Design of SHIVs Encoding HIV-1 Recombinase Brec1 Recognition Site for Nonhuman Primate Cure Studies

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Ryan J. Krause[1], Edward F. Kreider [1], Amie Albertus[1], Kevin Newcombe[1], Suvadip Mallick[1], Weimin Liu[1], Yingying Li[1], Emily Lewis[1], Himanshu Garg[1], Katharine Bar[1]
[1]University of Pennsylvania, Philadelphia, PA, USA

BACKGROUND

- Despite highly effective antiretroviral therapy (ART), a latent viral reservoir persists in people with HIV (PWH) in the form of integrated viral DNA known as a provirus.[1]
- A recombinase, Brec1, recognizes a highly conserved sequence (*loxBTR*) found in the long-terminal repeats (LTRs) that flank the provirus.[2]
- Simian-human immunodeficiency viruses (SHIVs) are chimeric viruses that recapitulate HIV-1 infection in nonhuman primate (NHP) models.[3] However, SHIVs are genetically distinct from HIV[4] and thus do not encode *loxBTR.*

Here, we generate and describe new SHIVs containing *loxBTR* for translational NHP cure studies using Brec1.

RATIONALE & METHODS

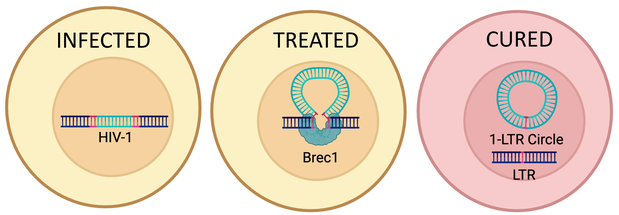


Figure 1: Mechanism of proviral excision. Brec1 facilitates a double stranded break and homologous end joining resulting in a 1-LTR circle and a 1-LTR "scar."

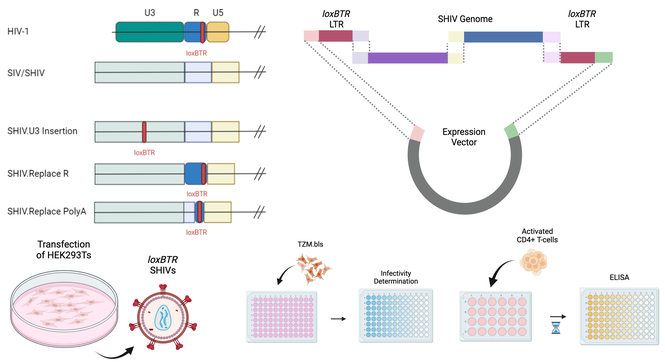


Figure 2: Design, construction, and phenotyping of *loxBTR* SHIVs. Three viruses were assembled into single plasmid infectious molecular clones (IMCs). Viruses were produced by transfecting HEK293T cells and were phenotyped with cell lines and primary cells. Viral RNA was extracted and sequenced.

loxBTR SHIVs offer a new platform for NHP studies studying Brec1 recombinase-based HIV-1 cure strategies

RESULTS

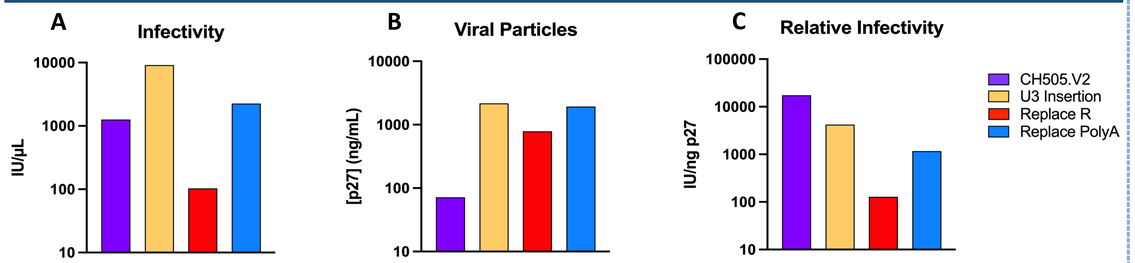


Figure 3: *loxBTR* SHIV IMCs produce infectious virus. (**A**) Infectivity titers on TZM.bl cells. (**B**) p27 Gag particle content by ELISA. (**C**) Infectivity per particle ratio. All measurements are compared to the parental "CH505.V2" strain.

