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

Pilar Mendoza[1,*], **Henning Gruell**[2,3,4,*], **Lilian Nogueira**[1], **Joy A. Pai**[1], **Allison L. Butler**[1], **Katrina Millard**[1], **Clara Lehmann**[3,4,5], **Isabelle Suárez**[3,4,5], **Thiago Y. Oliveira**[1], **Julio C.C. Lorenzi**[1], **Yehuda Z. Cohen**[1], **Christoph Wyen**[3,6], **Tim Kümmerle**[3,6], **Theodora Karagounis**[1], **Ching-Lan Lu**[1], **Lisa Handl**[7], **Cecilia Unson-O'Brien**[1], **Roshni Patel**[1], **Carola Ruping**[2], **Maike Schlotz**[2], **Maggi Witmer-Pack**[1], **Irina Shimeliovich**[1], **Gisela Kremer**[3], **Eleonore Thomas**[3], **Kelly E. Seaton**[8], **Jill Horowitz**[1], **Anthony P. West Jr.**[9], **Pamela J. Bjorkman**[9], **Georgia D. Tomaras**[8], **Roy M. Gulick**[10], **Nico Pfeifer**[7], **Gerd Fätkenheuer**[3,4], **Michael S. Seaman**[11], **Florian Klein**[2,4,5,#], **Marina Caskey**[1,#], and **Michel C. Nussenzweig**[1,12,#]

[1]Laboratory of Molecular Immunology, The Rockefeller University, New York, New York, USA

[2]Laboratory of Experimental Immunology, Institute of Virology, University Hospital Cologne, Cologne, Germany

[3]Department I of Internal Medicine, University Hospital Cologne, Cologne, Germany

[4]German Center for Infection Research, partner site Bonn–Cologne, Cologne, Germany

[5]Center for Molecular Medicine Cologne (CMMC), University of Cologne, Germany

[6]Praxis am Ebertplatz, Cologne, Germany

[7]Methods in Medical Informatics, Department of Computer Science, University of Tübingen, Tübingen, Germany

[8]Duke Human Vaccine Institute, Departments of Surgery, Immunology, and Molecular Genetics and Microbiology, Duke University, Durham, North Carolina, USA

Correspondence and requests for materials should be addressed to M.C. (mcaskey@rockefeller.edu), F.K. (florian.klein@uk-koeln.de), or M.C.N. (nussen@rockefeller.edu).
* or #equal contribution.

Data Availability Statement

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

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Author information

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The authors declare competing interest: there are patents on 3BNC117 and 10-1074 on which M.C.N is an inventor.

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[9]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, USA

[10]Division of Infectious Diseases, Weill Cornell Medicine, New York, New York, USA

[11]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, USA

[12]Howard Hughes Medical Institute, The Rockefeller University, New York, New York, USA

Abstract

HIV-1-infected individuals require lifelong antiretroviral therapy (ART) because treatment interruption leads to rapid rebound viremia. 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 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 15 to > 30 weeks (median = 21 weeks), and none developed viruses resistant to both antibodies. We conclude that the combination of anti-HIV-1 monoclonal antibodies 3BNC117 and 10-1074 can maintain long-term suppression in the absence of ART 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 pyroptosis1, a small percentage survive and harbor 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 therapy2. 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-lives3–5.

In human clinical trials, viremic individuals receiving either 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 responses6–8. Moreover, in HIV-1-infected individuals undergoing analytical treatment interruption (ATI) of antiretroviral therapy, four infusions of 3BNC117 maintained suppression for a median of 10 weeks as compared to 2.3 weeks in historical controls9,10. In contrast, six infusions of VRC01 maintained suppression for 5.6 weeks11. The difference in activity between VRC01 and 3BNC117 in pre-clinical experiments12,13, and clinical trials6,7,9,11 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 variants6–9,11. Like antiretroviral drugs, combinations of bNAbs are more effective than individual antibodies in humanized mouse and macaque models of infection14–16. In contrast, antibody combinations showed little if any efficacy in suppressing viremia during

ATI in humans17,18. However, these earlier studies were performed using bNAbs which were less potent than 3BNC117 and 10-1074. Here we re-examine the question of whether bNAb combinations 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 using the TZM-bl neutralization assay19. 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 (IC_{50} ≤ 2μg/ml, Extended Data Fig. 1a and Supplementary Table 1)8,9,20.

Study eligibility criteria included ongoing ART for at least 24 months with plasma HIV-1 RNA levels of < 50 copies/ml for at least 18 months (with one blip < 500 copies/ml allowed) and < 20 copies/ml at screening, as well as $CD4^+$ T cell counts > 500 cells/μl (Extended Data Fig. 1b and 2a). Enrolled participants received 3 infusions of 30 mg/kg each of 3BNC117 + 10-1074 at 3-week intervals beginning 2 days before treatment interruption (Fig. 1a). Individuals whose regimens contained non-nucleoside reverse transcriptase inhibitors were switched to an integrase inhibitor-based regimen 4 weeks before discontinuing ART (Extended Data Fig. 1b and 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 viremia of > 200 copies/ml was confirmed. Time of viral rebound was defined as the first of two consecutive viral loads > 200 copies/ml. Fifteen individuals were enrolled, but four of them showed viral loads of > 20 copies/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/μl at the time of first antibody infusion and at rebound, respectively (Extended Data Fig. 2b and Supplementary Table 2). Re-initiation of ART after viral rebound resulted in resuppression of viremia (Supplementary Table 2). We conclude that combination therapy with 3BNC117 + 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 ELISA (Extended Data Fig. 2c, d and Supplementary Table 2). 3BNC117 had a half-life of 12.5 and 17.6 days by TZM-bl and ELISA, respectively (Extended Data Fig. 2c, d). The half-life of 10-1074 was 19.1 and 23.2 days by TZM-bl and ELISA, respectively; longer than 3BNC117 in both assays ($P = 0.0002$ and $P = 0.02$, Extended Data Fig. 2c, d, e, f). These measurements are similar to those observed when each antibody was administered alone in ART-treated HIV-1-infected individuals6,8,9. We conclude that the pharmacokinetic profiles of 3BNC117 and 10-1074 are not altered when they are co-administered.

Combination bNAbs Maintain Viral Suppression

For the 11 individuals who had complete viral suppression (HIV-1 RNA < 20 copies/ml) during the screening period and at day 0, combination antibody therapy was associated with maintenance of viral suppression for 5 to > 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 non-interventional ATI studies10 and 6-10 weeks for monotherapy with 3BNC1179 (Fig. 1c). Altogether, 9 of the 11 participants maintained viral suppression for over 15 weeks, while 2 rebounded at weeks 5 and 7 (Fig. 1b, c).

Quantitative and qualitative viral outgrowth assays (Q^2VOA) were used to retrospectively analyze 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 rebounding early, 9245 and 9251, harbored 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 to one of the two bNAbs. Consistent with this idea, the delay in rebound in these two participants was within the range anticipated for antibody monotherapy9,11 (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 Fig. 4, 5 and Supplementary Table 4).

To examine the viruses arising in the early rebounding individuals, we performed single genome analysis (SGA) on rebound plasma. In addition to the pre-existing sequences associated with resistance in the 10-1074 target site (N332T + 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 individuals studied. This result is not surprising given that bulk cultures are dominated by a limited number of rapidly growing viral species which may not be representative of the diversity in the latent reservoir.

The median time to rebound in the 7 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 6-10 weeks for monotherapy with 3BNC1179 (Fig. 1c). In these participants, viral suppression was maintained for 15 to 26 weeks after ART discontinuation (Supplementary Table 2). The 2 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. The average 3BNC117 serum concentration (determined by TZM-bl assay)

at the time of rebound in sensitive individuals that rebounded during study follow-up was 1.9 μg/ml (Fig. 1b and Supplementary Table 2). In contrast, the average serum concentration of 10-1074 at rebound was 14.8 μg/ml (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, d, e, f and Supplementary Table 2). Finally, these 9 individuals showed little or no pre-existing neutralizing antibodies against a diagnostic panel of viruses before bNAb infusion (Supplementary Table 5).

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 sensitivity of rebound outgrowth viruses and/or pseudoviruses to 3BNC117 and 10-1074 by 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 analyzed 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 (Fig. 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 individuals20,21 (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 (Fig. 3, Extended Data Fig. 7 and 9)9. In contrast, rebound viruses are consistently polyclonal during ATI in the absence of antibody therapy22,23. Thus, the antibodies restrict the outgrowth of latent viruses *in vivo*.

The emerging viruses in 6 of the 7 individuals that rebounded when the mean 3BNC117 and 10-1074 concentrations were 1.9 and 14.8 μg/ml, respectively, carried resistance-associated mutations in the 10-1074 target site (Fig. 1b, 2a). Consistent with the sequence data, these rebound viruses were generally resistant to 10-1074 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, 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 9 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 clones20,24–26(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 the time points and some of these changes were significant (Extended Data Fig. 10a). To determine the number of infectious units per million (IUPM, http://silicianolab.johnshopkins.edu/), 6.0 x 10^7 - 6.2 x 10^8 $CD4^+$ T cells were assayed by Q^2VOA for each time point for each individual (Fig. 4b). The difference between the 2 time points was never greater than 6.5-fold for any individual, and the 2 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 receiving immunotherapy27.

