Development of a Multistrain CRISPR System for HIV-1 Elimination

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Abstract

Background: A key challenge to eliminating HIV-1 infection rests in removing the diverse array of integrated HIV-1 strain DNA from the genomes of CD4+ leukocytes. A means of achieving such lies in newer CRISPR-Cas9 technologies. Identifying a gRNA system to target the multitude of HIV-1 proviral DNA sequences and a delivery vector for CD4-specific delivery remain challenges.

Purpose: To develop a CRISPR-Cas9 system with broad abilities to cleave integrated HIV-1 proviral DNA delivered in CD4-targeted virus-like particles (VLPs).

Hypothesis: CRISPR-Cas9 inactivation of the HIV-1 transactivator of transcription (*tat*) gene in conserved subgenomic regions could reduce or eliminate viral replication. When loaded in HIV-1 envelope-pseudotyped VLPs, CRISPR-Cas9 could excise HIV-1 DNA in CD4+ T cells and macrophages in tissue viral reservoirs.

Methods: CRISPR-Cas9 guide RNAs (gRNAs) designed against HIV-1 *tat*, were developed against known HIV-1 sequences with limited to no off-target loci. The gRNAs were cloned into an spCas9 expression vector and screened for antiretroviral activity against seven HIV-1 strains. LTR- and *gag*- gRNAs served as reference controls. Delivery of CRISPR-Cas9 to CD4+ leukocytes in VLPS was assessed.

Results: Tat-directed gRNAs that also cleave *rev* (and *env*) exhibit greatest reduction in RT-activity in a 48-hour co-transfection screen. Reduction in RT-activity correlate with % sequence conservation. When delivered in VLP format, LTR/*gag* mediated excision was shown to be gp120::CD4 specific.

Targeting HIV-1*tat*

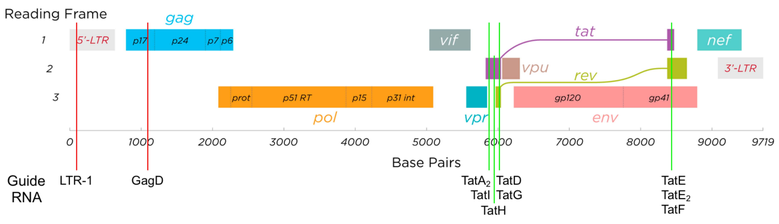


Figure 1. CRISPR-Cas9 gRNAs. Guide RNAs were designed against various positions of HIV-1 *tat*, *tat/rev*, or *tat/rev/env* are shown in green. Control gRNAs against HIV-1 LTR and *gag* previously demonstrated to show antiretroviral effects were also included in these studies as reference controls (red).

gRNA Sequence Conservation

gRNA Name	% Sequence Conservation (n = 4004 HIV-1 Strains)	CRISPR-OFF Score
LTR-1 (control)	27.12 %	6.937
GagD (control)	11.14 %	4.071
TatG	67.15 %	3.803
TatD	59.04 %	6.284
TatH	51.12 %	4.662
$TatA_2$	29.79 %	7.327
TatI	25.90 %	6.844
TatE	15.26 %	3.090
TatF	6.19 %	6.467
$TatE_2$	5.82 %	6.021

Figure 2. Sequence conservation and Off-target scores among gRNAs. Tat-directed or control gRNA sequences were screened against 4004 clinical HIV-1 DNA sequences (hiv.lanl.gov) for full sequence matches. Off-target cleavage potential of each gRNA against the human genome was assessed using CRISPR-OFF v1.1 tool (rth.dk/resources/crispr/crisproff), wherein high scores correlate with low off-target DNA breaks.

Combined Tat/Rev/gp41 gRNAs inactivate multiple HIV-1 strains

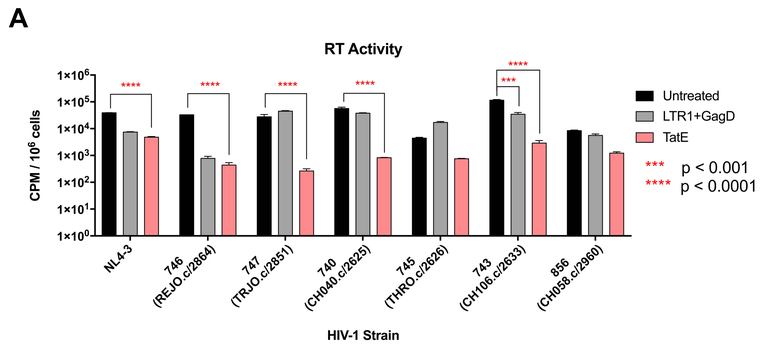
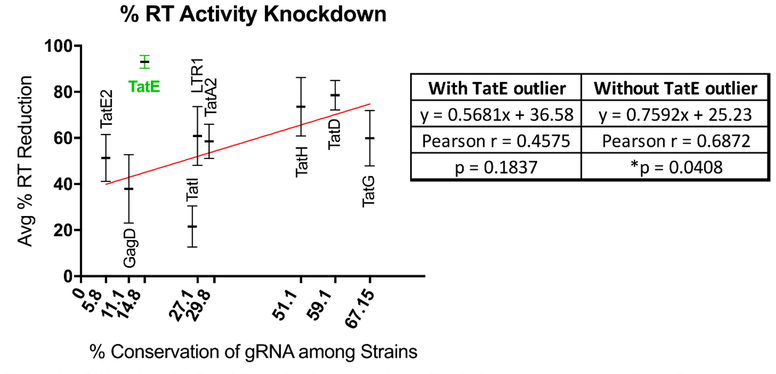


