Published in final edited form as:
Nat Med. 2018 November ; 24(11): 1701–1707. doi:10.1038/s41591-018-0186-4.

Safety and anti-viral activity of combination HIV-1 broadly neutralizing antibodies in viremic individuals

Yotam Bar-On[1,*], **Henning Gruell**[2,3,4,*], **Till Schoofs**[1,2], **Joy A. Pai**[1], **Lilian Nogueira**[1], **Allison L. Butler**[1], **Katrina Millard**[1], **Clara Lehmann**[3,4,5], **Isabelle Suárez**[3,4,5], **Thiago Y. Oliveira**[1], **Theodora Karagounis**[1], **Yehuda Z. Cohen**[1], **Christoph Wyen**[3,6], **Stefan Scholten**[7], **Lisa Handl**[8], **Shiraz Belblidia**[1], **Juan P. Dizon**[1], **Jörg J. Vehreschild**[3,4], **Maggi Witmer-Pack**[1], **Irina Shimeliovich**[1], **Kanika Jain**[2], **Kerstin Fiddike**[3], **Kelly E. Seaton**[9], **Nicole L. Yates**[9], **Jill Horowitz**[1], **Roy M. Gulick**[10], **Nico Pfeifer**[8,11,12,13], **Georgia D. Tomaras**[9], **Michael S. Seaman**[14], **Gerd Fätkenheuer**[3,4], **Marina Caskey**[1,#], **Florian Klein**[2,4,5,#], and **Michel C. Nussenzweig**[1,15,#]

[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]Praxis Hohenstaufenring, Cologne, Germany

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

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

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

[11]Medical Faculty, University of Tübingen, Tübingen, Germany

Correspondence should be addressed to Marina Caskey (mcaskey@rockefeller.edu), Florian Klein (florian.klein@uk-koeln.de) or Michel C. Nussenzweig (nussen@rockefeller.edu).
* or #equal contribution.

Author contributions

M.C. (principal investigator, US), F.K. (principal investigator, Germany) and M.C.N. designed the trial; Y.B.-O., H.G., M.C., F.K. and M.C.N. analyzed the data and wrote the manuscript; Y.B.-O., T.S. and T.K. performed single-genome sequencing; H.G., A.L.B., K.M., M.W.-P., K.F., J.H., M.C. and F.K. implemented the study; Y.Z.C., R.M.G. and G.F. contributed to study design and implementation; C.L., I. Suárez, C.W. and S.S. contributed to participant recruitment and clinical assessments; J.A.P. and T.Y.O. performed bioinformatics processing; H.G., L.N. and T.K. performed viral cultures; L.H. and N.P. contributed to statistical analyses; S.B., J.P.D., J.J.V., I. Shimeliovich and K.J. performed, coordinated or contributed to sample processing; K.E.S., N.L.Y. and G.D.T. performed anti-idiotypic ELISA; and M.S.S. performed neutralization assays.

Competing interests

There are patents on 3BNC117 and 10-1074 on which M.C.N. is an inventor.

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[12]German Center for Infection Research, partner site Tübingen, Tübingen, Germany

[13]Max Planck Institute for Informatics, Saarbrücken, Germany

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

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

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Monotherapy of HIV-1 infection with single antiretroviral agents is ineffective because error-prone HIV-1 replication leads to the production of drug-resistant viral variants1,2. Combinations of drugs can establish long-term control, however, antiretroviral therapy (ART) requires daily dosing, can cause side effects and does not eradicate the infection3,4. Although anti-HIV-1 antibodies constitute a potential alternative to ART5,6, treatment of viremic individuals with a single antibody also results in emergence of resistant viral variants7–9. Moreover, combinations of first generation anti-HIV-1 broadly neutralizing antibodies (bNAbs) had little measurable effect on the infection10–12. Here we report on a phase 1b clinical trial (NCT02825797) in which two potent bNAbs, 3BNC11713 and 10-107414, were administered to 7 HIV-1 viremic individuals in combination. Infusions of 30 mg/kg of each of the antibodies were well tolerated. In the 4 individuals with dual antibody-sensitive viruses, immunotherapy resulted in an average reduction in HIV-1 viral load of 2.05 $\log_{10}$ copies/ml that remained significantly reduced for 3 months following the first of up to three infusions. In addition, none of these individuals developed resistance to both antibodies. Larger studies will be necessary to confirm the efficacy of antibody combinations in reducing HIV-1 viremia and limiting the emergence of resistant viral variants.

3BNC117 and 10-1074 are potent broadly neutralizing antibodies (bNAbs) that target the CD4 binding site and the base of the V3 loop on the HIV-1 envelope spike, respectively13,14. Infusion of the combination of 3BNC117 and 10-1074 during ART interruption maintains suppression of viremia and prevents the emergence of resistant variants15.

Controlling infection in viremic individuals represents a much more difficult problem than maintaining suppression in ART-treated individuals undergoing treatment interruption simply because of the large diversity of circulating HIV-1 variants present during active infection. Thus, although monotherapy with any one of 3 different bNAbs reduced viremia by 1.1 - 1.5 $\log_{10}$, these effects were transient and superseded by the emergence of antibody-resistant viral variants7–9. To determine whether the combination of 3BNC117 and 10-1074 is safe and results in improved antiviral activity against HIV-1 as compared to monotherapy we conducted a phase 1b trial in viremic individuals.

Viremic participants were selected from a cohort that was screened for sensitivity to 3BNC117 and 10-1074 by TZM-bl assays performed on viruses derived from bulk $CD4^+$ T cell outgrowth cultures (Supplementary Fig. 1)16. In agreement with previous reports, 67% and 58% of the individuals tested showed IC_{50}s < 2μg/ml to 3BNC117 and 10-1074, respectively, and 40% were sensitive to both (Supplementary Table 1)8,17,18. The seven

viremic participants had been diagnosed for a median of 5 years and had a geometric mean viral load of 11,494 copies/ml on the day of the first infusion (Fig. 1b and Supplementary Tables 2 and 3). In addition, eight individuals on ART with viral loads below the limit of detection were included for safety and pharmacokinetic assessments (Fig. 1, Supplementary Fig. 1 and Supplementary Tables 2 and 3).

Participants received either a single intravenous infusion of 3BNC117 and 10-1074 at a dose of 30 mg/kg per antibody, or three infusions of 30 mg/kg per antibody every two weeks (Fig. 1a). Viral loads, antibody serum levels, $CD4^+$ T cell counts and clinical parameters were monitored for 24 weeks after the last antibody infusion (Fig. 1 and Supplementary Tables 3 and 4).

