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https://doi.org/10.1038/s41586-018-0531-2

Combination therapy with anti-HIV-1 antibodies maintains viral suppression

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Individuals infected with HIV-1 require lifelong antiretroviral therapy, because interruption of treatment leads to rapid rebound viraemia. Here we report on a phase 1b clinical trial in which a combination of 3BNC117 and 10-1074, two potent monoclonal anti-HIV-1 broadly neutralizing antibodies that target independent sites on the HIV-1 envelope spike, was administered during analytical treatment interruption. Participants received three infusions of 30 mg kg^{-1} of each antibody at 0, 3 and 6 weeks. Infusions of the two antibodies were generally well-tolerated. The nine enrolled individuals with antibody-sensitive latent viral reservoirs maintained suppression for between 15 and more than 30 weeks (median of 21 weeks), and none developed viruses that were resistant to both antibodies. We conclude that the combination of the anti-HIV-1 monoclonal antibodies 3BNC117 and 10-1074 can maintain long-term suppression in the absence of antiretroviral therapy in individuals with antibody-sensitive viral reservoirs.

During infection, HIV-1 is reverse transcribed and integrated as a provirus into the host genome. Although the vast majority of infected cells die by apoptosis or pyroptosis[1], a small percentage survive and harbour transcriptionally silent, integrated proviruses that comprise a reservoir that can be reactivated. Once established, the latent reservoir has an estimated half-life of 44 months, resulting in the lifelong requirement for antiretroviral therapy (ART)[2]. Passive administration of potent broadly neutralizing monoclonal anti-HIV-1 antibodies (bNAbs) represents a potential alternative to antiretroviral drugs because, in addition to neutralizing the virus, antibodies engage the host immune system and have long half-lives[3–5].

In human clinical trials, viraemic individuals who received 3BNC117 or VRC01, two related bNAbs that target the CD4 binding site on the HIV-1 envelope spike, or 10-1074, a bNAb that targets the base of the V3 loop and surrounding glycans, showed significant reductions in viremia[6–8]. Moreover, in HIV-1-infected individuals undergoing analytical treatment interruption (ATI) of antiretroviral therapy, four infusions of 3BNC117 maintained virus suppression for a median of 10 weeks compared to 2.3 weeks in historical controls[9,10]. By contrast, six infusions of VRC01 maintained suppression for 5.6 weeks[11]. The difference in activity between VRC01 and 3BNC117 in preclinical experiments[12,13] and clinical trials[6,7,9,11] is consistent with the lower relative neutralization potency of VRC01.

Across all bNAb clinical trials to date, and similar to monotherapy with antiretroviral drugs, treatment with any single bNAb was associated with the emergence of antibody-resistant viral variants[6–9,11]. Like antiretroviral drugs, combinations of bNAbs are more effective than individual antibodies in HIV-1 infected humanized mice and simian/human immunodeficiency virus (SHIV)-infected macaques[14–16]. By contrast, antibody combinations showed little if any efficacy in suppressing viraemia during ATI in humans[17,18]. However, these earlier studies were performed using bNAbs that were less potent than 3BNC117 and 10-1074. Here we investigate whether the bNAb combination of 3BNC117 and 10-1074 can maintain viral suppression during ATI in HIV-1-infected humans.

Combination bNAb infusion is well-tolerated

To evaluate the effects of the combination of 3BNC117 and 10-1074 on maintaining HIV-1 suppression during ATI, we conducted a phase 1b clinical trial (Fig. 1a). HIV-1-infected individuals on ART were pre-screened for 3BNC117 and 10-1074 sensitivity of bulk outgrowth culture-derived viruses in an in vitro neutralization assay using TZM-bl cells[19]. Consistent with previous results, 64% and 71% of the outgrowth viruses were sensitive to 3BNC117 and 10-1074, respectively, and 48% were sensitive to both[8,9,20] (half-maximum inhibitory concentration (IC_{50}) $\leq 2\,\mu g\,ml^{-1}$; Extended Data Fig. 1a and Supplementary Table 1).

Study eligibility criteria included ongoing ART for at least 24 months with plasma HIV-1 RNA levels of <50 copies per ml for at least 18 months (one blip <500 copies per ml was allowed) and <20 copies per ml at screening, as well as $CD4^+$ T cell counts >500 cells per μl (Extended Data Figs. 1b, 2a). Enrolled participants received three infusions of 30 mg kg^{-1} of 3BNC117 and 10-1074 each at

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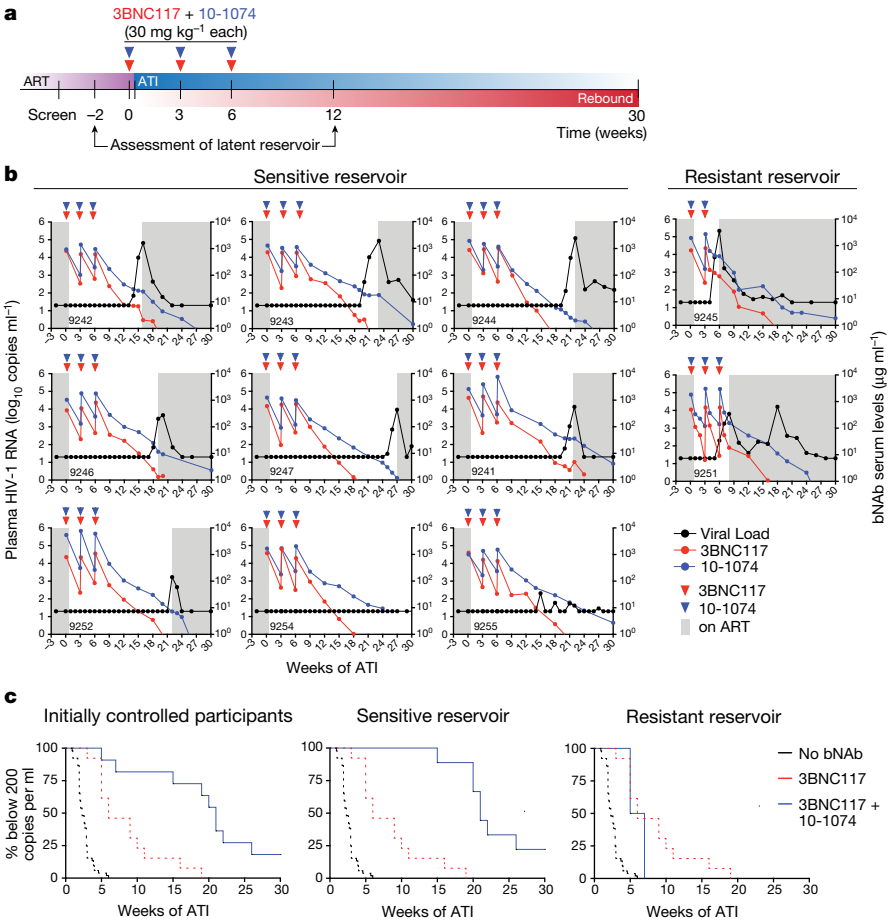

Fig. 1 | Delayed viral rebound with 3BNC117 and 10-1074 combination therapy during ATI. a, Study design. Red and blue triangles represent 3BNC117 and 10-1074 infusions, respectively. **b**, Plasma HIV-1 RNA levels (black; left *y* axis) and bNAb serum concentrations (3BNC117, red; 10-1074, blue; right *y* axis) in the nine bNAb-sensitive participants (left) and the two participants with pre-existing resistance against one of the antibodies (right). Red and blue triangles indicate 3BNC117 and 10-1074 infusions, respectively. Serum antibody concentrations were determined by TZM-bl assay. Grey-shaded areas indicate time on ART. Lower limit of detection of HIV-1 RNA was 20 copies per ml. **c**, Kaplan–Meier plots summarizing time to viral rebound for the participants with HIV-1 RNA <20 copies per ml two weeks before and at the start of ATI ($n = 11$, left), for the participants sensitive to both antibodies ($n = 9$, centre), and for the participants that showed pre-existing resistance to one of the antibodies ($n = 2$, right). The *y* axis shows the percentage of participants that maintain viral suppression. The *x* axis shows the time in weeks after start of ATI. Participants receiving the combination of 3BNC117 and 10-1074 are indicated by the blue line. Dotted red lines indicate a cohort of individuals receiving 3BNC117 alone during ATI[9] ($n = 13$) and dotted black lines indicate a cohort of participants who underwent ATI without intervention[10] ($n = 52$).

three-week intervals beginning two days before treatment interruption (Fig. 1a). Individuals whose regimens contained non-nucleoside reverse transcriptase inhibitors were switched to an integrase inhibitor-based regimen four weeks before discontinuing ART (Extended Data Figs. 1b, 2a). Viral load and $CD4^+$ T cell counts were monitored every 1–2 weeks (Supplementary Table 2). ART was reinitiated and antibody infusions were discontinued if viraemia of >200 copies per ml was confirmed. Time of viral rebound was defined as the first of two consecutive viral loads of >200 copies per ml. Fifteen individuals were enrolled, but four of them showed viral loads of >20 copies per ml two weeks before or at the time of the first bNAb infusion and they were excluded from efficacy analyses (Extended Data Fig. 1b and Supplementary Table 2).

Antibody infusions were generally safe and well-tolerated with no reported serious adverse events or antibody-related adverse events, except for mild fatigue in two participants (Supplementary Table 3). The mean $CD4^+$ T cell count was 685 and 559 cells per μl at the time of first antibody infusion and at rebound, respectively (Extended Data Fig. 2b and Supplementary Table 2). Reinitiation of ART after viral rebound resulted in resuppression of viraemia (Supplementary Table 2). We conclude that combination therapy with 3BNC117 and 10-1074 is generally safe and well-tolerated.

The serum half-life of each antibody was measured independently by TZM-bl assay and anti-idiotype enzyme-linked immunosorbent assay (ELISA, Extended Data Fig. 2c, d and Supplementary Table 2). 3BNC117 had a half-life of 12.5 and 17.6 days as measured using TZM-bl and ELISA, respectively (Extended Data Fig. 2c, d). The half-life of 10-1074 was 19.1 and 23.2 days as measured by TZM-bl and ELISA, respectively; significantly longer than 3BNC117 in both assays ($P = 0.0002$ and $P = 0.02$, Extended Data Fig. 2e, f). These measurements are similar to those observed when each antibody was administered alone in ART-treated HIV-1-infected individuals[6,8,9]. We conclude that the pharmacokinetic profiles of 3BNC117 and 10-1074 are not altered when they are used in combination.

The combination of bNAbs maintains viral suppression

For the 11 individuals who had complete viral suppression (HIV-1 RNA <20 copies per ml) during the screening period and at day 0, combination antibody therapy was associated with maintenance of viral suppression for between 5 and more than 30 weeks (Fig. 1b, c and Supplementary Table 2). The median time to rebound was 21 weeks compared to 2.3 weeks for historical controls who participated in previous non-interventional ATI studies[10] and 6–10 weeks for monotherapy with 3BNC117[9] (Fig. 1c). Together, 9 of the 11 participants maintained viral

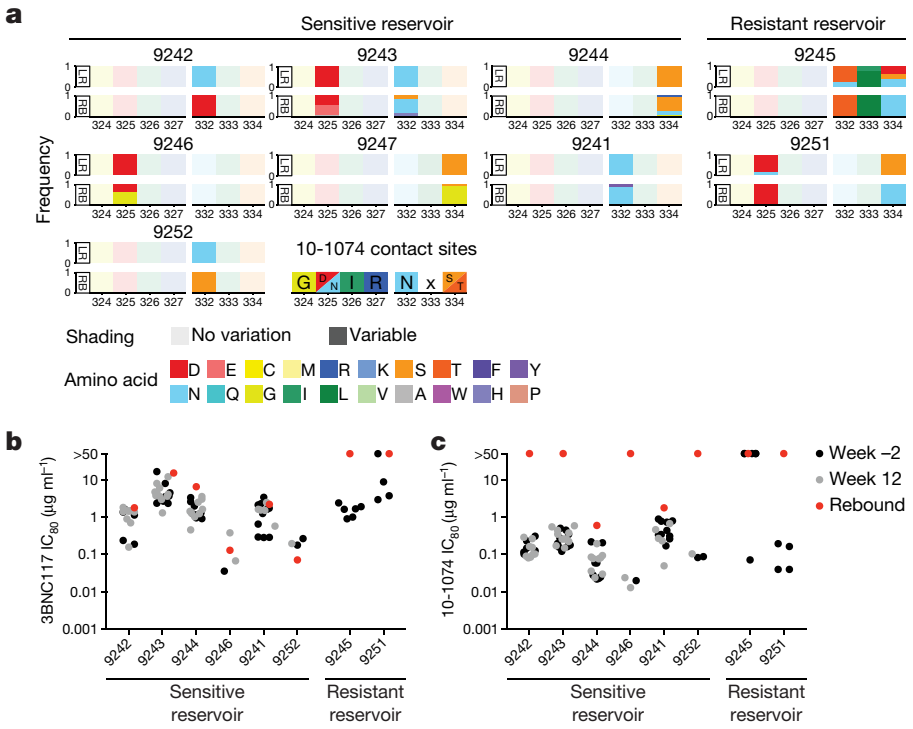

Fig. 2 | Amino acid variants at 10-1074 contact sites and bNAb sensitivity of reactivated latent and rebound viruses. a, Colour charts show Env contact sites of 10-1074 at the G(D/N)IR motif (positions 324–327, according to HXB2 numbering) and the glycan at the potential *N*-linked glycosylation site at position 332 (NxS/T motif at positions 332–334). Diagram shows the seven bNAb-sensitive participants that rebounded before week 30 (left) and the two individuals with pre-existing resistance to one of the two antibodies (right). LR, latent reservoir viruses isolated by Q^2VOA; RB, rebound viruses isolated by SGA (plasma) or viral outgrowth (PBMCs). Each amino acid is represented by a colour and the frequency of each amino acid is indicated by the height of the rectangle. Shaded rectangles indicate the lack of variation between latent reservoir virus and rebound virus at the indicated position. Full-colour rectangles represent amino acid residues with changes in distribution between reservoir and rebound viruses. **b**, **c**, Dot plots indicating IC_{80} (μg ml^{-1}) of 3BNC117 (**b**) and 10-1074 (**c**) against latent and rebound viruses determined by TZM-bl neutralization assay. Q^2VOA-derived latent viruses from week −2 and week 12 are shown as black and grey circles, respectively. For outgrowth culture-derived rebound viruses, the highest IC_{80} is shown as red circle. For 9246, 9252, 9245 and 9251 viruses could not be obtained from rebound outgrowth cultures and pseudoviruses were made from *env* sequences from Q^2VOA and plasma SGA.

suppression for at least 15 weeks, although two rebounded at weeks 5 and 7 (Fig. 1b, c).

