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Evaluation of HIV-specific T cell response in BEAT2 Clinical Trial

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BACKGROUND

The isolation and clinical development of broadly neutralizing HIV-specific monoclonal antibodies (bNAbs) have enabled promosing new strategies for HIV-1 prevention, therapy and cure. Human clinical trials of bNAbs administration have shown that while monotherapy temporarily delayed viral rebound, combination therapy more significantly delayed viral rebound following analytical antiretroviral treatment interruption (ATI)[1-3]. Interestingly, studies of bNAbs administration on non-human primates[4] and on PLWH have suggested enhancement of HIV-specific T cell responses, either during ATI or by preventing the contraction of the HIV-specific T cell response when administered in newly diagnosed individuals at ART initiation[5,6]. This "vaccinal" effect has been postulated to occur via immune complex formation, leading to dendritic cell activation and enhanced antigen processing and presentation to HIV-specific T cells, thus preserving or enhancing their function and frequency (Fig.1)[7].

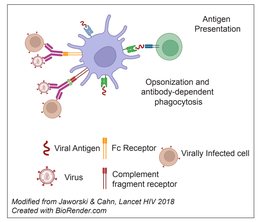


Figure 1: Vaccinal effect of bNAbs.

Immunomodulation through interferon alpha (IFNa) therapy has also been shown to delay post ATI viral rebound and reduce the viral reservoir[8]. However, it is unclear whether this therapeutic effect is due to enhancement of HIV-specific T cells (or NK cells) by the IFNa therapy. The BEAT2 clinical trial (ClinicalTrials.gov: NCT03588715) sought to partner combination bNAb therapy together with IFNa immunotherapy after ART interruption (see BEAT-HIV talk by Dr. Luis Montaner), finding a lack of re-treatment criteria with predominant viral loads <1000 HIV RNA copies/ml in 4/10 trial participants after several weeks of stopping all immunotherapy. Here we sought to determine whether HIV-specific CD8+ T cells were altered during viral suppression under immunotherapy and/or during continued control after stopping immunotherapy in the BEAT2 clinical trial.

AIM AND HYPOTHESIS

AIM: To determine the impact of bNAbs 3BNC117 and 10-1074 combined with type 1 IFN on HIV-specific T cell function in participants of the BEAT2 clinical trial.

HYPOTHESIS: combination bNAb and IFN-a2b therapy will enhance HIV-specific T cell function and/or frequency during ATI, leading to control or delay in viral rebound.

STUDY DESIGN

BEAT2 clinical trial inclusion criteria:
HIV+ individuals treated for at least 1 yr, with pVL<50 c/ml for at least 6 months, and CD4 > 450 cells/ul.

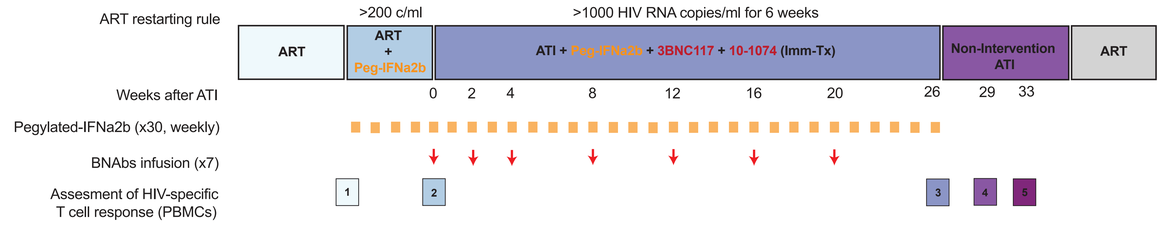
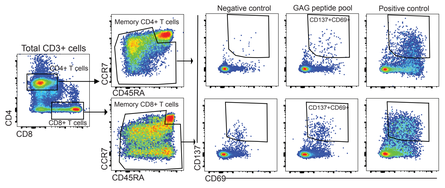


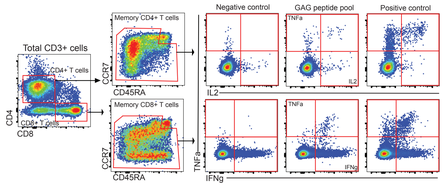
Figure 2: Peripheral mononuclear cells (PBMCs) were obtained from the **BEAT2 clinical study** in which baseline-sensitive PLWH received 30 weekly doses of pegylated IFNa2b (1.5ug/kg) (4 weeks on ART and 26 weks off ART), and seven IV infusions of bNAbs (30 mg/kg during the 26 weeks off ART). 10 participants received combined immunotherapy (Imm-Tx), and one received only bNAbs infusions.