Discussion

First generation anti-HIV-1 bNAbs were generally ineffective in suppressing viremia in animal models and humans leading to the conclusion that this approach should not be pursued17,18,28. The advent of new methods for anti-HIV-1 antibody cloning29 and subsequent discovery of a new more potent generation of bNAbs revitalized this area of research30,31.

bNAb monotherapy with 3BNC117 or VRC01 is not enough to maintain control during ATI in HIV-1-infected humans6–9,11. Similar results were obtained in participant 9251 who effectively received 10-1074 monotherapy due to pre-existing resistance to 3BNC117. In 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 in serum. Rebound occurred when 3BNC117 levels dropped below 10 μg/ml effectively leading to 10-1074 monotherapy, from which nearly all individuals rapidly escaped by mutations in the 10-1074 contact site. The observation that 9 individuals infected with distinct viruses were unable to develop double resistant viruses over a median 21 week period suggests that viral replication was severely limited by this antibody combination.

In human studies, monotherapy with 3BNC117 is associated with enhanced humoral immunity and accelerated clearance of HIV-1-infected cells5,32. In addition, when administered early to $SHIV_{AD8}$-infected macaques, combined 3BNC117 + 10-1074 immunotherapy induced host $CD8^+$ T cell responses that contributed to the control of viremia in nearly 50% of the animals3. However, virus-specific $CD8^+$ T cells responsible for control of viremia in these macaques were not detected in the circulation, and their

contribution to viral suppression was only documented after $CD8^+$ T cell depletion3. In most controller macaques, complete viral suppression was only established after rebound viremia that followed antibody clearance.

Two individuals in this study remained suppressed for over 30 weeks after ATI, 9254 and 9255. Neither one 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 control33. 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/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/ml and had an IUPM of 1.4 at the 12-week time point. A small fraction of individuals on ART10 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 infection34–38. Whether antibody-enhanced $CD8^+$ T cell responses contribute to the prolonged control in the 2 of 9 individuals receiving combination immunotherapy, and whether this effect can be enhanced by latency reactivating agents, or immune checkpoint inhibitors remains to be determined.

A significant fraction of the circulating latent reservoir is composed of expanded clones of infected T cells24,26,39–42. 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 time24,25. 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 harboring viruses sensitive to 3BNC117 and 10-1074 maintained viral suppression during ATI for a median of almost 4 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 antibodies14,15,43, or long-acting small molecule antiretroviral drugs.

In macaques, the therapeutic efficacy of anti-HIV-1 antibodies is directly related to their half-life4,12,13, which can be extended by mutations that enhance Fc domain interactions with the neonatal Fc receptor4,13,44. These mutations also increase the half-life of antibodies in humans by 2-4-fold45. Our data suggest that a single administration of combinations of bNAbs with extended half-lives could maintain suppression for 6-12 months in individuals harboring sensitive viruses.

Methods

Study design

An open-label phase 1b study was conducted in HIV-1-infected participants who were virologically suppressed on antiretroviral therapy (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 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 viral load increased to ≥ 200 copies/ml or $CD4^+$ T cell counts decreased to < 350 cells/μl in two consecutive measurements. Time of viral rebound was determined by the first viral load > 200 copies/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 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/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 < 20 copies/ml at the screening visit, and a current $CD4^+$ T cell count > 500 cells/μl. In addition, participants were pre-screened for sensitivity of latent proviruses against 3BNC117 and 10-1074 by bulk PBMC viral outgrowth culture as described below. Sensitivity was defined as an IC_{50} < 2 μg/ml for both 3BNC117 and 10-1074 against outgrowth virus. Participants on an NNRTI-based ART regimen were switched to an integrase inhibitor-based regimen (dolutegravir plus tenofovir disoproxil fumarate/emtricitabine) 4 weeks before treatment interruption due to the prolonged half-life of NNRTIs. Exclusion criteria included reported $CD4^+$ T cell nadir of < 200 cells/μl, 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 level of 30 mg/kg. 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 minutes. Study participants were observed at the Rockefeller University Hospital or the University Hospital Cologne for one hour 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

hematology, 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 to 2 weeks. After ART was re-initiated, participants returned for follow up every 2 weeks until viral re-suppression was achieved, and every 8 weeks thereafter. Study investigators evaluated and graded adverse events according to the DAIDS AE Grading Table (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. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation. The absolute number of PBMCs was determined by 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 quantitate HIV-1 RNA over a range of $2x10^1$ to $1×10^7$ copies/ml. These assays were performed at LabCorp or at the University Hospital Cologne.

$CD4^+$ T cells

$CD4^+$ T-cell counts were determined by a 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 previously5.

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.

3BNC117 and 10-1074 serum concentrations were measured by a validated sandwich ELISA. High bind polystyrene plates were coated with 4 μg/ml of an anti-idiotypic antibody specifically recognizing 3BNC117 (anti-ID 1F1-2E3 mAb) or 2μg/ml of an anti-idiotypic antibody specifically recognizing 10-1074 (anti-ID 3A1-4E11 mAb), 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, QCs 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 antihuman IgG Fc-specific antibody for 10-1074 (Jackson ImmunoResearch) and the HRP substrate tetra-methylbenzidine. 3BNC117 and 10-1074 concentrations were then calculated from a standard curve of 3BNC117 or 10-1074 run on the same plate using a 5-PL curve-fitting algorithm (Softmax Pro, v5.4.5). 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 mAbs were produced using a stable hybridoma cell line (Duke Protein Production Facility;6). The lower limit of quantitation for the 3BNC117 ELISA is 0.78 μg/ml and for the 10-1074 ELISA is 0.41 μg/ml. The lower limit of detection was determined to be 0.51 μg/ml and 0.14 μg/ml in HIV-1 seropositive serum for the 3BNC117 and 10-1074 ELISA, respectively. For values that were detectable (i.e., positive for mAb) but were below the lower limit of quantitation, values are reported as < 0.78 μg/ml and < 0.41 μg/ml 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 3-fold from background were excluded from the analysis of PK 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 described19. Briefly, serum samples were tested using a primary 1:20 dilution with a 5-fold 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 where serum ID_{50} titers against X2088_c9 were > 100,000, serum samples were also tested against a less sensitive strain, Du422 (Supplementary Table 2). To generate standard curves, 3BNC117 and 10-1074 clinical drug products were included in every assay set-up using a primary concentration of 10 μg/ml with a 5-fold titration series. Serum concentrations of 3BNC117 and 10-1074 for each sample were calculated as follows: serum ID_{50} titer (dilution) x 3BNC117 IC_{50} or 10-1074 IC_{50} titer (μg/ml) = serum concentration of 3BNC117 or 10-1074 (μg/ml). 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. Seaman unpublished data). Virus pseudotyped with the envelope protein of murine leukemia virus (MuLV) was utilized as a negative control. Antibody concentrations were calculated using the serum ID_{80} titer and monoclonal antibody IC_{80} if non-specific activity against MuLV was detected (ID_{50} > 20; 9246, week 30; 9248, baseline, d0, wk 18). All assays were performed in a laboratory meeting GCLP standards.

Pre-Screening Bulk PBMC culture

To test HIV-1 viral strains for sensitivity to 3BNC117 and 10-1074, we performed bulk viral outgrowth cultures by co-culturing isolated $CD4^+$ T cells with MOLT-4/CCR-5 cells or $CD8^+$ T cell-depleted donor lymphoblasts. PBMCs for pre-screening 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 μg/ml were deemed sensitive.

Quantitative and Qualitative Viral Outgrowth Assay (Q^2VOA)

The quantitative and qualitative viral outgrowth assay (Q^2VOA) was performed using isolated PBMCs from leukapheresis at week -2 and week 12 as previously described24. Briefly, isolated $CD4^+$ T cells were activated with 1 μg/ml phytohemagglutinin (Life Technologies) and 100 U/ml IL-2 (Peprotech) and co-cultured with 1 x 10^6 irradiated PBMCs from a healthy donor in 24-well plates. A total of 6 x 10^7 - 6.2 x 10^8 cells were assayed for each individual at each of the 2 time points. After 24 hours, PHA was removed and 0.1 x 10^6 MOLT-4/CCR5 cells were added to each well. Cultures were maintained for 2 weeks, splitting by half the MOLT- 4/CCR5 cells 7 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 infectious units per million (IUPM) algorithm developed by the Siliciano lab (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 x 10^6 beads per 10^6 $CD4^+$ T cells and 20 U/ml of IL-2. Rebound outgrowth was performed using PBMCS from the highest viral load sample (usually the repeat measurement ≥ 200 copies/ml). Viruses whose 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 described19.

Sequencing

HIV-1 RNA extraction and single genome amplification was performed as previously described46. 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 first PCR product 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′. *env* 3′ half-genome was amplified in a single PCR using B3F3 5′-TGGAAAGGTGAAGGGGCAGTAGTAATAC-3′ and R3B6R 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 and reverse primer 5′-CATAGGAGATGCCTAAGCCGGTGGAGCTCTGCTTATATAGACCTC. Individual *env* amplicons were amplified using forward primer 5’-CACC GGCTTAGGCATCTCCTATGGCAGGAAGAA and reverse primer 5’-GTCTCGAGATACTGCTCCCACCC. The CMV promoter amplicon was fused to individual purified *env* amplicons by PCR using forward primer 5′-AGTAATCAATTACGGGGTCATTAGTTCAT and reverse primer 5′-ACTTTTTGACCACTTGCCACCCAT. 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, 100 nM forward and reverse primers, 200 μM dNTP mix, 1 x 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 analyzed by gel electrophoresis, purified by gel extraction, and co-transfected with pSG3Δenv into HEK293T cells to produce pseudoviruses as described previously47.