Quantitative and qualitative viral outgrowth assays (Q^2VOA) were used to retrospectively analyse the replication-competent latent viral reservoir in all individuals. Phylogenetic analysis showed that the trial participants were infected with epidemiologically distinct clade B viruses (Extended Data Fig. 3). Q^2VOA analysis revealed that the pre-infusion latent reservoir in the two individuals who rebounded early, 9245 and 9251, harboured 10-1074- or 3BNC117-resistant viruses, respectively (Fig. 2 and Supplementary Table 4). Therefore, these two individuals were effectively subjected to antibody monotherapy, because there was pre-existing resistance in the reservoir of these individuals to one of the two bNAbs. Consistent with this idea, the delay in rebound in these two participants was within the anticipated range of antibody monotherapy[9,11] (Fig. 1c). In addition, all four of the individuals excluded from the analysis due to incomplete viral suppression showed pre-existing resistance or viruses that were not fully neutralized by one or both of the antibodies and these individuals rebounded before week 12 (Extended Data Figs. 4, 5 and Supplementary Table 4).

To examine the viruses that arose in the early rebounding individuals, we performed single genome analysis (SGA) of plasma viruses obtained at the time of rebound. In addition to the pre-existing sequences associated with resistance in the 10-1074 target site (N332T and S334N, Fig. 2a), rebound viruses in 9245 also carried an extended V5 loop and potential *N*-linked glycosylation sites that could interfere with 3BNC117 binding (Extended Data Fig. 6). Conversely, genetic features associated with resistance to 3BNC117 were found in the pre-infusion reservoir of 9251 and were accompanied by mutations in the 10-1074 target site in the rebounding viruses (S334N, Fig. 2a and Extended Data Fig. 6). For both individuals, resistance of rebound viruses to both antibodies was confirmed by the TZM-bl neutralization assay (Fig. 2b, c and Supplementary Table 4). Thus, bulk outgrowth cultures used for screening failed to detect pre-existing resistance in the reservoir of 2 of the 11 studied individuals. This result is not surprising given that bulk cultures are dominated by a limited number of rapidly growing viral species that may not be representative of the diversity of the latent reservoir.

The median time to rebound in the seven individuals that had no detectable resistant viruses in the pre-infusion latent reservoir, and rebounded during the study period, was also 21 weeks and different from the 6–10 weeks found for monotherapy with 3BNC117[9] (Fig. 1c). In these participants, viral suppression was maintained for 15–26 weeks after ART discontinuation (Supplementary Table 2). The two remaining participants (9254 and 9255) completed study follow-up at 30 weeks without experiencing rebound (Supplementary Table 2). Notably, viral rebound never occurred when the concentration of both administered antibodies was above 10 μg ml^{-1}. The average serum concentration of 3BNC117 (determined by TZM-bl assay) at the time of rebound in sensitive individuals that rebounded during study follow-up was 1.9 μg ml^{-1} (Fig. 1b and Supplementary Table 2). By contrast, the average serum concentration of 10-1074 at rebound was 14.8 μg ml^{-1} (Fig. 1b and Supplementary Table 2). The difference in the antibody concentrations at the time of rebound is consistent with the longer half-life of 10-1074, which resulted in a period of 10-1074 monotherapy (Fig. 1b, Extended Data Fig. 2c–f and Supplementary Table 2). Finally, these nine individuals showed little or no pre-existing neutralizing antibodies against a diagnostic panel of viruses before bNAb infusion (Supplementary Table 5).

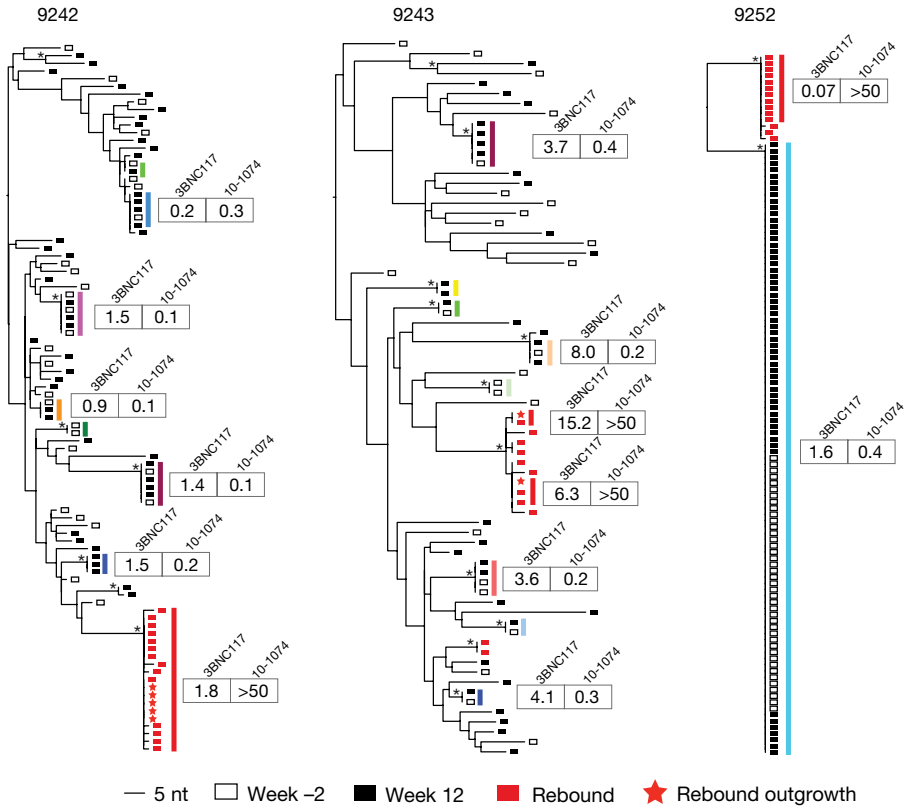

Fig. 3 | Comparison of the circulating latent reservoir and rebound viruses. Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^2VOA, rebound plasma SGA and rebound PBMC outgrowth cultures from three out of seven participants (9242, 9243 and 9252) that rebounded before week 30 (9241, 9244, 9247 and 9246 are depicted in Extended Data Fig. 7). Open and closed black rectangles indicate Q^2VOA-derived viruses from week −2 and week 12, respectively. Viruses obtained at the time of rebound are indicated by red rectangles (plasma SGA) and red stars (rebound PBMC outgrowth cultures). Asterisks indicate nodes with significant bootstrap values (bootstrap support ≥70%). Clones are denoted by coloured lines mirroring the colours of slices in Extended Data Fig. 10a. Boxes indicate IC_{80} values (μg ml^{-1}) of 3BNC117 and 10-1074 against representative viruses throughout the phylogenetic tree and clones, when possible (Supplementary Table 4). nt, nucleotide.

Rebound and latent viruses

To examine the relationship between rebound viruses and the circulating latent reservoir, we compared *env* sequences obtained from plasma rebound viruses by SGA with sequences obtained by Q^2VOA from both pre-infusion and week 12 samples. In addition, we measured the sensitivity of rebound outgrowth viruses and/or pseudoviruses to 3BNC117 and 10-1074 using the TZM-bl neutralization assay (Fig. 2b, c, 3, Extended Data Fig. 7 and Supplementary Table 4). A total of 154 viral *env* sequences obtained by plasma SGA were analysed and compared to 408 sequences obtained from the latent reservoir by Q^2VOA. Although rebound and reservoir viruses clustered together for each individual (Extended Data Fig. 3), we found no identical sequences between the two compartments in any of the individuals studied (Figs. 3, 4a and Extended Data Fig. 7). The difference could be accounted for by distinct requirements for HIV-1 reactivation in vitro and in vivo, compartmentalization of reservoir viruses, HIV-1 mutation during the course of the trial and/or by viral recombination in some individuals[20,21] (Extended Data Fig. 8). Whether or not bNAb therapy influences selection for recombination events remains to be determined.

Similar to 3BNC117 monotherapy, the vast majority of rebounding viruses clustered within low-diversity lineages consistent with expansion of 1–2 recrudescent viruses[9] (Fig. 3, Extended Data Figs. 7, 9). By contrast, rebound viruses are consistently polyclonal during ATI in the absence of antibody therapy[22,23]. Thus, the antibodies restrict the outgrowth of latent viruses in vivo.

The emerging viruses in 6 of the 7 individuals who rebounded when the mean 3BNC117 and 10-1074 serum concentrations were 1.9 and 14.8 μg ml^{-1}, respectively, carried resistance-associated mutations in the 10-1074 target site (Figs. 1b, 2a). Consistent with the sequence data, these rebound viruses were generally resistant to 10-1074, as shown by the TZM-bl neutralization assay, but remained sensitive to 3BNC117 (Fig. 2b, c and Supplementary Table 4). The level of sensitivity to 3BNC117 in these emerging viruses was similar to that found in the reservoir viruses in each of the individuals (Fig. 2b and Supplementary Table 4). One individual, 9244, showed rebound viruses that remained sensitive to both antibodies in TZM-bl neutralization assays. Rebound occurred when 3BNC117 and 10-1074 concentrations in serum of this individual were undetectable and 11.6 μg ml^{-1}, respectively (Fig. 1b and Supplementary Table 2). The sensitivity of the plasma rebound viruses was similar to that of latent pre-infusion and week 12 viruses obtained in viral outgrowth cultures (Fig. 2b, c and Supplementary Table 4). Therefore, this individual did not develop resistance to either of the antibodies despite prolonged exposure to both. In conclusion, none of the nine individuals with pre-infusion reservoirs containing viruses that were sensitive to both antibodies developed double resistance during the observation period.

The latent reservoir

To determine whether there were changes in the circulating reservoir during the observation period, we compared the results of Q^2VOA assays performed at entry and 12 weeks after the start of ATI for 8 of the 9 individuals that remained suppressed for at least 12 weeks (Fig. 4 and Extended Data Fig. 10). Similar to previous reports, 63% of all viruses obtained by Q^2VOA belonged to expanded clones[20,24–26] (Extended Data Fig. 10a, b). Comparison of the *env* sequences of the viruses that emerged in outgrowth cultures revealed that 60% of the sequences could be found at both time points. However, there were numerous examples of clones that appeared or disappeared between

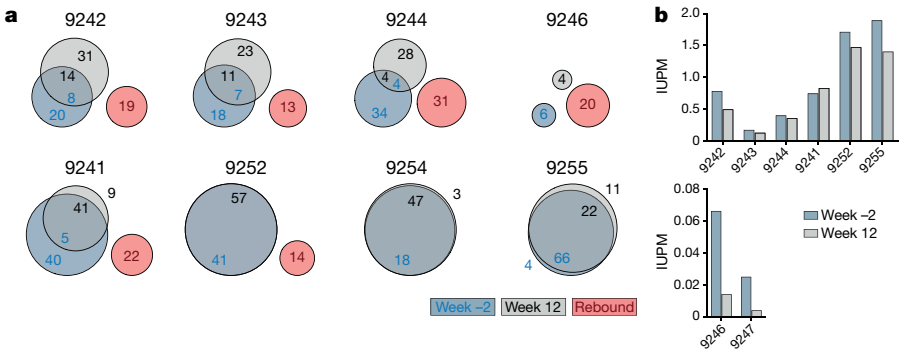

Fig. 4 | Distribution of the circulating latent reservoir and rebound viruses. a, Venn diagrams showing sequence identity between *env* sequences obtained from Q^2VOA at week −2 (blue) and week 12 (grey), and plasma SGA or rebound PBMC outgrowth culture at the time of viral rebound (red). Area of overlap is proportional to the number of identical sequences. The number of obtained sequences is indicated. **b**, IUPM $CD4^+$ T cells at weeks −2 and 12 as determined by Q^2VOA. Participants with IUPMs that were higher and lower than 0.1 are shown at the top and bottom, respectively. Participant 9254 is not shown owing to lack of sample availability. The two time points were not statistically different ($P = 0.078$ (paired Student's *t*-test)).

the time points and some of the changes were significant (Extended Data Fig. 10a). To determine the number of infectious units per million (IUPM, http://silicianolab.johnshopkins.edu/), 6.0×10^7–6.2×10^8 $CD4^+$ T cells were assayed by Q^2VOA for each time point for each individual (Fig. 4b). The difference between the two time points was never greater than 6.5-fold for any individual, and the IUPM values at the two time points were not statistically different ($P = 0.078$). Moreover, time to rebound was not directly correlated with IUPM (Extended Data Fig. 10c). Additional time points would be required to calculate the half-life of the reservoir in individuals who received immunotherapy[27].

