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LETTERS

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

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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 variants[1,2]. Combinations of drugs can establish long-term control, however, antiretroviral therapy (ART) requires daily dosing, can cause side effects and does not eradicate the infection[3,4]. Although anti-HIV-1 antibodies constitute a potential alternative to ART[5,6], treatment of viremic individuals with a single antibody also results in emergence of resistant viral variants[7–9]. Moreover, combinations of first-generation anti-HIV-1 broadly neutralizing antibodies (bNAbs) had little measurable effect on the infection[10–12]. Here we report on a phase 1b clinical trial (NCT02825797) in which two potent bNAbs, 3BNC117[13] and 10-1074[14], were administered in combination to seven HIV-1 viremic individuals. Infusions of 30 mg kg^{-1} of each of the antibodies were well-tolerated. In the four individuals with dual antibody-sensitive viruses, immunotherapy resulted in an average reduction in HIV-1 viral load of 2.05 $\log_{10}$ copies per ml that remained significantly reduced for three 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 bNAbs that target the CD4 binding site and the base of the V3 loop on the HIV-1 envelope spike, respectively[13,14]. Infusion of the combination of 3BNC117 and 10-1074 during ART interruption maintains suppression of viremia and prevents the emergence of resistant variants[15].

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 that are present during active infection. Thus, although monotherapy with any one of three different bNAbs reduced viremia by 1.1–1.5 $\log_{10}$ copies per ml, these effects were transient and superseded by the emergence of antibody-resistant viral variants[7–9]. To determine whether the combination of 3BNC117 and 10-1074 is safe and results in improved antiviral activity against HIV-1 compared to monotherapy, we conducted a phase 1b trial in viremic individuals (Fig. 1a).

Viremic participants were selected from a cohort that was screened for sensitivity to 3BNC117 and 10-1074 by TZM-bl cell neutralization assays performed on viruses derived from bulk CD4$^+$ T cell outgrowth cultures[16] (Supplementary Fig. 1). In agreement with previous reports, 67 and 58% of the individuals tested showed half-maximum inhibitory concentration (IC_{50}) values of $<2\,\mu g\,ml^{-1}$ to 3BNC117 and 10-1074, respectively, and 40% were sensitive to both[8,17,18] (Supplementary Table 1). The seven viremic participants had been diagnosed with HIV-1 infection for a median of five years and had a geometric mean viral load of 11,494 copies per ml on the day of the first infusion (Fig. 1b and Supplementary Tables 2, 3). In addition, eight individuals on ART with viral loads below the limit of detection were included for safety and pharmacokinetic assessments (Fig. 1a, Supplementary Fig. 1 and Supplementary Tables 2, 3).

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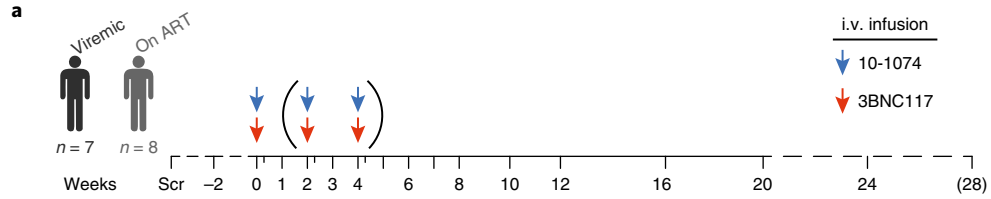

b

Baseline demographics

	All participants (n = 15)	
Male sex, n (%)	11 (73%)	
Age, median years (range)	47 (26–64)	
	Viremic (n = 7)	On ART (n = 8)
Years since HIV-1 diagnosis, median (range)	5 (0.2–22)	17.5 (10–25)
HIV-1 RNA levels (day 0)		
Mean copies per ml (range)	11,494 (730–97,800)	<20 (<20)
CD4$^+$ T cell count (day 0)		
Mean cells per µl (range)	570 (320–780)	662 (358–1,033)
Mean percentage (range)	30.2 (20.0–39.0)	37.0 (31.4–42.9)

