A New CD4mc Enables Fc-Mediated Reservoir Reduction for Durable Post-ART HIV-1 Control in Hu-Mice

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BACKGROUND

Persistently-infected HIV-1-positive cells are heterogeneous, rare, and inherently difficult to eliminate; shock-and-kill approaches have thus far proven inefficient in measurably reducing infected cell frequencies in clinical trials. CD4-mimetic compounds (CD4mcs) sensitize HIV-1-infected cells to antibody-dependent cellular cytotoxicity (ADCC) mediated by CD4-induced (CD4i) non-neutralizing antibodies (nnAb) found in plasma of people living with HIV (PLWH) [1, 2].

While ADCC can eliminate HIV-infected cells *in vivo,* this powerful immune mechanism is negated by the HIV-1 proteins, Vpu and Nef, that downregulate CD4, limiting exposure of the ADCC-vulnerable CD4-bound conformation of gp120 Env on the infected cell surface. CD4mc effectively promote Env recognition through sequential opening and binding of Abs to two categories of highly-conserved CD4i epitopes : 1) coreceptor-binding site (CoRBS) epitopes; and 2) “Cluster A” epitopes, which results in the stabilization of an asymmetric Env conformation (State 2A) that renders infected cells highly vulnerable to ADCC (**Fig 1**).

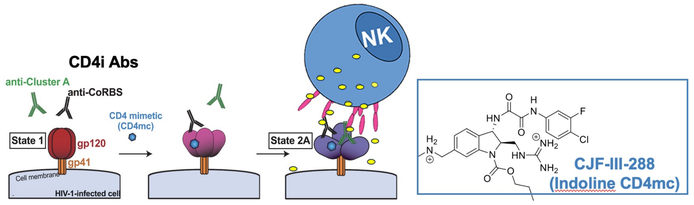


Fig. 1. Sequential opening of Env by CD4mc (CJF-III-288, inset) and CD4i Abs sensitizes of HIV-1-infected cells to ADCC.

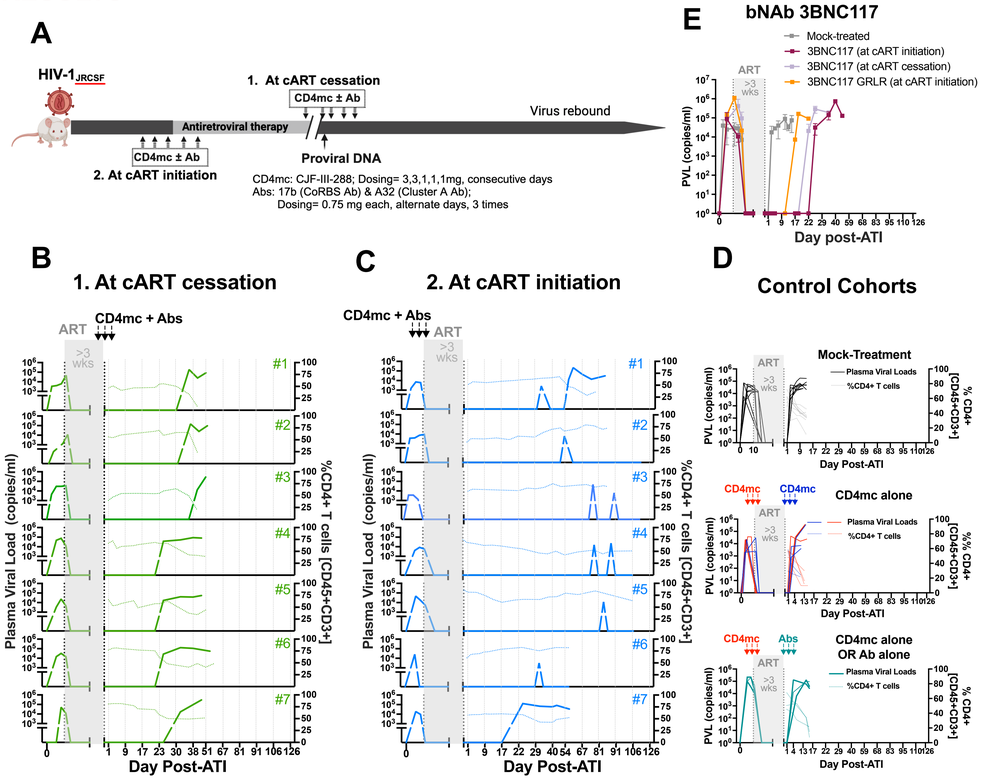
The development of a new indoline CD4mc, CJF-III-288, with improved potency in neutralization and ADCC [3], prompted us to investigate the impact of treatment at cART initiation or cART cessation, on viral tissue reservoirs and post-ART virus rebound dynamics in humanized mice (hu-mice) that support antibody effector function.

