A calculated risk: Evaluating HIV resistance to the broadly neutralising antibodies10-1074 and 3BNC117

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Purpose of this review
Broadly neutralising antibodies (bNAbs) are a promising new therapy for the treatment of HIV infection. However, the effective use of bNAbs is impacted by the presence of preexisting virological resistance and the potential to develop new resistance during treatment. With several bNAb clinical trials underway, sensitive and scalable assays are needed to screen for resistance. This review summarises the data on resistance from published clinical trials using the bNAbs 10-1074 and 3BNC117 and evaluates current approaches for detecting bNAb sensitivity as well as their limitations.

Recent findings
Analyses of samples from clinical trials of 10-1074 and 3BNC117 reveal viral mutations that emerge on therapy which may result in bNAb resistance. These mutations are also found in some potential study participants prior to bNAb exposure. These clinical data are further informed by ex-vivo neutralisation assays which offer an alternative measure of resistance and allow more detailed interrogation of specific viral mutations. However, the limited amount of publicly available data and the need for better understanding of other viral features that may affect bNAb binding mean there is no widely accepted approach to measuring bNAb resistance.

Summary
Resistance to the bNAbs 10-1074 and 3BNC117 may significantly impact clinical outcome following their therapeutic administration. Predicting bNAb resistance may help to lower the risk of treatment failure and therefore a robust methodology to screen for bNAb sensitivity is needed.

Keywords
10-1074, 3BNC117, broadly neutralising antibody, HIV, resistance

INTRODUCTION
The discovery and isolation of broadly neutralising antibodies (bNAbs) in the late 2000s [1,2] was a breakthrough with great promise for HIV prevention [3,4] and cure [5]. These antibodies are found only in a rare subpopulation of people living with HIV (PWH) [6]. Unlike strain-specific neutralising antibodies (NAbs), which arise in the majority of PWH, bNAbs can neutralise a wide range of viral strains circulating in the host by targeting multiple variants of the HIV Envelope protein (Env) [7]. bNAb neutralisation occurs through inhibiting different mechanisms of viral entry, such as steric interference with viral attachment to the cell, immobilisation of the Env trimer or acceleration of Env spike decay [8]. In clinical trials [9–15] passive administration of bNAbs significantly reduces viremia in participants who are not taking antiretroviral therapy (ART) and can maintain viral suppression in those on ART who subsequently undergo a treatment interruption. However, longer term viral suppression may be compromised due to the selection of preexisting resistant viruses emerging from the latent reservoir or the selection of newly formed escape variants generated by sub-optimal selection pressure [16]. Using dual rather than monotherapy with bNAbs helps overcome some issues around resistance and substantially extends the duration of viral remission in those stopping ART [13]. However, even when using two bNAbs together, resistance remains a barrier that needs to be removed to achieve truly long-term drug-free remission. In this article, we will...
review two of the most promising bNAbs, 3BNC117 and 10-1074, aiming to understand how bNAb resistance emerges, what its effect on viral fitness and functionality might be, and how best to measure it.

**10-1074 AND 3BNC117 IN THE CLINIC: ESCAPE SIGNALS**

Passive administration of 10-1074 and 3BNC117 – generally intravenously - alone or in combination is being tested as an HIV treatment in an increasing number of clinical trials, [11–13,15,17,18**] with hope for long-term drug-free remission, and even cure. Single infusions of 10-1074 or 3BNC117 in viraemic participants have been shown to transiently reduce viremia in two clinical trials; [10,12] however, sustained or full viral suppression was not achieved. BNAbs were administered to viraemic participants, who were screened for the bNAb 3BNC117. In an antiretroviral treatment interruption (ATI) setting - with participants who were screened for bNAb sensitivity - infusions of 3BNC117 resulted in longer periods of detectable viraemia compared to historical controls (average of 6.7 and 9.9 weeks of viral suppression off ART depending on the number of doses, vs. 2.9 weeks in controls) [15]. Moreover, a few participants did not rebound as long as bNAb levels remained therapeutic, which suggests that adequate serum levels of 3BNC117 may prevent the development or selection of escape mutations [15]. However, a 2018 study [14] showed that when 3BNC117 was administered in PWH who underwent ATI, preexisting resistance was a strong predictor of shorter time to viral rebound [14].