Administration of both antibodies was well tolerated. No serious adverse events or treatment-related adverse events graded as moderate or severe were observed (Supplementary Table 4). $CD4^+$ T cell counts did not change significantly during the observation period (Supplementary Fig. 2 and Supplementary Table 3). We conclude that the combination of 3BNC117 and 10-1074 is generally safe and well tolerated.

3BNC117 and 10-1074 antibody levels were determined by ELISA using anti-idiotypic antibodies and by the TZM-bl assay that measures the antibodies' neutralizing activities in serum. In viremic individuals, the half-lives of 3BNC117 and 10-1074 were 11.1 and 12.2 days when measured by ELISA, and 8.5 and 11.5 days when determined by the TZM-bl assay, respectively (Fig. 1, Supplementary Fig. 3, Supplementary Fig. 4 and Supplementary Tables 3 and 5). In ART-treated individuals, half-lives of 3BNC117 and 10-1074 were 14.5 and 19.0 days by ELISA, and 11.5 and 18.4 days in the TZM-bl assay, respectively (Supplementary Fig. 3, Supplementary Fig. 4 and Supplementary Table 5). Viremic individuals generally showed lower antibody half-lives than individuals on ART with suppressed viral loads, possibly due to an antigen sink effect7,8,19. Overall, these values are consistent with the results obtained when both antibodies were administered individually (Supplementary Fig. 3)7,8,17. Thus, pharmacokinetics of 3BNC117 and 10-1074 do not appear to be altered when the antibodies are administered in combination.

Plasma HIV-1 RNA levels were measured on a weekly basis for 4 weeks after antibody infusions and every 2-4 weeks thereafter (Fig. 2a-c, Supplementary Fig. 4 and Supplementary Table 3). The average drop in viral load for all viremic individuals was 1.65 $\log_{10}$ copies/ml and viremia remained significantly reduced until day 86 (Fig. 2d). The 4 individuals with sensitive viruses (see below) showed a more pronounced drop in viral load compared with the other individuals (average of 2.05 $\log_{10}$ copies/ml) and were significantly suppressed until day 94 (Fig. 2a,e-f). In comparison to a single infusion of either 3BNC1177 or 10-10748, viremic individuals receiving one or three infusions of the combination of both antibodies showed significantly prolonged viral suppression (P=0.00018) (Fig. 2d, Supplementary Fig. 5). We conclude that the combination of 3BNC117 and 10-1074 is more effective in suppressing viremia than either antibody alone.

Despite the pronounced difference in the duration of viremia reduction between monotherapy and combination therapy, there was considerable variation in the response of

individual participants receiving 3BNC117 and 10-1074 combination treatment (Fig. 2 and Supplementary Table 3). To define the relationship between individual responses to antibody therapy and circulating virus sensitivity to the antibodies we performed single genome amplification (SGA) of plasma viruses. 382 intact full-length *env* sequences were initially analyzed from the 7 viremic participants (Supplementary Fig. 6). All of these individuals were infected with epidemiologically distinct clade B viruses (Fig. 3a). In addition, sequences isolated from circulating virus at the time of viral rebound were polyclonal, and as expected for viremic individuals, recombination events were detected between circulating viruses in most individuals (Fig. 3b,c).

Pseudoviruses constructed from plasma SGA were tested for bNAb sensitivity in TZM-bl assay (Fig. 4a, and Supplementary Table 6). 91C33, the individual that failed to respond to therapy, had preexisting circulating viruses that were resistant to both antibodies. These viruses carried mutations in 3BNC117 contact sites (N280S and A281H) and in 10-1074 contact sites (N332T and S334N, Supplementary Fig. 6 and Supplementary Table 6). Two individuals, 91C35 and 9341, responded to antibody therapy with a decrease in viremia of -1.58 and -1.32 $\log_{10}$ copies/ml but HIV-1 RNA levels returned to baseline within 3 and 4 weeks, respectively (Fig. 2b). 91C35 was found to have pre-infusion circulating viruses with reduced sensitivity to 3BNC117, and carried a CD4 contact residue mutation (A281T) that was associated with viral escape from 3BNC117 (Fig. 4a, Supplementary Fig. 7 and Supplementary Table 6)20. Pre-infusion viruses derived from bulk $CD4^+$ T cell outgrowth cultures of 9341 showed a 10-1074 IC_{80} which was 1.3 $\log_{10}$ higher than the geometric mean IC_{80} of all other enrolled viremic individuals (Supplementary Table 1). In both of these cases, rebounding viruses were resistant to both antibodies and carried mutations resulting in the loss of the potential *N*-linked glycosylation site at position 332 that is critical for 10-1074 binding (Fig. 4a, b, Supplementary Fig. 6, Supplementary Fig. 7 and Supplementary Table 6). In addition, 91C35 and 9341 rebound viruses contained G471E and N276D mutations, respectively, that are associated with increased resistance to 3BNC1177,17,21,22. These mutations were not found in the pre-infusion circulating viruses described above or in additional 113 pre-infusion *env* sequences that were analyzed from these two participants (Supplementary Figure 8). Thus, 91C35 and 9341 were infected with viruses with reduced sensitivity to one of the two antibodies and resemble individuals that received antibody monotherapy, both in the magnitude of the drop in viremia and time required to return to baseline viremia7–9. We conclude that the bulk outgrowth cultures used for initial screening failed to detect partial or complete pre-existing resistance against one or both of the antibodies in 3 of the 7 individuals studied.

The four remaining individuals showed no detectable pre-existing resistant viruses in circulation and experienced significantly suppressed viremia until day 94 after the first antibody infusion with an average maximum drop in viral load of -2.05 $\log_{10}$ copies/ml (Fig. 2a, e, Fig. 4a and Supplementary Table 6). The individual in this group with the highest initial viral load (97,800 copies/ml; 9343) was the first to rebound at 8 weeks (Fig. 2a, Supplementary Table 3). The 2 individuals with the lowest initial viral loads, 91C22 and 9342 (750 and 2,550 copies/ml, respectively), demonstrated suppression to near or below the limit of detection for 12 and 16 weeks, respectively (Fig. 2a, Supplementary Table 3). Finally, viremia in participant 91C34 was reduced for a period of 12 weeks but never

dropped below 810 copies/ml. Despite the persistent viremia, no resistance against both antibodies developed in this individual for as long as bNAb serum levels were above 10 μg/ml (Supplementary Fig. 7, Supplementary Fig. 9 and Supplementary Table 3).