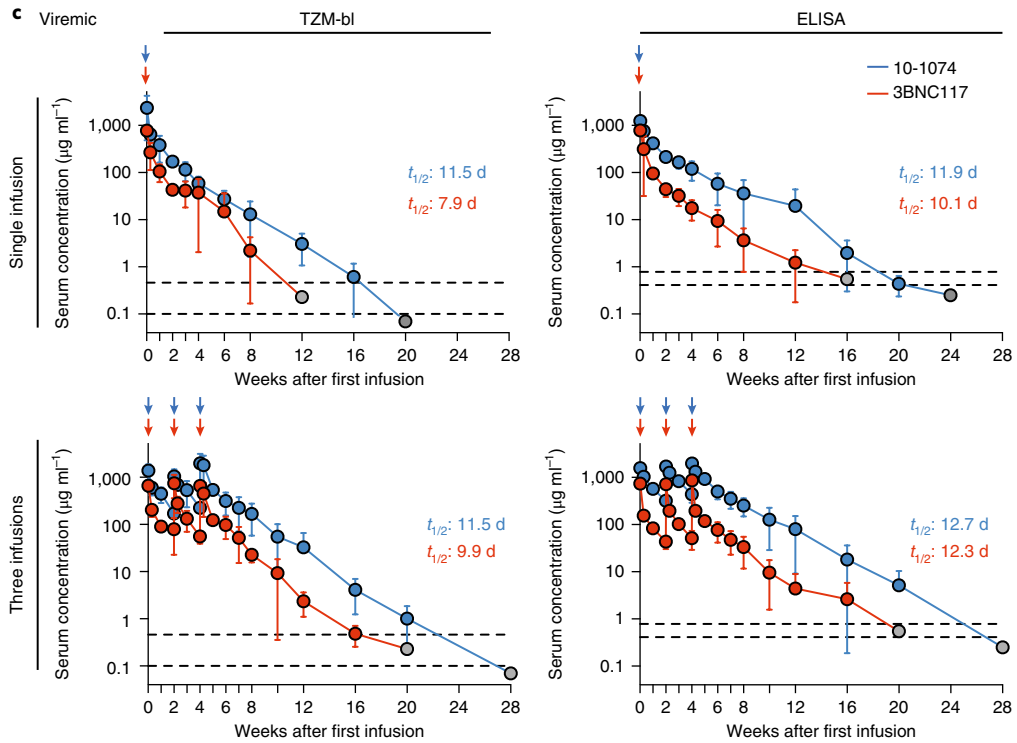

Fig. 1 | Study design and pharmacokinetics of 3BNC117 and 10-1074 in HIV-1-infected individuals. a, Schematic representation of the study design. i.v., intravenous. **b**, Baseline demographics of study participants. **c**, Serum concentrations (µg ml^{-1}) of 3BNC117 (red) and 10-1074 (blue) in viremic individuals after a single infusion (top) and three infusions given every two weeks (bottom) of 3BNC117 and 10-1074 (30 mg kg^{-1} 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 limits of quantitation (TZM-bl, 0.46 µg ml^{-1} and 0.1 µg ml^{-1} for 3BNC117 and 10-1074, respectively; ELISA, 0.78 µg ml^{-1} and 0.41 µg ml^{-1} for 3BNC117 and 10-1074, respectively). Grey circles indicate antibody levels below the limit of quantification. $t_{1/2}$, average half-life.

Participants received either a single intravenous infusion of 3BNC117 and 10-1074 at a dose of 30 mg kg^{-1} per antibody, or three infusions of 30 mg kg^{-1} 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, 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 in serum were determined via enzyme-linked immunosorbent assays (ELISAs) using anti-idiotypic antibodies and the TZM-bl assay, which measures the neutralizing activities of the antibodies in serum. In viremic individuals, the half-lives of 3BNC117 and 10-1074 were 11.1 and 12.2 days