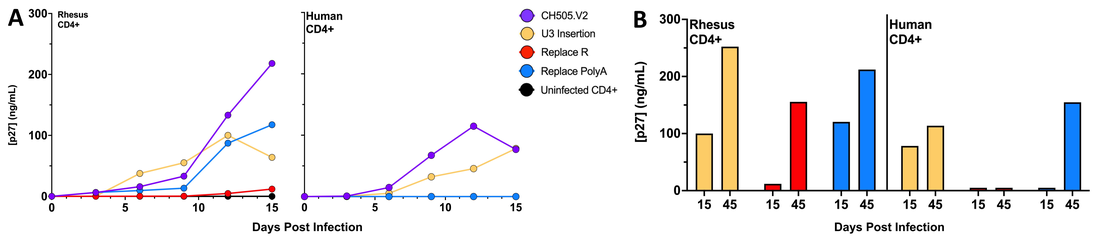


Figure 4: *loxBTR* SHIVs are replication competent. (**A**) Replication kinetics on infected CD4+ T-cells over 15 days. (**B**) Viral particle content at days 15 and 45.

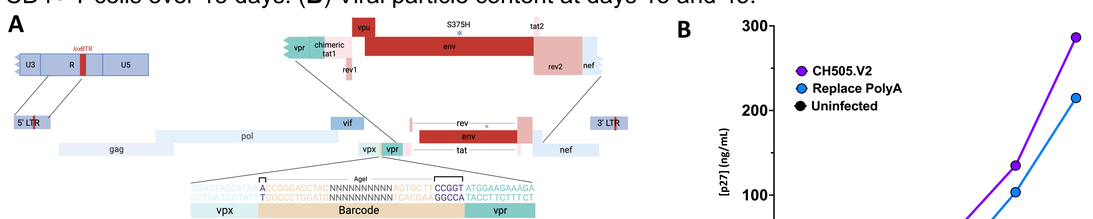


Figure 5: Barcoding virus does not affect fitness. (**A**) Schema of barcoded virus to produce a phenotypically identical yet genotypically identifiable swarm of virus. (**B**) Replace PolyA candidate barcoded virus replication compared to the parental strain.

RESULTS

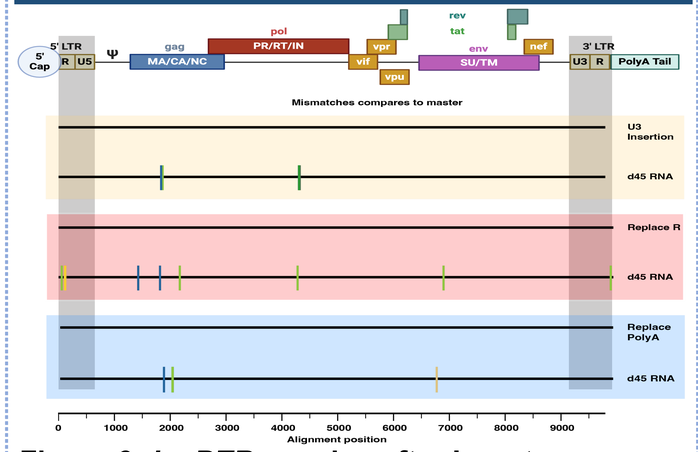


Figure 6: *loxBTR* remains after long-term replication. Day 45 viral RNA sequences compared to reference IMC. Ticks denote nucleotide changes. Gray overlays highlight the LTRs containing the *loxBTR* sites.

CONCLUSIONS

Full-length SHIVs encoding the Brec1 target sequence (*loxBTR*) within LTRs produce infectious, replication-competent viruses that persist *in vitro.*

FUTURE DIRECTIONS

- Delivery of Brec1 mRNA to infected cells *in vitro*
- Evaluate methods of quantifying viral excision in cell lines of latency modeling and in infected primary cells
- Test findings of *in vitro* viral kinetics and *loxBTR* fidelity in NHP models of HIV-1 infection
- Assess therapeutic efficacy of Brec1 mRNA *in vivo*

REFERENCES

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Email: ryan.krause@pennmedicine.upenn.edu