Discussion

First-generation anti-HIV-1 bNAbs were generally ineffective in suppressing viraemia in animal models and humans leading to the conclusion that this approach should not be pursued[17,18,28]. The advent of new methods for anti-HIV-1 antibody cloning[29] and subsequent discovery of a new, more potent generation of bNAbs revitalized this area of research[30,31].

bNAb monotherapy with 3BNC117 or VRC01 is not enough to maintain control during ATI in HIV-1-infected humans[9,11]. Similar results were obtained in participant 9251 who effectively received 10-1074 monotherapy due to pre-existing resistance to 3BNC117. By contrast the combination of 3BNC117 and 10-1074 is sufficient to maintain viral suppression in sensitive individuals when the concentration of both antibodies remains above of 10 μg ml^{-1} in serum. Rebound occurred when 3BNC117 levels dropped below 10 μg ml^{-1} effectively leading to 10-1074 monotherapy, from which viruses in nearly all individuals rapidly escaped by mutations in the 10-1074 contact site. The observation that nine individuals infected with distinct viruses were unable to develop viruses who were resistant to both antibodies over a median period of 21 weeks suggests that viral replication was severely limited by this combination of antibodies.

In human studies, monotherapy with 3BNC117 is associated with enhanced humoral immunity and accelerated clearance of HIV-1-infected cells[5,32]. In addition, when administered early to macaques infected with the chimeric simian/human immunodeficiency virus $SHIV_{AD8}$, combined 3BNC117 and 10-1074 immunotherapy induced host $CD8^+$ T cell responses that contributed to the control of viraemia in nearly 50% of the animals[3]. However, virus-specific $CD8^+$ T cells that were responsible for control of viraemia in these macaques were not detected in the circulation, and their contribution to viral suppression was only documented after $CD8^+$ T cell depletion[3]. In most macaques that maintained viral control, complete viral suppression was only established after rebound viraemia that followed antibody clearance[3].

Two individuals in this study remained suppressed for over 30 weeks after ATI, 9254 and 9255. Neither participant had detectable levels of ART in the blood or carried the B*27 and B*57 HLA alleles that are most frequently associated with elite control[33]. The first, 9254, reports starting ART within 4–5 months after probable exposure to the virus with an initial viral load of 860,000 copies per ml. Despite relatively early therapy, and excellent virological control for 21 years on therapy, this individual had an IUPM of 0.68 by Q^2VOA at the 12-week time point (Extended Data Fig. 10b). The second individual, 9255, showed several viral blips that were spontaneously controlled beginning 15 weeks after ATI when antibody levels were waning. This individual was infected for at least 7 months before starting ART with an initial viral load of 85,800 copies per ml and had an IUPM of 1.4 at the 12-week time point. A small fraction of individuals on ART[10] show spontaneous prolonged virologic control after ART is discontinued, and this number appears to increase when ART treatment is initiated during the acute phase of infection[34–38]. Whether antibody-enhanced $CD8^+$ T cell responses contribute to the prolonged control in the two out of nine individuals who received combination immunotherapy and whether this effect can be enhanced by latency reactivating agents or immune checkpoint inhibitors remains to be determined.

A substantial fraction of the circulating latent reservoir is composed of expanded clones of infected T cells[24,26,39–42]. These T cell clones appear to be dynamic in that the specific contribution of individual clones of circulating latently infected $CD4^+$ T cells to the reservoir of individuals receiving ART fluctuates over time[24,25]. Individuals that maintain viral suppression by antibody therapy appear to show similar fluctuations in reservoir clones that do not appear to be associated with antibody sensitivity. Whether the apparent differences observed in the reservoir during immunotherapy lead to changes in the reservoir half-life cannot be determined from the available data and will require reservoir assessments in additional individuals at multiple time points over an extended observation period.

Individuals harbouring viruses sensitive to 3BNC117 and 10-1074 maintained viral suppression during ATI for a median of almost four months after the final antibody administration. However, HIV-1 is a highly diverse virus with varying levels of sensitivity to specific bNAbs. As a result, maintenance therapy with just the combination of 3BNC117 and 10-1074 would only be possible for the approximately 50% of clade B-infected individuals that are sensitive to both antibodies. This problem may be overcome by addition of or substitution with other antibodies[14,15,43], or long-acting small-molecule antiretroviral drugs.

In macaques, the therapeutic efficacy of anti-HIV-1 antibodies is directly related to their half-life[4,12,13], which can be extended by mutations that enhance Fc domain interactions with the neonatal Fc receptor[4,13,44]. These mutations also increase the half-life of antibodies in humans by 2–4-fold[45]. Our data suggest that a single administration of combinations of bNAbs with extended half-lives could maintain suppression for 6–12 months in individuals harbouring sensitive viruses.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0531-2.

Received: 6 June 2018; Accepted: 30 July 2018;
Published online 26 September 2018.

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Acknowledgements We thank all study participants who devoted time to our research; members of the Klein and Nussenzweig laboratories for helpful discussions, especially Y. Bar-On, L. Cohn and M. Jankovic; R. Levin for study coordination and the Rockefeller University Hospital Clinical Research Support Office and nursing staff as well as K. Fiddike, C. Golder, S. Margane, M. Platten, E. Voigt and D. Weiland for help with recruitment and study implementation; K. Jain for help with sample processing; S. Kiss for ophthalmologic assessments; T. Keler and the Celldex Therapeutics team for 3BNC117 and 10-1074 manufacturing and regulatory support; C. Conrad for regulatory support; U. Kerkweg, R. Macarthur and A. Johnson for pharmaceutical services; H. Janicki, M. Ercanoglu, P. Schommers and R. Kaiser for help with virus cultures; C. Scheid and U. Holtick for leukaphereses; S. McMillan, S. Mosher, S. Sawant, D. Beaumont, M. Sarzotti-Kelsoe, K. Greene, H. Gao and D. Montefiori for help with PK assay development, validation, reporting, and/or project management; P. Fast and H. Park for clinical monitoring; and S. Schlesinger for input on study design. This work was supported by the Bill and Melinda Gates Foundation Collaboration for AIDS Vaccine Discovery (CAVD) grants OPP1092074, OPP1124068 (M.C.N.), CAVIMC OPP1146996 (G.D.T., M.S.S.); the Heisenberg-Program of the DFG (KL 2389/2-1), the European Research Council (ERC-StG639961), and the German Center for Infection Research (DZIF) (F.K.); the NIH grants 1UM1 AI100663 and R01AI-129795 (M.C.N.); the Einstein-Rockefeller-CUNY Center for AIDS Research (1P30AI124414-01A1); BEAT-HIV Delaney grant UM1 AI126620 (M.C.); and the Robertson fund of the Rockefeller University. M.C.N. is a Howard Hughes Medical Institute Investigator.

Reviewer information *Nature* thanks G. Silvestri and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions M.C. is the principal investigator for the work in the United States and F.K. is the principal investigator for Germany; M.C., F.K. and M.C.N. designed the trial; P.M., H.G., F.K., M.C. and M.C.N. analysed the data and wrote the manuscript; P.M., L.N. and T.Ka. performed Q^2VOA, rebound cultures and SGA; H.G., M.W.-P., G.K., E.T., J.H., M.C. and F.K. implemented the study; A.L.B., K.M., Y.Z.C., C.L., I.Su., C.W., T.Kü. and C.S. contributed to recruitment and clinical assessments; P.M., H.G. and L.N. performed bulk viral cultures; J.A.P. and T.Y.O. performed bioinformatics processing; K.E.S. and G.D.T. conducted anti-idiotype ELISA; M.S.S. conducted TZM-bl assays; C.U.-O., R.P., C.R., M.S. and I.Sh. coordinated and performed sample processing; L.H., A.P.W., P.J.B. and N.P. contributed to data analysis; J.C.C.L., C-L.L., R.M.G. and G.F. contributed to study design and implementation.

Competing interests : There are patents on 3BNC117 (PTC/US2012/038400) and 10-1074 (PTC/US2013/065696) that list M.C.N. as an inventor.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0531-2.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0531-2.
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METHODS

Study design. An open-label phase 1b study was conducted in HIV-1-infected participants who were virologically suppressed on ART (http://www.clinicaltrials.gov; NCT02825797; EudraCT: 2016-002803-25). Study participants were enrolled sequentially according to eligibility criteria. Participants received 3BNC117 and 10-1074 intravenously at a dose of 30 mg kg^{-1} body weight of each antibody, at weeks 0, 3 and 6, unless viral rebound occurred. ART was discontinued 2 days after the first infusion of antibodies (day 2). Plasma HIV-1 viral RNA levels were monitored weekly and ART was resumed if the viral load increased to $\geq$200 copies per ml or $CD4^+$ T cell counts decreased to <350 cells per μl in two consecutive measurements. Time to viral rebound was determined by the first of two consecutive viral loads of >200 copies per ml. Study participants were followed for 30 weeks after the first infusion. Safety data are reported until the end of study follow-up. All participants provided written informed consent before participation in the study and the study was conducted in accordance with Good Clinical Practice. The protocol was approved by the Federal Drug Administration in the USA, the Paul-Ehrlich-Institute in Germany, and the Institutional Review Boards (IRBs) at the Rockefeller University and the University of Cologne.

Study participants. Study participants were recruited at the Rockefeller University Hospital, New York, USA, and the University Hospital Cologne, Cologne, Germany. Eligible participants were adults aged 18–65 years, HIV-1-infected, on ART for a minimum of 24 months, with plasma HIV-1 RNA levels of <50 copies per ml for at least 18 months (one viral blip of >50 but <500 copies per ml during this 18-month period was allowed), plasma HIV-1 RNA levels <20 copies per ml at the screening visit, and a current $CD4^+$ T cell count >500 cells per μl. In addition, participants were prescreened for sensitivity of latent proviruses against 3BNC117 and 10-1074 by bulk PBMC viral outgrowth culture as described in 'Prescreening bulk PBMC cultures'. Sensitivity was defined as an $IC_{50} < 2\,\mu g\,ml^{-1}$ for both 3BNC117 and 10-1074 against outgrowth virus. Participants on an ART regimen that included a non-nucleoside reverse transcriptase inhibitor (NNRTI) were switched to an integrase inhibitor-based regimen (dolutegravir plus tenofovir disoproxil fumarate and emtricitabine) four weeks before treatment interruption due to the prolonged half-life of NNRTIs. Exclusion criteria included reported $CD4^+$ T cell nadir of <200 cells μl^{-1}, concomitant hepatitis B or C infection, previous receipt of monoclonal antibodies of any kind, clinically relevant physical findings, medical conditions or laboratory abnormalities, and pregnancy or lactation.

Study procedures. 3BNC117 and 10-1074 were administered intravenously at a dose of 30 mg kg^{-1}. The appropriate stock volume of 3BNC117 and 10-1074 was calculated according to body weight and diluted in sterile normal saline to a total volume of 250 ml per antibody. Monoclonal antibody infusions were administered sequentially and intravenously over 60 min. Study participants were observed at the Rockefeller University Hospital or the University Hospital Cologne for 1 h after the last antibody infusion. Participants returned for weekly follow-up visits during the ATI period for safety assessments, which included physical examination and measurements of clinical laboratory parameters such as haematology, chemistries, urinalysis and pregnancy tests (for women). Plasma HIV-1 RNA levels were monitored weekly during the ATI period and $CD4^+$ T cell counts were measured every 1–2 weeks. After ART was re-initiated, participants returned for follow-up every two weeks until viral re-suppression was achieved, and every eight weeks thereafter. Study investigators evaluated and graded adverse events according to the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2.0, November 2014) and determined causality. Leukapheresis was performed at the Rockefeller University Hospital or at the University Hospital Cologne at week –2 and week 12. Blood samples were collected before and at multiple times after 3BNC117 and 10-1074 infusions. Samples were processed within 4 h of collection, and serum and plasma samples were stored at −80 °C. PBMCs were isolated by density gradient centrifugation. The absolute number of PBMCs was determined using an automated cell counter (Vi-Cell XR; Beckman Coulter) or manually, and cells were cryopreserved in fetal bovine serum plus 10% DMSO.

Plasma HIV-1 RNA Levels. HIV-1 RNA levels in plasma were measured at the time of screening, at week –2, day 0 (before infusion), weekly during ATI, and every two weeks to every eight weeks after viral rebound had occurred. HIV-1 RNA levels were determined using the Roche COBAS AmpliPrep/COBAS TaqMan HIV-1 Assay (version 2.0) or the Roche COBAS HIV-1 quantitative nucleic acid test (COBAS 6800), which quantify HIV-1 RNA over a range of 2×10^1 to 1×10^7 copies per ml. These assays were performed at LabCorp or at the University Hospital Cologne.

$CD4^+$ T cells. $CD4^+$ T cell counts were determined by clinical flow cytometry assay, performed at LabCorp or at the University Hospital Cologne, at screening, week 0 (before infusion), weeks 2, 3, 5, 6, 8, 10, and weekly thereafter, while participants remained off ART.

Determination of baseline neutralizing antibody activity. Purified IgG (Protein G Sepharose 4 Fast Flow, GE Life Sciences) obtained before antibody infusions was tested against a panel of 12 HIV-1 pseudoviruses as described previously[5].

Measurement of 3BNC117 and 10-1074 serum levels. Blood samples were collected before, at the end of each 3BNC117 infusion and at the end of each 10-1074 infusion at weeks 0, 3 and 6, and weekly during the ATI period, up to week 30. Serum levels of 3BNC117 and 10-1074 were determined by a TZM-bl assay and by ELISA from samples obtained before and after each antibody infusion, and approximately every three weeks during follow-up as well as at the time of viral rebound.

