

## PE26

### Combinatorial CRISPR/Cas9 approaches targeting different steps in the HIV life cycle efficiently limits viral reactivation and halts viral replication

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**Background:** Currently available combination antiretroviral therapy can successfully control HIV replication. However, conventional treatment lacks the ability to stop viral production and clear the latent reservoir, which remains the major obstacle towards a cure. Novel strategies are required to permanently disrupt the HIV genome in the latently infected cells. In this study we have investigated the potential of the CRISPR/Cas9 system to prevent HIV reactivation from latently infected cells and to target different steps in the viral lifecycle to halt viral replication.

**Methods:** The CRISPR/Cas9 system is comprised of a Cas9 protein, which in combination with a guideRNA (gRNA), is able to cleave a complementary dsDNA sequence. gRNAs were designed to target HIV LTR, protease, reverse transcriptase, integrase and the structural matrix protein. The CRISPR/Cas9 system was cloned in a lentivirus vector and used to transduce SupT1 and Jurkat cells. The latter contains near full-length HIV and expresses GFP upon TNF $\alpha$  stimulation. SupT1 cells were transduced with the lentiviral constructs and subsequently infected with HIV using different MOIs and viral replication was monitored by HIV DNA quantification and HIV CA-p24 production. On and off targeting efficiency (three genes per CRISPR) was assessed by deep sequencing.

**Results:** Lentiviral transduction in SupT1 and Jurkat cells resulted in stable expression of the CRISPR/Cas9 system. Deep sequence analysis demonstrated efficient HIV genome editing (75-99%) and an off-target efficiency ranging between 0.4-1.7%. TNF $\alpha$ -induced HIV reactivation from latently infected T cells was significantly reduced after transduction with gRNAs. Single gRNA resulted in 50-95% loss of HIV expression and in cells targeted by a combination of two LTR gRNAs >98% loss of expression was shown. Subsequently, we investigated the potential of gRNAs to inhibit viral replication. HIV DNA quantification demonstrated up to 40-fold reduction in intracellular HIV DNA and a significant reduction in virus production. Most combinations of two gRNAs resulted in complete abrogation of viral replication, which could not even be rescued after months of in vitro selection.

**Conclusions:** The newly discovered CRISPR/Cas9 system is able to target HIV efficiently in both primary infection and latency models and may provide a specific, efficacious prophylactic and therapeutic anti-viral approach.