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Susceptibility to 3BNC117 and 10-1074 in ART suppressed chronically infected persons

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Abstract

Objective: The aim of this study was to assess the susceptibility of HIV to two HIV monoclonal antibodies (bnAbs), 3BNC117 and 10-1074, in individuals with chronically suppressed HIV infection.

Design: The susceptibility of bnAbs was determined using the PhenoSense mAb Assay, which is a cell-based infectivity assay designed to assess the susceptibility of luciferase-reporter pseudovirions. This assay is the only CLIA/CAP compliant screening test specifically developed for evaluating bnAb susceptibility in people with HIV infection.

Method: The susceptibility of luciferase-reporter pseudovirions, derived from HIV-1 envelope proteins obtained from PBMCs of 61 ART-suppressed individuals, to 3BNC117 and 10-1074 bnAbs was assessed using the PhenoSense mAb assay. Susceptibility was defined as an IC90 of less than 2.0 μg/mL and 1.5 μg/mL for 3BNC117 and 10-1074, respectively.

Results: About half of the individuals who were chronically infected and virologically suppressed were found to harbor virus with reduced susceptibility to one or both of the tested bnAbs.

Conclusions: The reduced combined susceptibility of 3BNC117 and 10-1074 highlights a potential limitation of using only two bnAbs for PREP or treatment. Further studies are needed to define and validate the clinical correlates of bnAb susceptibility.

Keywords: 3BNC117, 10-1074, HIV, ART suppression, PhenoSense, neutralizing antibody

Introduction

Broadly neutralizing antibodies (bnAbs) against the HIV-1 Env protein are being developed for prophylaxis, therapeutics and cure related strategies[1]. The recent development of long-acting antiretrovirals like cabotegravir/rilpivirine [2] and lenecapravir [3] has generated increased interest in HIV Env bnAbs as potential therapeutic agents to be used alone or in combination with other long-acting agents. Several passive transfer studies have demonstrated that bnAbs can maintain viral suppression in the absence of antiretroviral therapy in patients with bnAb sensitive virus[4, 5], but that long term benefits may be limited due to the potential for preexisting or developing bnAb resistance once classic antiretroviral therapy is discontinued. [4, 5]. bnAbs have also been evaluated as components of cure-related strategies for their Fc-associated activity (e.g. antigen-dependent cytotoxicity, ADCC). To date, the administration of bnAbs in ART-suppressed individuals has not demonstrated a clinically significant effect on the quantitative composition of the latent reservoir, most likely due to the lack of expression of envelope protein in the surface of infected cells that could mediate ADCC [6, 7].

In untreated people living with HIV (PLWH), anti-HIV-1 bnAbs develop over time through multiple iterative cycles of viral escape from autologous neutralizing antibodies and renewed cycles of B cell somatic hypermutation triggered by viral escape variants. HIV envelope evolves rapidly to avoid neutralizing responses[8]. Currently five primary epitope regions in the HIV trimer are targeted by available bnAbs including the CD4 binding site, trimer apex (V1/V2 loops), high-mannose patch, gp120-gp41 interface (including the fusion peptide) and the membrane proximal region (MPER) [9]. It is reasonable to assume that longer durations of HIV infection, particularly in settings of poorly controlled viral replication, would be associated with an increased Env diversity that, in turn, would elicit nAb responses that select for resistance to bnAbs. Thus, the main limitation for the use of bnAbs as either prophylactic or therapeutic agents is the potential for pre-existing viral resistance. The PhenoSense mAb Assay is a only CLIA/CAP compliant screening test that was specifically developed to assess bnAb susceptibility in people with HIV infection[10].

As a component of the screening process for the ongoing BEAT2 trial (NTC 03588715), we evaluated the susceptibility of HIV envelopes within the latent reservoir (PBMC) of suppressed participants against two bnAbs: a CD4 binding antibody 3BNC117 [11] and a V3 loop binding antibody 10-1074[12].

Methods

Study participants

Participants living with HIV-1 infection (PLWH) and receiving suppressive ART for a minimum of 6 months were recruited from the Hospital of the University of Pennsylvania or from Philadelphia FIGHT. The protocol was approved by the University of Pennsylvania Institutional Review Board. All subjects gave written informed consent.

Blood collection and processing

Venous blood (30-40 ml) was collected on Ethylenediaminetetraacetic Acid (EDTA) containing tubes and peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque PLUS (GE Healthcare, Chicago, IL). PBMC ($20x10^6$) were cryopreserved in freezing media consisting of 90% human serum (Gemini Bio, West Sacramento, CA) and 10% Dimethyl Sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO), stored in Liquid Nitrogen, and shipped to Monogram Biosciences, Inc. for analysis.