Serum concentrations of 3BNC117 and 10-1074 were measured by a validated sandwich ELISA. High-bind polystyrene plates were coated with 4 μg ml^{-1} of an anti-idiotypic antibody that specifically recognizes 3BNC117 (anti-ID 1F1-2E3 monoclonal antibody) or 2 μg ml^{-1} of an anti-idiotypic antibody that specifically recognizes 10-1074 (anti-ID 3A1-4E11 monoclonal antibody), and incubated overnight at 2–8 °C. After washing, plates were blocked with 5% Milk Blotto (w/v), 5% NGS (v/v) and 0.05% Tween 20 (v/v) in PBS. Serum samples, quality controls and standards were added (1:50 minimum dilution in 5% Milk Blotto (w/v), 5% NGS (v/v) and 0.05% Tween 20 (v/v) in PBS) and incubated at room temperature. 3BNC117 or 10-1074 were detected using a horseradish peroxidase (HRP)-conjugated mouse anti-human IgG kappa-chain-specific antibody (Abcam) for 3BNC117 or an HRP-conjugated goat anti-human IgG Fc-specific antibody for 10-1074 (Jackson ImmunoResearch) and the HRP substrate tetra-methylbenzidine. 3BNC117 and 10-1074 concentrations were then calculated from the standard curves of 3BNC117 or 10-1074 that were run on the same plate using a 5-PL curve-fitting algorithm (Softmax Pro, v.5.4.5). Standard curves and positive controls were created from the drug product lots of 3BNC117 and 10-1074 used in the clinical study. The capture anti-idiotypic monoclonal antibodies were produced using a stable hybridoma cell line (Duke Protein Production Facility[6]). The lower limit of quantification for the 3BNC117 ELISA is 0.78 μg ml^{-1} and for the 10-1074 ELISA is 0.41 μg ml^{-1}. The lower limit of detection was determined to be 0.51 μg ml^{-1} and 0.14 μg ml^{-1} in HIV-1 seropositive serum for the 3BNC117 and 10-1074 ELISA, respectively. For values that were detectable (that is, positive for the monoclonal antibodies) but were below the lower limit of quantification, values are reported as <0.78 μg ml^{-1} and <0.41 μg ml^{-1} for 3BNC117 and 10-1074 ELISA, respectively. If day 0 baseline samples had measurable levels of antibody by the respective assays, the background measured antibody level was subtracted from subsequent results. In addition, samples with antibody levels measured to be within threefold from background were excluded from the analysis of pharmacokinetic parameters.

Serum concentrations of active 3BNC117 and 10-1074 were also measured using a validated luciferase-based neutralization assay in TZM-bl cells as previously described[19]. In brief, serum samples were tested using a primary 1:20 dilution with a fivefold titration series against HIV-1 Env pseudoviruses Q769.d22 and X2088_c9, which are highly sensitive to neutralization by 3BNC117 and 10-1074, respectively, while fully resistant against the other administered antibody. In the case of the post-infusion time points of 10-1074, instances for which the serum 50% inhibitory dilution (ID_{50}) titres against X2088_c9 were >100,000, serum samples were also tested against a less sensitive strain, Du422 (Supplementary Table 2). To generate standard curves, clinical drug products of 3BNC117 and 10-1074 were included in every assay set-up using a primary concentration of 10 μg ml^{-1} with a fivefold titration series. Serum concentrations of 3BNC117 and 10-1074 for each sample were calculated as follows: serum ID_{50} titre (dilution) × 3BNC117 IC_{50} or 10-1074 IC_{50} titre (μg ml^{-1}) = serum concentration of 3BNC117 or 10-1074 (μg ml^{-1}). Env pseudoviruses were produced using an ART-resistant backbone vector that reduces background inhibitory activity of antiretroviral drugs if present in the serum sample (SG3ΔEnv/K101P.Q148H.Y181C; M.S.S., unpublished data). Virus that was pseudotyped with the envelope protein of murine leukaemia virus (MuLV) was used as a negative control. Antibody concentrations were calculated using the serum ID_{80} titre and monoclonal antibody IC_{80} if non-specific activity against MuLV was detected ($ID_{50} > 20$; 9246, week 30; 9248, baseline, day 0, week 18). All assays were performed in a laboratory that meets Good Clinical Laboratory Practice standards.

Prescreening bulk PBMC cultures. To test HIV-1 viral strains for sensitivity to 3BNC117 and 10-1074, we performed bulk viral outgrowth cultures by coculturing isolated $CD4^+$ T cells with the MOLT-4/CCR-5 cell line (NIH AIDS Reagent Program, Ca. No. 4984) or $CD8^+$ T cell-depleted healthy donor lymphoblasts. PBMCs for prescreening were obtained up to 72 weeks (range 54–505 days) before enrollment under separate protocols approved by the IRBs of the Rockefeller University and the University of Cologne. Sensitivity was determined by TZM-bl neutralization assay as described below. Culture supernatants with $IC_{50} < 2\,\mu g\,ml^{-1}$ were deemed sensitive.

Quantitative and qualitative viral outgrowth assay. The Q^2VOA was performed using isolated PBMCs from leukapheresis at week –2 and week 12 as previously described[24]. In brief, isolated $CD4^+$ T cells were activated with 1 μg ml^{-1} phytohaemagglutinin (PHA; Life Technologies) and 100 U ml^{-1} IL-2 (Peprotech) and cocultured with 1×10^6 irradiated PBMCs from a healthy donor in 24-well plates.

A total of 6×10^7–6.2×10^8 cells were assayed for each individual at each of the two time points. After 24 h, PHA was removed and 0.1×10^6 MOLT-4/CCR5 cells were added to each well. Cultures were maintained for two weeks, splitting the MOLT- 4/CCR5 cells in half seven days after the initiation of the culture and every other day after that. Positive wells were detected by measuring p24 by ELISA. The frequency of latently infected cells was calculated through the IUPM algorithm developed by the Siliciano laboratory (http://silicianolab.johnshopkins.edu).

Rebound outgrowth cultures. $CD4^+$ T cells isolated from PBMCs from the rebound time points were cultured at limiting dilution exactly as described for Q^2VOA. $CD4^+$ T cells were activated with T cell activation beads (Miltenyi) at a concentration of 0.5×10^6 beads per 10^6 $CD4^+$ T cells and 20 U ml^{-1} of IL-2. Rebound outgrowth cultures were performed using PBMCs from the highest viral load sample (usually the repeat measurement $\geq$200 copies per ml). Viruses for which the sequences matched the SGA *env* sequences, and therefore were identical to those present in plasma, as opposed to potentially reactivated PBMC-derived latent reservoir viruses, were selected to test for neutralization.

Viral sensitivity testing. Supernatants from p24-positive bulk PBMC cultures, rebound PBMC outgrowth cultures and Q^2VOA wells were tested for sensitivity to 3BNC117 and 10-1074 by TZM-bl neutralization assay as previously described[19].

Sequencing. HIV-1 RNA extraction and single-genome amplification was performed as previously described[46]. In brief, HIV-1 RNA was extracted from plasma samples or Q^2VOA-derived virus supernatants using the MinElute Virus Spin kit (Qiagen) followed by first-strand cDNA synthesis using SuperScript III reverse transcriptase (Invitrogen). cDNA synthesis for plasma-derived HIV-1 RNA was performed using the antisense primer envB3out 5′-TTGCTACTTGTGATTGCTCCATGT-3′. gp160 was amplified using envB5out 5′-TAGAGCCCTGGAAGCATCCAGGAAG-3′ and envB3out 5′-TTGCTACTTGTGATTGCTCCATGT-3′ in the first round and in the second round with nested primers envB5in 5′-CACCTTAGGCATCTCCTATGGCAGGAAGAAG-3′ and envB3in 5′-GTCTCGAGATACTGCTCCCACCC-3′. PCRs were performed using High Fidelity Platinum Taq (Invitrogen) and run at 94 °C for 2 min; 35 cycles of 94 °C for 15 s, 55 °C for 30 s and 68 °C for 4 min; and 68 °C for 15 min. Second-round PCR was performed with 1 μl of the PCR product from the first round as template and High Fidelity Platinum Taq at 94 °C for 2 min; 45 cycles of 94 °C for 15 s, 55 °C for 30 s and 68 °C for 4 min; and 68 °C for 15 min. cDNA synthesis for Q^2VOA-derived HIV-1 RNA was performed using the antisense primer R3B6R 5′-TGAAGCACTCAAGGCAAGCTTTATTGAGGC-3′. The *env* 3′ half-genome was amplified in a single PCR using B3F3 primer 5′-TGGAAAGGTGAAGGGGCAGTAGTAATAC-3′ and R3B6R primer 5′-TGAAGCACTCAAGGCAAGCTTTATTGAGGC-3′. PCR was performed using High Fidelity Platinum Taq and run at 94 °C for 2 min; 45 cycles of 94 °C for 15 s, 55 °C for 30 s and 68 °C for 5 min; and 68 °C for 15 min.

Pseudovirus generation. Selected single genome sequences from outgrowth culture supernatants and plasma were used to generate pseudoviruses that were tested for sensitivity to bNAbs in a TZM-bl neutralization assay. To produce the pseudoviruses, plasmid DNA containing the cytomegalovirus (CMV) promoter was amplified by PCR using forward primer 5′-AGTAATCAATTACGGGGTCATTAGTTCAT-3′ and reverse primer 5′-CATAGGAGATGCCTAAGCCGGTGGAGCTCTGCTTATATAGACCTC-3′. Individual *env* amplicons were amplified using forward primer 5′-CACC GGCTTAGGCATCTCCTATGGCAGGAAGAA-3′ and reverse primer 5′-GTCTCGAGATACTGCTCCCACCC-3′. The CMV promoter amplicon was fused to individual purified *env* amplicons by PCR using forward primer 5′-AGTAATCAATTACGGGGTCATTAGTTCAT-3′ and reverse primer 5′-ACTTTTTGACCACTTGCCACCCAT-3′. Overlapping PCR was carried out using the High Fidelity Platinum Taq (Invitrogen) in a 50-μl reaction consisting of 1 ng purified CMV promoter amplicon, 0.125 μl purified *env* SGA amplicon, 400 nM each forward and reverse primers, 200 μM dNTP mix, 1× Buffer HiFi and 1 μl DNA polymerase mix. PCR was run at 94 °C for 2 min; 25 cycles of 94 °C for 12 s, 55 °C for 30 s and 68 °C for 4 min; and 72 °C for 10 min. Resulting amplicons were analysed by gel electrophoresis, purified by gel extraction, and cotransfected with pSG3Δenv into HEK293T cells to produce pseudoviruses as described previously[47].

Sequence and phylogenetic analysis. Nucleotide alignments of intact *env* sequences were translation-aligned using ClustalW v.2.1[48] under the BLOSUM cost matrix. Sequences with premature stop codons and frameshift mutations that fell in the gp120 surface glycoprotein region were excluded from all analyses. Maximum likelihood phylogenetic trees were then generated from these alignments with PhyML v.3.1[49] using the GTR model with 1,000 bootstraps. For the combined analysis of sequences from all participants, *env* sequences were aligned using MAFFT v.7.309 and clustered using RAxML v.8.2.9[50] under the GTRGAMMA model with 1,000 bootstraps. To analyse changes between reservoir and rebound viruses, *env* sequences were aligned at the amino acid level to a HXB2 reference using ClustalW v.2.1.

Statistical analyses. For sample size considerations, one-sided Clopper-Pearson confidence intervals were calculated for varying number of observed rebounds. A sample size of 15 HIV-1 infected individuals was determined to allow for the rejection of the null hypothesis (rate = 0.85) with 80% power for an effect size equal to or higher than 0.33, if at least 6 out of 15 enrolled participants did not experience viral rebound by week 8 (2 weeks after the last antibody infusions). Pharmacokinetic parameters were estimated by performing a non-compartmental analysis using Phoenix WinNonlin Build 8 (Certara), using all available PK data starting with the time point after the last infusion of 10-1074 from either TZM-bl assay (using the X2088_c9 pseudovirus to determine 10-1074 levels) or ELISA, and compared by a two-tailed unpaired Student's *t*-test. $CD4^+$ T cell counts on day 0 and at the time of viral rebound were compared by two-tailed paired Student's *t*-test. IUPMs determined at week −2 and week 12 were compared using a two-tailed paired Student's *t*-test. Time to rebound in current trial participants (combination therapy with 3BNC117 and 10-1074), participants receiving 3BNC117 monotherapy[9] and participants in previous non-interventional ATI studies conducted by ACTG[10] were plotted using Kaplan–Meier survival curves. Potential correlation between IUPM and time to rebound was analysed by two-tailed Pearson's correlations.

Recombination analysis of *env* sequences. Multiple alignment of nucleotide sequences guided by amino acid translations of *env* sequences was performed by TranslatorX (http://translatorx.co.uk/). Latent and rebound sequences were analysed for the presence of recombination using the 3SEQ recombination algorithm (http://mol.ax/software/3seq/). Sequences that showed statistical evidence of recombination (rejection of the null hypothesis of clonal evolution) in which 'parent' sequences were derived from the latent reservoir and the 'child' sequence was a rebound sequence are represented in a circos plot (http://circos.ca/).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The sequences from all isolated viruses are available in GenBank, accession numbers MH575375–MH576416.