In 3 of the 4 initially sensitive individuals, rebound viremia was associated with the appearance of viruses that were resistant to 10-1074, but remained sensitive to 3BNC117 (Fig. 4a, and Supplementary Table 6). This is consistent with the relatively shorter half-life of 3BNC117, which means that participants were effectively exposed to 10-1074 monotherapy at the end of the observation period. In accordance with the increased resistance to 10-1074, rebound viruses carried mutations in 10-1074 contact sites (Fig. 4b, Supplementary Fig. 6 and Supplementary Fig. 7). In contrast, there was no accumulation of *de novo* mutations in 3BNC117 contact sites (Fig. 4b, Supplementary Fig. 6 and Supplementary Fig. 7). 91C22, the participant with the lowest initial viral load, only returned to baseline viremia after both antibodies were below the limit of detection, and rebound viruses remained sensitive to both antibodies (Fig. 2a, Supplementary Fig. 4, Supplementary Table 3 and Supplementary Table 6). Overall none of the 4 participants that were initially sensitive to the two antibodies developed *de novo* resistance to 3BNC117 over a cumulative observation period of over 1 year (56 weeks), despite the residual viremia observed in three of these participants and frequent recombination events between circulating viruses (Fig. 3c).

Combination bNAb therapy for HIV-1 in humans showed a number of similarities with bNAb therapy for macaques infected with $SHIV_{AD8}$. For example, suppression was incomplete in macaques with higher initial viral loads, but despite persistent low-level viremia there was no emergence of 3BNC117 and 10-1074 double resistant variants23. In contrast to the macaque infection with a clonal virus, each of the 4 antibody sensitive individuals in this study was infected with a uniquely diverse swarm of viruses. Thus, the relative difficulty of HIV-1 to develop resistance to the combination of 3BNC117 and 10-1074 is not limited to any particular strain of HIV-1. Macaque $CD8^+$ T cell responses can control viremia and this type of cellular immunity can be enhanced by bNAb therapy24. $CD8^+$ T cells have also been implicated in HIV-1 control in humans25. Whether such responses can also be enhanced by immunotherapy in humans remains to be determined.

3BNC117 and 10-1074 target distinct epitopes on the Env trimer. 3BNC117 interacts with the CD4 binding site, which is critical for HIV-1 binding to its cellular receptor CD4. Thus, escape mutations from 3BNC117 are limited by the requirement of continued affinity to CD4 and are associated with reduction in viral fitness7,17,26. Combinations of just 2 antibodies that synergize to further restrict viral escape may be even more effective than 3BNC117 and 10-107427.

Should antibodies enter clinical practice for HIV-1, adequate safeguards will be required to minimize the emergence of resistant variants. Reliable screening methods that identify viral resistance against individual drugs facilitate the selection of antiretroviral drug combinations with full activity. In contrast, the culture-based method used to screen for resistance in this study failed to detect partial or complete pre-existing antibody resistance in 3 of the 7 viremic participants. This is likely due to outgrowth of a limited set of viruses *in vitro* that

fails to represent the entire population that is circulating or archived *in vivo*8,15,17. Sequence-based screening methods that encompass a much larger group of viruses are currently being developed and should be far more effective than the bulk cultures.

This study highlights some of the limitations of immunotherapy with the combination of 3BNC117 and 10-1074 in viremic individuals. 3BNC117 and 10-1074 infusions failed to suppress viremia to undetectable levels in the two dual antibody-sensitive individuals with the highest pre-infusion viral load despite persistent reductions for up to 12 weeks. Sustained suppression of plasma HIV-1 RNA levels to below 20 copies/ml was only achieved in individual 91C22 who had the lowest pre-infusion viral load (730 copies/ml). Thus, whereas 2 antibodies may be sufficient to achieve and/or maintain suppression in sensitive individuals with very low levels of viremia or ART-suppressed individuals undergoing ATI15, additional antibodies or combinations of small molecule drugs and antibodies would be required if this type of therapy is to be considered for viremic individuals.

This trial was limited to 3 bNAb infusions but despite the small number of infusions, sensitive individuals maintained reductions in viral load for up to 3 months after the last infusion. In the case of anti-RSV antibodies and the anti-HIV-1 antibody VRC01, antibody half-life can be increased by up to more than a factor of 4 by mutations that alter binding to the neonatal Fc receptor (FcRN)28–30. In macaques, the same half-life extension mutations lead to a significant increase in the half-life and protective efficacy of 3BNC117 and 10-107431. Should they also do so in humans, intermittent infusions of combinations of antibodies or antibodies plus long acting antiretroviral drugs every 3-6 months might be an alternative to daily ART.

Online Methods

Study design

We conducted a dose-escalation phase 1b study in HIV-1-infected individuals to evaluate the safety, pharmacokinetics and antiretroviral activity of the combination of the antibodies 3BNC117 and 10-1074 (http://www.clinicaltrials.gov; NCT02825797; EudraCT: 2016-002803-25). Study participants were enrolled sequentially into groups 1A, 1B, 1C and 3 according to eligibility criteria (Supplementary Fig. 1). Participants in groups 1A and 1B were virologically suppressed on antiretroviral therapy (ART) and were randomized in a 2:1 ratio (6 participants per group) to receive one intravenous infusion of each 3BNC117 and 10-1074 (group 1A, 10 mg/kg per antibody; group 1B, 30 mg/kg per antibody) or placebo (sterile saline). Study participants and investigators were blinded to the assignment in groups 1A and 1B. Placebo recipients were not included in the data analysis. Viremic individuals off ART were enrolled in group 1C (4 participants) or group 3 (3 participants), and received one intravenous infusion (group 1C) or three intravenous infusions (group 3, every two weeks) of each 3BNC117 and 10-1074 at a dose of 30 mg/kg. Participation in groups 1C and 3 was open-label. All study participants were followed for 24 weeks after the last administration of the antibodies or placebo. Participants off ART were encouraged to initiate ART six weeks after the last antibody 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 trial was conducted in accordance with Good Clinical Practice. The study protocol was approved by the Food and 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 at the University Hospital Cologne, Cologne, Germany. Eligible participants were HIV-1-infected adults aged 18-65 years with a current $CD4^+$ T cell count > 300 cells/μl. Individuals on ART were eligible for participation and enrollment in groups 1A and 1B if HIV-1 RNA levels were < 20 copies/ml at screening. Viremic individuals were eligible for enrollment in groups 1C and 3, if they were off ART with detectable HIV-1 RNA plasma levels of < 100,000 copies/ml. Exclusion criteria included 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. Viremic participants were pre-screened for the sensitivity of bulk $CD4^+$ T cell outgrowth culture-derived virus against 3BNC117 and 10-1074 as described below. Antibody sensitivity was defined as an IC_{50} < 2 μg/ml for both 3BNC117 and 10-1074 measured in a TZM-bl neutralization assay.