As has been learned from the field of antiretroviral therapy, one approach to overcoming resistance is to give drugs together in combination. The joint administration of 10-1074 and 3BNC117 followed by ART interruption in PWH with bNAb sensitive viruses mediated viral suppression for an extended period (median 21 weeks) [13] – a longer period than monotherapy – and supporting the argument for combination therapy. These findings were confirmed in the most recent phase 1b clinical trial of 10-1074 and 3BNC117 combination given to PWH on or off antiretroviral treatment [18**]. Of note, when administered to viraemic participants, the combination of 3BNC117 and 10-1074 was not as effective; although a reduction in viremia was observed, full viral suppression was only seen in one participant with a low baseline viral load [11]. This suggests that for bNAbs to be fully effective, viral suppression may first need to be achieved with ART. Only participants with chronic infection were recruited in the clinical trials mentioned above, and it is that treatment in primary infection is more effective due to smaller HIV reservoirs and lower viral diversity.

The longer the duration for which bNAbs are above therapeutic levels, the greater the resulting period of virologic control. Figure 1 shows summary data from currently published clinical trials and shows that increasing numbers of doses of 3BNC117 alone or combined with 10-1074, result in longer periods of control in participants who interrupted ART immediately after receiving bNAbs (Fig. 1A, B). Cox regression analysis using combined data from studies that report both baseline sensitivity and time to viral rebound [13,18**,19**] showed a 37% decrease in viral rebound per increase in number of bNAb doses (hazard ratio, HR: 0.63, 95% confidence interval, CI: 0.50-0.78, p-value = 0.00004). Also, presence of resistance at baseline was associated with a 44% increase in expected viral rebound relative to absence of resistance (HR: 1.44, 95% CI: 0.60-3.45, p-value = 0.4), although this effect was not statistically significant. (The times to viral rebound for Sneller et al. [19**] are sampled by approximation from the paper figures.) One would expect, however, that these data will be strengthened by the results of clinical trials using long-acting ‘LS’ bNAb variants, which may result in 4-6 months suppression after single dosing.

**BROADLY NEUTRALISING ANTIBODIES TARGETING EPITOPES AND ESCAPE MUTATIONS**

Common features of bNAbs include extensive somatic hypermutation, the ability to recognise N-glycans as epitopes and long heavy chain complementary determining regions (CDRs) that reach protein epitopes concealed by the glycan shield or other Env protein loops. BNAbs target conserved HIV Env sites, which include the CD4 binding site (CD4bs),
the V3 glycan site, the V2 apex, the MPER site and the gp120-gp41 interface [20]. Notably, several bNAb epitopes, such as V3 and the CD4bs, have been shown to serve as strain-specific Nab targets [21].

10-1074 is an HIV Env V3 loop-targeting bNAb that interacts with the glycan attached on the potential N-glycosylation site (PNGS) at position 332 and binds the underlying $^{324}{GDIR}^{327}$ motif,
[12,22–24] which mediates CCR5 binding. The significance of this amino acid position was demonstrated by Caskey et al., [12] who observed that the 2 out of 19 PWH who had single N332T and D325E mutations in 100% of their preinfusion plasma viruses, did not respond to 10-1047 monotherapy. Interestingly, different residues at a given position are thought to affect 10-1074 escape heterogeneously. For instance, a serine or asparagine replacement of D325 on the BG505 viral strain was found to have no effect on 10-1074 binding, unlike D325E [25]. However, an intact PNG does not guarantee the presence of a glycan. Studies have indicated that the probability of glycan occupation was associated with the amino acid at the second position in the PNG sequon [26]. In their paper looking at how V3 bNAbs bind on the GDIR motif, Sok et al. showed that the absence of the glycan on either position 332 or 334 is associated with higher frequency of mutation atD325, R327 or H330 residues, which might affect bNAbsNeutralisation [23].

