BEAT2: PEG-INF-ALPHA + 3BNC117 + 10-1074 REBOUND VIRUS PHENOTYPE AND EVOLUTION

#326

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BACKGROUND

The study BEAT2 (NCT03588715) evaluated the safety, tolerability, and immune responses following the administration of the combination of pegylated Interferon alpha 2b (peg-IFN-α2b) with two broadly neutralizing antibodies (bnAbs; 3BNC117 and 10-1074) in the setting of a monitored analytical treatment interruption (ATI). Primary clinical outcomes are presented elsewhere (Tebas et al, abstract #431). Here we describe viral kinetics and antibody responses during ATI.

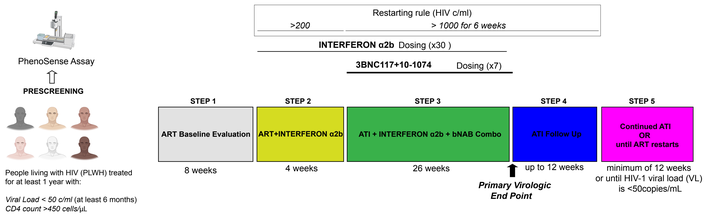

Figure 1. BEAT-HIV Study Design. 14 participants who met enrollment criteria, including virus reservoir sensitivity by Monogram Phenosense Assay (3BNC117 IC90 < 1.5 ug/ml; 10-1074 IC90 < 2 ug/ml), were enrolled. Two participants withdrew, n=12 were analyzed. **Step 1:** Baseline evaluation. **Step 2**: Participants on ART began weekly peg-IFN-α2b. **Step 3:** ART was discontinued, and participants received two bnAbs (30 mg/kg 3BNC117 and 10mg/kg 10-1074) every 2 weeks x 2, then every 4 weeks x 5, for a total of 7 doses over 26 weeks. ***The Primary Virologic End Point of the study was not meeting ART restart criteria at week 26 of Step 3***. **Step 4**: Peg-IFN-α2b, 3BNC117, and 10-1074 were discontinued, and ATI continues. **Step 5:** Continued ATI **or** ART restarts when participants have a confirmed return of HIV-1 viremia >1000 c/ml for 6 consecutive weeks or confirmed CD4+ T-cell count <300 cells/µl.

STUDY GOALS

- **To characterize the dynamics of plasma rebound virus longitudinally over ATI after administration of 2 bnAbs + IFN.**
- **To determine the role of administered bnAbs and evolving autologous neutralizing antibody response on virus evolution.**

METHODS

Plasma viral RNA extraction, cDNA synthesis, and SGS were performed to amplify the HIV-1 *env*. Phylogenetic analysis was performed, and selected *env* sequences from rebound timepoints were codon-optimized and molecularly cloned for pseudovirus production.

Autologous antibodies (plasma IgG) were extracted using Protein G GraviTrap™ columns.

TZM-bl assay: measurement of autologous antibodies and bnAb (3BNC117 and 10-1074) mediated neutralization of HIV-1 as a function of reductions in HIV-1 Tat-regulated firefly luciferase (Luc) reporter gene expression after a single round of infection with Env-pseudotyped viruses[1,2,3].

In the BEAT2 clinical trial, IFN + 2 bnAbs at ATI suppressed virus for the majority (10/12) of pre-screened participants. Analyses of viral dynamics reveal:

1. bnAb sensitivity:
- Rebound virus was oligoclonal with *increased resistance* to both bnAbs compared to pre-screening cutoffs.
- Rebound viruses' neutralization sensitivities were similar by lab and commercial assays and inversely correlated with time to rebound.

2. Virus evolution over weeks of ATI:
- Rebound virus populations evolved over 4+ weeks ATI, with within lineage evolution or replacement by genetically and phenotypically distinct lineages.