Study procedures

The monoclonal antibodies were prepared by the site pharmacist and provided to the investigators in indistinguishable infusion bags. The required stock volume of 3BNC117 or 10-1074 was calculated according to body weight and diluted in sterile normal saline to a total volume of 250 ml. Each monoclonal antibody was administered intravenously over 60 minutes. Both antibodies were administered individually and sequentially. Study participants were observed at the Rockefeller University Hospital or the University Hospital Cologne for four hours (groups 1A-C) or one hour (group 3) after the last antibody infusion. Participants returned for scheduled follow-up visits for safety assessments, which included physical examination as indicated and measurements of clinical laboratory parameters such as hematology, $CD4^+$ T cell counts, chemistries, urinalysis and pregnancy tests. Plasma HIV-1 RNA levels were monitored at each visit. 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 the causality of events. Blood samples were collected before and at multiple times after the infusions of 3BNC117 and 10-1074 or placebo. Samples were processed within 4 h of collection. Serum and plasma samples were stored at -80°C. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation and the absolute number of PBMCs was determined by an automated cell counter (Vi-Cell XR; Beckman Coulter) or manually. Isolated cells were cryopreserved in fetal bovine serum plus 10% DMSO.

Plasma HIV-1 RNA Levels

Plasma HIV-1 RNA levels were determined at every study visit, including the screening (day -49 to -7) and pre-infusion (day -42 to -2) visits, as well as before the first infusion on day 0 and two days after each infusion. Following the last infusion, HIV-1 RNA levels were

monitored weekly for four weeks, and continued to be monitored in two- to eight-week intervals until the end of study follow up. 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). These assays have a linear range of quantitation between 2×10^1 and 1×10^7 viral copies/ml and were performed at LabCorp or at the University Hospital Cologne.

CD4⁺ and CD8⁺ T cell counts

$CD4^+$ and $CD8^+$ T cell counts were determined by a clinical flow cytometry assay performed at LabCorp or at the University Hospital Cologne every two to four weeks and at the final study visit.

TZM-bl neutralization assay to measure 3BNC117 and 10-1074 serum levels

This assay was performed as previously described16. Briefly, serum samples were heat-inactivated for one hour at 56°C and tested using a primary 1:20 dilution and a 5-fold titration series against HIV-1 Env pseudoviruses Q769.d22 and X2088_c9. These pseudoviruses are highly sensitive to neutralization by 3BNC117 and 10-1074, respectively, and fully resistant against the other administered antibody. If serum titers exceeded 100,000 against X2088_c9, immediate post-infusion levels of 10-1074 were also determined using the less sensitive Du422 strain. 3BNC117 and 10-1074 clinical drug products were tested in parallel at a starting concentration of 10 μg/ml with a 5-fold titration series. Pseudoviruses were produced with an ART-resistant backbone vector that reduces the inhibitory activity of antiretroviral drugs (SG3ΔEnv/K101P.Q148H.Y181C, M. S. Seaman unpublished data). In viremic indivduals, serum concentrations of 3BNC117 and 10-1074 were calculated by multiplying the determined ID_{50} titer of the respective serum sample and the determined IC_{50} concentration of each monoclonal standard antibody. In individuals on ART serum bNAb concentrations were calculated using the ID_{80} serum titers and IC_{80}s of the monoclonal antibodies as described above to minimize the impact of nonspecific ART-mediated background activity. Viruses pseudotyped with the envelope protein murine leukemia virus (MuLV) were used as negative control and measurements were excluded if nonspecific serum activity against MuLV-pseudotyped viruses was observed (ID_{50} or ID_{80} > 20 in viremic individuals or individuals on ART, respectively). All assays were performed in a laboratory meeting GCLP standards. The lower limit of detection was determined to be 0.24 μg/ml and 0.10 μg/ml for the 3BNC117 and 10-1074 TZM-bl assay, respectively.

ELISA-based measurement of 3BNC117 and 10-1074 serum levels

3BNC117 and 10-1074 serum concentrations were measured by a validated sandwich ELISA. High bind polystyrene plates were coated overnight at 2-8°C with 4 μg/ml of an anti-idiotypic antibody that specifically recognizes 3BNC117 (anti-ID 1F1-2E3 mAb) or 2μg/ml of an anti-idiotypic antibody specifically recognizing 10-1074 (anti-ID 3A1-4E11 mAb). 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. A horseradish peroxidase (HRP)-conjugated mouse anti-human IgG kappa-chain-specific antibody (Abcam) was used to detect 3BNC117

and an HRP-conjugated goat antihuman IgG Fc-specific antibody (Jackson ImmunoResearch) to detect 10-1074. For detection, the HRP substrate tetra-methylbenzidine was added. A 5-PL curve fitting-algorithm (Softmax Pro, v. 5.4.5) was used to calculate serum 3BNC117 and 10-1074 concentrations from respective standard curves run on the same plate. Standards and positive controls were created from the drug product lots of 3BNC117 and 10-1074 that were used in the clinical study. The capture anti-idiotypic mAbs were produced in a stable hybridoma cell line (Duke Protein Production Facility7). If day 0 samples had measurable levels of antibody by the respective assays, the background measured antibody level was subtracted from subsequent results. In addition, samples with measured antibody levels within 3-fold from background were excluded from the analysis of PK parameters. 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 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.

Single genome amplification (SGA) of viral *env* genes

Single genome amplification and sequencing of HIV-1 env genes was performed for plasma samples as described previously17,33. All *env* sequences were translated to amino acids and aligned using ClustalW34. Sequences containing premature stop codons or large internal deletions that would compromise Env functionality were removed from downstream analysis. Frequency plots were produced to analyze changes in 3BNC117 and 10-1074 binding sites between day 0 and rebound viruses. Amino acids were numbered according to the HXB2 *env* sequence (GenBank accession no. K03455). Logo plots were generated using the Longitudinal Antigenic Sequences and Sites from Intra-host Evolution tool (LASSIE)35. Maximum likelihood phylogenetic trees were generated from the alignments with PhyML v3.136 using the GTR model37 with 1,000 bootstraps. For the combined analysis of sequences from all participants, *env* sequences were aligned using MAFFT v7.30938 and clustered using RAxML v8.2.9 under the GTRGAMMA model37 with 1,000 bootstraps.

