TCR-mimic bispecific antibody reduces HIV provirus and delayed the viral rebound post ATI in a novel HLA allele specific BLT hu-mice

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BACKGROUND

Despite the efficient suppression of HIV-1 replication with combined antiretroviral therapy, viral latency is a barrier to HIV cure. Bispecific antibodies against Env and CD3 have resulted in ex vivo killing of HIV containing cells from PLWH. However, the low level of Env expression on cell surface and the sequence variation in env protein highlight the need for alternative approaches. We previously generated novel bispecific TCR mimic (TCRm) reagents against CD3 and HIV-1 Pol region peptides bound to human leukocyte antigen (HLA) *02:01 complexes, capable of linking CTLs to infected target cells and promoting target cell lysis. Based on this ex vivo killing capability, we investigated whether the TCR-mimic single-chain diabodies (ScDb) could reduce provirus and delay viral rebound post ATI in the ART suppressed HIV-infected humanized mouse model.

METHODS

We build a novel HLA-A allele specific BLT mouse model. BLT hu-mice generated from donors with the HLA-A*02:01 allele were infected with transmitted/ founder (T/F) virus HIVsuma. Viremia was suppressed on cART (FTC+TDF+RAL). ScDb HI12 directed against the Pol region of HIV and isotype control ScDb H2 against a p53 epitope were used to treat the animals from 3 to 5 weeks post inoculation. ScDb in vivo kinetics were measured in BLT humanized mice. Fourteen daily infusion of HI12 or H2 were administered to animals with partially suppressed viremia after early cART started intraperitoneally over two weeks from day 21-35 (Fig 1). Independent experiments addressed weekly plasma viral load and (a) intact and defective proviral reservoir size by the intact proviral DNA assay (IPDA) were measured on cART, or (b) viral rebound after cART interruption. CD4 depletion and leukocyte subsets activation were also evaluated. Analysis was done with Prism software.

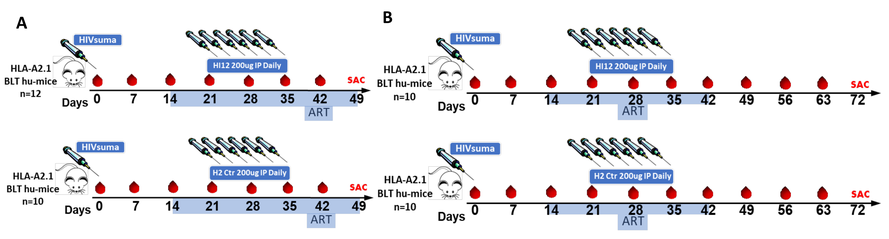


Figure 1. Study design for cohort I: reservoir size assessment (A) and cohort II: the rebound delay post ATI (B). Blood drops indicated the bleeding time points; blue shades indicated the period of ART treatment.

TCR-mimic ScDb reduced HIV provirus and delayed the viral rebound in HLA specific BLT hu-mice.

RESULTS

Using an HLA-A allele specific BLT humanized mice, we found the in vivo half-life of TCRm ScDb post IP injection was 3.9 hrs, peaking at ~2.4 ug/ml. With stable bodyweight post treatment (Fig 2A), TCRm ScDb was well tolerated in BLT hu-mice. TCRm ScDb HI12 added to early cART achieved suppression quicker than the H2 control group (Fig 2D). CD38+HLA-DR+ double positive population were significantly elevated in both CD4+ and CD8+ T cells during the HI12 treatment period (Fig 2C). Intact (P=0.026), 3' defective (p=0.002) and total (p=0.007) HIV proviruses in splenocytes were significantly decreased post HI12 treatment, whereas the 5' (p=0.295) defective proviruses did not show a significant decrease (Fig 3). In independent experiments, viral rebound time was significantly delayed (p=0.0026) in HI12 treated animals over H2 control treated ones (Fig 4).

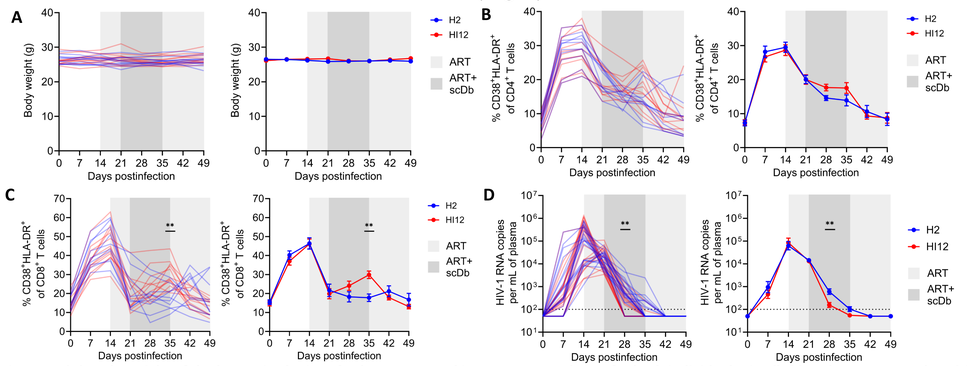


Figure 2. (A) Body weight of the humanized mice of cohort I reported in grams. Data shown for both individual mice (left) and as average values (right, mean, SD). (B & C) Activation of peripheral blood T cells for cohort I. Data shown as the percent of CD38+HLA-DR+ cells within the CD3+CD4+CD8- (B) or CD3+CD4-CD8+ (C) populations. (D) HIV-1 plasma RNA before and during ART administration (day 0 to day 49 post-infection) for cohorts I and II. Significance determined by two-way ANOVA followed by Šídák's test for multiple comparisons, **P<0.01.