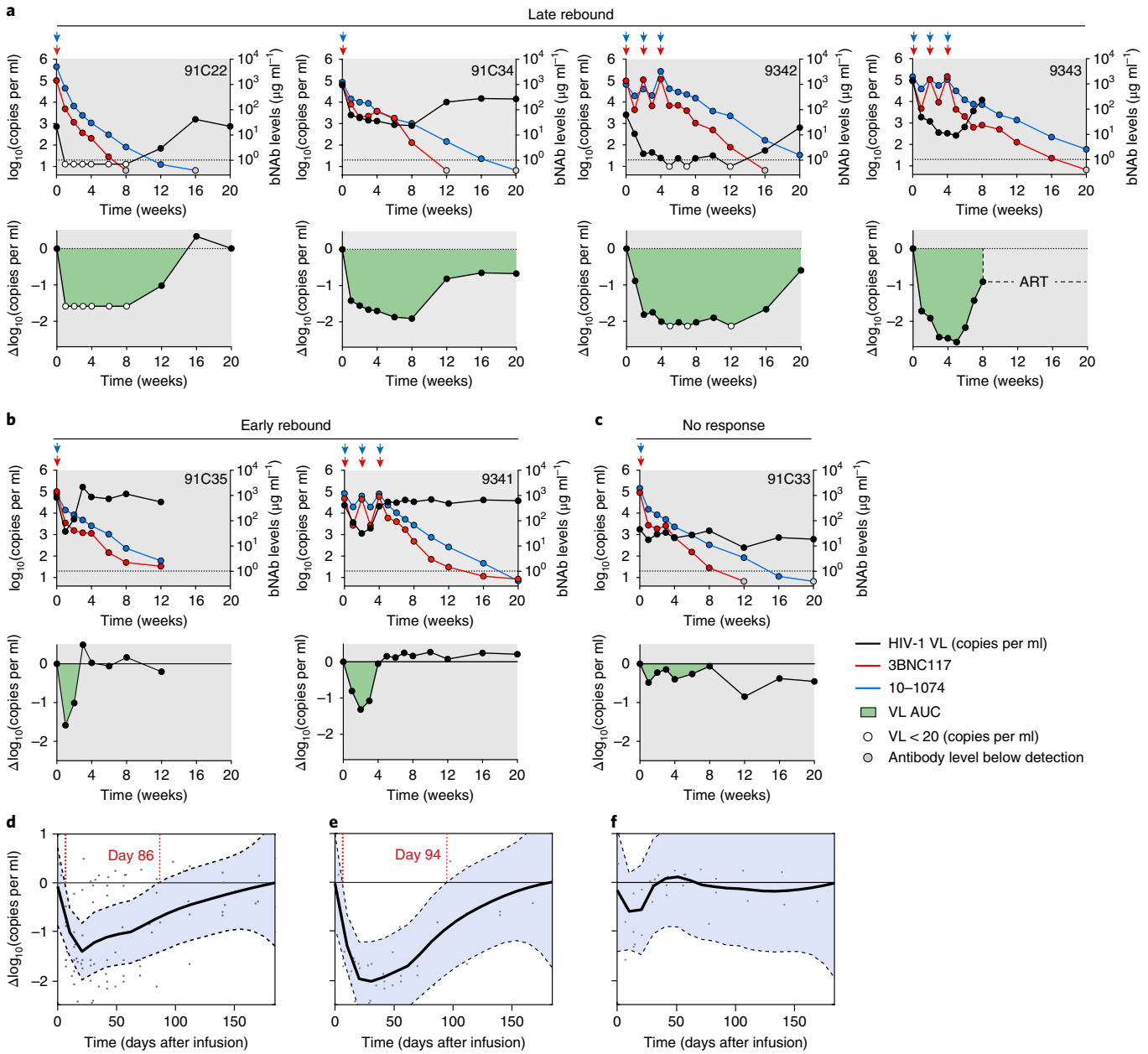

Fig. 2 | Viral load following 3BNC117/10-1074 infusions in HIV-1-infected participants. a–c, Changes in viremia and bNAb serum concentrations in HIV-1-infected participants showing late rebound (**a**), early rebound (**b**) or no response (**c**) after 3BNC117 and 10-1074 combination therapy. Top, HIV-1 RNA in $\log_{10}$ copies per ml (black, left *y* axis), and 3BNC117 (red) and 10-1074 (blue) serum levels (right *y* axis, determined by TZM-bl). The *x* axis shows the time in weeks after the first antibody infusion. The dashed line indicates the lower limit of detection of HIV-1 RNA (20 copies per ml). Arrows indicate antibody infusions. Bottom, $\log_{10}$ changes per ml in HIV-1 RNA copies compared to day 0. Green shading depicts viral suppression compared to day 0. VL, viral load. **d–f**, Simultaneous confidence band estimation to determine time of significant suppression (red dotted lines) of HIV-1 viremia in all viremic participants (**d**; $n = 7$, participants shown in **a**–**c**), individuals harboring 3BNC117- and 10-1074-sensitive viruses (**e**; $n = 4$, participants shown in **a**), and participants carrying viruses with partial or full bNAb resistance (**f**; $n = 3$, participants shown in **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).

when measured using ELISA, and 8.5 and 11.5 days when determined by the TZM-bl assay, respectively (Fig. 1, Supplementary Figs. 3, 4 and Supplementary Tables 3, 5). In ART-treated individuals, half-lives of 3BNC117 and 10-1074 were 14.5 and 19.0 days as measured using ELISA, and 11.5 and 18.4 days in the TZM-bl assay, respectively (Supplementary Figs. 3, 4 and Supplementary Table 5). Viremic individuals generally showed lower antibody half-lives than individuals on ART with suppressed viral loads, possibly owing to an antigen sink effect[7,8,19]. Overall, these values are consistent with the results obtained when both antibodies were administered individually[7,8,17] (Supplementary Fig. 3). 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 four weeks after antibody infusions and every 2–4 weeks thereafter (Fig. 2a–c, Supplementary Fig. 4 and Supplementary Table 3). The