Pseudovirus production

Selected viral sequences which were isolated from participant's plasma by SGA were used to generate CMV promoter-based pseudoviruses as previously described33,39. The CMV promoter was amplified using the forward primer 5′AGTAATCAATTACGGGGTCATTAGTTCAT and the reverse primer 5′CATAGGAGATGCCTAAGCCGGTGGAGCTCTGCTTATATAGACCTC. Individual *env* amplicons were amplified using the forward primer 5'CACC GGCTTAGGCATCTCCTATGGCAGGAAGAA and the reverse primer 5'GTCTCGAGATACTGCTCCCACCC. To fuse the individual purified *env* amplicons to the CMV promoter, an overlapping PCR was performed by using the forward primer 5′AGTAATCAATTACGGGGTCATTAGTTCAT and the reverse primer 5′ACTTTTTGACCACTTGCCACCCAT. Pseudoviruses were generated by transfecting 293T cells as previously described39.

Pre-Screening bulk PBMC culture

Candidate viremic individuals were pre-screened for sensitivity of bulk culture-derived outgrowth virus against 3BNC117 and 10-1074 as described previously7,8,15,17. PBMCs for pre-screening were obtained by a median of 27 weeks (range 4.9 – 38 weeks) before enrollment under separate protocols approved by the IRBs of The Rockefeller University and the University of Cologne. Briefly, isolated $CD4^+$ T cells were co-cultured with MOLT-4/CCR-5 cells or $CD8^+$ T cell-depleted donor lymphoblasts and culture supernatants were regularly monitored for p24 levels. Viral supernatants from p24-positive cultures were tested for sensitivity against 3BNC117 and 10-1074 by the TZM-bl neutralization assay as described below. Cultures were deemed sensitive if the determined individual IC_{50}s for 3BNC117 and 10-1074 were < 2 μg/ml.

Virus neutralization assays

Supernatants from p24-positive bulk $CD4^+$ T cell cultures and pseudoviruses were tested for sensitivity to antibodies as previously described16.

Pharmacokinetic analyses

PK-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 infusion of 3BNC117 from either TZM-bl assay or ELISA.

Viral *env* recombination analysis

Multiple sequence alignment of *env* genes guided by amino acid translations *of env* sequences was done by TranslatorX (http://translatorx.co.uk/). The 3SEQ recombination algorithm (http://mol.ax/software/3seq/) was used to detect recombination between day 0 viruses and rebound viruses or between different rebound viruses. Instances in which statistical evidence of recombination was found (rejection of the null hypothesis of clonal evolution) are indicated in a circos plot (http://circos.ca/).

Statistical analyses

The sample size to detect > 0.9 $\log_{10}$ copies/ml decline in viremia with 80% power at 5% of significance level *P* of 0.05 was determined to be 6 viremic HIV-1-infected individuals, assuming that the standard deviation would be similar to 3BNC117 or 10-1074 monotherapy in humans (SD 0.75 and 0.6, respectively)7,8. To measure the effect of the combination treatment on viral load (VL), we estimated simultaneous confidence bands (SCBs) for the Δlog10 VLs. The VL was considered significantly suppressed whenever the two dashed lines representing the SCBs at 95% certainty level excluded zero (Fig. 2d-f). We computed SCBs with the R package locfit (version 1.5-9.1) using the Gaussian family for the local likelihood function (Fig. 2d-f). To estimate whether there is a significant difference between 3BNC117 + 10-1074 combination therapy and 3BNC117 or 10-1074 monotherapy in viremic individuals off antiretroviral therapy, we fit a linear mixed effects model to the data, using time and treatment as fixed effects and a random intercept for each participant. Data for 3BNC117 and 10-1074 monotherapy have been published previously and only time points from viral load measurements off antiretroviral therapy and subjects responding to

antibody infusions by a drop in viremia were included7,8. We compared it to a model without treatment as predictor using a likelihood ratio test. The time point of VL measurement was modeled as an ordered factor and correlation structure between measurements from the same individual was modeled based on the order of measurements using different options available in nlme (exponential, linear, rational quadratic, and spherical correlation structure, as well as different combinations of autocorrelation and moving average). The models were fit maximizing the log-likelihood with the lme function of the R package nlme (version 3.1-131). We decided on the best model using AIC (see Supplementary Fig. 5). Time points were restricted to day0, week1, week2, week3, week4, week6, week8, week12, week16, week20 and week24 to have a sufficient number of measurements per time point. Marginal means (a.k.a. least-squares means) are shown in Supplementary Fig. 5. $CD4^+$ T cell counts before and after 3BNC117 plus 10-1074 infusions were compared by one-way ANOVA using GraphPad Prism (version 7.0).

Data Availability Statement

All requests for raw and analyzed data and materials are promptly reviewed by the Rockefeller University to verify if the request is subject to any intellectual property or confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient confidentiality. Any data and materials that can be shared will be released via a Material Transfer Agreement. HIV-1 envelope SGA data are available in GenBank, accession numbers MH632763 - MH633255.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank all study participants who devoted time to our research, and members of the Klein and Nussenzweig labs for helpful discussions, especially P. Mendoza, C.-L. Lu, J.C.C. Lorenzi, L. Cohn and M. Jankovic. We thank R. Levin, G. Kremer and D. Weiland for study coordination; the Rockefeller University Hospital Clinical Research Support Office and nursing staff as well as C. Golder, E. Thomas, M. Platten, S. Margane and T. Kümmerle for help with recruitment and study implementation; C. Ruping and M. Schlotz 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 for pharmaceutical services; H. Janicki, M. Ercanoglu, P. Schommers and R. Kaiser for help with virus cultures; P. Fast and H. Park for clinical monitoring; S. Schlesinger for input on study design; 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. 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 NIH grants 1UM1 AI100663 and R01AI-129795 (M.C.N.); 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 Einstein-Rockefeller-CUNY Center for AIDS Research (1P30AI124414-01A1); BEAT-HIV Delaney grant UM1 AI126620 (M.C.); and the Robertson fund. M.C.N. is a Howard Hughes Medical Institute Investigator.