3BNC117 is a VRC01-class bNAb that mimics CD4 binding [27] CD4 binding site bNAb epitopes need to be conserved and lie within an HIV Env cavity, protected by the Env V1V2 and V3 regions and the glycan shield [28]. The contact residues of 3BNC117 on Env are located in Loop D, the CD4 binding loop and the β23 V5 loop (HXB2 amino acid positions 274–283, 364–374 and 455–471, respectively) [9,14]. Studies indicate that mutations emerging both inside and outside the known CD4 binding site contact residues are associated with VRC01-class bNAbs resistance and may confer different replication fitness costs, compensating each other to overcome resistance [29,30]. In particular, mutations G459D, Q363H, S461D, and S274Y have been associated with different levels of 3BNC117 resistance due to bNAb binding interference [10]. These resistance mutations were, however, not observed in the rebound viruses of PWH who received 3BNC117 infusions; R456S and atypical residues in K272 were found in the majority of rebound viruses in two participants, which may affect the structure of V5 and disrupt 3BNC117 binding [15]. A mutation in residue 459, where glycine was replaced by isoleucine, was also detected in 100% of rebound viruses, although 459I was present in a minority of pre3BNC117 infusion plasma viruses in PWH [14]. Notably, no single mutations that affect the sensitivity of both bNAbs were detected in clinical trials where a combination of 3BNC117 and 10-1074 was administered in PWH [11,13]. Intrinsic bNAb sensitivity may also differ among HIV subtypes. For example, 3BNC117 is less inhibitory against C clade viruses, and 10-1074 is less effective against CRF01-AE, A and C viruses due to either complete lack or under-representation of the 332 PNGS [29].

Structural changes caused by insertions and/or deletions in the hypervariable HIV Env regions may also mediate bNAb escape. Some studies propose that the main role of the HIV V1 and V2 Env regions is as a defence mechanism against the host immune response [31]. Concordant with this, increased V1 and V2 length correlates with V3 and CD4bs bNAb resistance [29,32,33]. Changes in the length of the HIV Env V4 region have been reported to affect the structure of the glycan shield that surrounds the CD4 binding site [34]. A longer V5 loop has also been associated with 3BNC117 resistance, due to steric Env changes that cause clashing with the bNAb heavy and light chains [10,13]. Moreover, high viral diversification during untreated infection is thought to be more likely to give rise to bNAb escape mutations [35], especially within the neutralising antibody epitopes that overlap with bNAb binding sites [36].

Lastly, it has been proposed that the adaptation of HIV to humoral responses over time may lead to increased resistance to neutralisation by bNAbs at a population level, and across time and different clades [37]. To explore this further we ran a bNAb sensitivity prediction algorithm [18] on B clade env protein sequences downloaded from the los Alamos database (https://www.hiv.lanl.gov/) which had been collected from PWH since the beginning of the pandemic (2816 sequences). For each year since 1986, we estimated the number of sequences that carried residues associated with resistance to 10-1074. Interestingly, the analysis reveals a trend of increasing frequency of residues associated with 10-1074 resistance over time $\left( R^2=0.17, P < 0.05 \right)$ (Fig. 2A). A similar trend of increasing resistance to 3BNC117 over time was observed in the dataset using the same algorithm (Fig. 2B), although it should be noted that the algorithm for 3BNC117 may be less reliable at predicting resistance.

As bNAbs have only recently become available as a therapy, this would suggest that overlapping pressures from circulating antibodies may result in immune escape, which is potentially transmitted to new hosts resulting in an increase in population level resistance. Should this pattern continue at the current rate – or even accelerate – this may have future implications for the broader utility of bNAbs therapy.

LIMITATIONS OF CURRENT BROADLY NEUTRALISING ANTIBODY SENSITIVITY SCREENING ASSAYS. WHAT DOES THE FUTURE HOLD?