Sequence and phylogenetic analysis

Nucleotide alignments of intact *env* sequences were translation-aligned using ClustalW v2.148 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 v3.149 using the GTR model with 1,000 bootstraps. For the combined analysis of sequences from all participants, *env* sequences were aligned using MAFFT v7.309 and clustered using RAxML v8.2.950 under the GTRGAMMA model with 1,000 bootstraps. To analyze changes between reservoir and rebound viruses, *env* sequences were aligned at the amino acid level to a HXB2 reference using ClustalW v2.1.

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Statistical analyses

Pharmacokinetic parameters were estimated by performing a non-compartmental analysis (NCA) using Phoenix WinNonlin Build 8 (Certara), using all PK data available 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 t-test. $CD4^+$ T cell counts on day 0 and at the time of viral rebound were compared by two-tailed paired t-test. IUPM determined at week -2 and week 12 compared using a two-tailed paired t-test. Time to rebound in current trial participants (combination therapy with 3BNC117 + 10-1074), participants receiving 3BNC117 monotherapy9 and participants in previous non-interventional ATI studies conducted by ACTG10 were plotted using Kaplan-Meier survival curves. Potential correlation between IUPM and time to rebound was analyzed by two-tailed Pearson correlation test.

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 analyzed for the presence of recombination using the 3SEQ recombination algorithm (http://mol.ax/software/3seq/). Sequences presenting 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/).

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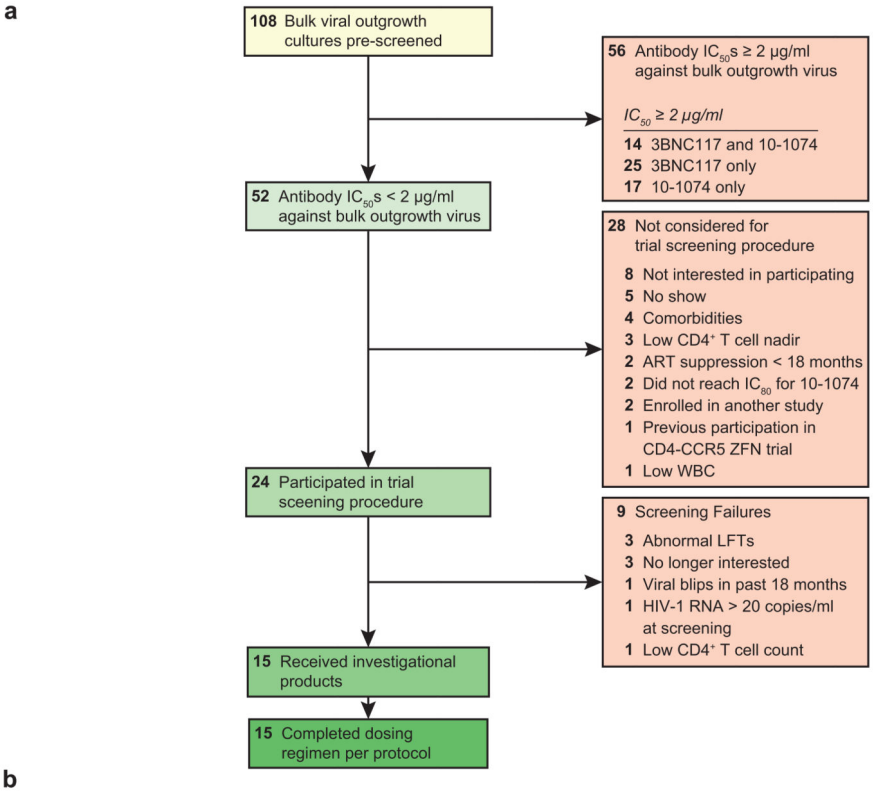
Extended Data

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 IC50	3BNC117 IC80	10-1074 IC50	10-1074 IC80	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	<20	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 D	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	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	<20 D	>30

Extended Data Figure 1. Study participant selection and demographics.

a, Flow diagram indicating the selection of study participants. **b**, Table showing individual participant demographics and baseline clinical characteristics. * EVG – elvitegravir, cobi - cobicistat, TDF - tenofovir disoproxil fumarate, FTC - emtricitabine, RPV - rilpivirine, EFV - efavirenz, TAF - tenofovir alafenamide fumarate, D, RTV – ritonavir, ABC – abacavir, 3TC - lamivudine, DTG – dolutegravir. ** NNRTI-based regimens were switched four

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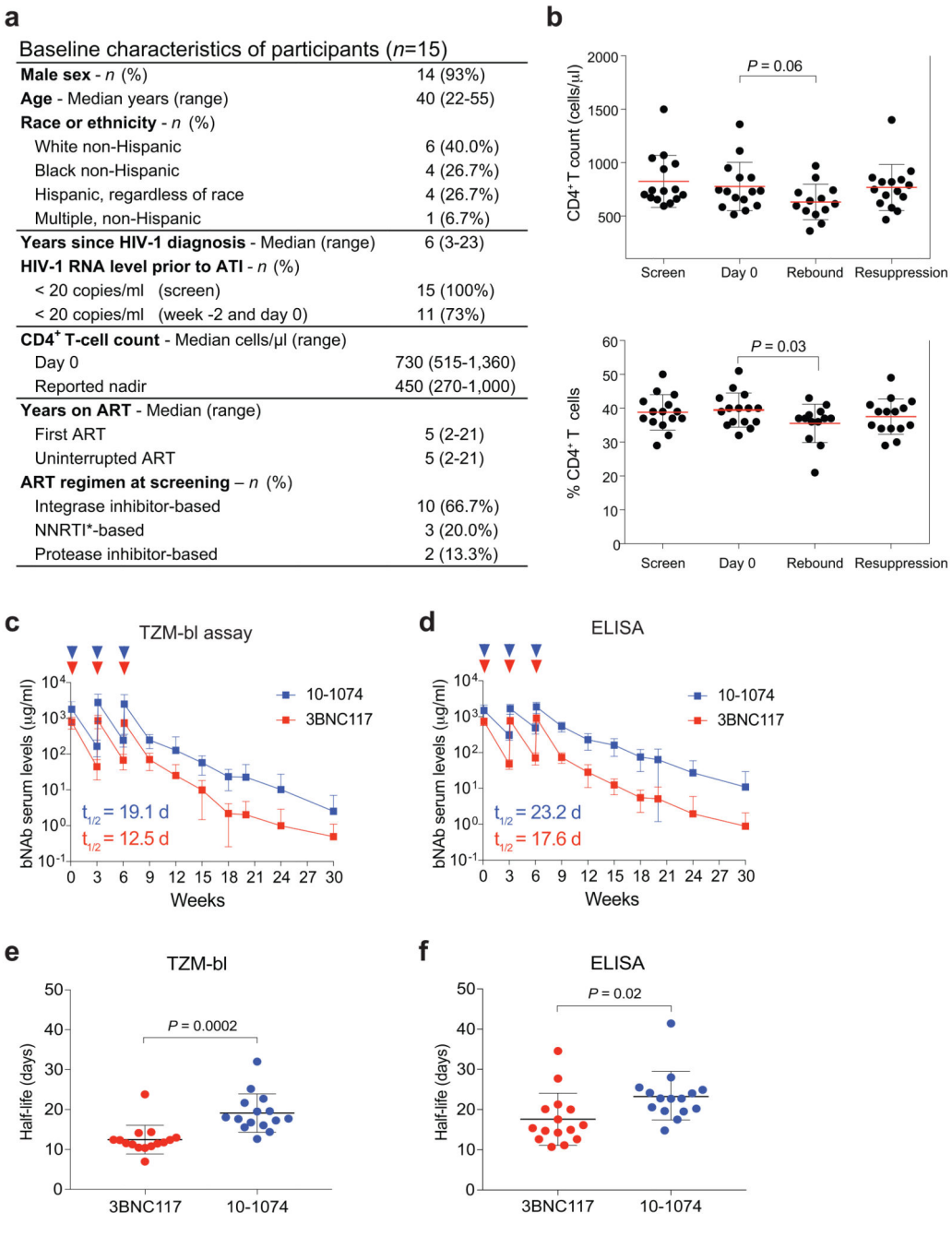
weeks before ART interruption due to longer half-lives of NNRTIs. *** Pre-screening of bulk outgrowth virus obtained from PBMC culture by TZM-bl assay. # All participants harbored clade B viruses. Viral load < 20 D: Plasma HIV-1 RNA detected but not quantifiable by clinical assay. Dx, diagnosis; Scr, screening; Wk -2, week -2; d0, day 0. Grey shaded rows indicate participants who were found to have detectable viremia (HIV-1 VL > 20 copies/ml) at week -2 or day 0. These participants were not included in the efficacy analyses given lack of viral suppression at baseline.

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%)



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Extended Data Figure 2. Demographics, $CD4^+$ T cells during study period in participants and pharamacokinetics of 3BNC117 and 10-1074.

a, Table showing baseline participant demographics. *NNRTI - Non-nucleoside reverse transcriptase inhibitor. **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 and error bars indicate standard deviation. *P* values were obtained using a two-tailed paired 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**). Curves indicate mean serum antibody concentrations and error bars represent standard deviation. Red and blue triangles indicate 3BNC117 and 10-1074 infusions, respectively. In the TZM-bl assay, lower limits of detection were 0.46 μg/ml and 0.01 μg/ml for 3BNC117 and 10-1074, respectively (**c**). In the ELISA, lower limits of detection were 0.78 μg/ml and 0.41 μg/ml, respectively (**d**). In cases where 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. **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 t-test comparing the two antibodies.