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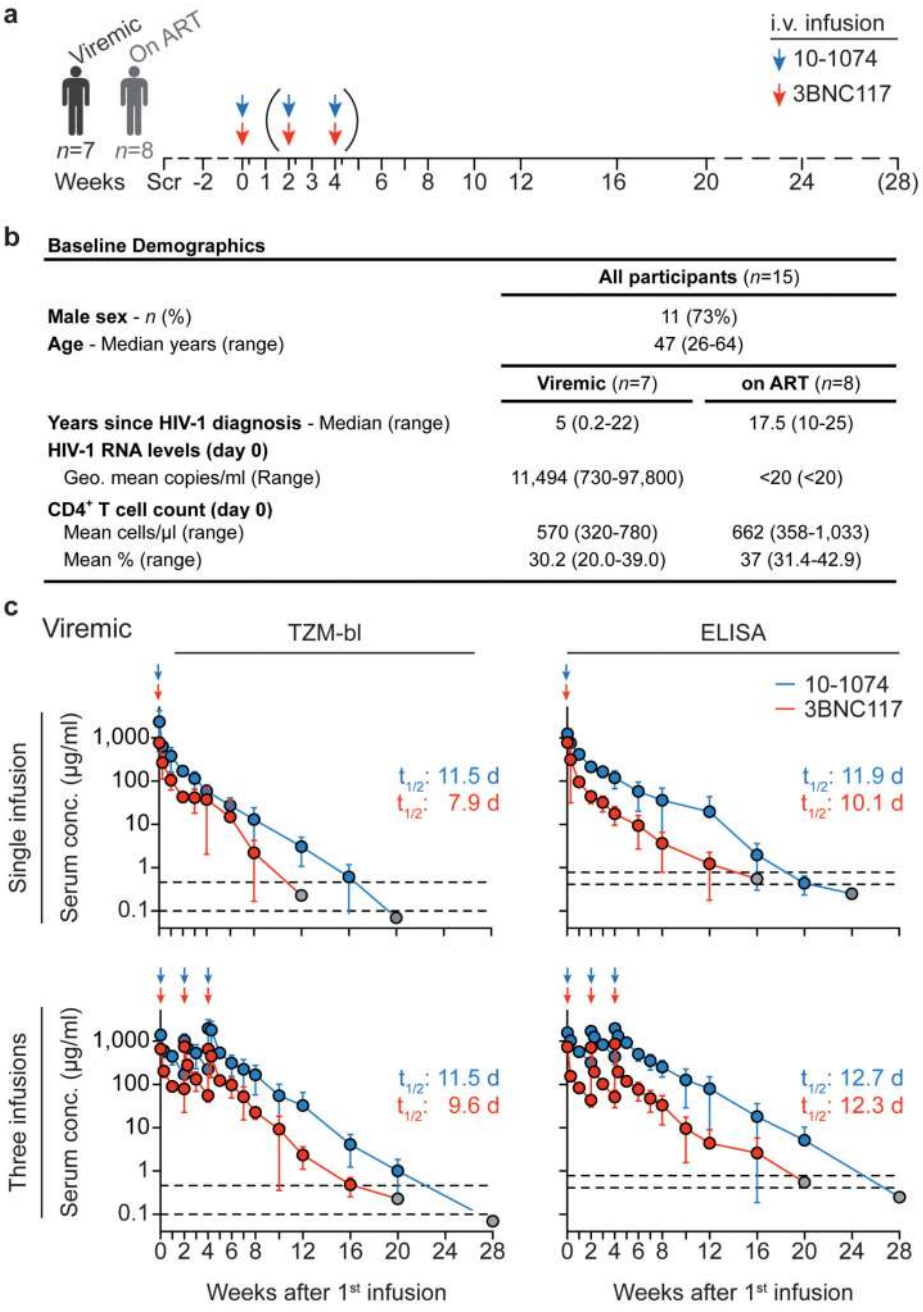

Figure 1. Study design and pharmacokinetics of 3BNC117 and 10-1074 in HIV-1-infected individuals.

(**a**) Schematic representation of the study design. (**b**) Baseline demographics of study participants. (**c**) Serum concentrations (µg/ml) of 3BNC117 (red) and 10-1074 (blue) in viremic individuals after a single infusion (upper panels) and three infusions given every two weeks (lower panels) of 3BNC117 and 10-1074 (30 mg/kg of each antibody). bNAb concentrations were determined by TZM-bl assay (left) and ELISA (right). Lines indicate arithmetic mean concentration and standard deviation. Dotted grey lines indicate lower

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limits of quantitation (TZM-bl, 0.46 μg/ml and 0.1 μg/ml for 3BNC117 and 10-1074, respectively; ELISA, 0.78 μg/ml and 0.41 μg/ml for 3BNC117 and 10-1074, respectively). Grey circles indicate antibody levels below the limit of quantitation. Numbers show average half-life.

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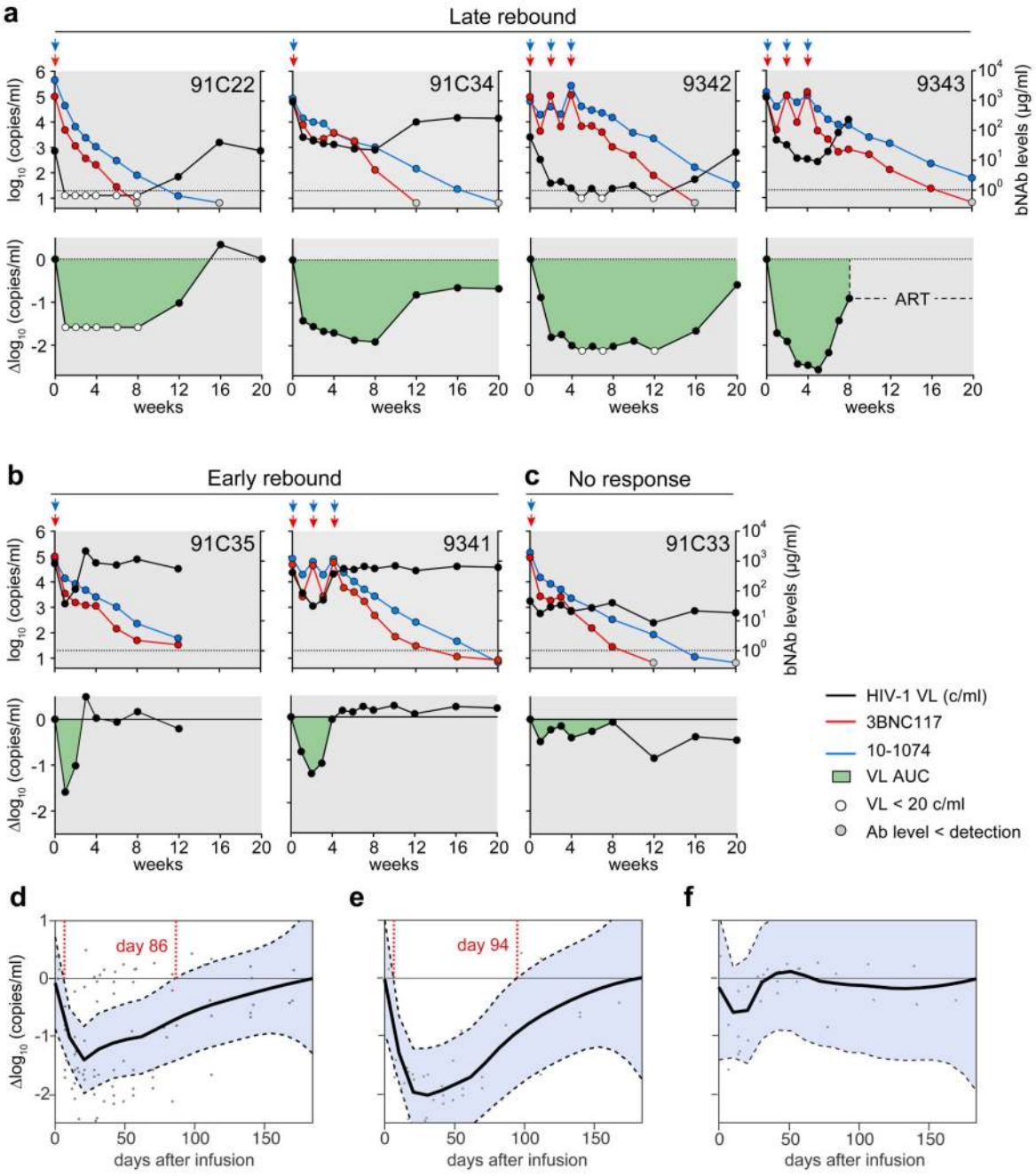