MATERIALS AND METHODS

Blood was collected periodically to determine plasma viral load and evaluate HIV-specific T cell function.
=> T cell function was assessed longitudinally at baseline (time point 1 -TP1-: during ART, pre peg-IFNa2b; TP2: at the end of ART+Peg-IFNa2b treatment; TP3: at the end of Peg-IFNa2b+bNABs treatment during ATI; and TP4/5: during ATI with no immunotherapy). Activation-induced marker (AIM) assay and intracellular cytokine staining (ICS) were utilized to evaluate specific T cell responses against and array of HIV antigens. Briefly, PBMCs were stimulated with 1 ug/ml of GAG, ENV, NEF or POL peptide pools[9] and anti-CD49d/anti-CD28 as co-stimulation. For the AIM assay, cells were incubated for 18 hours. For the ICS assay, 1 h after initiation of stimulation, protein transport inhibitors brefeldin A and monensin were added, and cells were incubated for a total of 6 h. Staphylococcal enterotoxin B (SEB) was used as a positive control.



A) AIM assay:
HIV-specific CD4+ or CD8+ T cells were identified as the sum of CD137+CD69+, CD137+PDL1+ or CD69+PDL1+ cells after stimulating them with the corresponding HIV antigens peptide pools.

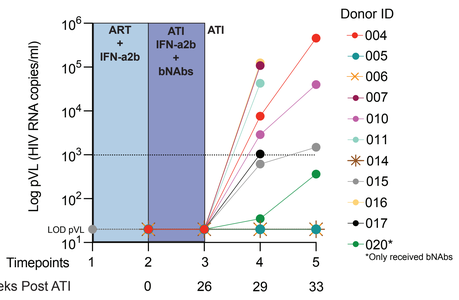


B) ICS assay:
HIV-specific CD4+ or CD8+ T cells were identified as the sum of cytokine+ cells after stimulating them with the corresponding HIV antigens peptide pools.
CD4: TNFa, IFNg, IL2
CD8: TNFa, IFNg, MIP1b.

Figure 3: Forward scatter height (FSC-H) vs forward scatter area (FSC-A) and side scatter area (SSC-A) vs FSC-A plots were used to exclude doublets and focus on singlet small lymphocytes. Dead cells, monocytes and B cells were excluded via de Aqua Blue-CD14/19 dump gate. CD4+ and CD8+ T cells were gated within CD3+ cells. To determine the memory phenotype, CCR7 vs CD45RA were used, and naive T cells were excluded from the analysis. Representative flow plots indicating the gating strategy used to analyze the proportion of HIV-specific CD4+ or CD8+ T cells in the AIM assay (B) or ICS (B) are shown.

RESULTS

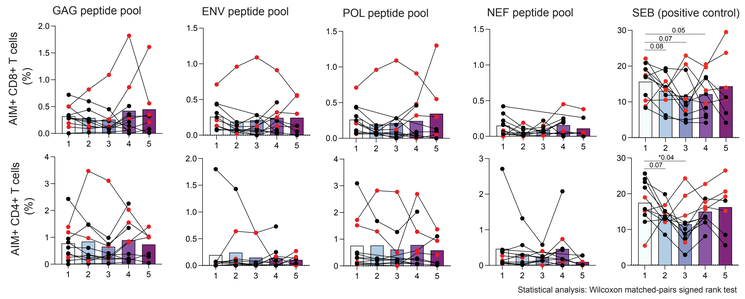
1- 4/10 individuals that received Peg-IFN-a2b and bNAbs showed sustained control of viremia by not meeting protocol ART restart criteria (pVL>1000 copies/ml over 6 weeks)



