Characterizing how the novel recombinase Brec1 acts on HIV-1 DNA in vitro

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Background: Human immunodeficiency virus (HIV-1) establishes viral reservoirs during acute infection following transmission and evades conventional therapeutics by integrating into the host genome. Although antiretroviral therapy suppresses viral replication, analysis of tissues and cells from people with HIV demonstrate HIV-1 persistence, continued HIV-1 transcription, and viral protein production. Thus, HIV-1 cure strategies aim to eliminate these complications by targeting the viral reservoirs. The most important facet of the HIV reservoir is integrated proviruses found in immune cells, specifically long-lived CD4+ T cells and myeloid cells (monocytes/macrophages). Brec1, a novel evolutionary derivative of Cre, is an HIV-1-specific designer recombinase which excises the provirus from the host's genome at *loxBTR* sites in HIV-1 long terminal repeat motifs. Brec1 is reportedly effective against over 80% of HIV-1 isolates and targets up to 94% of HIV-1 subtype B viruses. However, questions and challenges remain regarding the characterization and efficacy of Brec1, and entering cells constituting the viral reservoir.

Results: J-Lat 10.6 cells containing a transcriptionally silent, but inducible *loxBTR*-flanked GFP expression vector were treated with *Brec1* mRNA-LNPs to determine if delivery and vector excision was achievable. Following unsuccessful *Brec1* mRNA delivery with this strategy, J-Lat 10.6 cells were electroporated with *Brec1* or *GFP* mRNA and analyzed by flow cytometry to determine treatment outcomes. Reduced *Brec1* mRNA-treated culture viability precluded analysis beyond recovery of *GFP* mRNA or electroporated negative control conditions, which respectively demonstrated equivalent cell viability, and *Brec1*-treated conditions showed no difference after induced GFP expression, suggesting unsuccessful vector excision.

To further investigate Brec1 in vitro, recombinant Brec1 was titrated upon circular or linearized plasmid vectors and infectious molecular clones (IMCs) containing *loxBTR*s, such as HIV-1 IMC pNL4.3 and *loxBTR*-containing novel simian-human immunodeficiency viruses (SHIVs). An EC50 of ~45 nM Brec1 was determined for in vitro reactions with pNL4.3 by gel electrophoresis. Reaction products retained their respective topology, determined after treatment with exonuclease V, which degrades linear DNA.

Conclusion: We continue to characterize and validate the application of Brec1 as a novel HIV-1 cure strategy, which will inform future animal studies in humanized mouse and NHP models of infection with *loxBTR*-containing IMCs and SHIVs.