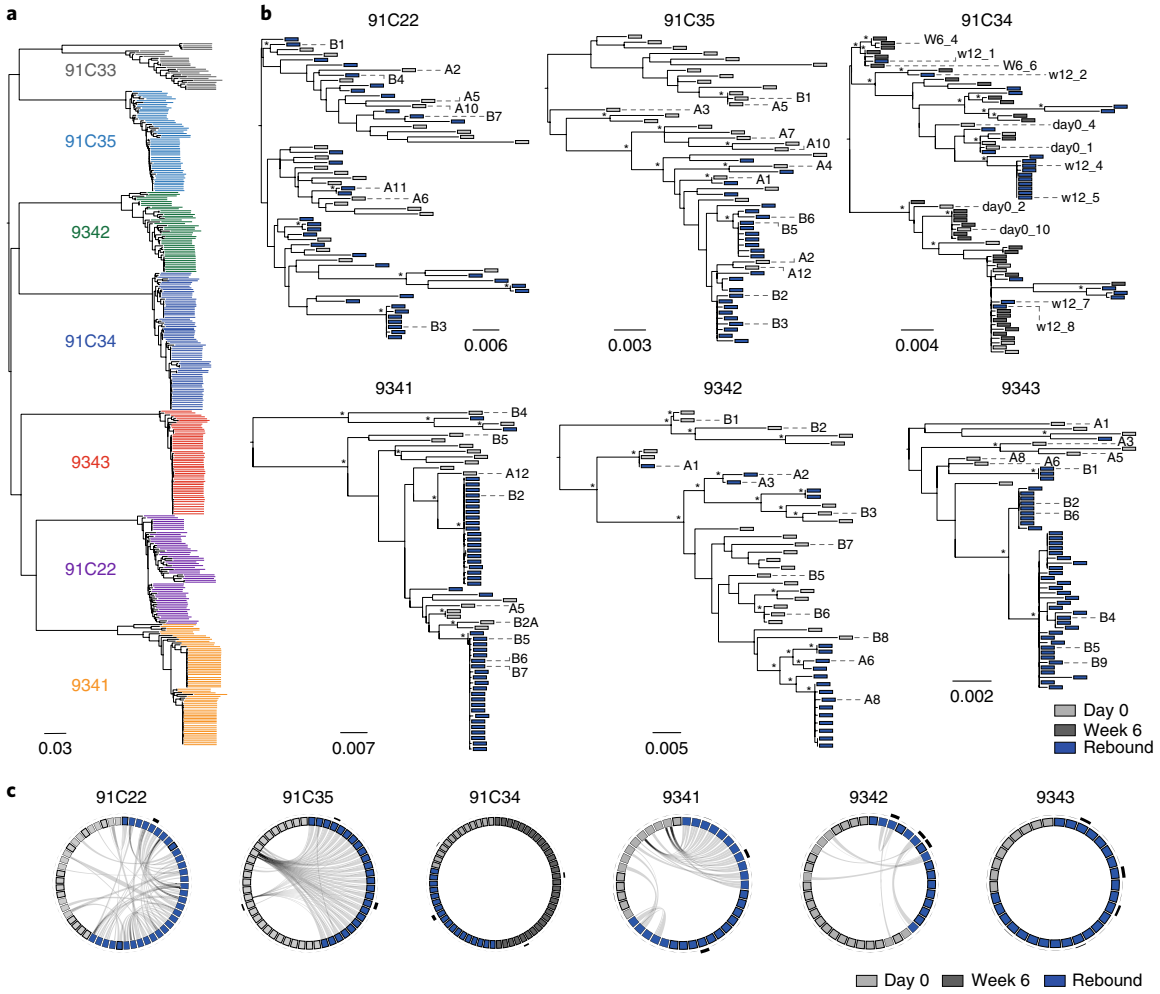

Fig. 3 | Phylogenetic sequence analysis of viremic participants. a, Maximum likelihood phylogenetic tree of all SGA-derived *env* gene sequences ($n=382$) obtained from plasma of viremic study participants ($n=7$). **b**, Maximum likelihood phylogenetic trees of *env* sequences ($n=356$, 91C33 not shown) 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 antibody infusion (participant 91C34). *env* sequences that were used to produce pseudoviruses for neutralization testing are indicated. Asterisks indicate nodes with significant bootstrap values (bootstrap support of ≥70%). **c**, Circos plots indicating the relationship between parent sequences and recombinant sequences 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 viruses. Thickness of the black outer bars represents the number of sequences obtained from that particular clone.

average drop in viral load for all viremic individuals was 1.65 $\log_{10}$ copies per ml and viremia remained significantly reduced until day 86 (Fig. 2d). The four individuals with sensitive viruses (see below) showed a more pronounced drop in viral load compared to the other individuals (average of 2.05 $\log_{10}$ copies per ml) and were significantly suppressed until day 94 (Fig. 2a,e,f). In comparison to a single infusion of either 3BNC117[7] or 10-1074[8], viremic individuals receiving one or three infusions of the combination of both antibodies showed significantly prolonged viral suppression ($P=0.00018$) (Fig. 2d and 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. Initially, 382 intact full-length *env* sequences were analyzed from the seven viremic participants (Supplementary Fig. 6). All of these individuals were infected with epidemiologically distinct clade B virus (Fig. 3a). In addition, sequences of circulating viruses 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 the TZM-bl assay (Fig. 4a and Supplementary Table 6). Participant 91C33, who failed to respond to antibody infusions, had preexisting circulating viruses that were resistant to both antibodies (Fig. 4a and Supplementary Table 6). These viruses carried mutations in 3BNC117 contact sites (N280S and A281H) and in 10-1074 contact sites (N332T and S334N, Supplementary Fig. 6). Two individuals, 91C35 and 9341, responded to antibody therapy with a decrease in viremia of −1.58 and −1.32 $\log_{10}$ copies per 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

