

Broadly neutralizing antibodies in adult males living with HIV undergoing analytical treatment interruption: secondary and exploratory outcomes of the phase II randomized controlled RIO trial

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RIO is an ongoing phase 2 double-blind randomized placebo-controlled human trial. Sixty-eight adult men living with human immunodeficiency virus (HIV), who were predominantly white and initiated antiretroviral therapy (ART) during primary or early-stage infection, underwent treatment interruption and were randomly assigned to a group receiving one or two doses of two long-acting broadly neutralizing antibodies (bNAbs) 3BNC117-LS and 10-1074-LS (arm A, $n = 34$) or saline (arm B, $n = 34$). The primary clinical study met its primary endpoint and was published elsewhere, demonstrating significantly delayed viral rebound and prolonged ART-free control in participants receiving bNAbs compared with placebo. The infusions were generally safe and well tolerated, with no study-related serious adverse events reported. The most common reported treatment-related adverse events were fatigue, lethargy and somnolence. Here we report on prespecified secondary analyses examining viral rebound, antibody sensitivity and reservoir dynamics, as well as prespecified exploratory analyses of rebound virus evolution and autologous antibody activity. Preinfusion reservoir measurements showed low levels of intact proviral HIV-1 DNA in circulating CD4⁺ T cells in both arms. The rebounding viruses in participants who received the antibodies showed significant selection for resistance to 10-1074-LS but not to 3BNC117-LS. Notably, there was a significant correlation between greater initial reservoir sensitivity to autologous antibodies and 10-1074 and time to rebound. Finally, comparison of preinfusion and prerebound HIV-1 proviral reservoir showed a decrease in intact but not defective proviruses in arm A but not in arm B. Together, these findings suggest that baseline humoral immunity and prolonged exposure to LS-bNAbs jointly shape post-treatment viral rebound dynamics in individuals who initiate ART during primary infection. ClinicalTrials.gov identifier: [NCT04319367](https://clinicaltrials.gov/ct2/show/study/NCT04319367).

Upon entry into CD4⁺ T cells, the human immunodeficiency virus (HIV-1) RNA genome is reverse transcribed and preferentially integrated into transcriptionally active sites in the host genome¹. High-level transcription of replication-competent proviruses leads to cell death and, if untreated, almost invariably results in lethal immunodeficiency². Antiretroviral therapy (ART) prevents the spread of infection and immunodeficiency but fails to eliminate a reservoir of latent proviruses found primarily in long-lived, resting memory CD4⁺ T cells^{3–6}. These reservoir cells express viral RNA at levels below the threshold required to either induce cytopathic effects or elicit robust immune-mediated clearance, yet they can retain the capacity to reinstate systemic infection upon ART interruption^{7–9}.

The HIV-1 reservoir contains both intact and defective proviruses and has been characterized by several different methods including viral outgrowth assays, digital droplet polymerase chain reaction (ddPCR) and whole-genome amplification and sequencing (quadruplex four-probe PCR (Q4PCR))^{10–13}. Whereas the size of the defective reservoir is relatively stable over time on ART, the intact reservoir shows early, rapid decay that slows to a half-life of approximately 4.5 years, with possible further prolongation after 7 years on therapy^{14–17}. Intact proviruses remaining in the reservoir after long-term therapy tend to reside in areas of the genome that are less transcriptionally active^{18–20}. Although the mechanism of selection against the reservoir viruses integrated into transcriptionally active parts of the genome is not precisely understood, selection is thought to be mediated by a combination of HIV-1 cytopathic effects and host immune responses.

Passively administered antibodies can enhance host immune responses and induce simian HIV control by a CD8⁺ T cell dependent mechanism^{21–23}. In addition, several small studies in humans have reported that broadly neutralizing antibody (bNAb) therapy is associated with accelerated clearance of infected cells, delayed viral rebound and a higher-than-expected rate of prolonged control^{24–29}. Control has been associated with increased HIV-1-specific CD8⁺ T cell responses in some but not all studies^{29–32}. In addition, observational studies in post-treatment controllers, people living with HIV (PLWH) and tissue culture experiments suggest that autologous antibodies may also put pressure on the reservoir^{33–36}. Together, these findings provided the rationale for evaluating whether prolonged exposure to long-acting bNAbs could enhance post-treatment viral control and for investigating the virologic and immunologic correlates associated with this outcome.