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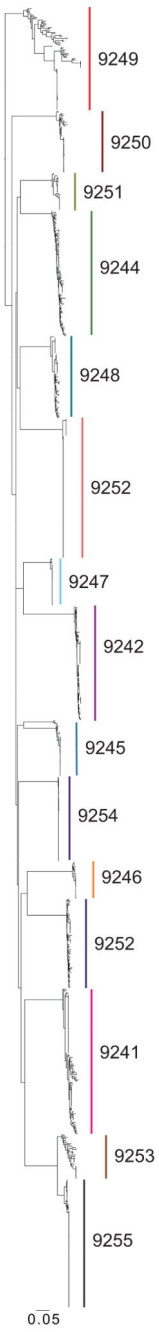
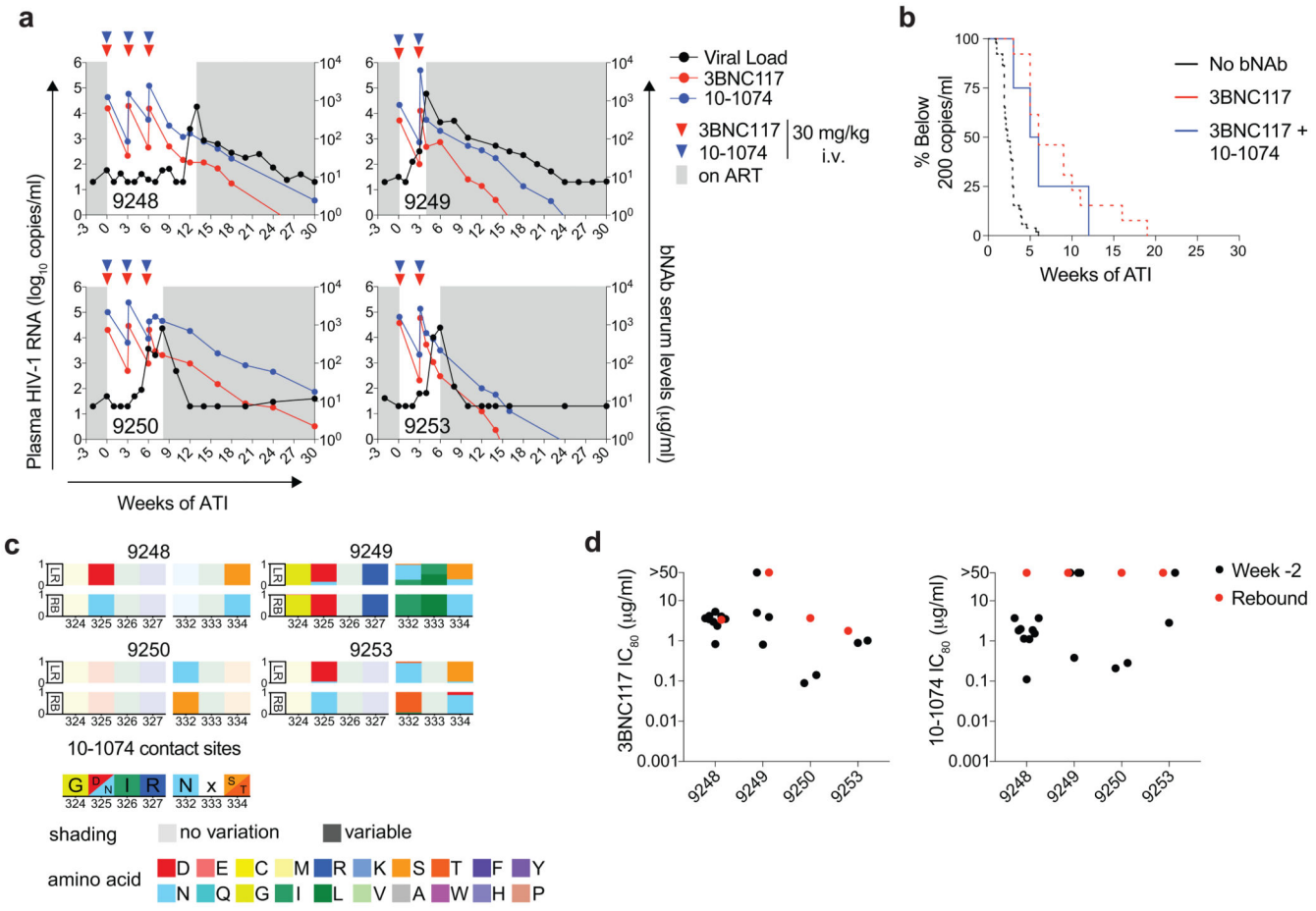


Extended Data Figure 3. Phylogenetic tree of viruses from all enrolled participants.

Maximum likelihood phylogenetic trees of full-length *env* sequences containing all the 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 colors.



Extended Data Figure 4. Viral rebound, amino acid variants at 10-1074 contact sites and sensitivities of latent and rebound viruses in the participants with detectable viremia >20 copies/ml 2 weeks prior to 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/ml. **b**, Kaplan-Meier plots summarizing time to viral rebound. Y-axis indicates percentage of participants that maintain viral suppression. X-axis indicates weeks after start of ATI. Participants receiving the combination of 3BNC117 + 10-1074 are indicated by the blue line (n=4). Dotted red line indicates a cohort of individuals receiving 3BNC117 alone during ATI9 (n=13) and dotted black line indicates a cohort of participants who underwent ATI without any intervention10 (n=52). **c**, Color 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 indicates latent reservoir viruses isolated by Q^2VOA (week -2) and RB indicates rebound viruses isolated by SGA (plasma) or viral outgrowth (PBMCs). Each amino acid is represented by a color 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-color rectangles represent amino acid residues

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with changes in distribution between reservoir and rebound viruses. **d**, Dot plots indicating IC_{80} (μg/ml) 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). 9248 and 9250 had pre-existing viruses that failed to reach an IC_{100} when tested up to 50 μg/ml for 3BNC117 (Extended Data Fig. 5). Rebound viruses of all 4 participants showed IC_{80} or IC_{100} of > 50 μg/ml for both 3BN117 and 10-1074.

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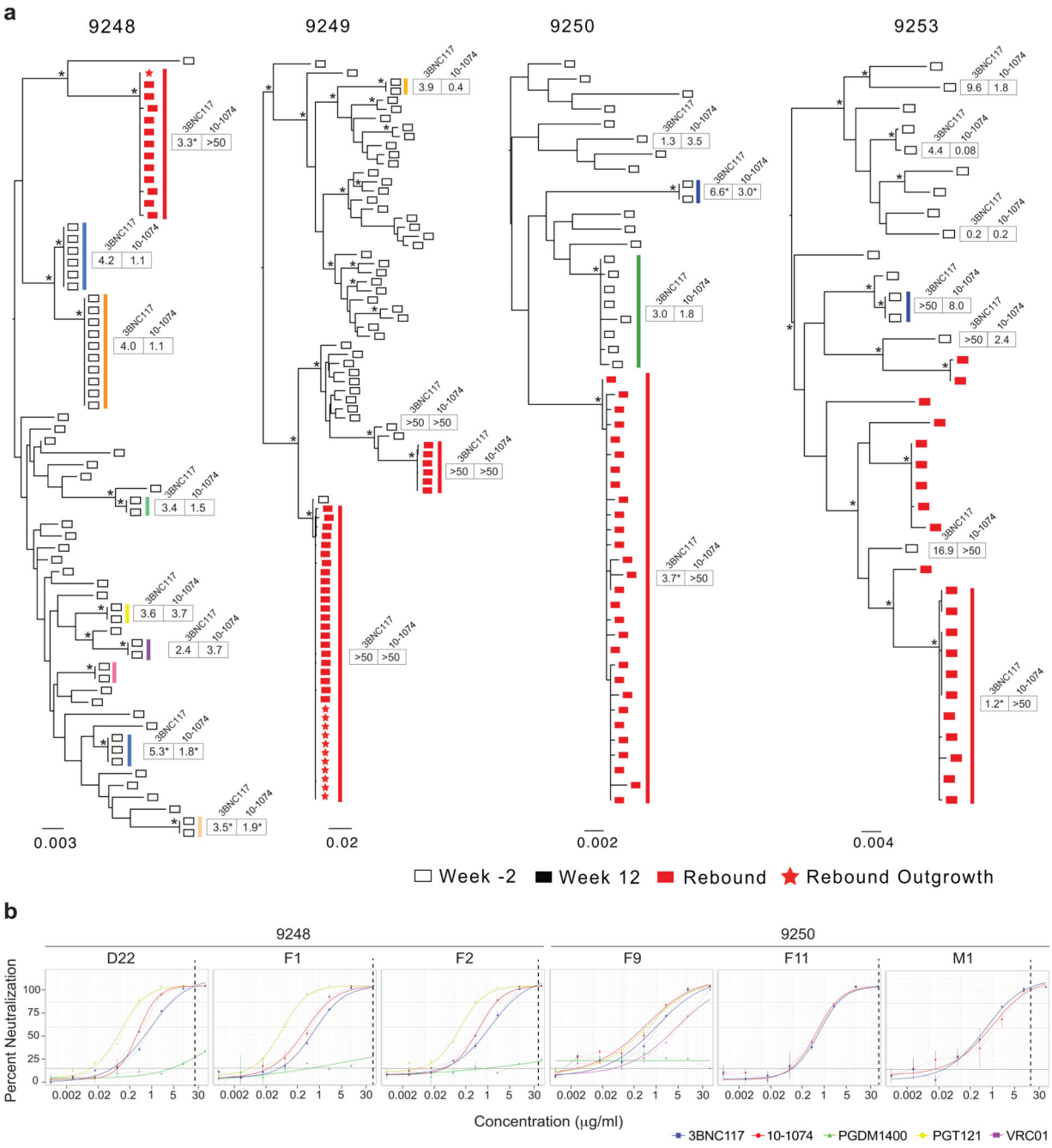

Extended Data Figure 5. Phylogenetic *env* trees and TZM-bl neutralization curves for individuals with viral blips.

a, Circulating reservoir and viral rebound in study participants with detectable viremia 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 4 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 colored lines. Boxes indicate IC_{80}s (μg/ml) of 3BNC117 and 10-1074 against individual clones, with asterisks indicating IC_{100} values of > 50 μg/ml. **b**, Latent reservoir virus TZM-bl neutralization curves for two participants that had a viral load > 20 copies/ml at day 0 (9248 and 9250). Curves show neutralization titers 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 (top) and 9250 (bottom) are shown, respectively. Note that, although these viruses had low 3BNC117 and 10-1074 IC_{50} or IC_{80} titers, IC_{100} (black dotted line) is reached only at a high concentration or not reached at all. The neutralization titer was measured by TZM-bl neutralization assay using a 5-parameter curve fit method.