HIV bnAb susceptibility testing

The PhenoSense Monoclonal Antibody (mAb) Assay (Labcorp-Monogram Biosciences) assesses the susceptibility of pseudo-virions bearing plasma or PMBC derived HIV-1 envelope proteins to anti-envelope mAbs. The assay is a modification of the Phenosense assay described elsewhere [8]. Full-length envelope sequences, amplified from PBMC-associated HIV DNA were cloned into an envelope expression vector which uses the cytomegalovirus immediate-early promoter enhancer to drive env insert expression in transfected cells, Virus particles containing patient virus envelope proteins were produced by cotransfecting HEK293 cells with pCXAS-env libraries plus an HIV genomic vector that contains a firefly luciferase indicator gene. HIV pseudo-virions carrying the firefly luciferase reporter gene are harvested 48h post-transfection and evaluated for neutralization sensitivity to 3BNC117 and 10-1074 bnAbs after incubation for 1 h at 37°C with serial 4-fold dilutions of the bnAbs in U87 cells that express CD4 plus the CCR5 and CXCR4 coreceptors. Virus infectivity is determined 72 h post-inoculation by measuring the amount of luciferase activity expressed in infected cells. The concentration of bnAb required to inhibit virus infectivity by 50%, 80%, 90% and 95% (IC50, IC80, IC90 and IC95, respectively), as well as the maximum percent inhibition (MPI) was assessed. Clinically validated cut-off values for specific bnAb sensitivities have not yet been established for the PhenoSense mAb Assay, thus for the purpose of the BEAT 2 study, we elected to define mAb sensitivity as follows: IC90 < 2 µg/mL for 3BNC117 and < 1.5 µg/mL for 10-1074 and a maximum percent inhibition (MPI) > 98%.

Results

Sixty-one participants with HIV were recruited. All enrolled participants were chronically suppressed on ART at <20 HIV-1 copies/mL and had $CD4^+$ T cell counts ≥450 cells/mm^3 and nadir $CD4^+$ T cell counts >200 cells/mm^3. Figure 1A summarizes the baseline characteristics of the individuals: 85% were male, 87% African American and the median age was 50 years.

PCR amplification of HIV envelope sequences to enable bnAb testing failed for nine of the 61 individuals (15%) at the first attempt; two of the nine failures were successfully amplified on a 2nd attempt (Figure 1B). Distribution of bnAb IC90 values and the percentage of isolates with IC90 above the 10-1074 and 3BNC117 IC90 cutoffs, and the Spearman correlation between bnAbs susceptibility are shown in Figure 2A and 2B. The baseline viruses of 24% of the participants exhibited an IC90 greater than 2 μg/mL against 3BNC17 and the baseline viruses of 30% of the participants exhibited an IC90 greater than 1.5 μg/mL against 10-1074. Seven individuals (13%) were resistant to both. Paired IC90 values were independent of one another, e.g.: viruses resistant to 3BNC117 were neither more nor less likely to be resistant to 10-1074 (Figure 2B). The overall distribution of IC50, 1C80, IC90 and MPI for each one of the isolates is shown in Figure 2C and D. These parameters were highly correlated within each participant (Supplementary Figure 1, http://links.lww.com/QAD/C867), for example the r^2 of the IC80 and IC90 for 3BNC117 was 0.95, $p<0.0001$, allowing for the calculation of a given parameter knowing another (IC90 = 1.083*IC80 + 0.7851 by linear regression).

Using logistic regression, no demographic characteristics (age, gender, nadir CD4, race) were associated with sensitivity to bnAbs.

Discussion

Based on our convenience sample of the greater Philadelphia population, 50% of the chronically infected, virologically suppressed individuals harbored virus sensitive to both 3BNC117 and 10-1074. Although our definition of sensitivity is arbitrary given the lack of larger data correlating sensitivity testing of bnAbs to clinical outcomes, some clinical observations are emerging. For example, in the recently published HVTN 704/HPTN 085 and HVTN 703/HPTN 081 studies more than 5000 participants (2699 in HVTN 704/HPTN 085 and 1924 in HVTN 703/HPTN 081) received two different doses of a CD4 binding antibody VRC01 [13]. Protection from infection by this antibody was limited to viruses that exhibited a sensitivity to the antibody< 1 μg/mL. Among the 64 infected individuals in the combined placebo groups only 19 had an IC80 less than 1 μg/mL (30%), suggesting a significant amount of pre-existing resistance to this antibody among virus circulating in the communities where the study was conducted (mainly sub-Saharan Africa). The IC80 threshold of 1 μg/mL used in the HVN/HPTN studies corresponds to an IC90 of 1.9 μg/mL based on the high correlation IC80 and IC90 values reported here. Although that study concluded that a combination of antibodies will be necessary for the use of bnAbs in a prevention strategy, our study suggest that a combination of two antibodies as potent as 3BNC117 and 101074 may also be insufficient, given that approximately 50% of the chronically infected individuals harbor virus resistant to at least one of the two bnAbs tested (11% were resistant to both bnAbs). Note we did not attempt to genotype or correlate the env sequence in the reservoir with Phenosense data because the genotype mAb sensitivity testing has not been clinically validated and the genotypic correlates of resistance have not been established, particularly for CD4 binding antibodies.