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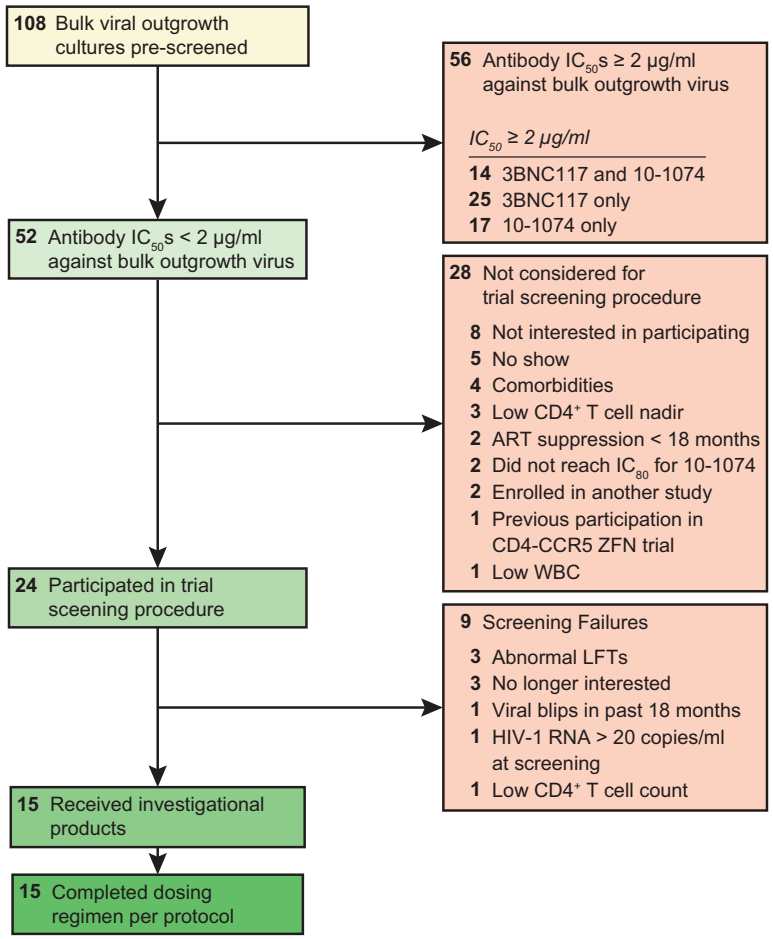
a



b

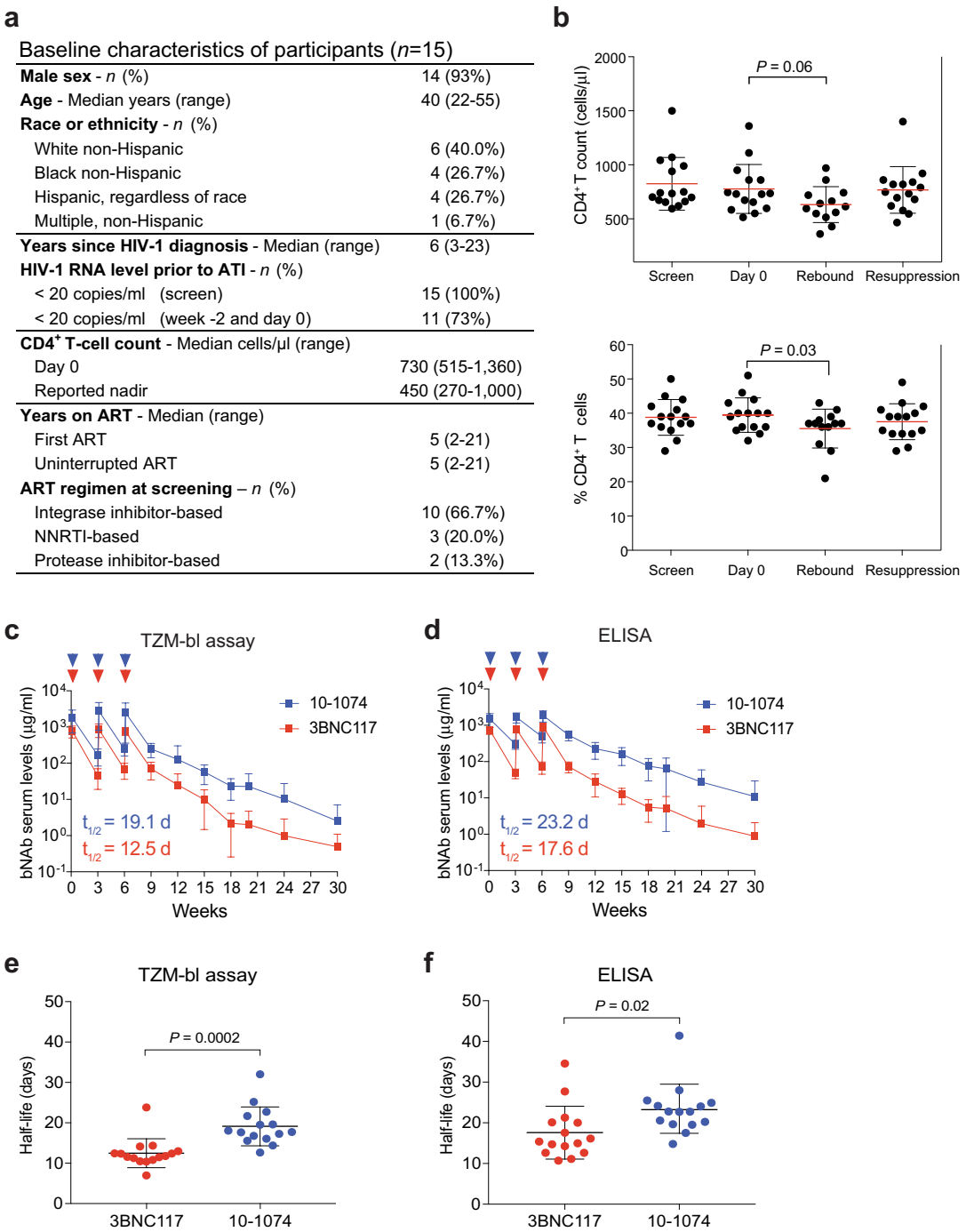
ID	Age	Gender	Race	Years since HIV-1 dx	Years since first ART	Uninterr. ART before ATI (yrs)	ART at Screening*	Switched ART**	Reported CD4 nadir	HLA alleles	Pre-Screen Sensitivity (µg/ml)*** 3BNC117 IC_{50}	3BNC117 IC_{80}	10-1074 IC_{50}	10-1074 IC_{80}	CD4 count (d0)	HIV-1 RNA (cp/ml)# Scr	Wk -2	d0	Weeks to viral rebound
9241	40	M	White/Hisp	6	5	5	EVG/cobi/ TDF/FTC	-	500	n.d.	0.809	2.212	0.090	0.243	515	<20	<20	<20	21
9242	43	M	White/Hisp	3	3	2	EVG/cobi/ TDF/FTC	-	450	n.d.	0.160	0.433	0.144	0.389	654	<20	<20	<20	15
9243	29	M	Amer Indian/Hisp	5	5	5	RPV/TDF/FTC	DTG/TDF/ FTC	350	n.d.	0.641	2.913	0.072	0.241	583	<20	<20 D	<20 D	20
9244	36	M	Amer Indian/not Hisp	9	5	5	EFV/TDF/FTC	DTG/TDF/ FTC	730	n.d.	0.277	0.966	0.025	0.068	1,110	<20	<20	<20	21
9245	22	M	White/Hisp	5	5	5	EVG/cobi/ TAF/FTC	-	360	n.d.	0.417	1.423	0.038	0.089	736	<20	<20	<20	5
9246	30	M	Black	5	5	5	EVG/cobi/ TAF/FTC	-	500	n.d.	0.144	0.387	0.040	0.105	745	<20	<20	<20 D	19
9247	31	M	Black	6	6	6	EVG/cobi/ TAF/FTC	-	600	n.d.	0.556	1.930	0.072	0.326	728	<20	<20	<20	26
9248	52	M	White	11	11	11	DRV/RTV/ TAF/FTC	-	310	n.d.	1.863	9.738	0.676	2.252	730	<20 D	<20 D	58	12
9249	49	M	White	23	20	6	DRV/RTV ABC/3TC	-	426	n.d.	0.562	2.095	0.260	0.983	860	<20 D	<20 D	32	3
9250	55	M	White	7	5	5	EVG/cobi/ TAF/FTC	-	350	n.d.	0.558	3.174	0.447	2.644	550	<20	40	50	6
9251	40	M	Black	6	2	2	EVG/cobi/ TDF/FTC	-	1,000	n.d.	1.200	3.125	0.073	0.153	672	<20	<20	<20	7
9252	51	F	Black	11	11	11	EFV/TDF/FTC	DTG/TDF/ FTC	270	n.d.	0.630	3.074	0.243	0.640	598	<20	<20	<20	22
9253	41	M	White	5	2	2	DTG/TAF/FTC	-	387	n.d.	0.558	2.644	0.020	0.317	950	<20	41	<20 D	5
9254	48	M	White	21	21	21	EVG/cobi/ TAF/FTC	-	590	A1,29 B38,44	0.142	0.386	0.085	0.240	860	<20	<20	<20	>30
9255	30	M	White	5	4	4	EVG/cobi/ TAF/FTC	-	779	A3,25 B18,44	0.324	0.833	0.006	0.015	1,360	<20	<20 D	<20	>30

Extended Data Fig. 1 | Study participant selection and demographics. **a**, Flow diagram indicating the selection of study participants. **b**, Individual participant demographics and baseline clinical characteristics. Grey-shaded rows indicate participants who were found to have detectable viraemia (HIV-1 viral load of >20 copies ml^{-1}) at week −2 or day 0. These participants were not included in the efficacy analyses given the lack of viral suppression at baseline. Amer Indian, American Indian; Hisp, Hispanic. *3TC, lamivudine; ABC, abacavir; cobi, cobicistat; DRV, darunavir; DTG, dolutegravir; EFV, efavirenz; EVG, elvitegravir; FTC, emtricitabine; RPV, rilpivirine; RTV, ritonavir; TAF, tenofovir alafenamide fumarate; TDF, tenofovir disoproxil fumarate. **NNRTI-based regimens were switched four weeks before ART interruption due to longer half-lives of NNRTIs. ***Pre-screening of bulk outgrowth virus obtained from PBMC cultures by TZM-bl assay. #All participants harboured clade B viruses. Viral load <20 D, plasma HIV-1 RNA detected but not quantifiable by clinical assay. d0, day 0; Dx, diagnosis; Scr, screening; Wk −2, week −2.

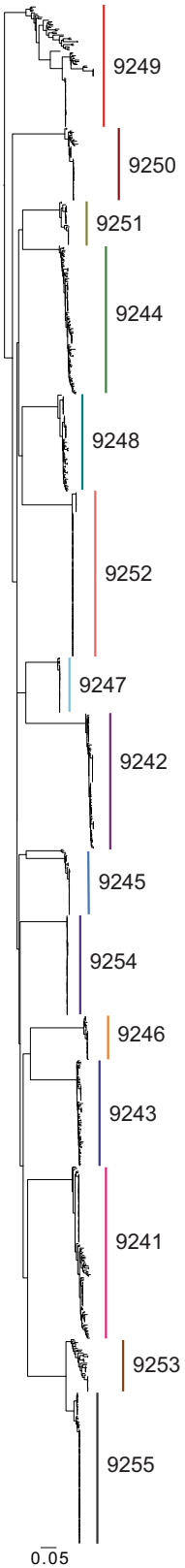
a

Baseline characteristics of participants (n=15)

