CRISPRs are able to efficiently target latent HIV and halt new infections

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Background: Currently available combination antiretroviral therapy (cART) can successfully control HIV replication. However, conventional treatment lacks the ability to clear the latent reservoir and stop viral production, which remains the major obstacle in achieving 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 RNA guided DNA endonucleases system to edit the HIV genome, prevent re-activation from latently infected cells and halt new infections.

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 (PR), reverse transcriptase (RT), integrase (IN) and the structural matrix protein (MA).
- The CRISPR-Cas9 system was cloned in a lentivirus vector (Figure 1) and used to transduce SupT1 and Jurkat cells. The latter is latently infected with near full-length HIV and expresses GFP upon TNFα stimulation (J.Lat Full Length Clone 15.4).
- SupT1 cells are transduced with the lentiviral constructs and infected with HIV using different MOI and viral replication was monitored by HIV DNA quantification, HIV induced cell-killing and luminescence.
- On- and off-target efficiency of the different CRISPRs was assessed by deep sequence analysis.

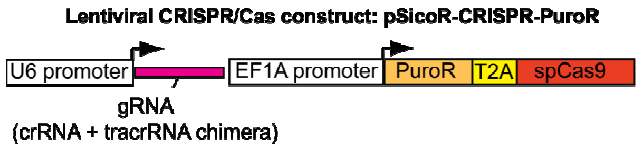


Figure 1. Schematic overview of the lentiviral pSicoR-CRISPR-PuroR vector

Results:

- Lentiviral transduction in SupT1 and Jurkat cells resulted in stable expression of the CRISPR-Cas9 system.
- Deep sequence analysis of transduced Jurkat cells demonstrated efficient HIV genome editing (75-100%) and an off-target efficiency ranging between 0.4-1.7% (Table 1).

Table 1. On- and off-target analysis of HIV gRNAs.

On-target Analysis

	gRNA positive cells sequences total	Sequences (%) Mutant*	WT	gRNA negative cells (controls) sequences total	Sequences (%) Mutant*	WT	gRNA pos - neg
LTR6	1715	100,0	0,00	620	0.81	99.19	99.19
MA3	582	75,95	24,05	469	1.28	98.72	74.67
IN5	2588	90,11	9,89	1193	1.76	98.24	88.35

Off-target Analysis

		gRNA positive cells sequences total	Sequences (%) Mutant*	WT	gRNA negative cells (controls) sequences total	Sequences (%) Mutant*	WT	gRNA pos- neg
LTR6	off1	2491	0.60	99.40	1241	1.37	98.63	-0.77
	off2	4177	1.01	98.99	1166	0.86	99.14	0.15
	off3	2426	0.91	99.09	951	1.05	98.95	-0.14
MA3	off1	2259	1.73	98.27	1111	0.81	99.19	0.92
	off2	2007	0.90	99.10	977	0.34	99.66	0.56
	off3	1841	0.43	99.57	770	1.17	98.83	-0.74
IN5	off1	1743	1.09	98.91	1019	0.59	99.41	0.50
	off2	2487	0.88	99.12	1087	0.65	99.35	0.23
	off3	2903	0.76	99.24	1374	0.66	99.34	0.10

*Number of sequences displaying alterations as compared to the reference sequence. A sequence is scored as 'mutant' when there was a mismatch/indel 3bp up and downstream of the Cas9 cleavage site.

Efficient targeting of provirus in latently infected T-cells:

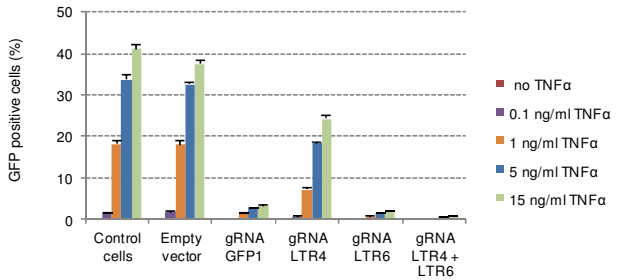


Figure 2. The effect of different gRNAs targeting the HIV LTR region on reactivation of provirus from latently infected T-cells (J.Lat Full Length Clone 15.4). In this latency model for T-cells, the integrated HI"V-genome is complemented with an HIV LTR driven GFP sequence which is inducible upon TNFα stimulation. For all experiments, measurements for quadruplicate experiments are presented + STD.

- HIV reactivation was reduced significantly after transduction with a single gRNA (50-95% loss of expression) or two gRNAs (>98% loss of expression) (Figure 2).

Efficient inhibition of viral replication in T cells:

- CRISPRS targeting the LTR, PR, RT, IN and MA inhibit viral replication in SupT1 cells. HIV DNA quantification demonstrated up to 40-fold reduction in intracellular HIV DNA (data not shown) and a significant reduction in virus production was observed (Figure 4/5).

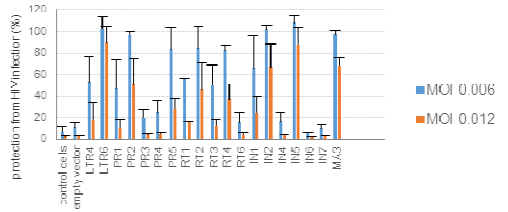


Figure 4. The effect of different gRNAs targeting the HIV LTR, protease, reverse transcriptase, integrase and matrix region on viral replication in SupT1 cells. Protection from viral replication by the gRNAs is measured after five days. For all experiments, measurements for triplicate experiments are presented + STD.

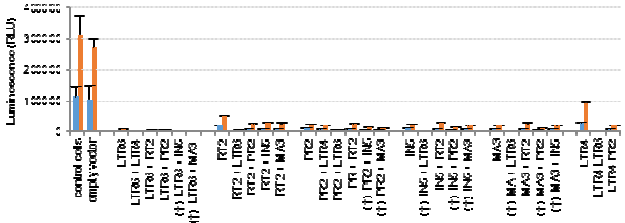


Figure 5. The effect of different gRNAs targeting the HIV LTR, protease, reverse transcriptase, integrase and matrix region on viral replication in SupT1 cells. Luminescence is measured after six days (MOI 0.003 in blue and MOI 0.006 in orange). For all experiments, measurements for duplicate experiments are presented + STD. († indicates abortive infection)

Conclusion: 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.

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