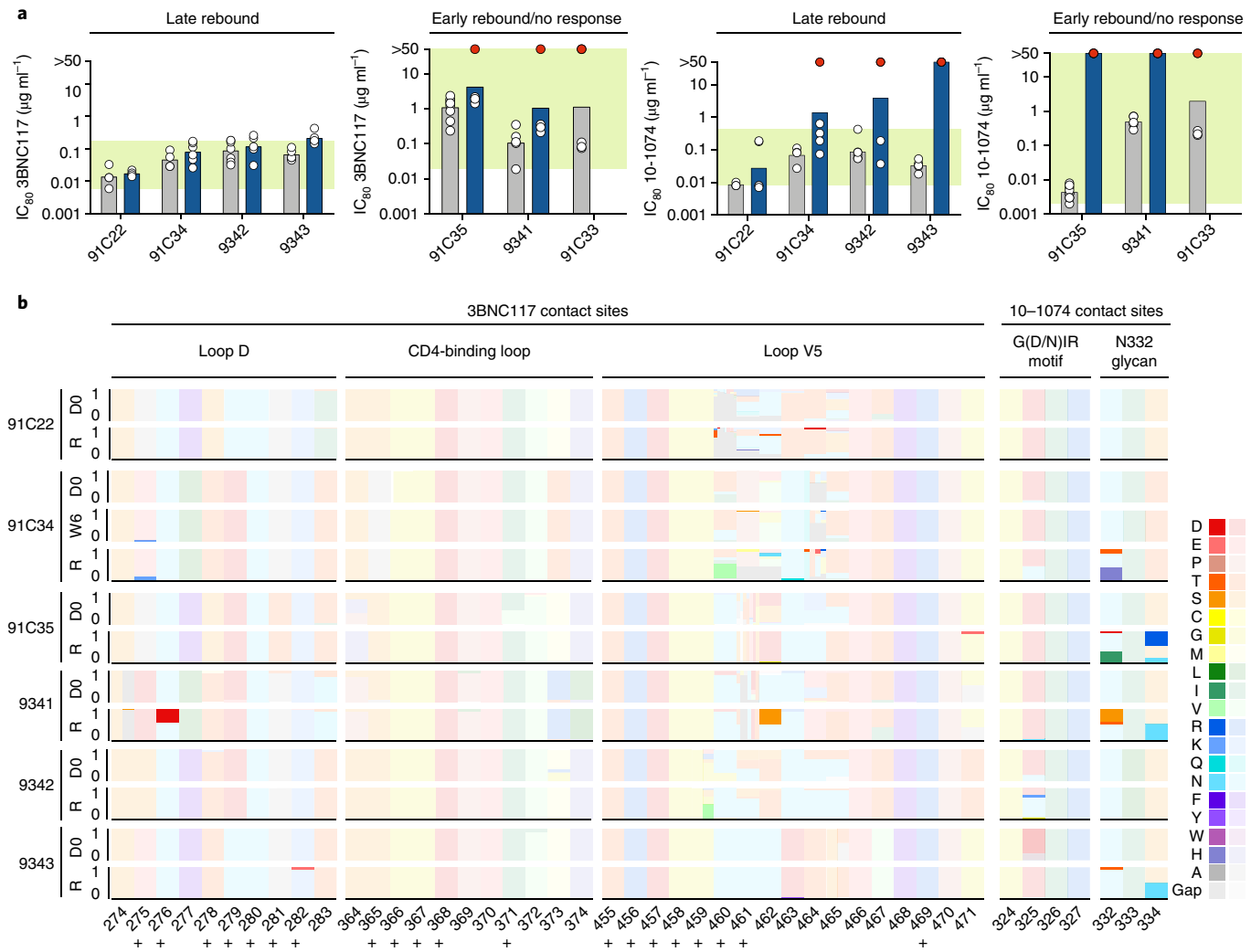

Fig. 4 | HIV-1 escape analysis of individuals receiving 3BNC117 and 10-1074 therapy. a, Viral sensitivities (IC_{80}, μg ml^{-1}) of pseudoviruses constructed from SGA-derived *env* sequences obtained on day 0 (grey) and at the time of rebound (blue). Columns reflect geometric mean IC_{80} values of viruses tested against 3BNC117 (left) and 10-1074 (right). Each circle represents one viral isolate. Fully resistant viruses ($IC_{80} > 50$ μg ml^{-1}) are depicted by red circles. Green shading indicates the range of IC_{80} values. **b**, Frequency of amino acids in and around known 3BNC117 and 10-1074 contact residues in Env (3BNC117, amino acids 274–283, 364–374 and 455–471; 10-1074, amino acids 324–327 and 332–334). Amino acids are numbered according to HXB2. 3BNC117 contact sites are indicated by '+'[32]. D0 indicates viruses isolated from plasma by SGA before antibody infusions (day 0); R indicates rebound viruses isolated by SGA; W6 indicates viruses isolated at week 6. 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.

a CD4 contact residue mutation (A281T) that was associated with viral escape from 3BNC117[20] (Fig. 4a, Supplementary Figs. 6 and 7 and Supplementary Table 6). Pre-infusion viruses derived from bulk CD4$^+$ T cell outgrowth cultures of 9341 showed a 10-1074 IC_{80} that 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 Figs. 6, 7 and Supplementary Table 6). In addition, rebound viruses from 91C35 and 9341 contained G471E and N276D mutations, respectively, that are associated with increased resistance to 3BNC117 (Supplementary Fig. 6)[7,17,21,22]. These mutations were not found in the pre-infusion circulating viruses described above or in the additional 113 pre-infusion *env* sequences that were analyzed from these two participants (Supplementary Fig. 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 viremia[7–9]. 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 three of the seven 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 per ml (Figs. 2a,e, 4a and Supplementary Table 6). The individual in this group with the highest initial viral load (97,800 copies per ml; patient 9343) was the first to rebound at eight weeks (Fig. 2a and Supplementary Table 3). The two individuals with the lowest

initial viral loads, 91C22 and 9342 (750 and 2,550 copies per ml, respectively), demonstrated suppression to near or below the limit of detection for 12 and 16 weeks, respectively (Fig. 2a and Supplementary Table 3). Finally, viremia in participant 91C34 was reduced for a period of 12 weeks, however it never dropped below 810 copies per 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^{-1} (Supplementary Figs. 7, 9 and Supplementary Table 3).