The RIO trial is a randomized, double-blind, placebo-controlled phase 2 study evaluating the long-acting bNAbs 3BNC117-LS and 10-1074-LS administered during analytical treatment interruption (ATI) in individuals who initiated ART during primary or early HIV infection^{37,38}. Here we report on prespecified secondary and exploratory analyses including viral reservoir composition and antibody sensitivity that shape rebound dynamics and post-treatment control in the RIO trial.

Results

Trial overview

Between 17 May 2021 and 30 July 2024, the RIO trial enrolled 68 participants who were randomized 1:1 to receive two bNAbs, 3BNC117-LS and 10-1074-LS (arm A) or placebo (arm B) 2 days before ATI. Baseline demographic and clinical characteristics were balanced between the two study arms. All participants were men, with a median age of 40 years, and most identified as white (58/68, 85.3%) (Extended Data Table 1). The primary outcome, reported elsewhere³⁸, was time to viral rebound within 20 weeks after ATI. Prespecified secondary outcomes were safety, delayed viral rebound and prolonged ART-free viral control after ATI. Additional prespecified secondary outcomes, including CD4⁺ T cell recovery, pharmacokinetic analyses, antiretroviral antibody responses and quality-of-life assessments, have also been reported in the primary clinical report³⁸. The infusions were generally safe.

ART was restarted if the viral load (VL) was >100,000 HIV-1 copies per ml for 2 consecutive weeks or >1,000 HIV-1 copies per ml for 6 weeks or CD4⁺ T cell count <350 cells per ml or clinical symptoms or participant preference³⁸. Early rebound before week 20 occurred in 8 arm A and 30 arm B participants: 75% versus 11% ($P < 0.001$; Extended Data Fig. 1). The median time to ART restart was 45.4 weeks in arm A, where 7 of 29 participants (24%) remained off ART at 96 weeks, compared with 4.6 weeks in arm B, where only 2 of 32 (6%) maintained undetectable HIV-1 levels throughout 96 weeks of observation ($P < 0.001$; Extended Data Fig. 1)³⁸. Notably, 13 of the 29 individuals in arm A restarted ART before meeting virological restart criteria³⁸.

Prespecified secondary outcomes presented in this study include quantitation of viral reservoir size and dynamics, time to ART restart and reservoir viral sensitivity and resistance to the administered bNAbs. Prespecified exploratory outcomes include rebound virus sequencing, phylogenetic analyses, reservoir characterization and virologic correlates of post-treatment viral control. Additional prespecified exploratory investigations, including immune phenotyping, host gene expression and HIV integration site analyses, are beyond the scope of the present study and will be reported separately. Analyses of autologous antibody activity were not prespecified in the protocol or Statistical Analysis Plan and are presented here as non-prespecified exploratory analyses. Post hoc analyses were limited to subgroup analyses of participants with distinct rebound patterns following bNAb therapy.

Baseline reservoir

To examine the HIV-1 proviral reservoir in individuals enrolled in the RIO trial, we assayed preintervention peripheral blood mononuclear cells (PBMCs). Reservoir size was estimated by ddPCR and Q4PCR^{10,11}. ddPCR documents the presence of two conserved regions in the provirus: the packaging signal and the envelope¹⁰. It is more sensitive, less expensive and scalable than Q4PCR¹¹. However, it does not allow sequence confirmation, and therefore some of the proviruses that are assigned as intact are in fact defective, a problem that can increase with time because the intact reservoirs decay more quickly than the defective ones³⁹. Q4PCR provides sequence information and direct confirmation of whether a provirus is intact or defective, but it is less sensitive than ddPCR because it requires amplification of the nine-kilobase proviral genome⁴⁰.