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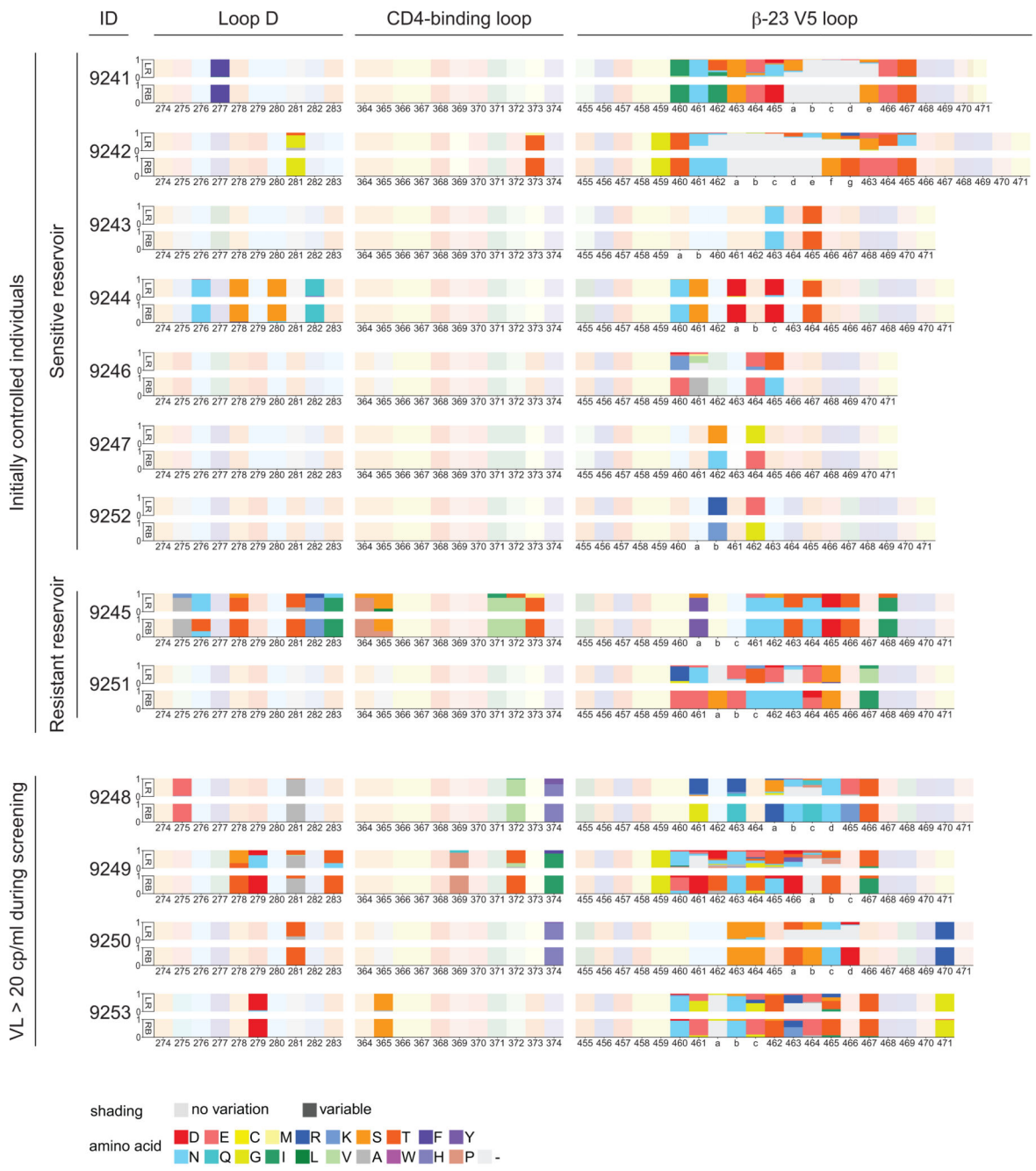
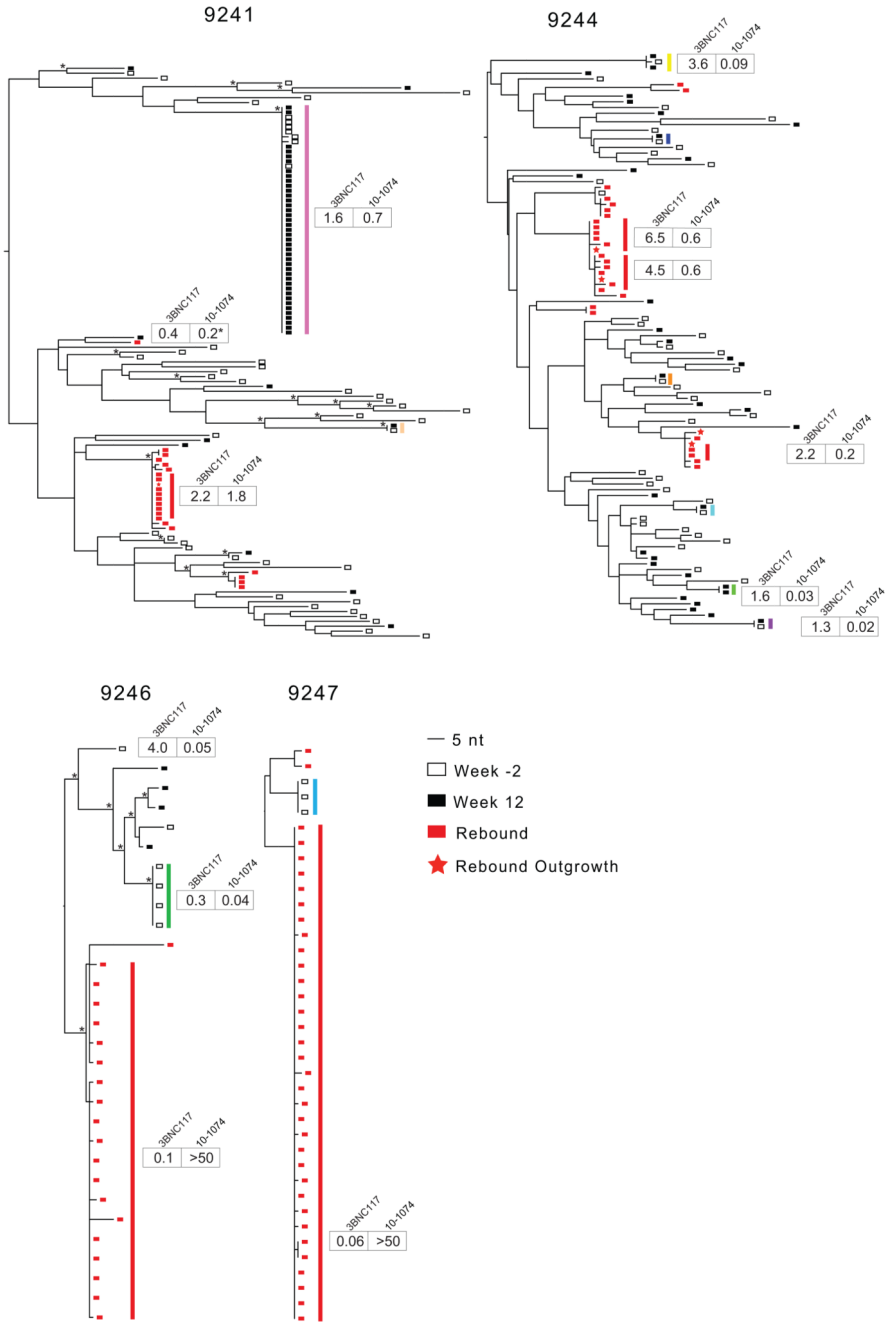


Extended Data Figure 6.

Color charts show 3BNC117 contact sites in Env according to HXB2 numbering. Diagram shows the 13 participants that experienced viral rebound before week 30. LR indicates latent reservoir viruses isolated by Q^2VOA (on weeks -2 and 12 when available). RB indicates rebound viruses isolated by SGA (plasma) and viral outgrowth (PBMCs). Each amino acid is represented by a color and the frequency of each amino acid is indicated by the height of the rectangle. Shaded rectangles indicate the lack of variation and full-color rectangles

represent amino acid residues with changes in the distribution between the reservoir and rebound.