3. Autologous neutralizing antibody sensitivity:
- Rebound viruses were resistant to baseline autologous nAb responses.
- Autologous nAb responses increased after rebound to titers greater than combined plasma neutralization potency with peak bnAb levels.

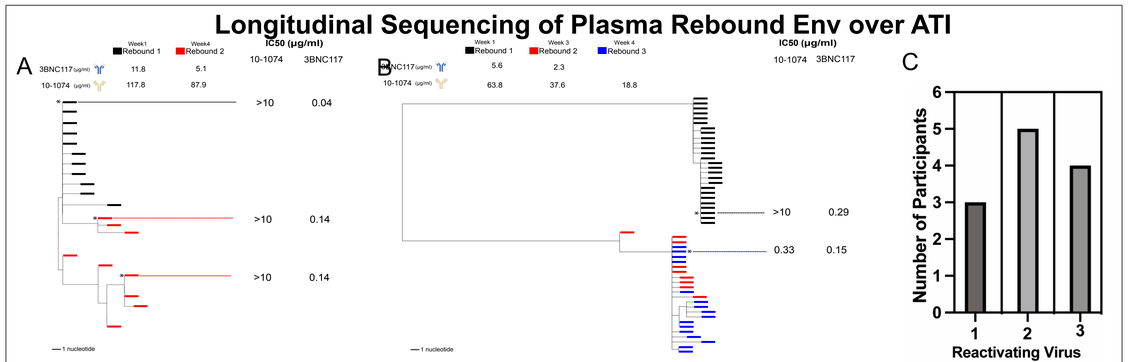

Figure 2. Rebound viruses evolve over Analytical Treatment Interruption. Maximum likelihood phylogenetic trees of SGS–derived gp160 envs from 2 representative participants' rebound plasma. The scale bar indicates genetic distance. Fifty percent inhibitor concentration (IC50) neutralization titers are shown aligned to the specific envelope glycoprotein cloned and tested for sensitivity to administered bnAbs. In both participants, first detectable rebound aligns in one low diversity lineage representing a single sampled virus population that was resistant to 10-1074 and marginally sensitive to 3BNC117. In **A**, virus evolves with few changes in gp120 conferring modest increase in resistance to 3BNC117. In **B**, the original virus lineage is no longer sampled at later timepoints (17 and 28 days later), replaced by a distinct virus population (and recombinants), that is more sensitive to both bnAbs.

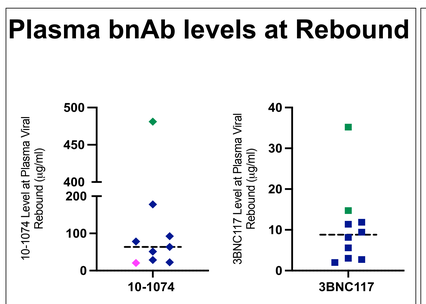

Figure 3. BnAb levels at time of virus rebound, color-coded for Study Step in which participant experienced rebound. Early rebound in Step 3 (green), Rebound during Step 4 (blue), and Late rebound in Step 5 (magenta).

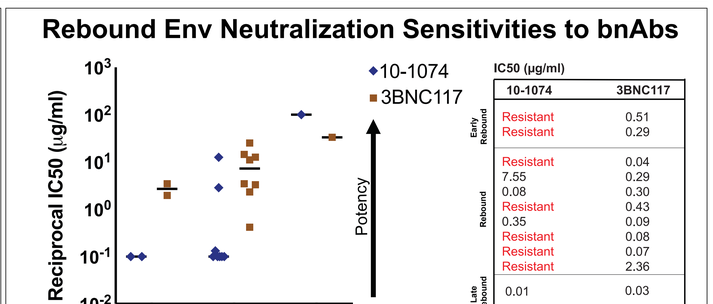

Figure 4. Neutralization resistance of Rebound Envs to bnAbs & time to rebound. IC50 of rebound virus measured stratified by the Study Step in which virus rebound occurred. Early Rebound during Step 3 occurred through high bnAb levels (n=2); Rebound during Step 4 occurred through waning bnAb levels (n=8); Late rebound during Step 5 occurred after bnAbs had waned from circulation (n=1 analyzed).