To examine the preintervention reservoir in the RIO participants, we performed both assays. Individuals in the two arms (ddPCR: arm A $n = 32$, arm B $n = 29$; Q4PCR: arm A $n = 30$, arm B $n = 28$) showed similar numbers of intact and defective proviruses in ddPCR and Q4PCR assays (Extended Data Fig. 2a,b and Supplementary Table 1). The geometric mean number of intact proviruses per million CD4⁺ T cells was 8.58 and 6.94 by ddPCR ($P = 0.5$) and 0.27 and 0.28 by Q4PCR ($P = 0.5$) for arms A and B, respectively (Extended Data Fig. 2a). The relatively small size of the reservoir in this cohort is consistent with early therapy^{41–43}.

We were able to recover 141 and 91 intact proviral reservoir sequences from baseline samples from 15 and 11 individuals in arms A and B, respectively (Fig. 1a,b and Supplementary Table 2). We were unable to recover intact proviruses from the remaining participants due to their small reservoirs. As expected, each participant harbored a separate non-overlapping group of intact HIV-1 proviral sequences (Fig. 1a,b). Despite early therapy and the small reservoir sizes, intact proviruses showed a median level of clonality of 57% when considering all individuals from whom we were able to obtain three or more intact proviral sequences ($n = 10$ for arm A and $n = 8$ for arm B)^{12,44–46} (Fig. 1c and Supplementary Table 2).

To determine whether baseline reservoir size was associated with virologic outcomes after ATI, we compared intact proviral reservoir measurements with time to ART restart and peak viremia. There was no significant correlation between the size of the intact proviral reservoir and time to ART restart in the placebo or bNAb arm of the study, as measured by either ddPCR or Q4PCR (Extended Data Fig. 3). In addition,