Figure 2. Viral load following 3BNC17/10-1074 infusions in HIV-1-infected participants.

(**a**-**c**) Changes in viremia and bNAb serum concentrations in HIV-1-infected participants showing (**a**) late rebound, (**b**) early rebound or (**c**) no response after 3BNC117 and 10-1074 combination therapy. Upper graphs show HIV-1 RNA in copies/ml (black, left y-axis), and 3BNC117 (red) and 10-1074 (blue) serum levels (right y-axis, as determined by TZM-bl). X-axis shows weeks after the first antibody infusion. Dashed line indicates the lower limit of detection of HIV-1 RNA (20 copies/ml). Arrows indicate antibody infusions. Lower graphs show $\log_{10}$ changes of HIV-1 RNA copies compared to day 0. Green shading depicts viral

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suppression compared to day 0. (**d-f**) Simultaneous confidence band estimation to determine time of significant suppression (red dotted lines) of HIV-1 viremia in (**d**) all viremic participants (n=7, a-c), (**e**) individuals harboring 3BNC117- and 10-1074-sensitive viruses (n=4, a), and (**f**) participants carrying viruses with partial or full bNAb resistance (n=3, b-c). Each dot represents a viral load measurement. Solid and dashed lines represent the regression fit and simultaneous confidence bands at 95% certainty level, respectively, and were computed using the Gaussian family for the local likelihood function using R package locfit (version 1.5-9.1).

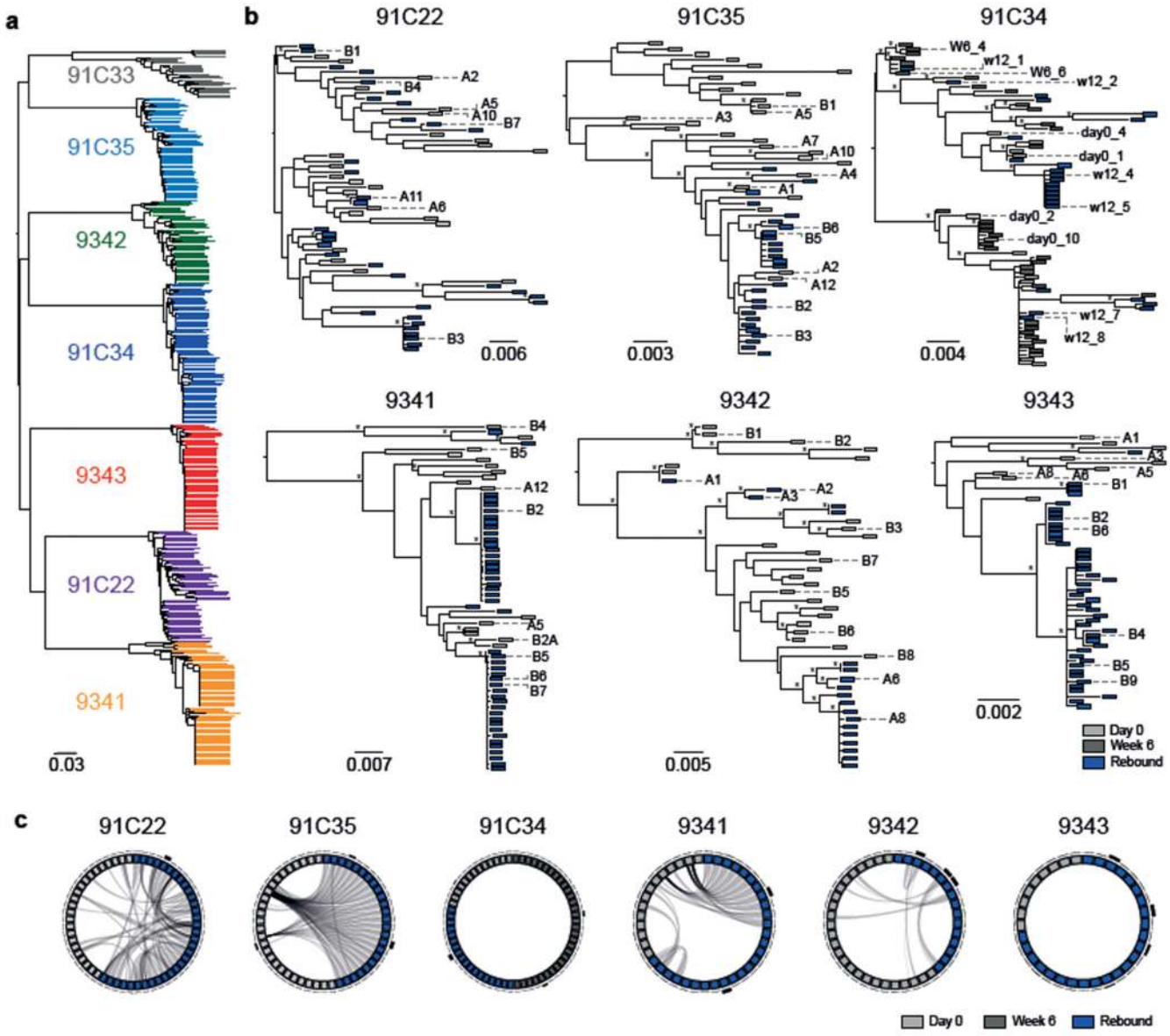

Figure 3. Phylogenetic sequence analysis of viremic participants.

