HIV-1 Env Markers of Prevention Efficacy in HVTN 704/HPTN 085, the Antibody-Mediated Prevention (AMP) Trial of Broadly Neutralizing Antibody (bnAb) VRC01 in the Americas and Europe: Genotypic Sieve Analysis

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Background

AMP was two harmonized prevention efficacy trials, with the goal of evaluating HIV prevention efficacy (PE)* of VRC01, a bnAb against the CD4 binding site. The two AMP trials were: (1) HVTN 703/HVTN 081, enrolling 1,924 women in sub-Saharan Africa; and (2) HVTN 704/HPTN 085, enrolling 2,699 HIV-uninfected MSM and transgender adults in the Americas and Switzerland

$$^{*}PE = 1 - \frac{p\,(HIV{-}1\ diagnosis\ by\ Week\ 80)_{VRC01\ arms}}{p\,(HIV{-}1\ diagnosis\ by\ Week\ 80)_{placebo\ arm}} \times 100\%$$

AMP participants were randomized 1:1:1 to receive ten infusions (10mg/kg or 30mg/kg of VRC01, or placebo) every eight weeks, with HIV testing every four weeks, over the 80-week study period.

AMP's primary result: PE depended on in vitro neutralization resistance of the acquired virus. No efficacy was observed against resistant viruses, whereas against sensitive viruses (IC_{80} < 1 µg/ml), 73.0% efficacy (95% C.I.: 27.6% - 89.9%) was observed.

In the sieve analysis for AMP, we evaluate how PE may be influenced by Env amino acid (AA) sequence features.

Due to data availability, the scope of this poster is constrained to our initial SAP-specified sieve analyses of HVTN 704. Additional sieve analyses are ongoing.

Methods

Sequencing: 127 HVTN 704 participants who acquired an HIV-1-primary endpoint were sequenced using the PacBio Single Molecule Real-Time platform. We sequenced the first RNA+ sample and a follow-up sample, for each participant, acquiring a mean of 173 sequences with universal molecular identifiers for each sample.

Two regions were sequenced: (1) a 3 kb region covering *rev*, *env*, and partial *nef* (REN) and (2) a 2.5 kb covering *gag* and partial *pol* (GP).

The sieve analysis presented here only includes primary endpoints; i.e., participants diagnosed HIV-1+ by the Week 80 study visit (n = 98).

PAR Score: LANL's CATNAP database was used to build a superlearner model to predict the probability of IC_{80} > 1 µg/ml from an Env protein sequence. We refer to this output as the PAR (Proteomic Antibody Resistance) score. (Magaret et al., 2019, *PLoS Comp Biol*; Williamson, Benkeser et al., 2021, *Bioinformatics.*)

Prespecified Env Features: To preserve our statistical power, we used the predictive analysis mentioned above, and three other approaches (Bricault et al., 2019, *Cell Host Microbe*; Williamson et al., 2020, *Biometrics*), to pre-select twenty-three sequence features based on their importance for predicting *in vitro* VRC01 neutralization resistance. These features include Env residues at 12 specific positions, the presence of a PNGS at five specific sites, and six features pertaining to viral geometry. The selected features are outlined in Table 1.

Statistics: PE against acquisition of HIV-1 with specific Env features were analyzed by mark-specific proportional hazards models (Juraska and Gilbert, 2013, *Biometrics*).

Table 1: Pre-Selected Sequence Features

Position	In VRC01 BS	In CD4 BS	Notes
60			C1
170			V1
230			Lead residue of Glycosite 230
279	Yes	Yes	Loop D; CD4 mid/side-chain BS
280	Yes	Yes	Loop D; CD4 mid/side-chain BS
317			V3; Coreceptor (R5/X4) BS
365	Yes	Yes	CD4 mid/side-chain BS
429	Yes	Yes	CD4 mid/side-chain BS
456	Yes	Yes	CD4 BS, side-chain only
458	Yes	Yes	CD4 BS, main-chain only
459	Yes	Yes	CD4 BS, main-chain only
471		Yes	CD4 BS, main-chain only; one residue downstream of V5

PNGS at Position	Viral Geometry Feature
156	Length of gp120
229	Length of V1V2 Region
234	Length of V5 Loop
616	Total PNGS Count in V1V2 Region
824	Total PNGS Count in V5 Loop
	Total Cysteines in gp120

Results

PE vs. PAR Score: We found that PE decreased with the predicted probability of IC_{80} >1 µg/ml (p = 0.017) (Figure 1). Viruses with a resistance probability of 0.3 had estimated PE=80%, while the PE decreases to 0% against viruses with resistance probability of 0.72.

