PRIMATES BY CRISPR-CAS9

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Background:
Antiretroviral therapy (ART) has increased survival, but is a non-curative approach as replication competent proviral DNA, with high risk for reactivation upon ART cessation, remains. As such, HIV is now a chronic disease with a broad range of co-morbidities and drug toxicity. Curative strategies to eradicate the infected cells or viral genome without further treatment are vital. Here, we develop and test the ability of the CRISPR-Cas9 gene editing method for elimination of the SIV viral genome in rhesus macaques.

Methods:
We employed AAV-9 as a vector to deliver CRISPR-Cas9 designed to target sequences spanning the LTR and Gag genes and permanently inactivating proviral DNA by excising intervening DNA fragments. Adult Chinese rhesus macaques (n=8) were i.v. infected with SIVmac239. At 8 weeks post infection, animals were treated daily with a drug regimen of tenofovir, emtricitabine and dolutegravir (5.1/50/2.5mg/kg daily by s.q.). Ex vivo gene editing was performed in PBMCs by AAV9-CRISPR-Cas9 transduction, PCR amplification and Sanger sequencing of the amplicons to assess the potency and precision of viral DNA elimination. In a proof of concept in vivo study, 4 animals, 3 were given an i.v. infusion of AAV-9-CRISPR-Cas9 (10^13GC/kg), and after three weeks, animals were necropsied, blood and tissues were harvested virological and gene excision evaluations.

Results:
In all SIV-infected animals, ex vivo excision of viral DNA was confirmed by the detection of distinct DNA fragments of 464bp and 358bp resulting from the removal of intervening DNA sequences between 5'LTR to Gag and 3'LTR to Gag, respectively. Results from Sanger sequencing confirmed the breakpoint of the viral DNA. Delivery was confirmed by the presence of Cas9 and expression of both gRNAs. In vivo, both 5'LTR to Gag and 3'LTR to Gag excision were confirmed in blood of animals that received AAV-9-CRISPR-Cas9 infusion. In contrast to the control animal, which displayed rapid viral outgrowth, no outgrowth was detected in PBMC/CEM co-cultures after 30 days from animals with AAV-9-CRISPR-Cas9.

Conclusion:
We demonstrated, for the first time, high specificity and efficacy of the CRISPR technology for targeting SIV proviral LTR and Gag regions, which led to both ex vivo and in vivo editing of SIV DNA. These observations support the potential use of CRISPR/Cas9 technology as a curative strategy that warrants further investigation.