Extended Data Figure 7. Comparison of the circulating latent reservoir and rebound viruses.
Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^{2}VOA, rebound plasma SGA, and rebound PBMC outgrowth cultures from participants 9241, 9244, 9246 and 9247, that rebounded before week 30. Open and closed black rectangles indicate Q^{2}VOA-derived viruses from week -2 and week 12, respectively.

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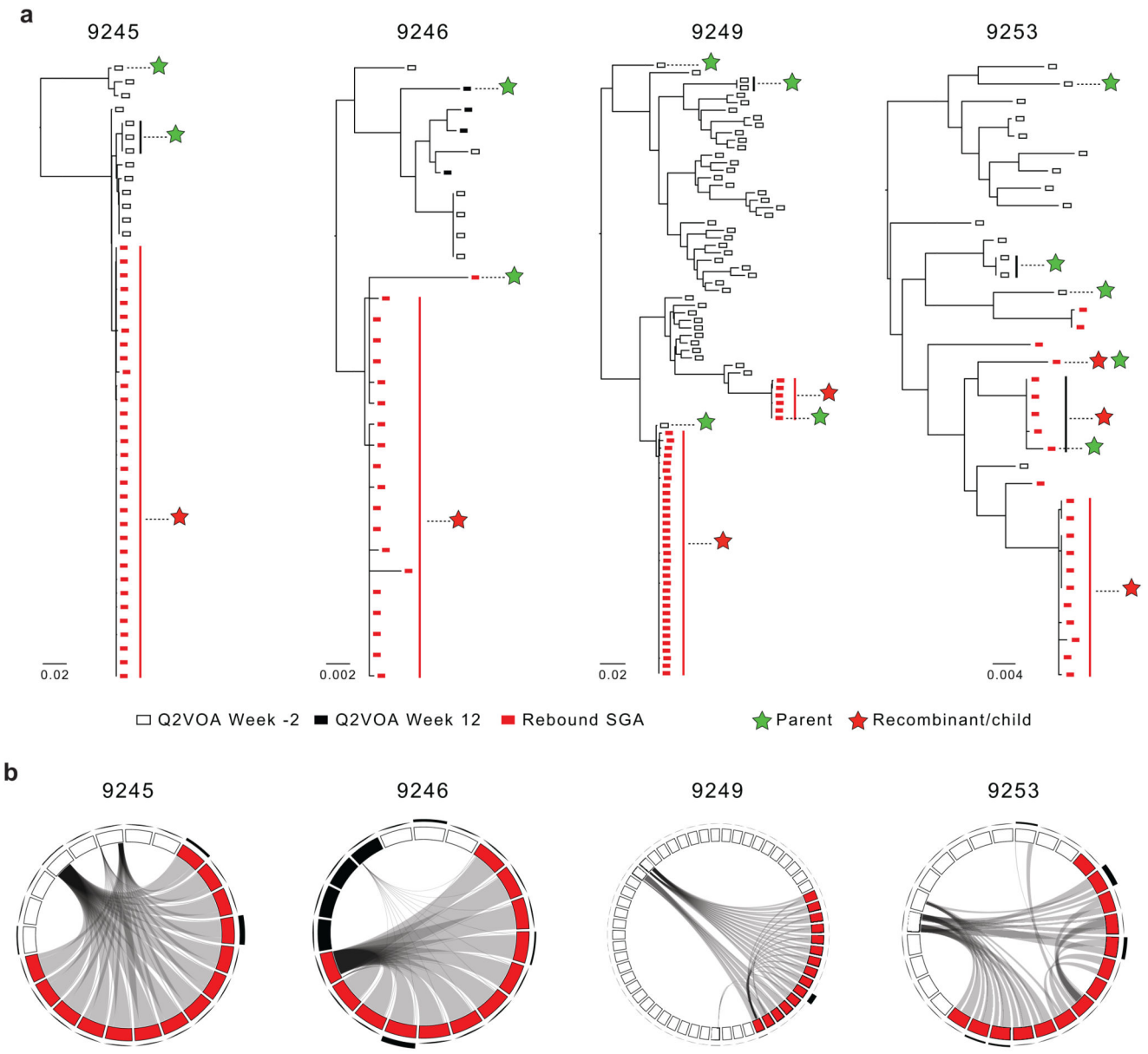
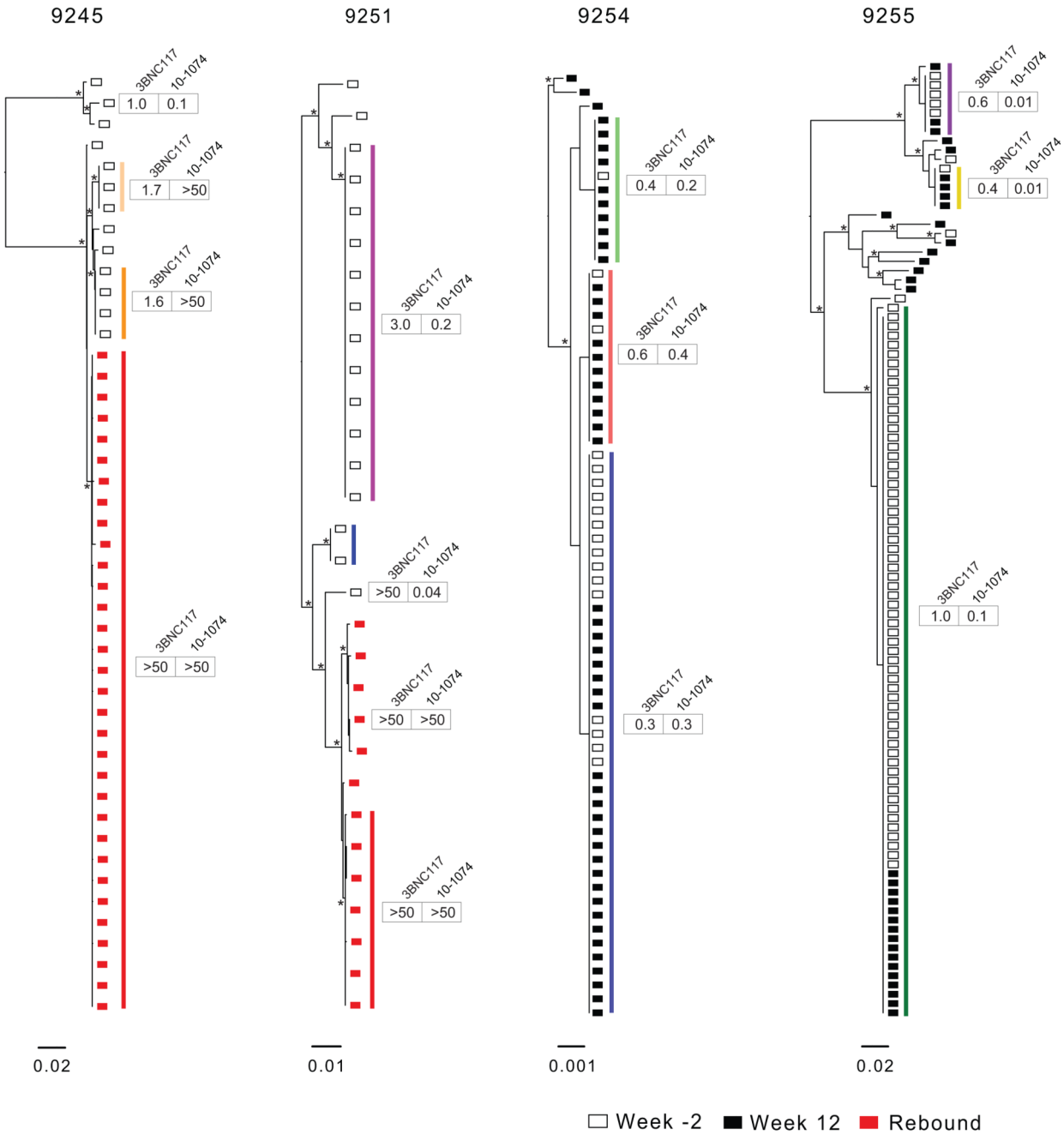
Viruses obtained at the time of rebound are indicated by red rectangles (plasma SGA) and red stars (rebound PBMC outgrowth cultures). Asterisks indicate nodes with significant bootstrap values (bootstrap support ≥ 70%). Clones are denoted by colored lines mirroring the colors of slices in Figure 5. Boxes indicate IC_{80}s (μg/ml) 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} values of > 50 μg/ml.



Extended Data Figure 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 4 participants where rebound viruses showed recombination events. Open and closed black rectangles indicates 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.



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Extended Data Figure 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 2 participants with pre-existing resistance to one of the 2 antibodies (9245 and 9251) and the 2 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 colored lines beside the phylogenetic tree. Numbers correspond to 3BNC117 and 10-1074 IC_{80} neutralization titers.