In three of the four initially sensitive individuals, rebound viremia was associated with the appearance of viruses that were resistant to 10-1074, but these individuals 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 and Supplementary Figs. 6, 7). By contrast, there was no accumulation of de novo mutations in 3BNC117 contact sites (Fig. 4b and Supplementary Figs. 6, 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 and Supplementary Tables 3, 6). Overall none of the four participants that were initially sensitive to the two antibodies developed de novo resistance to 3BNC117 over a cumulative observation period of over one 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 chimeric simian/human immunodeficiency virus AD8. For example, suppression was incomplete in macaques with higher initial viral loads; however, despite persistent low-level viremia, there was no emergence of 3BNC117 and 10-1074 double-resistant variants[23]. In contrast to the macaque infection with a clonal virus, each of the four 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 therapy[24]. CD8$^+$ T cells have also been implicated in HIV-1 control in humans[25]. 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 a reduction in viral fitness[26]. Combinations of just two antibodies that synergize to further restrict viral escape may be even more effective than 3BNC117 and 10-1074[27].

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. By contrast, the culture-based method used to screen for resistance in this study failed to detect partial or complete pre-existing antibody resistance in three of the seven 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 per ml was only achieved in individual 91C22, who had the lowest pre-infusion viral load (730 copies per ml). Thus, whereas two antibodies may be sufficient to achieve and/or maintain suppression in sensitive individuals with very low levels of viremia or ART-suppressed individuals undergoing analytical treatment interruption[15], 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 three bNAb infusions. However, despite the small number of infusions, sensitive individuals maintained reductions in viral load for up to three 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[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 10-1074[31]. 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 content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41591-018-0186-4.

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Author contributions

M.C. (principal investigator in the United States), F.K. (principal investigator in 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.Su., 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.Sh. and K.J. performed, coordinated or contributed to sample processing; K.E.S., N.L.Y. and G.D.T. performed anti-idiotypic ELISAs; 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.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41591-018-0186-4.

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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 ART and were randomized in a 2:1 ratio (six participants per group) to receive one intravenous infusion of 3BNC117 and 10-1074 (group 1A, 10 mg kg^{-1} per antibody; group 1B, 30 mg kg^{-1} 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 (four participants) or group 3 (three participants), and received one intravenous infusion (group 1C) or three intravenous infusions (group 3, every two weeks) of 3BNC117 and 10-1074 at a dose of 30 mg kg^{-1}. 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 per µl. Individuals on ART were eligible for participation and enrollment in groups 1A and 1B if HIV-1 RNA levels were <20 copies per 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 per 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 prescreened for the sensitivity of bulk CD4$^+$ T cell outgrowth culture-derived viruses to 3BNC117 and 10-1074 as described below. Antibody sensitivity was defined as an $IC_{50} < 2$ µg ml^{-1} for both 3BNC117 and 10-1074 measured in a TZM-bl neutralization assay.

Study procedures. 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 min. Both antibodies were administered individually and sequentially. Placebo recipients received equivalent volumes of sterile normal saline. Study participants were observed at the Rockefeller University Hospital or the University Hospital Cologne for 4 h (groups 1A–C) or 1 h (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 using an automated cell counter (Vi-Cell XR; Beckman Coulter) or manually. Isolated cells were cryopreserved in fetal bovine serum and 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 with two- to four-week intervals and at the final study visit. 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 quantification range between 2×10^1 and 1×10^7 viral copies per 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 using a clinical flow cytometry assay performed at LabCorp or at the University Hospital Cologne every 2–4 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 described[16]. In brief, serum samples were heat-inactivated for 1 h at 56 °C and tested using a primary 1:20 dilution and a fivefold 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 half-maximum inhibitory dilution (ID_{50}) 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^{-1} with a fivefold 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.S., unpublished data). In viremic individuals, 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} values of the monoclonal antibodies as described above to minimize the influence 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 that met Good Clinical Laboratory Practice standards. The lower limit of detection was determined to be 0.24 µg ml^{-1} and 0.10 µg ml^{-1} for the 3BNC117 and 10-1074 TZM-bl assay, respectively. The lower limit of quantification was 0.46 mcg/ml for 3BNC117 and 0.1 mcg/ml for 10-1074.

ELISA-based measurement of 3BNC117 and 10-1074 serum levels. Serum concentrations of 3BNC117 and 10-1074 were measured by a validated sandwich ELISA. High bind polystyrene plates were coated overnight at 2–8 °C with 4 µg ml^{-1} of an anti-idiotypic antibody that specifically recognizes 3BNC117 (anti-ID 1F1-2E3 monoclonal antibody) or 2 µg ml^{-1} of an anti-idiotypic antibody that specifically recognizes 10-1074 (anti-ID 3A1-4E11 monoclonal antibody). After washing, plates were blocked with 5% Milk Blotto (w/v), 5% normal goat serum (v/v), and 0.05% Tween 20 (v/v) in PBS. Serum samples, quality controls and standards were added (1:50 minimum dilution in 5% Milk Blotto (w/v), 5% normal goat serum (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 anti-human 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, Molecular Devices) was used to calculate serum concentrations of 3BNC117 and 10-1074 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 monoclonal antibodies were produced in a stable hybridoma cell line (Duke Protein Production Facility[7]). If day 0 samples had measurable levels of antibody by the respective assays, the measured background antibody level was subtracted from subsequent results. In addition, samples with measured antibody levels within threefold of background values were excluded from the analysis of pharmacokinetic (PK) parameters. The lower limit of detection was determined to be 0.51 µg ml^{-1} and 0.14 µg ml^{-1} in HIV-1 seropositive serum for the 3BNC117 and 10-1074 ELISA, respectively. For values that were detectable (that is, positive for monoclonal antibodies) but below the lower limit of quantification, values are reported as <0.78 µg ml^{-1} and <0.41 µg ml^{-1} for 3BNC117 and 10-1074 ELISA.