Currently, the gold standard for assessing bNAb resistance in viral strains is the in-vitro neutralisation assay, [15] a process that is resource- and time-expensive but also potentially difficult to interpret
clinically [13,15,16]. Epitope mapping for 10-1074 and 3BNC117 has identified resistance mutations that have been used to give a binary outcome of bNAb sensitivity, mirroring the role of genotyping for drug resistance [24,25,30]. However, as shown in a recent study by Gaebler et al. [18] compared to neutralisation assays, genotypic prediction only poorly identified resistant clones in prebNAb samples from participants who subsequently rebounded with 10-1074 resistant viruses. Of note, post-hoc neutralisation assays from participants with primary HIV infection recruited to a 10-1074 and 3BNC117 clinical trial [19], showed a correlation of baseline bNAb sensitivity with a better response to

**FIGURE 2.** BNAAb sensitivity in B clade env sequences across time, based on a bNAb sensitivity prediction algorithm. Resistance is defined based on the detection of mutations associated with a.10-1074 resistance (HXB2 sites: 325, 330, 332, 333 and 334), b. 3BNC117 resistance (HXB2: 279, 280, 456, 457, 458, 459). [13] Each point represents the percentage of resistant sequences, and the grey ribbon is the confidence interval.
treatment. The improvements in next-generation sequencing and bioinformatics methods over the last few years have offered a range of \textit{in silico} bNAbs sensitivity prediction methods which may allow genotypic assays to become more predictive [38,39].

There are increasing numbers of computational models designed to predict inhibitory 10-1074 and 3BNC117 concentrations (IC50 and IC80) [29, 40–46] based on genotypic data. The main limitation of these models lies in the short supply of neutralisation datasets to use for model training, especially for the very potent bNAbs like 3BNC117. This may explain the lower prediction performance of models when compared to the in-vivo response to bNAb treatment [41, 42, 45]. In addition, feature selection in most algorithms is primarily built upon genomic data of bNAb epitopes and only a few approaches incorporate additional features that influence bNAb binding, such as PNs [45], or even regions outside the predefined epitope sites [42]. Interestingly, Meijs et al. [46] employed a bNAb resistance evolution model that factors in the fitness cost of resistance-associated mutations. The model was trained on a small dataset, but it suggested that viral escape from bNAbs can be predicted based on intra-host parameters (such as antibody levels and the degree of viraemia) rather than solely on resistance-conferring mutations.

Prediction accuracy notwithstanding, obtaining an adequate number of single HIV sequences that represent the full diversity of circulating viruses or latent proviral sequences is yet another challenge. Single genome HIV Env amplification and sequencing (using limiting dilution approaches) is the most commonly used method to do this, despite the long, laborious process and high cost [38]. Incorporating high-throughput elements in the assay, such as capture of single, intact viruses prior to amplification [47, 48], would increase both the time and cost efficiency of the process. The combination of improved techniques to achieve high numbers of single genomes, greater sequencing read-length technologies, improved bioinformatic and machine learning approaches and larger, robust training datasets with clinical metadata are all key to developing a reproducible and accurate resistance-prediction methodology.

CONCLUSION

There is growing evidence that bNAbs, such as 10-1074 and 3BNC117, are an exciting new treatment option which may be used alone or in conjunction with antiretroviral treatment to confer long-term virological control. Their use in hard-to-reach groups and regions with poor healthcare infrastructure could deliver viral suppression to individuals where it is currently challenging. However, viral resistance to bNAbs could set back the success of the intervention and so developing tools to accurately predict bNAb sensitivity has become a point of focus in the field. In this review, we outlined the signatures associated with resistance \textit{in vivo} and the available tools for predicting sensitivity to 10-1074 and 3BNC117. However, as this is such a new area, knowledge of the virological genotypic and phenotypic features that predict resistance is still limited. As more clinical bNAb trials enrol, and more data are collected on outcomes, it will hopefully become possible to develop the necessary more robust methodologies needed to help guide clinical decision-making.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest

