Targeted disruption of essential HIV proviral genes by rare cutting nucleases

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BACKGROUND

- Over the years Highly Active Antiretroviral Therapy (HAART) has played a crucial role in saving millions of lives. The success of HAART in repressing HIV viral load to nearly undetectable levels is formidable. However, the therapy is limited by its inability to penetrate viral reservoir that is hidden, in most part, in long-lived central memory cells. Lifelong consistent adherence to the HAART regime is paramount in controlling viral loads. If interruption of therapy occurs, HIV will rebound to pre-HAART viral loads within weeks.
- Subsequently, the need for a curative HIV therapy that will eliminate or inactivate viral reservoirs remains vital to global HIV eradication.

Hypothesis

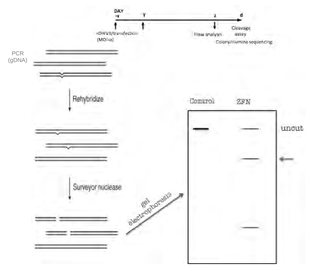
- Here we propose targeted disruption of essential proviral genes using rare cutting endonucleases as a curative therapy for latent HIV infection.



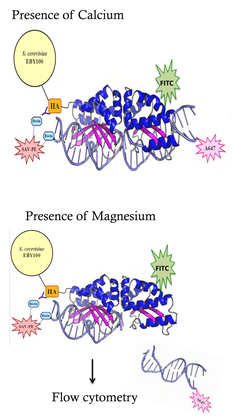
Upon reactivation, No viable progeny is produced.

Materials & Methods

T7 cleavage assay

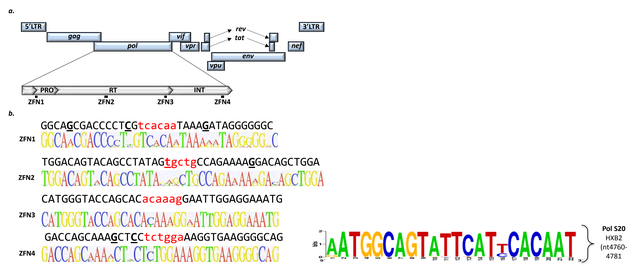


Yeast surface display catalytic activity assay

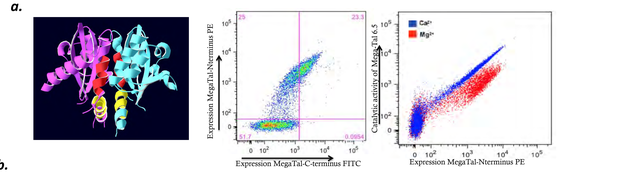


RESULTS

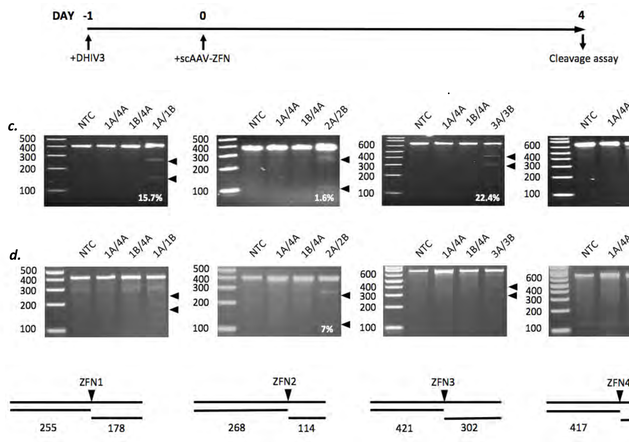
conservation of ZFN and mega nuclease target sites across 2843 HIV *pol* sequences



HEs and ZFNs are able efficiently introduce various mutations containing deletions and insertions into both integrated HIV provirus and readily available POL substrate