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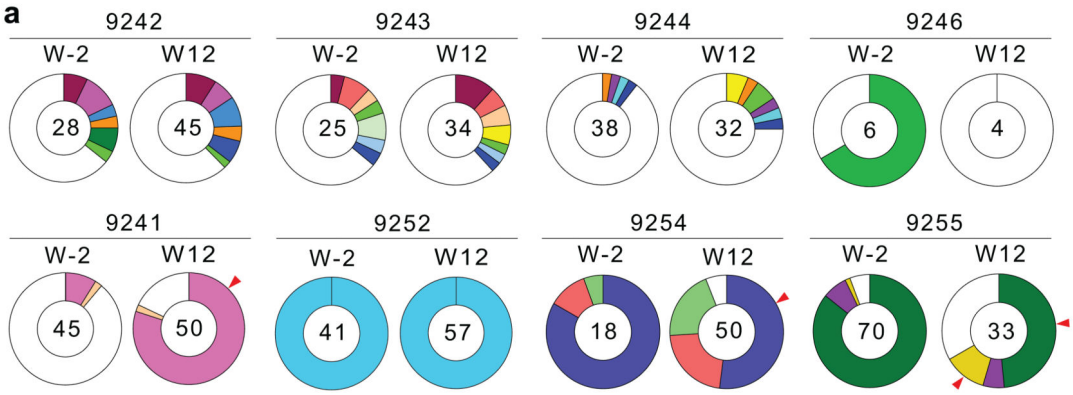
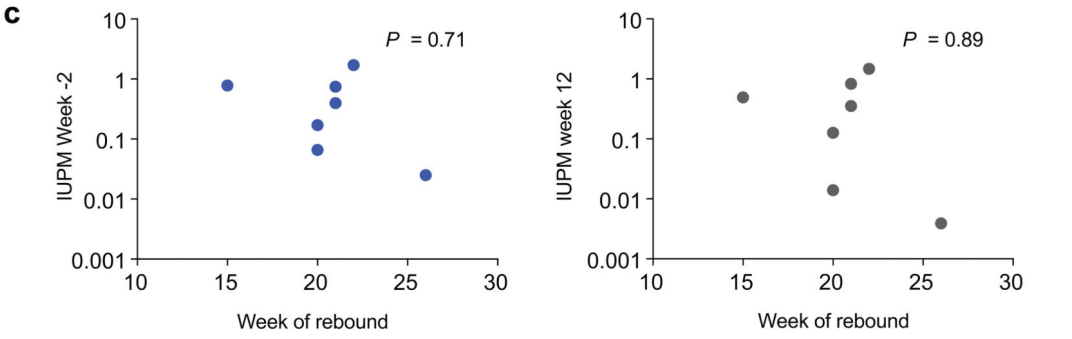


b

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**		



Extended Data Figure 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 *env* sequences analyzed. White represents sequences isolated only once across both time points and colored 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**, Table indicating the summary of clonal *env* sequences and IUPM in the 9 individuals with an antibody-sensitive reservoir. **c**, IUPM versus time of viral rebound in the antibody-sensitive individuals (n=7) that rebounded within the study observation period (30 weeks). *P* values were obtained using a two-tailed Pearson correlation test comparing the two variables.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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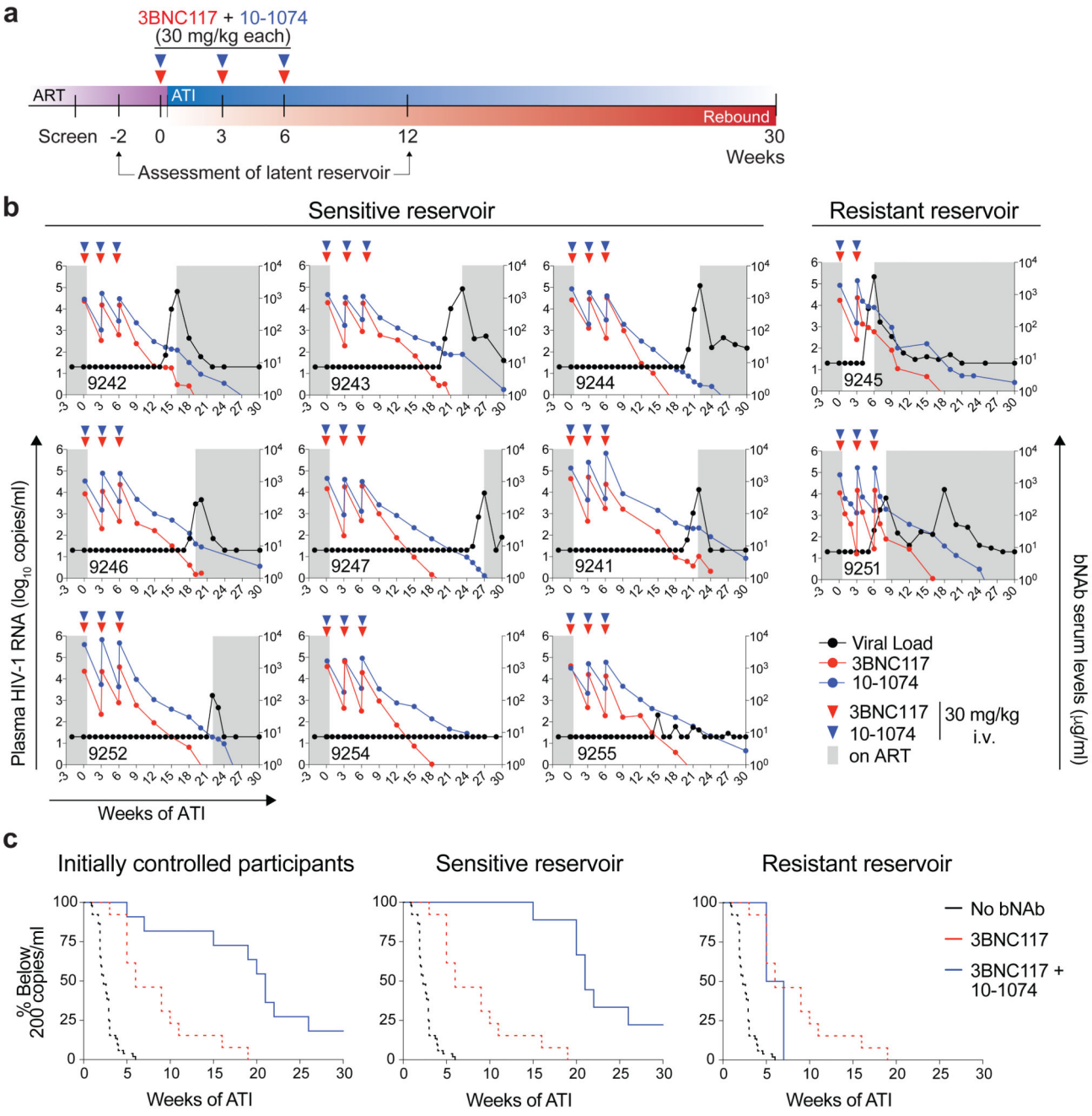

Figure 1. Delayed viral rebound with 3BNC117 and 10-1074 combination therapy during ATI.

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 9 bNAb-sensitive participants (left) and the 2 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/ml. **c**,

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Kaplan-Meier plots summarizing time to viral rebound for the participants with HIV-1 RNA < 20 copies/ml 2 weeks before and at the start of ATI (n=11, left), for the participants sensitive to both antibodies (n=9, center), and for the participants that showed pre-existing resistance to one of the antibodies (n=2, right). Y-axis indicates percentage of participants that maintain viral suppression. X-axis indicates weeks after start of ATI. Participants receiving the combination of 3BNC117 + 10-1074 are indicated by the blue line. Dotted red lines indicate a cohort of individuals receiving 3BNC117 alone during ATI9 (n=13) and dotted black lines indicate a cohort of participants who underwent ATI without intervention10 (n=52).

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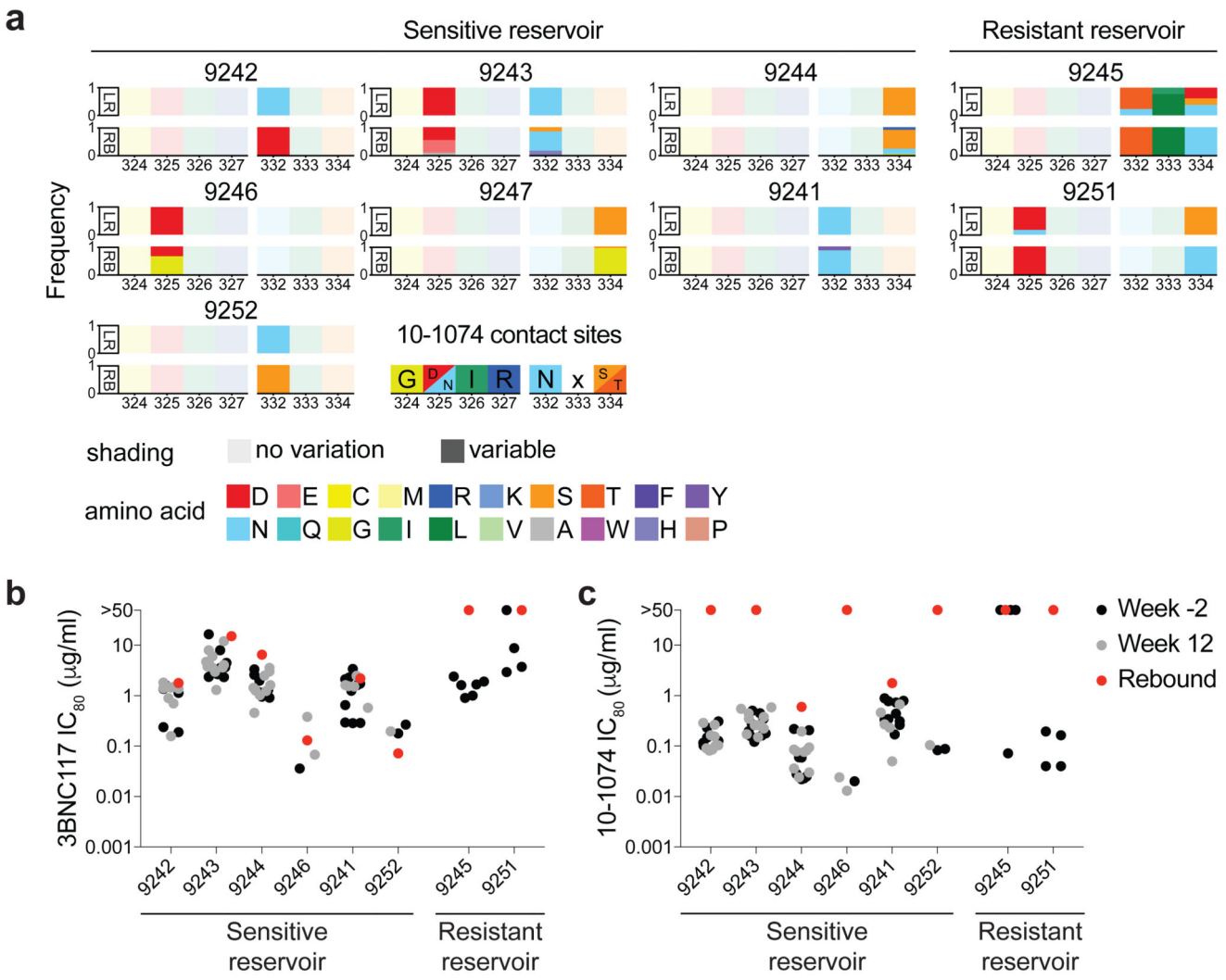