SGA of viral *env* genes. SGA and sequencing of HIV-1 *env* genes was performed for plasma samples as described previously[17,33]. All *env* sequences were translated to amino acids and aligned using ClustalW[34]. 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 number 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 v.3.1[36] using the GTR model[37] with 1,000 bootstraps. For the combined analysis of sequences from all participants, *env* sequences were aligned using MAFFT v.7.309[38] and clustered using RAxML v.8.2.9 using the GTRGAMMA model[37] with 1,000 bootstraps.

Pseudovirus production. Selected viral sequences that were isolated from the plasma of each participant by SGA were used to generate CMV-promoter-based pseudoviruses as previously described[33,39]. The CMV promoter was amplified using the forward primer 5′-AGTAATCAATTACGGGGTCATTAGTTCAT-3′ and the reverse primer 5′-CATAGGAGATGCCTAAGCGGTGGAGCTCTGCTTATA TAGACCTC-3′. Individual *env* amplicons were amplified using the forward primer 5′-CACCGGCTTAGGCATCTCCTATGGCAGGAAGAA-3′ and the reverse primer 5′-GTCTCGAGATACTGCTCCCACCC-3′. To fuse the individual

purified *env* amplicons to the CMV promoter, overlapping PCR was performed using the forward primer 5′-AGTAATCAATTACGGGGTCATTAGTTCAT-3′ and the reverse primer 5′-ACTTTTTGACCACTTGCCACCCAT-3′. Pseudoviruses were generated by transfecting HEK293T cells as previously described[39].

Prescreening bulk PBMC culture. Candidate viremic individuals were prescreened for sensitivity of bulk culture-derived outgrowth viruses against 3BNC117 and 10-1074 as described previously[7,8,15,17]. PBMCs for prescreening were obtained a median of 27 weeks (range 4.9–38 weeks) before enrollment under separate protocols approved by the Institutional Review Boards of the Rockefeller University and the University of Cologne. In brief, isolated $CD4^+$ T cells were cocultured with the MOLT-4/CCR-5 cell line (NIH AIDS Reagent Program, cat. no. 4984) 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} values for 3BNC117 and 10-1074 were $<2\,\mu g\,ml^{-1}$.

Virus neutralization assays. Supernatants from p24-positive bulk $CD4^+$ T cell cultures and pseudoviruses were tested for sensitivity to antibodies as previously described[16].

Pharmacokinetic analyses. PK parameters were estimated by performing a non-compartmental analysis 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 shown in a circos plot (http://circos.ca/).

Statistical analyses. The sample size to detect a decline in viremia of >0.9 log_{10} copies per ml with 80% power at 5% significance level with P of 0.05 was determined to be six viremic HIV-1-infected individuals, assuming that the standard deviation would be similar to 3BNC117 or 10-1074 monotherapy in humans (s.d. of 0.75 and 0.6, respectively)[7,8]. To measure the effect of the combination treatment on viral load, we estimated simultaneous confidence bands for the Δlog_{10} viral loads. The viral load was considered significantly suppressed whenever the two dashed lines representing the simultaneous confidence bands at 95% certainty level excluded zero (Fig. 2d–f). We computed simultaneous confidence bands 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 the 3BNC117 and 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 included[7,8]. We compared it to a model without treatment as predictor using a likelihood ratio test. The time point of viral load measurement was modeled as an ordered factor and the 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 fitted maximizing the log-likelihood with the lme function of the R package nlme (version 3.1–131). We decided on the best model using Akaike information criterion (see Supplementary Fig. 5). Time points were restricted to day 0, week 1, week 2, week 3, week 4, week 6, week 8, week 12, week 16, week 20 and week 24 to have a sufficient number of measurements per time point. Marginal means (also known as 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).

Reporting Summary. Further information on research design can be found in the Nature Research Reporting Summary linked to this article.

Data availability

All requests for raw and analyzed data and materials are promptly reviewed by the Rockefeller University to verify whether 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.

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☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Software for obtaining the sequencing data was MiSeq Control Software 2.6 2.1. Software for tracking the clinical samples was IRIS by iMedRIS.
Data analysis	- Pharmacokinetics analysis was performed with Phoenix WinNonlin Build 8 (Certara). - To measure the effect of the combination treatment on viral load (VL), we estimated simultaneous confidence bands (SCBs) for the delta log10 VLs. . We computed SCBs with the R package locfit (version 1.5-9.1) using the Gaussian family for the local likelihood function. 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 were included. 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). Time points were restricted to {day0, week1, week2, week3, week4, week6, week8, week12, week16, week20, week24} to have a sufficient number of measurements per time point. CD4+ T cell counts before and after 3BNC117 plus 10-1074 infusions were compared by one-way ANOVA using GraphPad Prism. Logo plots were generated using the longitudinal antigenic sequences and sites from intra-host evolution tool (LASSIE) - Analysis of HIV-1 envelope sequences was performed before infusion of the antibodies (day 0) and at viral rebound. Multiple alignment

of nucleotide sequences guided by amino acid translations of env sequences was performed by TranslatorX (http://translatorx.co.uk/). Sequences were analyzed for the presence of recombination using the 3SEQ recombination algorithm (http://mol.ax/software/3seq/).