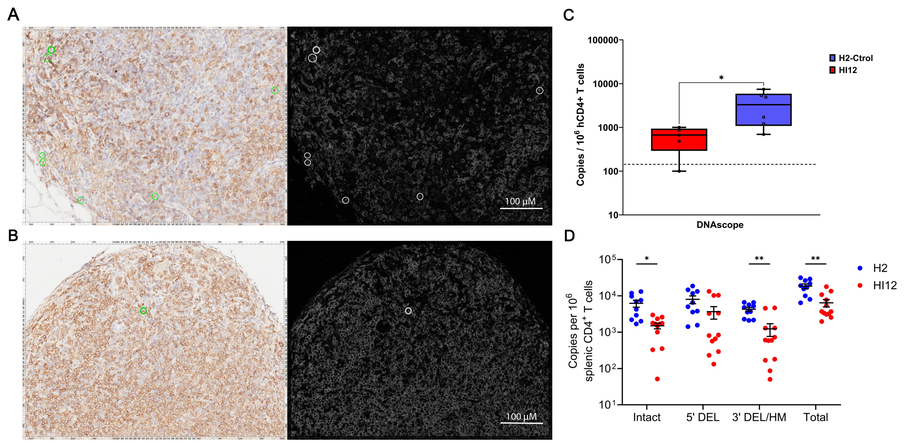


Figure 3. (A & B) HIV DNAscope signal in LN of study animals and vDNA+ cells frequency by the end of study in cohort I. CD4 signal in brown, cDNA in red with green circles highlighted in H2 (A) and HI12 (B) treated animals. (C) HIV proviral DNA size in LN quantification based on the DNAscope results. (D) Frequencies of splenic CD4+ T cells harboring intact, 5' deleted, 3' deleted/hypermutated, or total HIV-1 provirus (cohort I). Data reported as HIV-1 copies per million splenic CD4+ T cells. Significance determined by two-way ANOVA followed by Šídák's test for multiple comparisons, *P<0.05, **P<0.01.

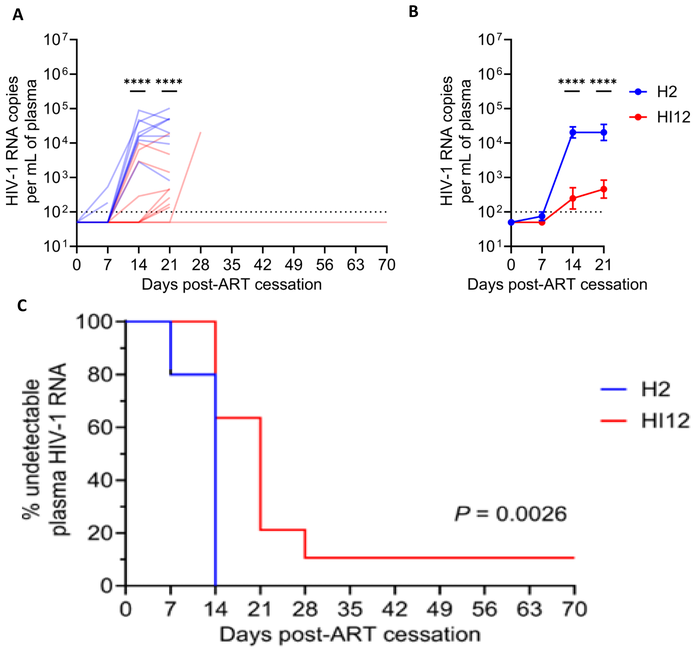


Figure 4. (A & B) HIV-1 plasma RNA rebound following ART cessation (day 49 to day 119 post-infection) for cohort II. HIV-1 RNA copies/mL plasma shown for both individual mice (A) and as average values (B, mean, SD) for the H2 scDb (n=10, blue) and HI12 scDb (n=11, red) treatment groups. Significance determined by two-way ANOVA followed by Šídák's test for multiple comparisons, ****P<0.0001. (C) Survival analysis of mice with undetectable HIV-1 plasma RNA (<100 copies/mL) post-ART cessation (day 49 to day 119 post-infection). Significance determined by log-rank test.

CONCLUSIONS

Using HLA-A matched BLT mice, the addition of TCRm ScDb targeting Pol epitope in corresponding HLA complexes during early ART increased T cell immune activation and reduced the intact provirus burden on ART resulting in a delay of viral rebound after cART interruption. The use of TCRm ScDb strategies warrants further clinical studies towards HIV cure.

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