Figure 2. Amino acid variants at 10-1074 contact sites and bNAb sensitivity of reactivated latent and rebound viruses.

a, Color 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 7 bNAb-sensitive participants that rebounded before week 30 (left) and the 2 individuals with pre-existing resistance to one of the 2 antibodies (right). LR indicates latent reservoir viruses isolated by Q^2VOA and RB indicates rebound viruses isolated by SGA (plasma) or viral outgrowth (PBMCs). Each amino acid is represented by a color 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-color rectangles represent amino acid residues with changes in distribution between reservoir and rebound viruses. **b,c**, Dot plots indicating IC_{80} (μg/ml) of 3BNC117 (**b**, left panel) and 10-1074 (**c**, right panel) 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} determined 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.

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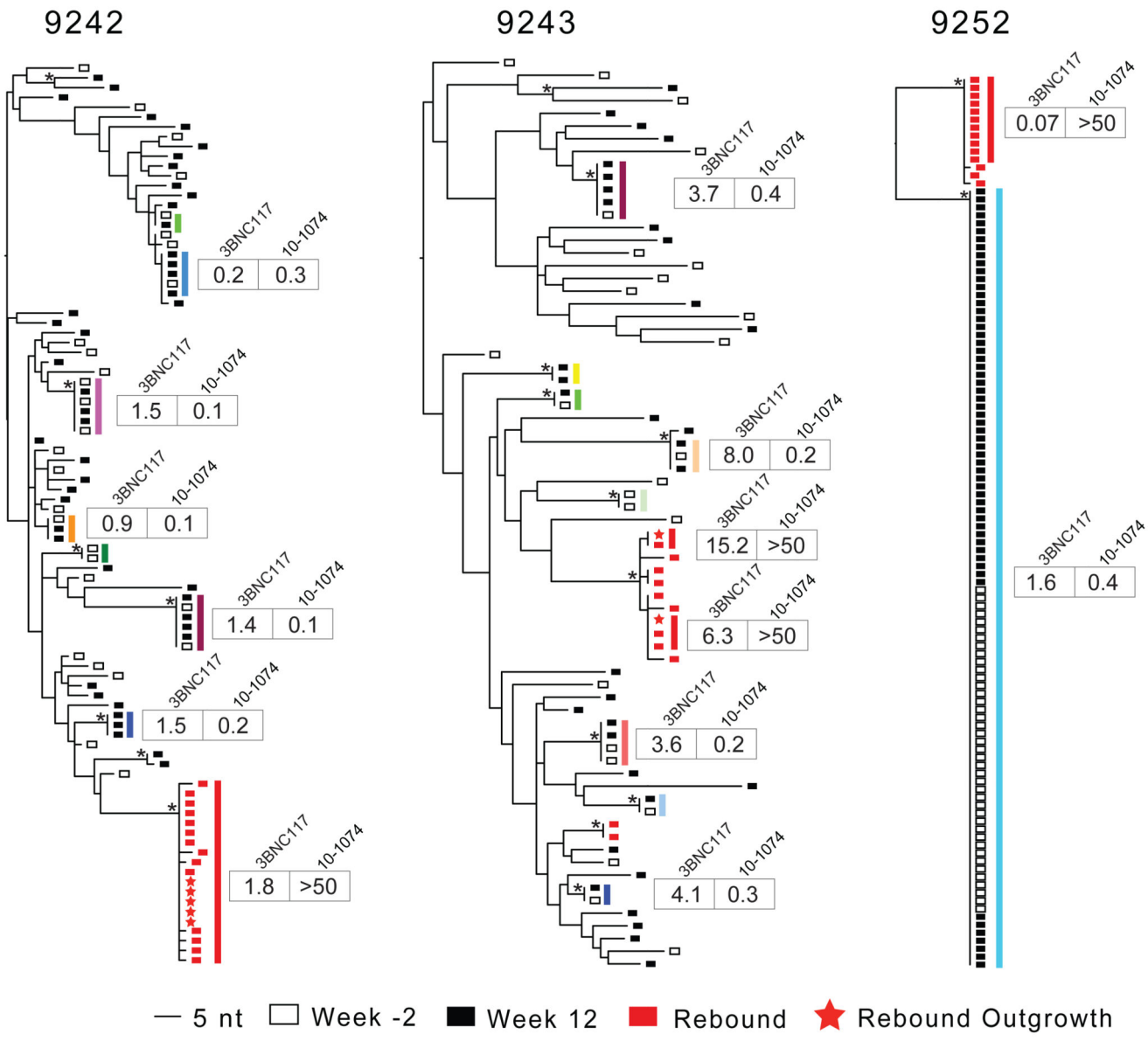

Figure 3. Comparison of the circulating latent reservoir and rebound viruses.

Maximum likelihood phylogenetic trees of full-length *env* sequences of viruses isolated from Q^2VOA, rebound plasma SGA, and rebound PBMC outgrowth cultures from 3 out of 7 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 colored lines mirroring the colors of slices in Extended Data Fig. 10a. Boxes indicate IC_{80}s (μg/ml) 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} values of > 50 μg/ml.

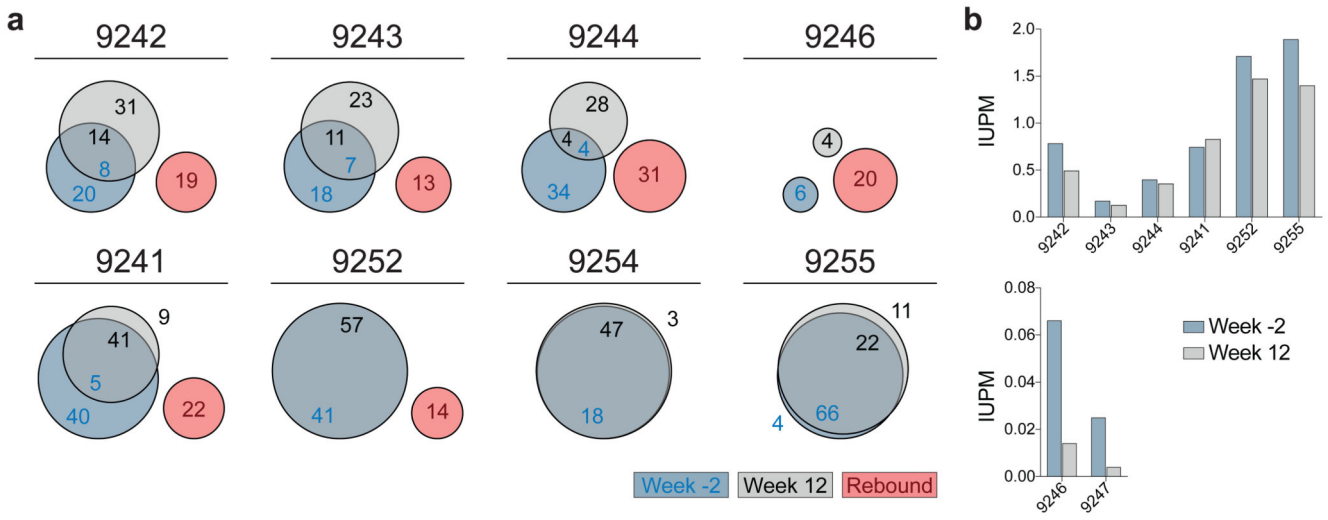

Figure 4. Distribution of the circulating latent reservoir and rebound viruses.

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. Number of sequences obtained is indicated. **b**, Infectious units per million (IUPM) $CD4^+$ T cells at weeks -2 and 12 as determined by Q^2VOA. Participants with IUPMs higher and lower than 0.1 are shown on the top and bottom, respectively. Participant 9254 is not shown due to lack of sample availability. The 2 time points were not statistically different ($P = 0.078$ (paired t-test)).

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