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

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- A list of figures that have associated raw data
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Sequences from all isolated viruses will be made available in GenBank.

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Life sciences study design

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

Sample size

A single infusion of 3BNC117 at 30 mg/kg in 10 viremic participants led to an average decline in plasma HIV-1 RNA levels of 1.48 log10 copies/ml (SD = 0.6 log10 copies/ml). When administered at 30 mg/kg, 10-1074 led to similar decline in plasma HIV-1 RNA (average decline of 1.48 log10 copies/ml, SD = 0.62 log10 copies/ml). When the combination of 3BNC117 and 10-1074 was administered to SHIV-infected non-human primates average decline in plasma viremia was 2 log10 copies/ml. (SD = 0.75 log10 copies/ml).

The table below shows the minimum average decline in plasma HIV-1 RNA levels (log10) that can be detected with 80% power and a one-sided 0.05 test, assuming a standard deviation of 0.6 (log10 copies/ml) from the 3BNC117 single infusion data and 10-1074 at 30 mg/kg data in humans, and 0.75 (log10 copies/ml) from the 3BNC117 plus 10-1074 data in non-human primates. The calculations were performed for n=4 to 6 subjects off ART in Groups 1C and 3.

Table 1. Minimum average decline in plasma HIV-1 RNA levels

N	standard deviation (Log10)	Minimum average decline in plasma HIV-1 RNA (Log10)
4	0.6	0.99
4	0.75	1.24
5	0.6	0.82
5	0.75	1.02
6	0.6	0.71
6	0.75	0.89

Data exclusions

Data from individuals on ART that were randomized to receive placebo are not included in this manuscript.

Replication

This study was a clinical trial and the analyses were performed on individual trial participants. Experiments did not include replicates as all participants and data points are unique. All available data is included in the manuscript.

Randomization

Two groups of the study, Groups 1A and 1B of ART-treated individuals, were randomized 2:1 to receive the antibodies or placebo (sterile saline).

Blinding

Enrollment in all study groups was sequential. Enrollment in the ART-treated groups was double-blinded and placebo-controlled. Enrollment in the viremic groups was open label and not placebo-controlled. Upon careful consideration of risk-benefit and in view of current recommendations to start HIV-infected participants on ART at diagnosis or as soon as possible, we concluded that the information to be gained by including placebo comparators in the viremic groups did not appropriately justify delaying initiation of standard of care in participants who might be randomized to receive sterile saline, at this early stage of clinical development.

Reporting for specific materials, systems and methods

Materials & experimental systems

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

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials: The study included the analyses of blood samples collected from enrolled HIV-infected trial participants. These samples were obtained under an IRB approved protocol for the purposes of this study and associated analytical plan.

Antibodies

Antibodies used:

3BNC117 and 10-1074 are investigational anti-HIV-1 neutralizing antibodies manufactured for clinical use. They are being investigated under US FDA INDs 118225 and 123713, respectively.
Antibodies for the Ab detection in serum by ELISA included:
- Anti-ID 3BNC117: DHVI Protein Production Facility
  Lot #: 3BNC 29Nov2017
  Dilution: 4 ug/ml coating concentration
  Clone name: anti-ID 1F1-2E3 mAb
- Anti-ID 10-1074: DHVI Protein Production Facility
  Lot #: 3Aug2016
  Dilution: 2 ug/ml coating concentration
  Clone name: anti-ID 3A1-4E11 mAb
- (HRP)-conjugated mouse anti-human IgG kappa-chain-specific antibody (Abcam), Catalog #: ab79115
  Dilution: 1:15,000
  Clone name: SB81a
- HRP-conjugated goat anti-human IgG Fc-specific antibody from Jackson ImmunoResearch.

Validation:

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s): The 293T cell line was obtained from ATCC. the MOLT-4/CCR-5 cell line (obtained through the NIH AIDS Reagent Program)

Authentication: The cell line was not authenticated

Mycoplasma contamination: The cell line was not tested for mycoplasma

Commonly misidentified lines (See ICLAC register): None

Human research participants

Policy information about studies involving human research participants

Population characteristics: HIV-infected participants, male and female, aged 18-65 enrolled in the study. Participants were either off ART, with HIV viral loads < 100,000 copies/ml, or were fully suppressed on ART with viral loads < 20 copies/ml. CD4 counts at screening was > 300 cells/ml in all study groups.

Recruitment: Viremic participants were pre-screened for sensitivity of circulating viruses against 3BNC117 and 10-1074 antibodies by bulk PBMC viral outgrowth. Sensitivity was defined as an IC50 <2 µg/ml for both 3BNC117 and 10-1074 against outgrowth virus.

Participants harboring sensitive viruses were invited for screening and were enrolled in the study sequentially. Participants were enrolled at two clinical sites at the Rockefeller University (New York, US) and Cologne University Hospital (Germany).