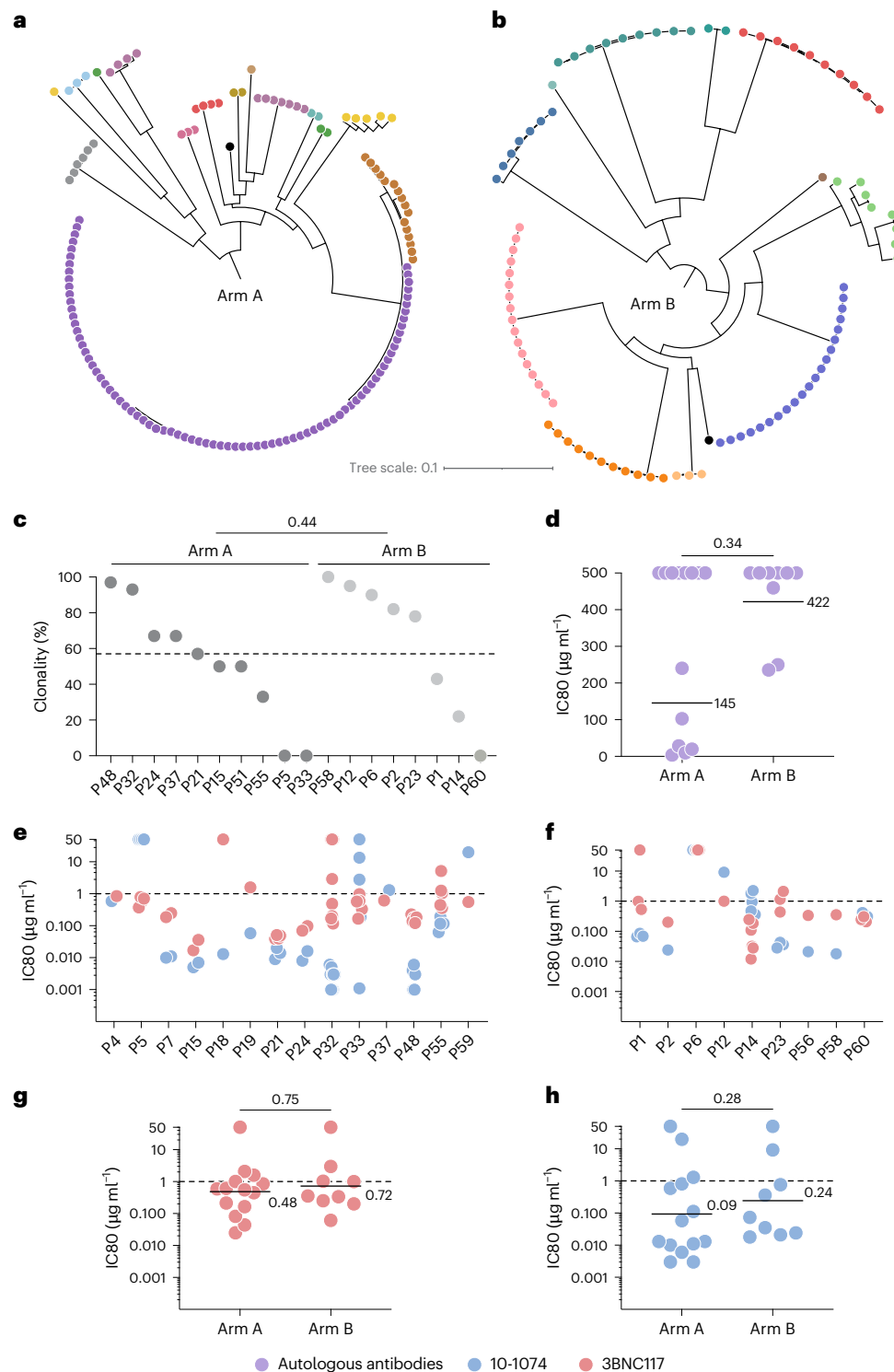


Fig. 1 | Baseline reservoir characteristics. a, b, Circular phylogenetic trees using the generalized time-reversible (GTR) model depict clonal distribution of intact proviral nucleotide sequences based on *env* in arm A (**a**) and arm B (**b**). Colors represent different participants. Black dot is HXB2. **c,** Intact reservoir clonality for the indicated participants, calculated based on amino acid sequences of *env* for individuals with ≥ 3 intact proviral sequences. Dashed line indicates combined average clonality of both arms. **d,** Baseline reservoir sensitivity to preinfusion autologous IgG antibodies, expressed as IC80. Each dot represents one participant (one biological replicate), using the geometric mean IC80 of all reservoir-derived pseudoviruses from that participant. Lines and numbers

show overall geometric mean for all participants in arms A ($n = 13$) and B ($n = 9$). **e, f,** Baseline reservoir neutralization sensitivities to 3BNC117 (red) and 10-1074 (blue), expressed as IC80, for individual pseudovirus IC80s for the indicated participants in arm A (**e**) and arm B (**f**). Dotted lines indicate 1 mg ml^{-1} . **g, h,** Each dot represents one participant (one biological replicate), using the geometric mean IC80 of all reservoir-derived pseudoviruses from that participant against 3BNC117 (**g**) and 10-1074 (**h**). Arm A $n = 14$ and arm B $n = 9$. Group comparisons in **c, d** and **g, h** were performed using a two-sided Mann-Whitney test, with statistical significance defined as $P < 0.05$.

there was no correlation between the reservoir size and peak viremia before ART restart in either group (Extended Data Fig. 3).

Sixty-five pseudotype viruses from distinct intact reservoir-derived *env* sequences from 14 and 9 individuals in arms A and B, respectively, were tested for neutralization sensitivity to autologous baseline IgG, 3BNC117 and 10-1074 in TZM-bl assays⁴⁷ (Fig. 1d–h and Supplementary Tables 2 and 4). Analysis of autologous antibody responses was performed as a non-prespecified exploratory analysis. Consistent with early therapeutic intervention, only 8 of these 23 individuals showed autologous antibody neutralizing activity, with an 80% inhibitory concentration (IC80) of $<250 \mu\text{g ml}^{-1}$ of purified IgGs (corresponding to $\geq 1:100$ dilution of serum) against their reservoir proviruses (Fig. 1d and Supplementary Tables 2 and 4). To assess whether autologous neutralizing activity changed over time, we performed longitudinal neutralization assays following depletion of residual infused bNAbs (Methods). Control experiments demonstrated that the depletion procedure efficiently removed therapeutic bNAbs while preserving autologous neutralizing activity (Extended Data Fig. 4a). Autologous IgG was tested both against baseline reservoir-derived viruses and against a panel of HIV strains resistant to 3BNC117 and 10-1074 to evaluate neutralization breadth. We found no increase in autologous neutralizing activity against baseline reservoir viruses and no measurable expansion of neutralization breadth in samples collected at a mean of 68 weeks after ATI from individuals in arm A (Extended Data Fig. 4).