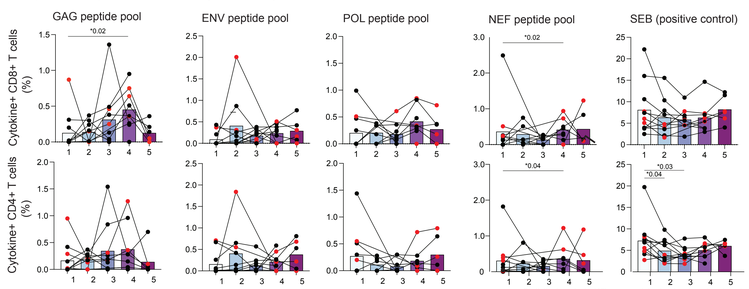
- Shown are time point 1 (baseline), 2 (end of 4 doses of weekly IFN-a2b on ART), 3 (end of 26 weeks of combined bNAbs + IFN-a2b), 4 and 5 (period after end of immunotherapy).
- All eleven bNAbs-sensitive BEAT2 clinical trial participants maintained viral loads below the limit of detection (LOD) during 26 weeks after ART interruption while receiving bNAbs infusions and peg-IFN-a2b (one of eleven, 020, did not receive IFN-a2b).
- Up to week 33 post ATI, 4/10 individuals showed sustained control of viremia after immunotherapy (either below LOD or less than 1000 c/ml over 6 weeks). Donor 020 was excluded from summaries of trial to date because the primary end-point was based on the analysis of participants that received combined immunotherapy.
- As last bNAb infusion was at week 20 with a reported half-life of ~12-15 weeks, decreasing bNAb levels are expected through week 33 (i.e, week 13 from last infusion).

2- bNAbs administration during peg-IFN-a2b treatment did not enhance T cell response

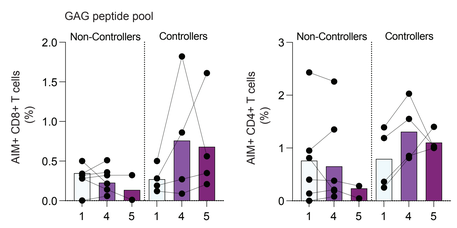
A) T cell response evaluated by AIM assay



B) T cell response evaluated by ICS assay



B) T cell response evaluated by AIM assay

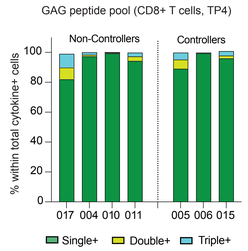


A) and B): HIV-specific T cell response analyzed by AIM assay: Background-subtracted T cell CD4+ and CD8+ T cell responses after peptide stimulation are shown:

A) Individuals that showed sustained control of viremia are highlighted in red (005,006,014 and 015).
No overall increase in HIV-specific T cell responses were observed during (TP2) or immediatly after immunotherapy (TP3), or during the non-interventional ATI (TP4 and TP5) compared to baseline (TP1). We also observed a decrease in the proportion of SEB-activated CD4+ T cells at TP3.

B) Heightened GAG-specific CD4+ and CD8+ T cells in controllers individuals during the post immunotherapy non-intervention ATI timepoints (TP4 and/or 5) compared to TP1. Participant 020 was excluded from the analysis.

D) T cell response evaluated by ICS assay



C) and D): HIV-specific T cell response analyzed by ICS assay: Background-subtracted T cell CD4+ and CD8+ T cell responses after peptide stimulation are shown (TP4 and TP5 not available for donors #007 and #014):

C) Individuals that showed sustained control of viremia are highlighted in red (005,006,014 and 015). No overall increase in HIV-specific T cell responses were observed during (TP2) or immediatly after immunotherapy (TP3). Following immunotherapy, and coordinated with viral rebound in the noncontrollers, we observed an increase in the proportion of GAG-specific cytokine-producing CD8+ T cells (TP4).

D) Limited polyfunctional properties of GAG-specific CD8+ T cell responses at TP4 (donor #016 was excluded due to low event counts). Most GAG-specific cells are single cytokine producers (IFNg) regardless of control status. Participant 020 was excluded from the analysis.

CONCLUSIONS

- We were unable to observe evidence of a vaccinal effect on HIV-specific CD8+ T cells in response to 3BNC117+10-1074 bNAb administration combined with IFN-a2b.
- By ICS assay, we observed increased HIV GAG-specific CD8+ T cell responses following cessation of immunotherapy and coincident with viral rebound in the non-controller individuals.
- Sustained control of viremia following immunotherapy cessation was only associated with heightened HIV-specific CD8+ T cell responses in 2/4 controller individuals.

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