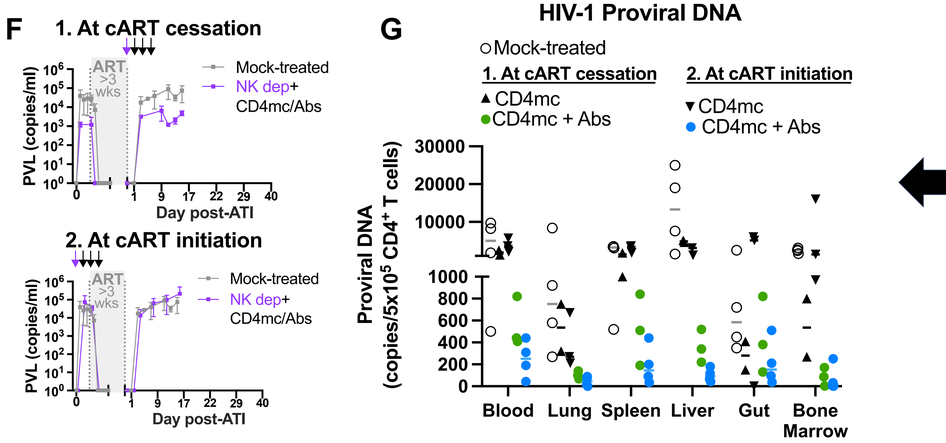
METHODS

NSG-Tg(IL15) hu-mice were treated with CJF-III-288 in combination with CD4i A32 (anti-cluster A) and 17b (anti-coreceptor binding site) nnAbs either at cART initiation or at cART cessation (analytical treatment interruption or ATI). Post-ART plasma viremia and tissue viral reservoir size were analyzed by longitudinal measurements of HIV-1 RNA and DNA respectively. We also analyzed the contributions of natural killer (NK) cells to the observed effects by *in vivo* cell-depletion studies. Treatment with the broadly-neutralizing antibody (bNAb) 3BNC117 was similarly studied for comparison.

A new indoline CD4-mimetic compound CJF-III-288 has therapeutic potential, when administered at early stages of infection, in the presence of HIV-1 anti-Env CD4i non-neutralizing antibodies that are found in plasma of most people living with HIV-1. In a humanized-mouse (hu-mouse) model of HIV-1 infection with natural killer (NK) cell function, administration of a “cocktail” of CJF-III-288 and CD4i Abs at the time of cART initiation resulted in sustained virus control after analytical treatment interruption.

RESULTS





CONCLUSIONS

- Treatment of HIV-1_{JRCSF}-infected NSG-Tg(IL15) hu-mice with a cocktail of CJF-III-288 and the CD4iAbs CoRBS 17b/Cluster A A32 at the time of cART initiation can lead to durable control of viral rebound. Indeed, plasma viral loads were repeatedly suppressed in a large subset of animals for >100 days after ATI, despite intermittent blips!
- Dramatic (>3-log) reductions in proviral DNA copies in CD4 T cells occurred in various tissues upon CD4mc/CD4iAb treatment at cART initiation.
- Depletion of NK cells abrogated CD4mc/CD4iAb effects, resulting in rapid viral rebound after ATI indicating a key role for Ab Fc-effector function and ADCC.
- Treatment with bNAb 3BNC117 at initiation of cART did not result in durable control of rebound viremia.

CJF-III-288/CD4i-Abs at the time of cART initiation has can serve as an early cure intervention for HIV-1.

REFERENCES CITED

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Fig. 2. CD4mc/CD4iAb intervention at cART initiation durably controls viral rebound and significantly reduces the size of the HIV-1 reservoir in NSG15 hu-mice. (**A**) HIV-1_{JRCSF}-infected NSG15 hu-mice were initiated on cART comprising raltegravir, emtricitabine and tenofovir disoproxil fumarate when plasma VLs were ~10^4-10^5/ml and treated with a cocktail of CD4mc (CJF-III-288) + CD4iAbs (Abs = CoRBS 17b/Cluster A A32) either at (1) cART cessation or (2) cART initiation, as indicated (**B** and **C**). (**D**) Control cohorts received only cART (Mock-Treatment) or CD4mc or Abs individually, along with cART. (**E**) Separate cohorts were treated similarly with the bNAb 3BNC117 or its Fc-null variant 3BNC117 GRLR. (**F**) Some cohorts were administered the NK cell-depleting Ab NKp46 (Clone B-L46, 50 µg/mouse, two consecutive days) two days prior to CD4mc/CD4iAb administration. (**G**) Proviral DNA (copies) in CD4+ T cells were measured in tissues at day 3 post-ATI corresponding to the time of viral rebound in mock-treated mouse cohorts. Plasma VL (left Y axis, RNA copies/ml plasma, solid lines, detection limit 350 copies/ml, **B-F**) and peripheral CD4+ T cell percentages (right Y axis, dotted lines, **B-D**) were measured longitudinally after ATI. Gray shading indicates cART treatment, arrows indicate the timing and interventions applied and each line/symbol corresponds to an individual mouse in **B**, **C**, **D**, **G** and average data (Mean ± S.D.) in **E**, **F**.