(**a**) Maximum likelihood phylogenetic tree of all SGA-derived *env* gene sequences (*n*=382) obtained from plasma of viremic study participants (*n*=7). **(b)** Maximum likelihood phylogenetic trees of *env* sequences (*n*=356) obtained from plasma of single participants before antibody therapy (light grey) and at viral rebound (blue). Dark grey indicates sequences amplified at week 6 after the antibody infusion (participant 91C34). *Env* sequences that were used to produce pseudoviruses for neutralization testing are indicated. Black asterisks indicate nodes with significant bootstrap values (bootstrap support ≥70%). (**c**) Circos plots indicating the relationship between parent sequences and recombinants in single participants (n=6). SGA sequences are depicted by light grey (day 0), dark grey (week 6) and blue (rebound) rectangles. Grey lines indicate recombination events between different

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viruses. Thickness of the black outer bars represents the number of sequences obtained from that particular clone.

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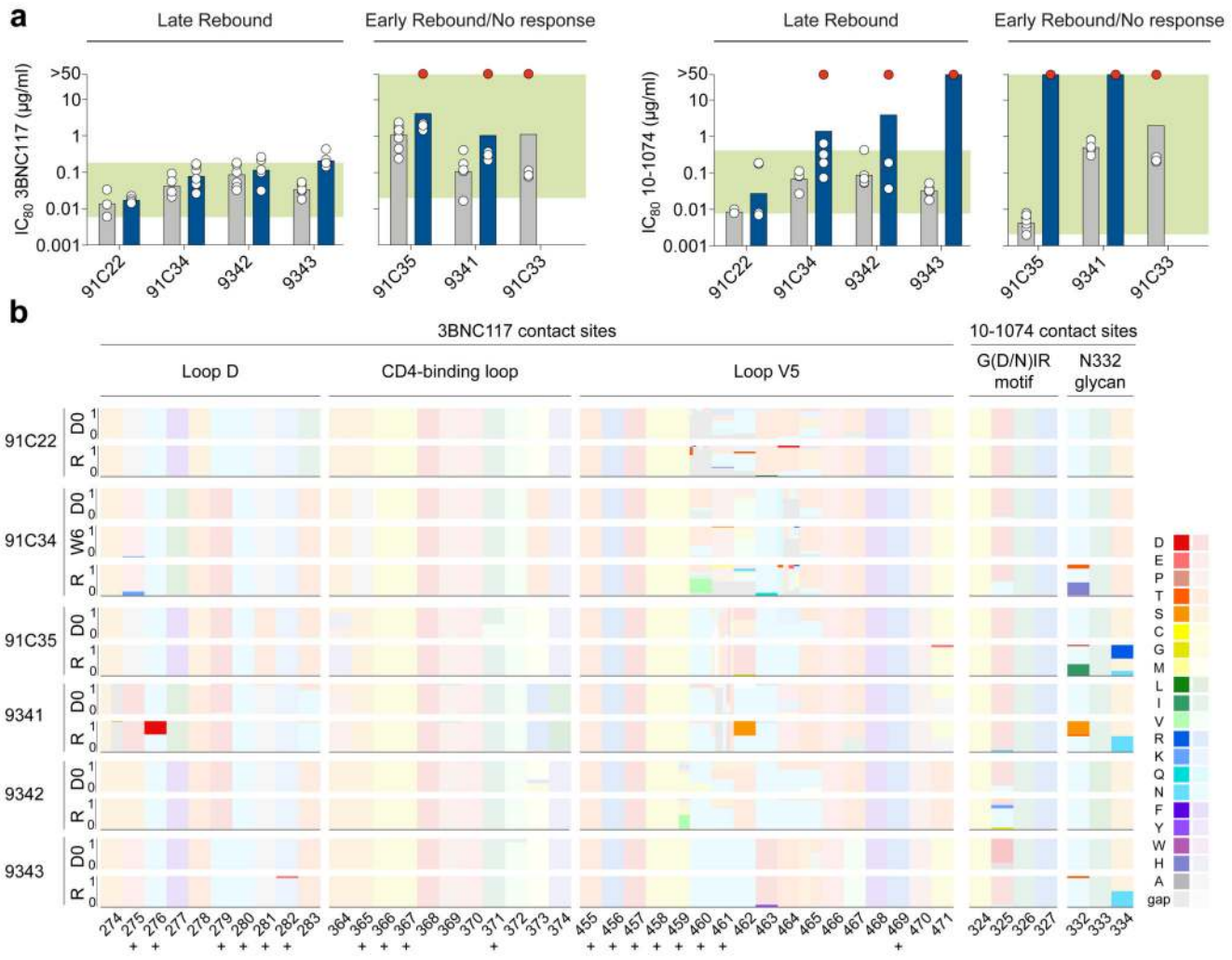

Figure 4. HIV-1 escape analysis of individuals receiving 3BNC117/10-1074 therapy.

(**a**) Viral sensitivities (IC_{80}, μg/ml) of pseudoviruses constructed from SGA-derived *env* sequences obtained on day 0 (grey) and at the time of rebound (blue). Columns reflect geometric mean IC_{80}s of viruses tested against 3BNC117 (left panel) and 10-1074 (right panel). Each dot represents one viral isolate. Fully resistant viruses (IC_{80} > 50 μg/ml) are depicted by red circles. Green shading indicates the range of IC_{80}s. (**b**) Frequency of amino acids in and around known 3BNC117 and 10-1074 contact residues in Env (3BNC117, aa 274–283, 364–374, and 455 to 471; 10-1074, aa 324-327 and 332-334). Amino acids are numbered according to HXB2. + indicate 3BNC117 contact sites32. D0 indicates viruses isolated from plasma by SGA before antibody infusions (day 0) and R indicates rebound viruses isolated by SGA. 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 represent instances in which amino acids that were found in rebound viruses were also found in day 0 viruses at the indicated position. Full-color rectangles represent instances in which an amino acid was found in rebound sequences but not in day 0 sequences.