Figure 3. Screen of tat-directed gRNA reduction in RT-activity. A transfection screen of gRNAs cloned into spCas9 expression plasmid pX333 (Addgene #64073) was performed in HEK293FT cells co-transfected with HIV-1 encoding plasmids. LTR-1 and GagD were both cloned onto the same plasmid. After 48 hours, supernatant was assessed for reverse-transcriptase (RT) activity (A). Percent reduction from untreated controls is represented in heat-map form (B). DNA was extracted from cells co-transfected with $pHIV_{NL4-3}$ and assayed by PCR for excision of the first exon of *tat* by dual *tat*-directed gRNAs (C). CPM: counts per minute ^{3}H-thymidine incorporation.

gRNAs and HIV-1 suppression



With TatE outlier	Without TatE outlier
y = 0.5681x + 36.58	y = 0.7592x + 25.23
Pearson r = 0.4575	Pearson r = 0.6872
p = 0.1837	*p = 0.0408

Figure 4. gRNA induced RT activity reductions correlate with viral sequence conservations. The average percent RT-activity reduction against seven HIV-1 molecular clones was determined. Pearson correlation was determined to be significant when TatE (a positive outlier) is excluded from metanalysis.

HIV-1 VLPs target CD4

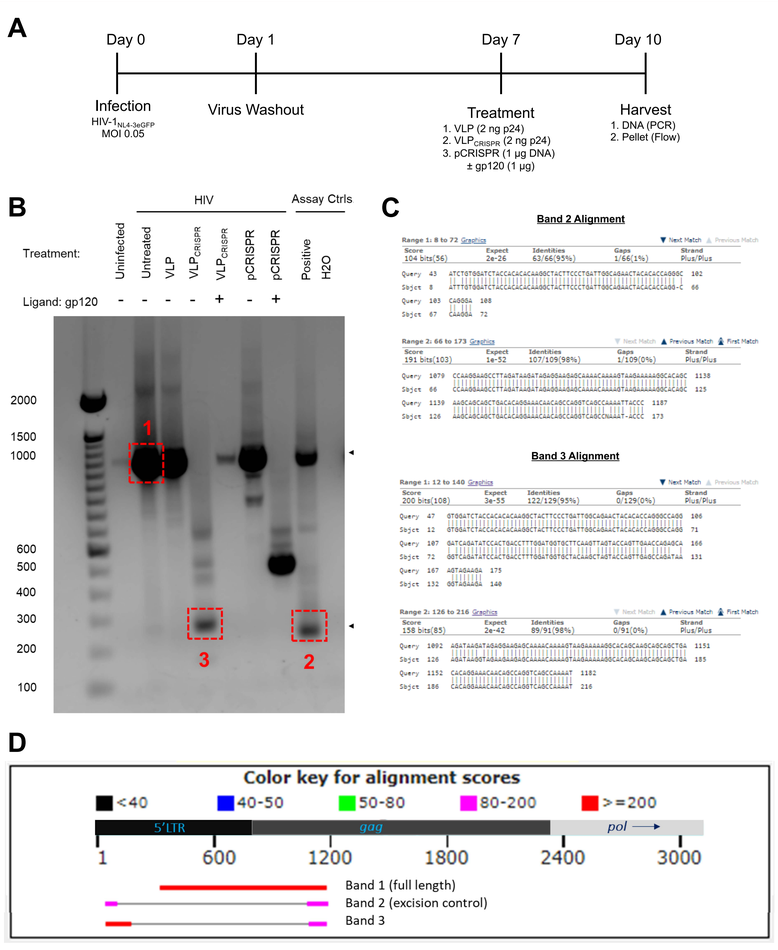


Figure 5. VLPs for CRISPR-Cas9 delivery and viral excision. CD4+ T cells were infected with HIV-1 for 7 days then treated with HIV-1envelopef VLPs (2 ng HIV-1 p24 / 10^6 cells) or appropriate controls for 72 hours according the timeline depicted in (A). As a control for specificity to CD4+ T cells, excess recombinant HIV-1gp120 (1 µg / 10^6 cells) was added during treatment. (B) PCR for CRISPR-Cas9-mediated excision of 5' LTR and *gag* sequences. (C) PCR amplicons outlined in (B) were extracted, Sanger sequenced, and aligned using NCBI BlastN. (D) Summary alignment charts for sequenced DNA amplicons. VLP: virus-like particles synthesized by pHIV89.6env, $pCRISPR_{LTR\text{-}Gag}$, and psPAX2 co-transfection.

Conclusions

1. HIV-1 *tat* gRNAs can maintain >50% sequence conservation.
2. TatD and TatE CRISPR-Cas9 reduce progeny virion production in diverse HIV-1 molecular clones.
3. TatD and TatE cleavage of *tat/rev(/env)* lead to greatest viral suppression.
4. HIV-1 virus-like particles can delivery CRISPR-Cas9 payloads to infected CD4+ T cells and macrophages.

Acknowledgments

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