Larger studies on clinical outcomes are merited as recent findings based on a small study of 10 persons showed limited correlation between suppression with 3BNC117 pls 10-1074 during an extended ART interruption and baseline PhenoSense assay results with more conservative sensitivity

thresholds (IC90 <1 μg/ml) and a study combining 3BNC117 with romidepsin (a latency reversing agent) was associated with enhanced HIV-1 Gag responses and virological control during an analytical treatment interruption in participants whose HIV-1 env sequences were bnAb sensitive[14]. The differences in frequency of preexisting resistance to VRC01 (70%) in the HVTN/HPTN study vs only 24% to 3BNC117 in our study suggest that the frequency of pre-existing resistance may vary geographically, and/or based on different HIV variants and within or across different bnAb classes.

On the positive side, our study demonstrates that sensitivity to these two distinct bnAbs is independent; implying that the inclusion of one or more antibodies in a combination regimen will broaden coverage against resistant viruses. Given the high degree of correlation between IC50, IC80, IC90 and MPI, it may not be necessary to provide all these values in the definition of standard thresholds when testing sensitivity to bnAbs. Interestingly, MPI values identify virus resistant to 10-1074.

The inability of the PhenoSense mAb assay to amplify envelope sequences from the HIV reservoir in 15% of the study candidates is not surprising given the established sequence diversity of the HIV envelope region, but may complicate screening protocols in switch-and-cure strategies and will be an important consideration when contemplating the number of individuals that may need to be screened to enroll future studies. This problem is not unique to our cohort: in recent studies using the same Phenosense mAb assay in a smaller study the envelope sequences of 6/16 (38%) were not successfully amplified, whereas a separate study evaluating a phenotypic and a genotypic sensitivity method to evaluate resistance in a cohort of acutely infected individuals showed 18 of 96 sequences could not be phenotypically characterized (19%). [15]

No demographic characteristics were associated with sensitivity to bnAbs in our cohort, however the broader implication of this observation is limited given the small sample size. Duration of infection (using current CD4 T cell count as a potential surrogate marker) did not predict sensitivity to bnAbs, suggesting that resistance to bnAbs may emerge early after infection, a conclusion corroborated in the HVTN/HPTN studies[13]and in the Zurich Primary HIV Infection (ZPHI) Study [15].

Our complete data (see Supplementary Table 1, http://links.lww.com/QAD/C866) may be useful to support sample size calculations both for screening failure rate (15% 95%CI 7-26%) as well as the prevalence of resistance to one or both antibodies when using combination bnAbs for maintenance or curative strategies.

Given the high rate of preexisting resistance, the expense in manufacturing, and the variability of the pharmacokinetics of the different products both at the individual and the product level, accurate estimates of susceptibility to bnAbs versus clinical response are needed as well as alternative methods of delivery of multiple of these molecules (mRNA, DNA, multispecificity[16], AAV [17], etc.) to make these therapeutic interventions sustainable, particularly when compared to use of long-acting, low molecular weight antiretroviral agents.

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Figure 1. Participant Demographics and screening flowchart. Pane A: Demographic characteristics. Panel B: Screening subjects and amplification results.

(a)

Ethnicity	Hispanic	3 (5%)
Race	Caucasian	8 (13%)
	Black	53 (87%)
Gender	Male	52 (85%)
	Female	9 (15%)
Age (y) (median, IQR)	50 (40-57)	

(b)

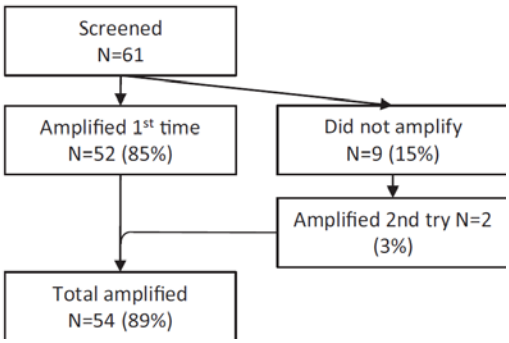

Figure 2. 3BNC117 and 10-1074 susceptibility, correlation of susceptibility and distribution of inhibitory concentrations. Panel A: Range of bNAb susceptibility of the isolates and proportion of subjects excluded based on reduced susceptibility to 3BNC117 and 10-1074. Panel B: Correlation of 3BNC117 and 10-1074 bnAb susceptibility. Panel C: Distribution of IC50, 1C80, IC90 IC95 and MPI (Maximum Percent Inhibition) against 3BNC117. Panel D: Distribution of IC50, 1C80, IC90 IC95 and MPI against 10-1074 for each one of the isolates.