1. Scheid JF, Mouquet H, Feldhahn N, et al. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature 2009; 458:636–640.
2. Mascola JR, Haynes BF. HIV-1 neutralizing antibodies: understanding nature’s pathways. Immuno Rev 2013; 254:225–244.
3. Walsh SR, Seaman MS. Broadly Neutralizing Antibodies for HIV-1 Prevention. Front Immunol 2021; 12:712122.
4. Mahomed S, Garrett N, Baxter C, et al. Clinical trials of broadly neutralizing monoclonal antibodies for human immunodeficiency virus prevention: a review. J Infect Dis 2021; 223:370–380.
5. Caskey M. Broadly neutralizing antibodies for the treatment and prevention of HIV infection. Curr Opin HIV AIDS 2020; 15:49–55.
6. Russet P, Kouyou RD, Kadela C, et al. Determinants of HIV-1 broadly neutralizing antibody induction. Nat Med 2016; 22:1280–1287.
7. Derdeyn CA, Moore PL, Morris L. Development of broadly neutralizing antibodies from autologous neutralizing antibody responses in HIV infection. Curr Opin HIV AIDS 2014; 9:210–216.
8. Burton DR, Hangartner L. Broadly neutralizing antibodies to HIV and their role in vaccine design. Annu Rev Immunol 2016; 34:635–659.
9. Schoofs T, Klein F, Braunschweig M, et al. HIV-1 therapy with monoclonal antibody 10-1074 elicits host immune responses against HIV-1. Science 2016; 352:997–1001.
10. Caskey M, Klein F, Lorenz JCC, et al. Viral immunity in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. Nature 2015; 522:487–491.
11. Bar-On Y, Gruell H, Schoofs T, et al. Safety and antiviral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals. Nat Med 2018; 24:1701–1707.
12. Caskey M, Schoofs T, Gruell H, et al. Antibody 10-1074 suppresses viremia in HIV-1-infected individuals. Nat Med 2017; 23:185–191.
13. Mendoza P, Gruell H, Nogueira L, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. Nature 2018; 561:479–484.
These observations may provide a biological background for the evolution of escape mutations, the cost on viral fitness and the mechanisms to revert this. This study looks at VRC01-class bNAb sensitivity at a protein sequence level and provides sustained virological suppression. Nature 2022; 606:376–381. Regardless of baseline sensitivity to bNAbs, 76% of participants who received a combination of 10-1074 and 3BNC117 in this clinical trial, viral suppression for longer than 20 weeks of ART. These findings suggest that improvements are needed in current methods to predict bNAb resistance at baseline.

These findings show that PWH treated in the early/acute phase of infection may have a smaller reservoir consisting of bNAb sensitive viruses. Env diversity is a feature that may be useful in predicting bNAb sensitivity at baseline.

Bar KJ, Sneller MC, Harrison LJ, et al. Effect of HIV antibody VRC01 on viral rebound after treatment interruption. N Engl J Med 2016; 375:2037–2050.

Stefic K, Bouvin-Pley M, Brabant M, Barn F. Impact of HIV-1 diversity on its sensitivity to neutralization. Vaccines (Basel) 2019; 7:1472.

Salazar-Gonzalez JF, Bales E, Pham KT, et al. Deciphering human immunodeficiency virus type 1 transmission and early envelope diversification by single-genome amplification and sequencing. J Virol 2008; 82:3952–3970.

Travers KJ, Chin C-S, Rank DR, et al. A flexible and efficient template format for circular consensus sequencing and SNP detection. Nucleic Acids Res 2010; 38:e159–e1159.

Hepler NL, Scheffler K, Weaver S, et al. IDEPI: rapid prediction of HIV-1 antibody epitopes and other phenotypic features from sequence data using a flexible machine learning platform. PLOS Comput Biol 2014; 10:e1003842.

Hake A, Pfifer N. Prediction of HIV-1 sensitivity to broadly neutralizing antibodies shows a trend towards resistance over time. PLOS Comput Biol 2017; 13:e1005789.

Ravi R, Mall R, Shen C-H, et al. Accurate prediction for antibody resistance of clinical HIV-1 isolates. Sci Rep 2019; 9:14696.

Williamson BD, Magaret CA, Gilbert PB, Nizam S, et al. Super LeAmer prediction of NAB panels (SLAPNAP): a containerized tool for predicting combination monoclonal broadly neutralizing antibody sensitivity. Bioinformatics 2021; 37:4187–4192.