Parallel analyses of rebound Envs sensitivity to bnAbs in TZM.bl vs. PHENOSENSE^{GT} assay.

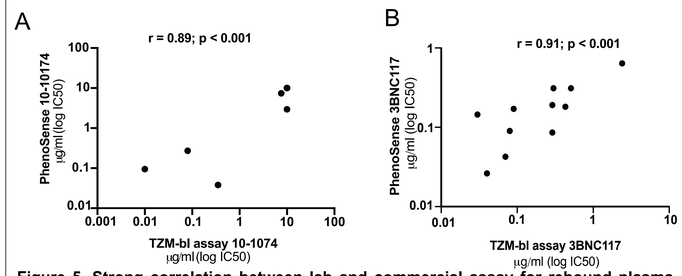

Figure 5. Strong correlation between lab and commercial assay for rebound plasma Envs. Spearman correlation between IC50 of first detectable rebound plasma virus to 10-1074 (A) and 3BNC117 (B) measured by TZM-bl vs PhenoSense^{GT} assay.

Neutralization potency of autologous plasma IgG against rebound variants

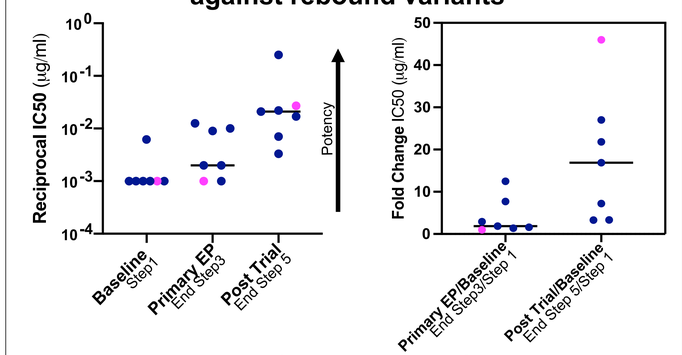

Figure 6. Neutralization potency of autologous plasma IgG against rebound variants. Neutralization potency of plasma IgG from 3 timepoints: Baseline (pre-trial), Primary End Point (EP) Week 26 of Step 3 (with administered bnAbs), and post-trial (after ART re-initiation), against matched rebound Envs displayed as reciprocal IC50 evaluated in 7 participants (**A**). Baseline anAbs have minimal potency vs. the viruses that would rebound later in the study. At Week 26 of Step 3, plasma contains both anAbs & administred bnAbs. Post-trial, plasma IgG does not contain bnAbs, but anAb responses boosted after rebound viremia. Fold change in neutralization titers from baseline to Step 3 (+ bnAbs; median 2-fold) and baseline to post-trial (boosted anAbs; median 17-fold) (**B**).

CONCLUSIONS

- **Rebound envs were oligoclonal (1-3 initial virus populations), revealed a high frequency of resistance to administered bnAbs (10-1074 > 3BNC117), and evolved longitudinally over weeks of ATI.**
- **Earlier time to rebound was associated with greater bnAb resistance in rebound virus; Rebound Env neutralization sensitivity was highly concordant between lab and commercial assays.**
- **Rebound virus was resistant to baseline anAb responses, but anAbs rose substantially (median 17-fold) after rebound to titers greater than seen in plasma at peak bnAb concentrations.**
- **Ongoing work includes evaluation of interplay of anAb + bnAbs, patterns of bnAb escape, and evaluation of the selective pressures restricting reservoir provirus reactivation at ATI.**

[1]Keele BF, et al. Proc Natl Acad Sci U S A 2008;105:7552-7; [2]Li H, et al. PLoS pathogens 2010;6:e1000890; [3]Wei, et al. Nature 2003 422:307-312