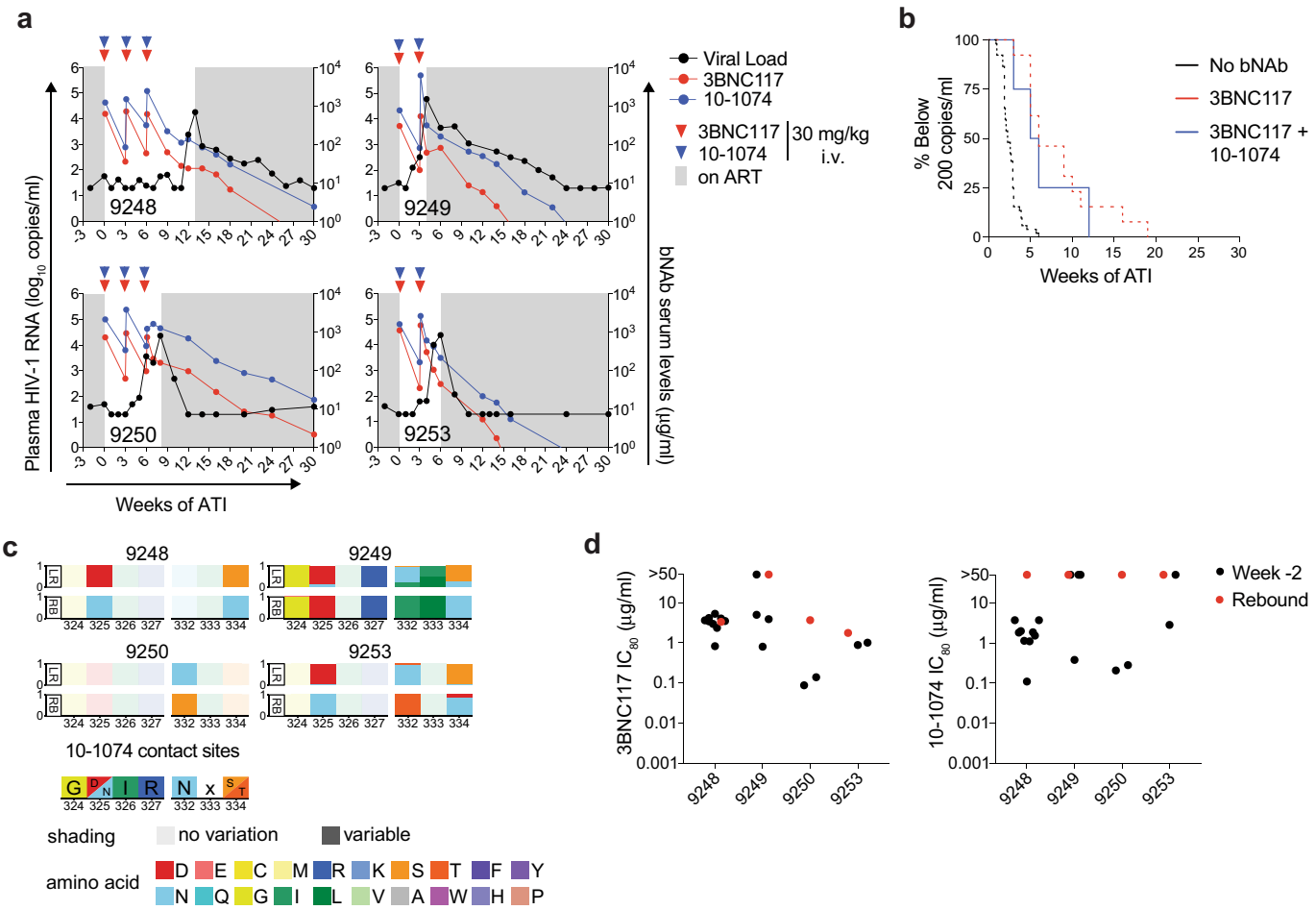
Male sex - *n* (%)	14 (93%)
Age - Median years (range)	40 (22-55)
Race or ethnicity - *n* (%)	
White non-Hispanic	6 (40.0%)
Black non-Hispanic	4 (26.7%)
Hispanic, regardless of race	4 (26.7%)
Multiple, non-Hispanic	1 (6.7%)
Years since HIV-1 diagnosis - Median (range)	6 (3-23)
HIV-1 RNA level prior to ATI - *n* (%)	
< 20 copies/ml (screen)	15 (100%)
< 20 copies/ml (week -2 and day 0)	11 (73%)
CD4$^+$ T-cell count - Median cells/µl (range)	
Day 0	730 (515-1,360)
Reported nadir	450 (270-1,000)
Years on ART - Median (range)	
First ART	5 (2-21)
Uninterrupted ART	5 (2-21)
ART regimen at screening – *n* (%)	
Integrase inhibitor-based	10 (66.7%)
NNRTI-based	3 (20.0%)
Protease inhibitor-based	2 (13.3%)



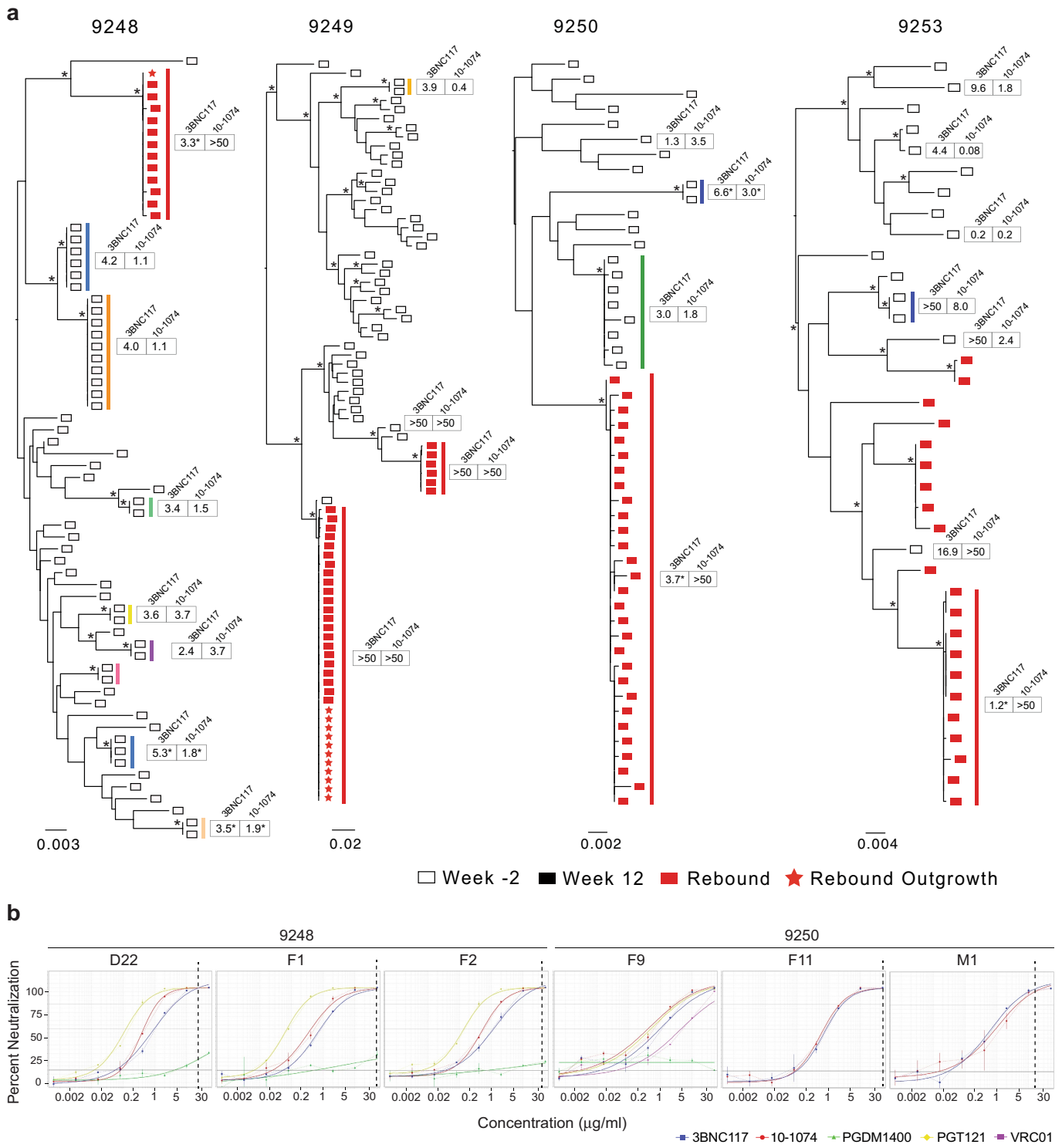
Extended Data Fig. 2 | Demographics, CD4$^+$ T cells during study period in participants and pharmacokinetics of 3BNC117 and 10-1074. **a**, Baseline participant demographics. **b**, Absolute CD4$^+$ T cell counts and percentage of CD4$^+$ T cells among CD3$^+$ T cells at screening (n = 15), day 0 (n = 15), at the time of viral rebound (n = 13) and at the end of the study are shown (n = 15) (see also Supplementary Table 2). The last available time point after resuppression was used as end of the study time point for the participants that reinitiated ART. Red lines indicate mean, error bars indicate standard deviation and individual participants are shown as dots. P values were obtained using a two-tailed paired Student's t-test comparing CD4$^+$ T cell counts between day 0 and the time of viral rebound. **c**, **d**, 3BNC117 (red) and 10-1074 (blue) levels in serum (n = 15) as determined by TZM-bl assay (**c**) and ELISA (**d**). In cases in which participants only received 2 infusions due to early viral rebound (9245, 9249 and 9253), only antibody concentrations up to the second infusion were included. Half-life of each bNAb is indicated in days. Curves indicate mean serum antibody concentrations and error bars represent standard deviation. Red and blue triangles indicate 3BNC117 and 10-1074 infusions, respectively. **c**, In the TZM-bl assay, lower limits of quantification were 0.46 µg ml^{-1} and 0.10 µg ml^{-1} for 3BNC117 and 10-1074, respectively. **d**, In the ELISA, lower limits of detection were 0.78 µg ml^{-1} and 0.41 µg ml^{-1}, respectively. **e**, **f**, Half-lives of both antibodies as measured by TZM-bl assay (**e**) and ELISA (**f**). Each dot represents a single participant. The half-lives of both antibodies from the 15 participants enrolled in the study are represented. Black lines indicate the mean value and standard deviation (n = 15). P values were obtained using a two-tailed unpaired Student's t-test comparing the two antibodies.



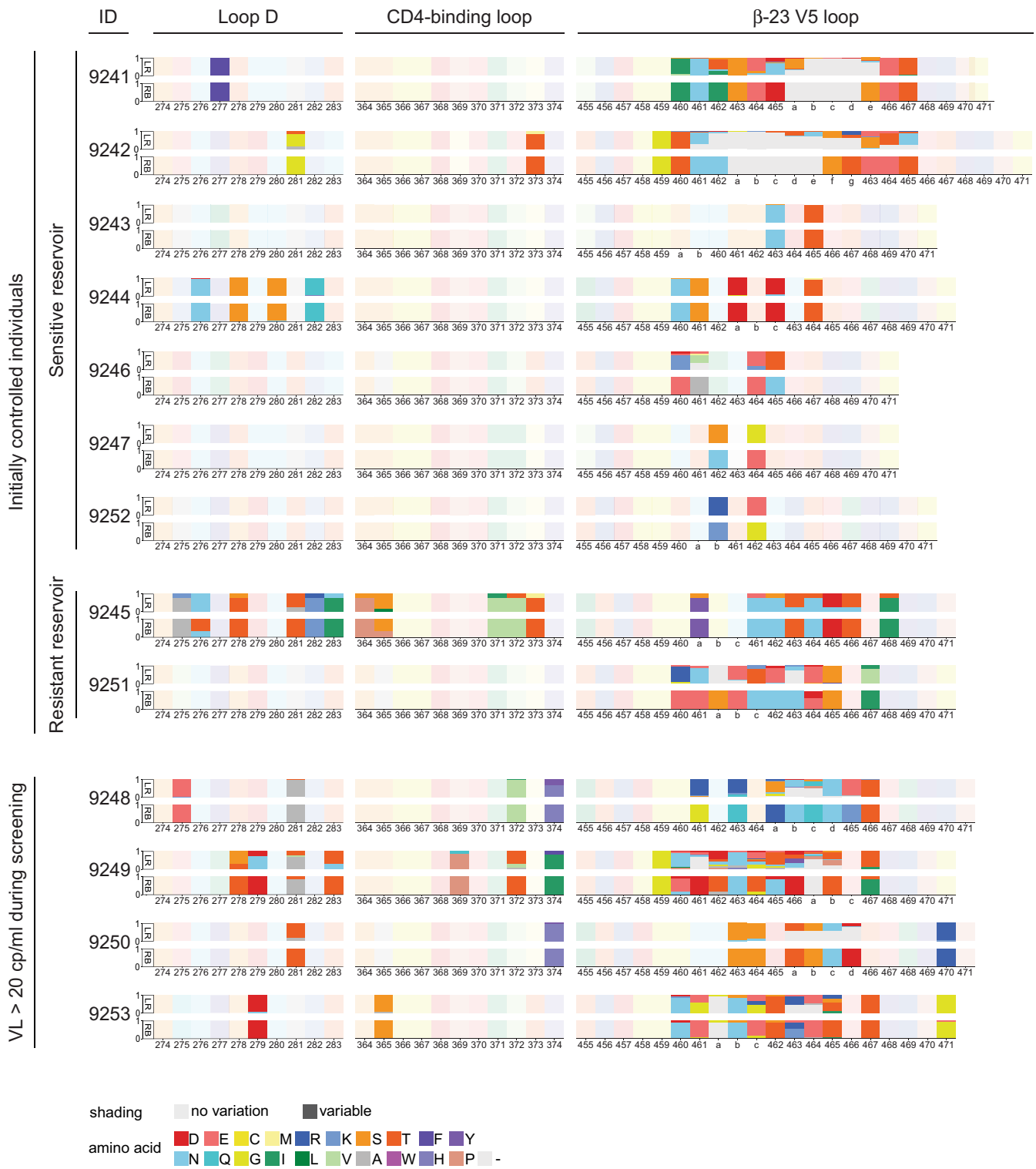
Extended Data Fig. 3 | Phylogenetic tree of viruses from all enrolled participants. Maximum likelihood phylogenetic trees of full-length *env* sequences containing all sequences obtained from Q^2VOA cultures and rebound viruses from SGA or rebound outgrowth of the 15 participants enrolled in the study. Participants are indicated by individual colours.