West AP Jr, Scharf L, Horwitz J, Klein F, et al. Computational analysis of anti-HIV-1 antibody neutralization panel data to identify potential functional epitope residues. Proc Natl Acad Sci U S A 2013; 110:10598–10603.

Mejers M, Varshyila K, Gruell H, et al. Predicting in vivo escape dynamics of HIV-1 from a broadly neutralizing antibody. Proc Natl Acad Sci USA 2021; 118:e2103153.

Cohen YZ, Lorenzi JCC, Krassnig L, et al. Relationship between latent and rebound viruses in a clinical trial of anti-HIV-1 antibody 3BNC117. J Exp Med 2018; 215:2011–2024.

Scheid JF, Horwitz JA, Bar-On Y, et al. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. Nature 2016; 535:556–560.

Hsu DC, Mellors JW, Vasen S. Can broadly neutralizing HIV-1 antibodies help achieve an ART-free remission? Front Immunol 2021; 12:710044.

Caskey M, Klein F, Nussenzevitch MC. Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. Nat Med 2019; 25:547–553.

Gaebler C, Nogueira L, Stoffel E, et al. Prolonged viral suppression with anti-HIV-1 antibody therapy. Nature 2022; 368–374.

This study demonstrated that the combination of 10-1074 and 3BNC117 effectively suppressed HIV viral load for up to 43 weeks, compared to 8 weeks for participants who received placebo, provided that the baseline samples were not resistant to bNAbs, providing a rationale for prospective bNAb resistance screening.

Sok D, Burton DR. Recent progress in broadly neutralizing antibodies to HIV. Nat Immunol 2018; 19:1179–1188.

Moody MA, Gao F, Guryel TC, et al. Strain-specific V3 and CD4 binding site autologous HIV-1 neutralizing antibodies select neutralization-resistant viruses. Cell Host Microbe 2015; 18:354–362.

Baron ET, Cope HM, Fredrickson E, et al. Structural characterization of a highly-potent V3-glycan broadly neutralizing antibody bound to native-glycosylated HIV-1 envelope. Nat Commun 2018; 9:1251.

Sok D, Pauthner M, Briney B, et al. A Prominent site of antibody vulnerability on HIV envelope incorporates a motif associated with CCR5 binding and its camouflaging glycans. Immunity 2016; 45:31–45.

Mouquet H, Scharf L, Euler Z, et al. Complex-type N-glycan recognition by broadly neutralizing HIV antibodies. Proc Natl Acad Sci U S A 2012; 109:E3268–E3277.

Dirgens AS, Arent D, Weigend H, et al. An antigenic atlas of HIV-1 escape from broadly neutralizing antibodies distinguishes functional and structural epitopes. Immunity 2019; 50:520–532.

van den Herkhoft TLGM, van Gils MJ, Boesser-Nunnink BD, et al. Probability of N332 glycan occupancy on HIV-1 gp120 modulates sensitivity to broadly neutralizing antibodies. AIDS 2016; 30:2179–2184.

Scheid JF, Mouquet H, Ueberehnis B, et al. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science 2011; 333:1633–1637.

Parker Miller E, Finkelstein MT, Erdman MC, et al. A structural update of neutralizing epitopes on the HIV envelope, a moving target. Viruses 2021; 13:11774.

Bricault CA, Yuxun K, Seaman MS, et al. HIV-1 neutralizing antibody signatures and application to epitope-targeted vaccine design. Cell Host Microbe 2019; 25:59–72.

Lynen RM, Patrick W, Lillian T, et al. HIV-1 fitness cost associated with escape from the VRC01 class of CD4 binding site neutralizing antibodies. J Virol 2022; 86:4201–4213.

This study looks at VRC01 class bNAb sensitivity at a protein sequence level and the evolution of escape mutations, the cost on viral fitness and the mechanisms to revert this. These observations may provide a biological background for forerunners selected as critical, by some bNAb sensitivity prediction algorithms.