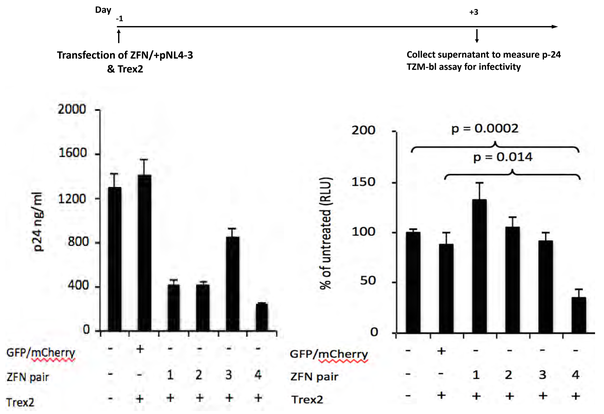


		Total reads	Unique reads	WT GTATTC	indels	dels	ins	del -4 GTAT----	del -5 G----	mod GTATTT
SupT1-DHIV3	(Neg Control)	72	3	95.8%	4.2%	2.8%	0	0	0	1.4%
	MT6.5	1023	24	93.5%	6.5%	2.2%	0.6%	0	0.2%	1.9%
pDHIV3	MT7.5	7585	272	80%	20%	13.6%	1.9%	2.0%	0.7%	2.1%
	MT7.5 + Trex2	5301	210	73.7%	26.3%	19.3%	2.1%	2.9%	0.04%	1.6%
	MT6.5 (B)	18481	590	75.9%	24.1%	17.5%	2.0%	3.0%	0.8%	1.7%
	MT6.5 + Trex2 (A)	17236	549	52.4%	47.6%	39.1%	2.1%	8.5%	0.06%	1.2%
	(Neg Control)	15112	32	97.3%	2.7%	0.013%	0	0	0	2.2%

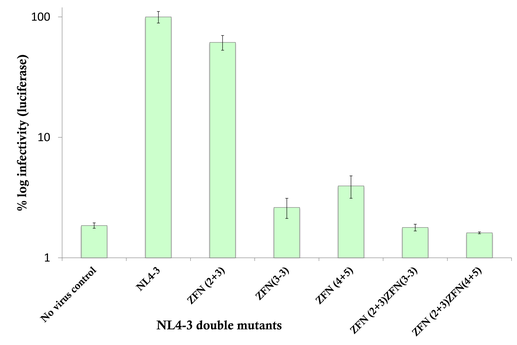


a. Catalytic activity of integrase specific (target site shown in red) MegaTal is measured using yeast surface display assay.***b***. Efficiency of error prone repair is measured by transfecting HEK293 cells with pDHIV3 and plasmids expressing each ZFN subunits or infecting sup-T1 cells with DHIV3-WT at an moi=2 and then treating the cells with respective MegaTals. % mutagenesis and nature of mutational landscape assessed by illumina sequencing. ***C & d,*** Each ZFN pairs' activity in ***c. plasmid sustrate & d. intergrated provirus*** is measured in the same manner as ***b***. % mutagenesis is measured using T7 endonuclease assay.

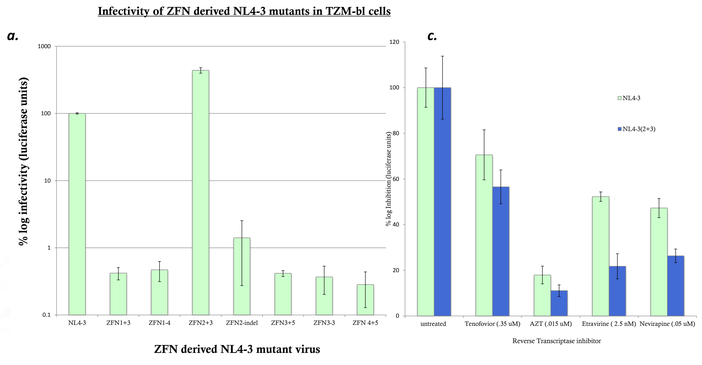
Viruses containing ZFN mediated target site mutations are able to knock down global viral infectivity



Targeting multiple sites for mutagenesis can overcome treatment resistance



Potentially treatment resistant infectious virus clone was isolated among many clones





a. In order to assess the impact of individual mutations on viral infectivity number of mutations discovered through clonal sequencing from previous experiments were cloned into NL4-3 backbone and % infectivity was measured by TZM-bl assay ***b***. Each mutant virus tested is aligned to WT target sequence. A variety of mutants including some that are in frame and small deletions and insertions are selected. ***C.*** *ZFN2(+3) which tested against a panel of NRTi and NNRTis.*

Conclusions

- We demonstrate that meganuclease and ZFNs targeted toward HIV *pol* gene exhibit activity on both NL4-3 derived plasmid substrate and integrated provirus in T-cell line
- we are able to demonstrate that the mutations derived from ZFNs are able to knockdown overall virus infectivity
- Most importantly, we are able to isolate potentially treatment resistant mutant virus that retained infectivity to WT levels. However, it confer sensitivity against NRTi and NNRTis.
- Lastly, we are able to abrogate the ZFN2(+3) "treatment resistant" virus by introducing mutations at other locations of POL

Future directions

- Demonstrate that the NL4-3 mutants can no longer be cleaved by their respective ZFNs
- Assess the fitness NL4-3-ZFN2(+3) and the other mutants in primary CD4+

References

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