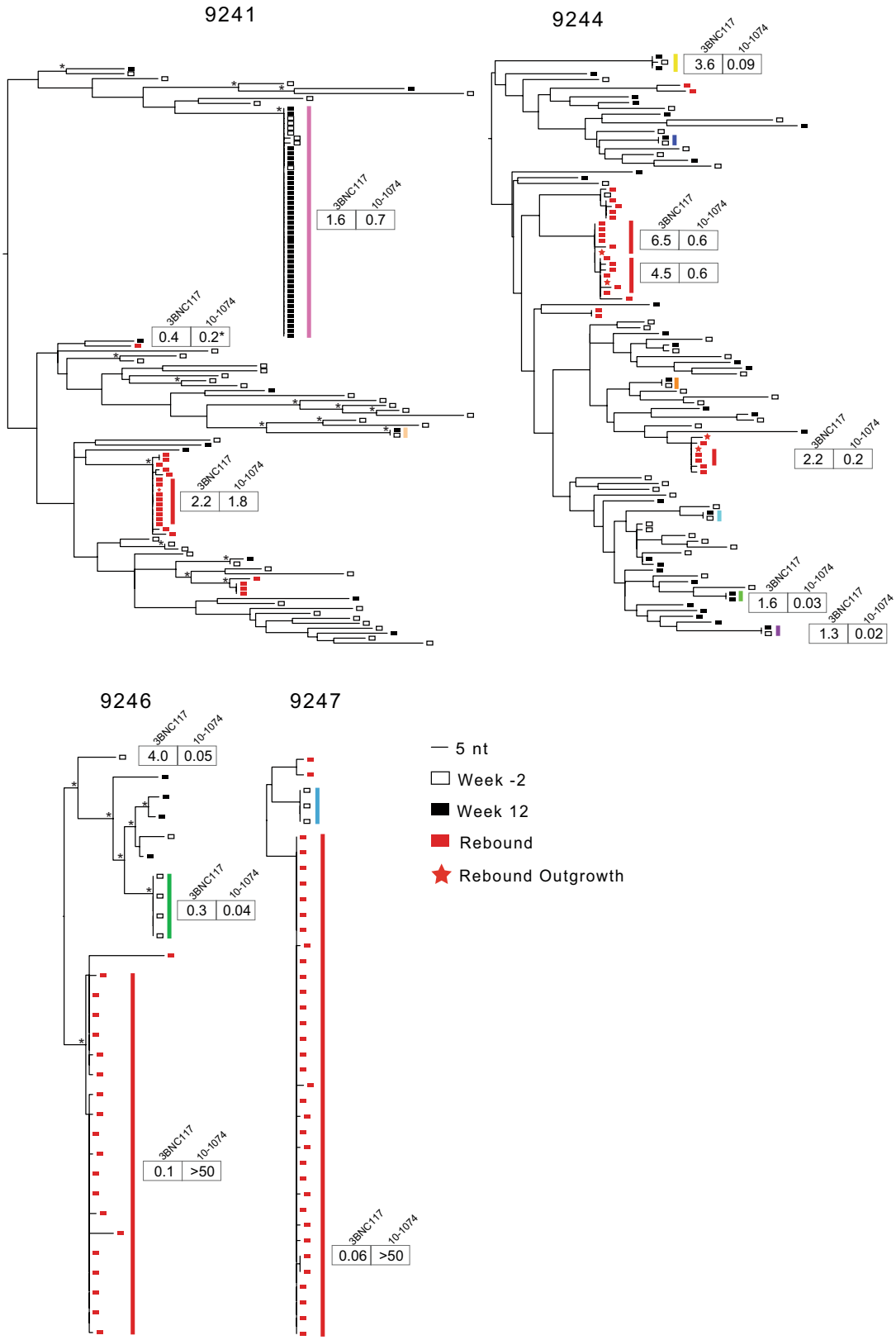
Extended Data Fig. 4 | Viral rebound, amino acid variants at 10-1074 contact sites and sensitivities of latent and rebound viruses in the participants with detectable viraemia (>20 copies per ml) two weeks before or at the start of ATI. a, Plasma HIV-1 RNA levels (black; left *y* axis) and bNAb serum concentrations (3BNC117, red; 10-1074, blue; right *y* axis). Red and blue triangles indicate 3BNC117 and 10-1074 infusions, respectively. Serum antibody concentrations were determined by TZM-bl assay. Grey-shaded areas indicate time on ART. Lower limit of detection of HIV-1 RNA was 20 copies per ml. **b**, Kaplan–Meier plots summarizing time to viral rebound. The *y* axis shows the percentage of participants that maintained viral suppression. The *x* axis shows the time in weeks after the start of ATI. Participants receiving the combination of 3BNC117 and 10-1074 are indicated by the blue line ($n = 4$). The dotted red line indicates a cohort of individuals receiving 3BNC117 alone during ATI[9] ($n = 13$) and the dotted black line indicates a cohort of participants who underwent ATI without any intervention[10] ($n = 52$). **c**, Colour charts show Env contact sites of 10-1074 at the G(D/N)IR motif (positions 324–327, according to HXB2 numbering) and the glycan at the potential *N*-linked glycosylation site at position 332 (NxS/T motif at positions 332–334). LR, latent reservoir viruses isolated by Q^2VOA (week −2); RB, rebound viruses isolated by SGA (plasma) or viral outgrowth (PBMCs). Each amino acid is represented by a colour and the frequency of each amino acid is indicated by the height of the rectangle. Shaded rectangles indicate the lack of variation between latent reservoir and rebound viruses at the indicated position. Full-colour rectangles represent amino acid residues with changes in distribution between reservoir and rebound viruses. **d**, Dot plots showing the IC_{80} (μg ml^{-1}) of 3BNC117 (left) and 10-1074 (right) against latent and rebound viruses determined by TZM-bl neutralization assay. Q^2VOA-derived latent viruses from week −2 are shown as black circles. For outgrowth culture-derived rebound viruses, the highest IC_{80} determined is shown as red circle. For 9250 and 9253, no viruses could be obtained from rebound outgrowth cultures and pseudoviruses were made from *env* sequences of the latent reservoir (Q^2VOA) and rebound viruses (plasma SGA). Note that 9249 and 9253 had pre-existing resistant viruses in the reservoir ($IC_{50} > 2$ μg ml^{-1}). 9248 and 9250 had pre-existing viruses that failed to reach an IC_{100} when tested up to 50 μg ml^{-1} for 3BNC117 (Extended Data Fig. 5). Rebound viruses of all four participants had an IC_{80} or IC_{100} of >50 μg ml^{-1} for both 3BN117 and 10-1074.



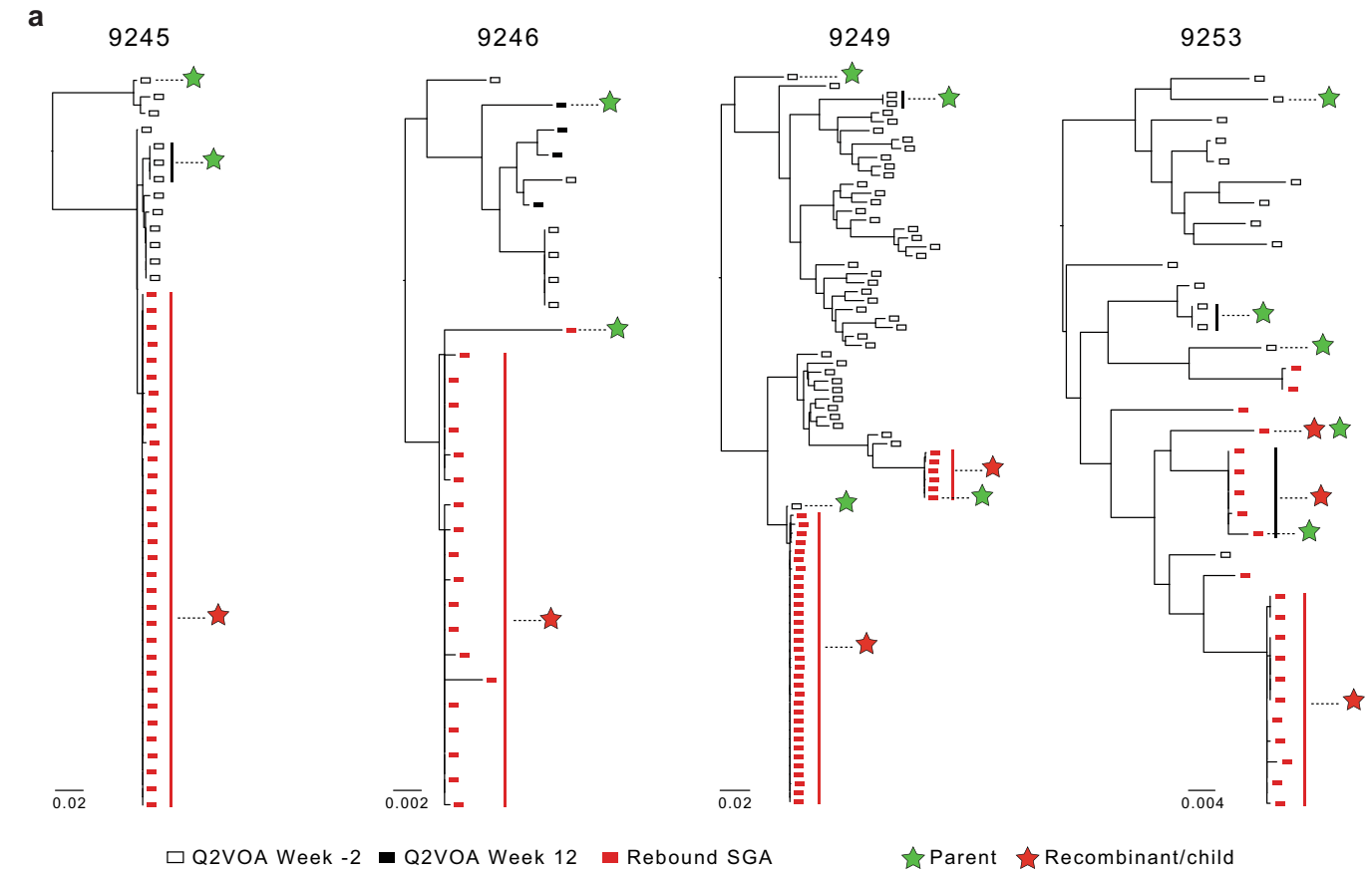
Extended Data Fig. 5 | Phylogenetic *env* trees and TZM-bl neutralization curves for individuals with viral blips. a, Circulating reservoir and viral rebound in study participants with detectable viraemia at week −2 or day 0. Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from week −2 Q^2VOA cultures, rebound plasma SGA and rebound outgrowth from the four participants with viral blips. Open black rectangles indicate Q^2VOA-derived viruses from week −2. Viruses obtained at the time of rebound are indicated by red rectangles (plasma SGA) and red stars (rebound PBMC outgrowth cultures), respectively. Asterisks indicate nodes with significant bootstrap values (bootstrap support ≥70%). Clones are denoted by coloured lines. Boxes indicate IC_{80} values (μg ml^{-1}) of 3BNC117 and 10-1074 against individual clones, with asterisks indicating $IC_{100} > 50$ μg ml^{-1}. **b**, Latent reservoir virus TZM-bl neutralization curves for two participants that had a viral load of >20 copies per ml at day 0 (9248 and 9250). Curves show neutralization titres by 3BNC117 (blue), 10-1074 (red) and other bNAbs, when available, for week −2 Q^2VOA-derived viruses present in the circulating reservoir. Three representative viruses from 9248 (left) and 9250 (right) are shown. Although these viruses had low 3BNC117 and 10-1074 IC_{50} or IC_{80} titres, the IC_{100} (black dotted line) is reached only at a high concentration or not reached at all. The neutralization titre was measured by TZM-bl neutralization assay using a five-parameter curve fit method.



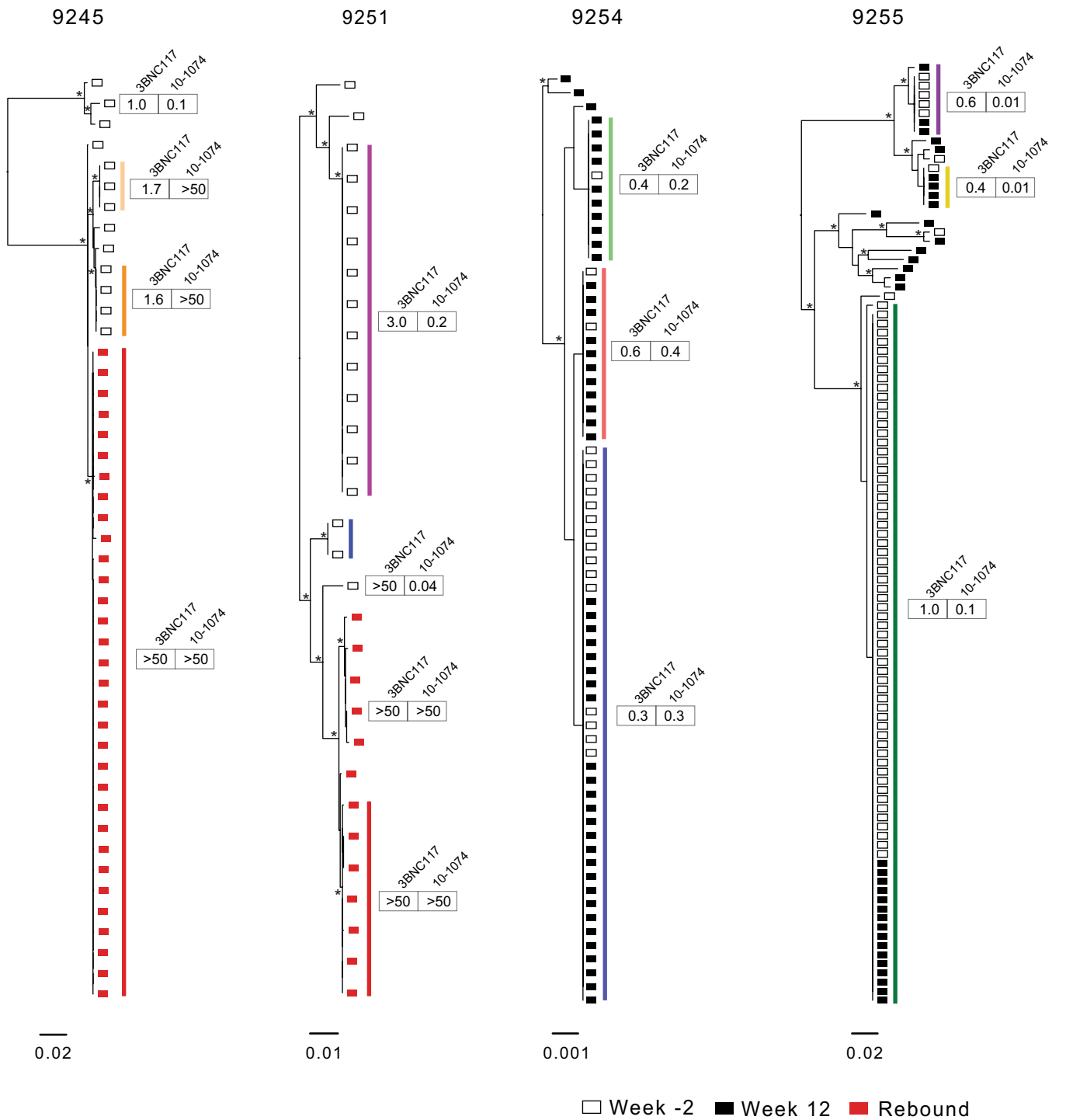
Extended Data Fig. 6 | Amino acid variants at 3BNC117 contact sites of reactivated latent and rebound viruses. Colour charts show 3BNC117 contact sites in Env according to HXB2 numbering. Diagram shows the 13 participants that experienced viral rebound before week 30. LR, latent reservoir viruses isolated by Q^2VOA (on weeks −2 and 12 when available); RB, rebound viruses isolated by SGA (plasma) and viral outgrowth (PBMCs). Each amino acid is represented by a colour and the frequency of each amino acid is indicated by the height of the rectangle. Shaded rectangles indicate the lack of variation and full-colour rectangles represent amino acid residues with changes in the distribution between the reservoir and rebound.



