In Vivo Replication of a *loxBTR*-SHIV for Nonhuman Primate Cure Studies

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Background: Preclinical evaluation of HIV cure strategies requires models that faithfully recapitulates human and HIV-1 biology. One strategy delivering an engineered Cre recombinase (Brec1) to reservoir cells has resulted in proviral excision *ex vivo* and in humanized mice. Although present in >90% of HIV-1 strains, the Brec1 recognition site (*loxBTR*) is absent in SIVs used in HIV translational primate studies. Here, we design and phenotype three distinct SIV/HIV-1 chimeras (SHIVs) encoding *loxBTR* and evaluate replication of a candidate SHIV in rhesus macaques (RMs).

Methods: *loxBTR* was incorporated into three positions of either U3 or R in the long terminal repeats of SHIV.C.CH505.v2. Virion content and infectivity was compared to parental SHIV.C.CH505.v2. Growth kinetics in human and rhesus CD4+ T cells over 15+ days was measured by viral capsid (p27) assay. Viral RNA from culture supernatants were sequenced for virus adaptation. Ten RMs were infected with either the lead candidate *loxBTR*- or parental-SHIV.C.CH505.v2 to compare viral kinetics and reservoir seeding prior to antiretroviral therapy (ART) initiation at week 16.

Results: All *loxBTR*-SHIVs generated infectious virions, with two of three demonstrating substantial infectious particle production (43% and 52% of parental IU/ng p27) and viral fitness (66% and 75% of parental [p27] at day 12). Long-term cultures led to high peak p27 levels in all *loxBTR*-SHIVs (143-252ng/mL) at day 42 and no adaptation in *loxBTR*. RMs were intravenously challenged with SHIV.C.CH505.v2 containing the HIV-1 *loxBTR* in the polyA stem-loop of R. 3/5 RMs displayed peak viral loads >50,000 copies/mL (median, 55,728 copies/mL), a range of setpoint viremias (median, 720 copies/mL), and were suppressed following two weeks of ART. Cell-associated DNA was detected in pre-ART PBMCs (median, 16.0 copies/10^6 cells) and week 20 lymph node mononuclear cells (median, 109.0 copies/10^6 cells). Week 20 DNA sequencing demonstrated no adaptation of *loxBTR*.

Conclusions: *loxBTR*-SHIVs confer productive infection in rhesus macaques while retaining the Brec1 target site both in vitro and in vivo. To our knowledge, this is the first example of a primate lentivirus that is HIV/SIV chimeric in the essential, polyfunctional regulatory region of R. *loxBTR*-SHIVs offer a new platform for primate studies investigating Brec1 recombinase-based HIV cure strategies.