Site 230: PE significantly differed with the residue content at one site: the presence of an aspartic acid ("D") at site 230 (p = 0.008) (Figure 2). Site 230 is the lead position in glycosite 230, and a glycand bound to this site is known to facilitate VRC01 binding. Disrupting the PNGS motif (by changing the asparagine ("N") to aspartic acid ("D")) promotes escape from VRC01 (Figure 3). We found that VRC01 had a PE against D230 of -24% (-118% - 29%, p = 0.45), while it had a PE of 64% (26% - 82%, p = 0.004) against viruses with notD230.

Figure 1: PE by PAR Score

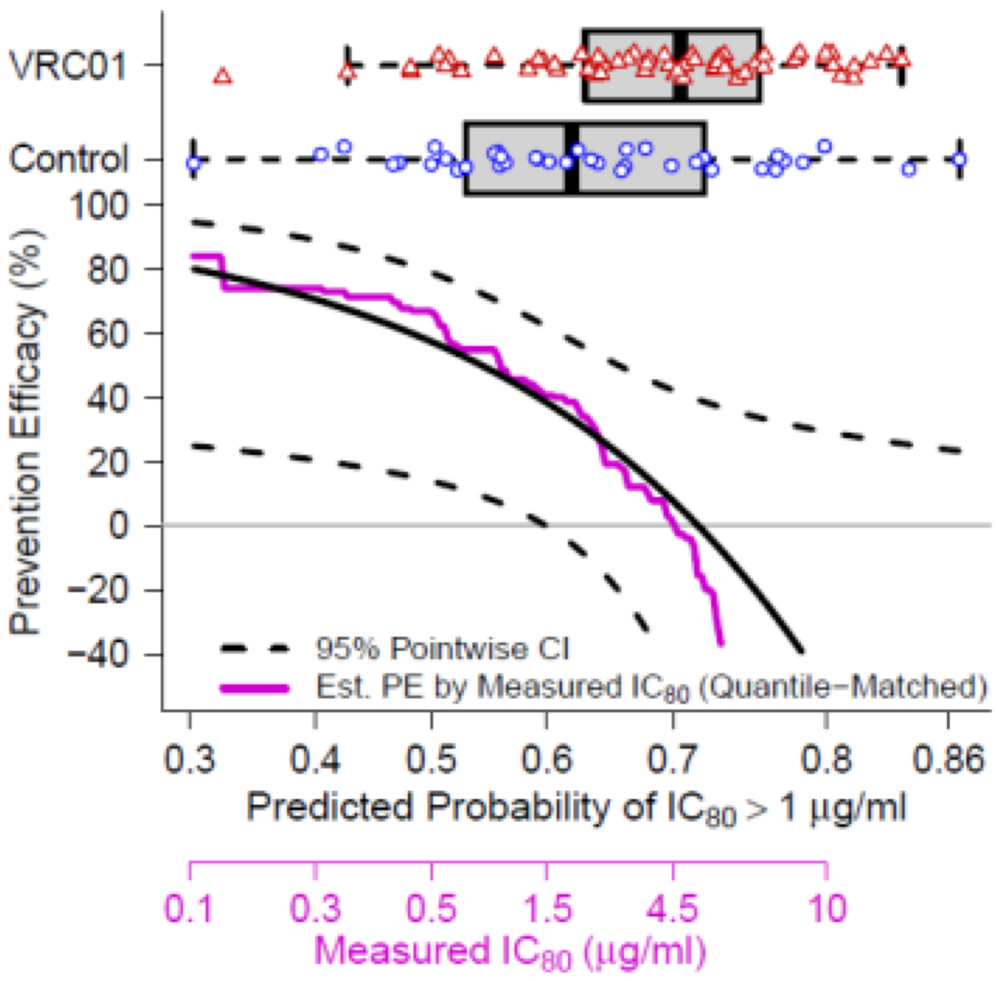

Figure 1. HIV-1 Prevention Efficacy (PE) by Proteomic Antibody Resistance (PAR) score (black) recapitulates PE by measured IC80 (pink).