Extended Data Fig. 7 | Comparison of the circulating latent reservoir and rebound viruses. Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^2VOA, rebound plasma SGA and rebound PBMC outgrowth cultures from participants 9241, 9244, 9246 and 9247, who rebounded before week 30. Open and closed black rectangles indicate Q^2VOA-derived viruses from week −2 and week 12, respectively. Viruses obtained at the time of rebound are indicated by red rectangles (plasma SGA) and red stars (rebound PBMC outgrowth cultures). Asterisks indicate nodes with significant bootstrap values (bootstrap support ≥70%). Clones are denoted by coloured lines mirroring the colours of slices in Extended Data Fig. 10a. Boxes indicate IC_{80} values (μg ml^{-1}) of 3BNC117 and 10-1074 against representative viruses throughout the phylogenetic tree and clones, when possible (Supplementary Table 4). Asterisks in boxes indicate $IC_{100} > 50$ μg ml^{-1}.

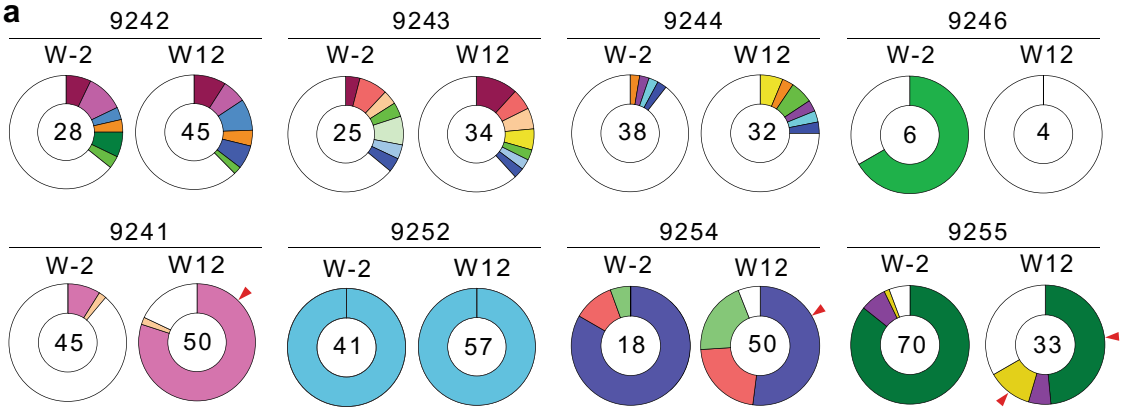


Extended Data Fig. 8 | Recombination events in rebound viruses. **a**, Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^2VOA cultures and rebound SGA in the four participants for whom rebound viruses showed recombination events. Open and closed black rectangles indicate Q^2VOA-derived viruses from week −2 and week 12, respectively. Rebound plasma SGA- or outgrowth-derived viruses are indicated by closed red rectangles. Green stars represent parent sequences that underwent recombination to produce the child sequences (red stars). **b**, Circos plots indicating the relationship between the parent sequences and the recombinants. Open and closed black rectangles indicate Q^2VOA-derived sequences from week −2 and week 12, respectively. Rebound virus sequences are indicated by red rectangles. The thickness of the black outer bars represents the number of sequences obtained from that particular clone.



Extended Data Fig. 9 | Phylogenetic trees of participants 9245, 9251, 9254 and 9255. Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^2VOA cultures and rebound plasma SGA and rebound outgrowth from the two participants (9245 and 9251) with pre-existing resistance to one of the two antibodies and the two sensitive participants (9254 and 9255) who maintained viral suppression for >30 weeks (end of the study). Open and closed black rectangles indicate Q^2VOA-derived viruses from week −2 and week 12, respectively. Rebound plasma SGA viruses are indicated by closed red rectangles. Asterisks indicate nodes with significant bootstrap values (bootstrap support ≥ 70%). Clones are denoted by coloured lines beside the phylogenetic tree. Numbers correspond to 3BNC117 and 10-1074 IC_{80} neutralization titres.

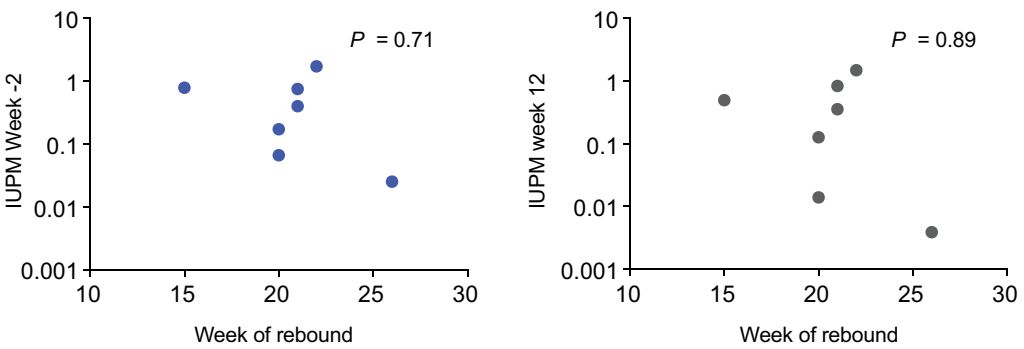
a



b

Study ID	Total *env* seqs by Q^2VOA	Clonal *env* sequences		IUPM	
	(no.)	(no.)	(%)	week -2	week 12
9242	73	27	37.0	0.781	0.493
9243	59	22	37.3	0.170	0.126
9244	70	12	17.1	0.397	0.354
9246	10	4	40.0	0.066	0.014
9247	3	3	100.0	0.025	0.004
9241	95	46	48.4	0.743	0.828
9252	98	98	100.0	1.709	1.470
9254	68	65	95.6	N/A	0.680
9255	103	88	85.4	1.890	1.400
Total	**579**	**365**	**63.0**		

c



Extended Data Fig. 10 | Clonal distribution of the circulating latent reservoir and IUPM changes. a, Pie charts depicting the distribution of Q^2VOA-derived *env* sequences obtained at weeks −2 (W−2) and week 12 (W12). Number in the inner circle indicates the total number of analysed *env* sequences. White represents sequences isolated only once across both time points and coloured slices represent identical sequences that appear more than once (clones). The size of each pie slice is proportional to the size of the clone. Red arrows denote clones that significantly change in size ($P \leq 0.05$ (two-sided Fisher's exact test)) between the two time points. **b**, Summary of clonal *env* sequences and IUPM in the nine individuals with an antibody-sensitive reservoir. **c**, IUPM versus time of viral rebound in the antibody-sensitive individuals ($n = 7$) who rebounded within the study observation period (30 weeks). *P* values were obtained using a two-tailed Pearson correlation test comparing the two variables.

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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Software for obtaining the sequencing data was MiSeq Control Software 2.6 2.1. Software for tracking the clinical samples was IRIS by iMedRIS.
Data analysis	- Pharmacokinetics analysis was performed with Phoenix WinNonlin Build 8 (Certara). - Effects of antibody combination on time to viral rebound was compared to previous published data with antibody monotherapy or in the antibody treatment. R (version 3.4.2) with the package coin (version 1.2-2) was used for exact Wilcoxon tests in the presence of ties, as well as flexsurv (version 1.1) and fitdistrplus (version 1.0-9) for parametric survival regression. The weighted log-rank test was implemented in Matlab (version R2018a). Class probabilities were estimated with a lasso logistic regression model (Matlab function lassoglm) using five values for lambda and threefold cross-validation. - Analysis of HIV-1 envelope sequences was performed at two time points during viral suppression and at viral rebound. Nucleotide alignments of intact env sequences were translation-aligned using ClustalW v2.1. Maximum likelihood phylogenetic trees were then generated from these alignments with PhyML v3.1. Multiple alignment of nucleotide sequences guided by amino acid translations of env sequences was performed by TranslatorX (http://translatorx.co.uk/). Latent and rebound sequences were analyzed for the presence of recombination using the 3SEQ recombination algorithm (http://mol.ax/software/3seq/). - Levels of antibody in serum by ELISA were calculated with Softmax Pro, v5.4.5 (Molecular Devices).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The sequences will be available in GenBank upon publication with the accession numbers MH575375-MH576416.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	One of the primary outcomes of the study was the percentage of participants who met ART reinitiation criteria prior to week 8. A one-sided upper confidence interval was constructed for the probability of meeting ART reinitiation criteria using the Clopper-Pearson method. As such, a sample size of 15 HIV-infected individuals would allow the rejection of the null hypothesis (rate = 0.85) with 80% power for an effect size equal or higher than 0.33, if at least 6 out of 15 participants enrolled in Group 2 did not experience viral rebound by week 8 (2 weeks after last mAb infusion). If 10 or more participants experienced viral rebound prior to week 5 [5 weeks after ART interruption and weeks after second 3BNC117 and 10-1074 infusions], additional participants would not undergo ART interruption.
Data exclusions	No data were excluded.
Replication	This study was a clinical trial and the analyses were performed on individual trial participants. Experiments did not include replicates as all participants and data points are unique. All available data is included in the manuscript.
Randomization	The study was single arm.
Blinding	The study was open label.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	The study included the analyses of blood samples collected from enrolled HIV-infected trial participants. These samples were obtained under an IRB approved protocol for the purposes of this study and associated analytical plan, therefore they cannot be shared without appropriate IRB review and future studies would be limited to what was covered under the original informed consent.

Antibodies

Antibodies used

3BNC117 and 10-1074 are investigational anti-HIV-1 neutralizing antibodies manufactured for clinical use. They are being investigated under US FDA INDs 118225 and 123713.

Antibodies for the Ab detection in serum by ELISA included:
- Anti-ID 3BNC117: DHVI Protein Production Facility
  Lot #: 3BNC 29Nov2017
  Dilution: 4 ug/ml coating concentration
  Clone name: anti-ID 1F1-2E3 mAb
- Anti-ID 10-1074: DHVI Protein Production Facility
  Lot #: 3Aug2016
  Dilution: 2 ug/ml coating concentration
  Clone name: anti-ID 3A1-4E11 mAb
- (HRP)-conjugated mouse anti-human IgG kappa-chain-specific antibody (Abcam), Catalog #: ab79115
  Dilution: 1:15,000
  Clone name: SB81a

Validation

3BNC117 and 10-1074 that were administered to the participants were manufactured by Celldex Therapeutics under Good Manufacturing Practice and have been fully characterized in terms of biophysical properties and potency (INDs 118225 and 123713). Both drug products are under long term stability monitoring.
Anti-idiotypic antibodies from the Duke Human Vaccine Institute (DHVI) Protein Production facility have been validated for their use in ELISA against human antibodies.
HRP-mouse monoclonal anti-human IgG kappa-chain-specific antibody has been validated for its use in ELISA and ICC/IF, reactivity against Human Kappa Chain. This product has been referenced in Scheid JF et al. Nature 535:556-60 (2016).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

MOLT-4/CCR5 cell lines obtained from NIH AIDS Reagent Program, catalog number 4984.
A stable hibridoma cell line from the Duke Protein Production Facility was utilized for the production of anti-idiotypic mAbs for the quantification of Ab levels in serum by ELISA.

Authentication

The cells were analyzed by flow cytometry to determine the levels of CCR5 and CD4 expression.

Mycoplasma contamination

The cell lines were not tested for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Eligible participants were adults aged 18-65 years, HIV-1-infected, on ART for a minimum of 24 months, with plasma HIV-1 RNA levels of 50 copies/ml for at least 18 months (one viral blip of >50 but <500 copies/ml during this 18-month period was allowed), plasma HIV-1 RNA levels of 20 copies/ml at the screening visit, and a current CD4+ T cell count 500 cells/µl.

Recruitment

Participants were pre-screened for sensitivity of latent proviruses against 3BNC117 and 10-1074 antibodies by bulk PBMC viral outgrowth. Sensitivity was defined as an IC50 <2 µg/ml for both 3BNC117 and 10-1074 against outgrowth virus. Participants harboring sensitive viruses were invited for screening and were enrolled in the study sequentially. Participants were enrolled at two clinical sites at the Rockefeller University (New York, US) and Cologne University Hospital (Germany).