Figure 2: AA Distributions at Site 230

Figure 2. Distributions of amino acids (AAs) at HIV-1 Env site 230. Each column along the x-axis represents a case (i.e., a participant who acquired a primary HIV-1 endpoint). Within each column, the color(s) reflect(s) the AA(s) at position 230 in all viruses sequenced for a given case. The mean number of viruses sequenced per case,per timepoint = 173.

Figure 3: Visualization of Glycosite 230 in Env

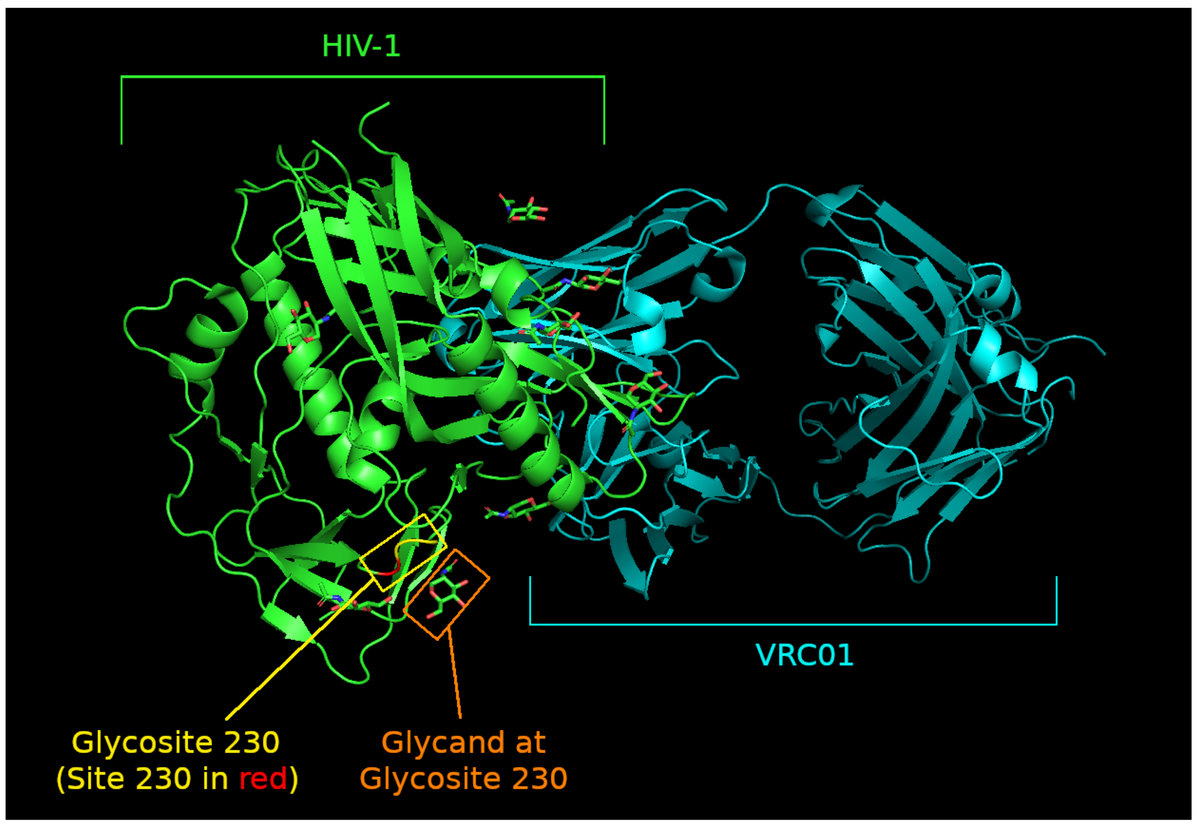

Figure 3. A crystal structure of trimeric HIV-1 in complex with VRC01, illustrating the position of glycosite 230 and its associated glycand. The glycosylation motif is shown in yellow, with site 230 in red. Disrupting this PNGS promotes escape from VRC01.

Conclusions

The Env protein sequence can predict the neutralization IC_{80} and the PE of VRC01.

For future studies, we assess circulating viral sequences to inform the identification and development of bnAb regimens and vaccine immunogens that may prevent HIV-1 acquisition.

Future Work

These same analyses are currently underway for HVTN 703, and additional sieve analyses are planned for both HVTN 703 and HVTN 704.