Reservoir sensitivity to each of the two bNAbs ranged from an IC80 of <0.001 to $>50 \mu\text{g ml}^{-1}$ for 10-1074 and 0.012 to $>50 \mu\text{g ml}^{-1}$ for 3BNC117, with no significant differences between the two study arms (Fig. 1e–h). The geometric mean IC80s were 0.48 and $0.72 \mu\text{g ml}^{-1}$ for 3BNC117 and 0.09 and 0.24 for 10-1074 for arms A and B, respectively (Fig. 1g,h and Supplementary Table 4). All individuals in the bNAb arm showed reservoir proviral sensitivity to at least one of the two bNAbs (Supplementary Tables 2 and 4).

Rebound viremia

We were able to obtain 323 and 370 rebound viral *env* sequences by single-genome amplification from 19 and 16 individuals who experienced viral rebound in arms A and B, respectively (Supplementary Table 3). To examine the nature of rebound viremia and its relationship to the proviral reservoir, we compared reservoir and rebound *env* sequences from 18 individuals from whom we were able to obtain both intact reservoir and rebound sequences (Fig. 2a,b). Pseudotype viruses obtained from participants in arms A (96) and B (119) were produced and tested for sensitivity to the two bNAbs and to autologous antibodies obtained at the time of entry (Figs. 2c–f and 3a–f and Supplementary Table 3).

Rebound virus sequences rarely overlap with the dominant latent intact proviral clones found in the circulating reservoir^{48,49}. This phenomenon remains poorly understood but has been attributed in part to suppression of reservoir viruses by autologous antibodies^{35,50}. Among 33 intact latent proviral reservoir clones (91 sequences) obtained from 9 control-arm participants, we found only 2 that overlapped with rebound sequences (Fig. 2b). There was no overlap between the reservoir and rebound sequences in the remaining seven control participants, only three of whom had measurable levels of autologous neutralizing antibodies to the reservoir (Fig. 2e and Supplementary Tables 2 and 4). Thus, autologous antibody neutralizing activity cannot entirely account for the disparity between intact proviruses found in the reservoir and the dominant viruses found at the time of rebound in this study of individuals receiving early ART therapy. Nevertheless, rebound viruses in the three individuals from the control group who had measurable autologous neutralizing antibodies to the reservoir were resistant to their own antibodies (Fig. 2d–f). As expected, when autologous antibodies are present at baseline (during ART), rebound viruses are enriched in variants that are autologous antibody resistant^{33,34}.

Pseudotype viruses obtained from rebound were also tested for sensitivity to the two bNAbs (Fig. 3). Consistent with the absence of selective pressure in the control arm, the geometric mean bNAb sensitivity of all reservoir and rebound pseudotype viruses obtained from arm B participants was not significantly different for either antibody (3BNC117 $P = 0.76$, 10-1074 $P = 0.98$; Fig. 3b,e,f).

In contrast, selection for bNAb resistance was evident among rebound viruses obtained from arm A participants (Fig. 3a,c,d). The geometric mean neutralizing IC80s for 10-1074 against reservoir and rebound pseudoviruses was 0.09 and 14.7 mg ml^{-1} , respectively, with the majority of the rebound viruses being completely resistant ($\text{IC80} > 50 \mu\text{g ml}^{-1}$) ($P < 0.001$; Fig. 3d and Extended Data Fig. 5b). In contrast, there was a much smaller difference in the geometric mean IC80s between reservoir and rebound for 3BNC117 that did not reach statistical significance ($P = 0.53$; Fig. 3c and Extended Data Fig. 5a). Only 3 of 19 participants who received bNAbs showed rebound viruses that were uniformly resistant to 3BNC117 (Fig. 3a and Supplementary Tables 3 and 4). In conclusion, 10-1074 escape variants were far more likely to emerge than 3BNC117 resistant variants during dual bNAb therapy, as reported in earlier studies^{25,27,51}.

Viral control and reservoir antibody sensitivity

To determine whether reservoir sensitivity to either autologous antibodies or bNAbs was associated with control of viremia, we compared baseline autologous neutralizing titers and bNAb IC80s to reservoir viruses and time of ART restart (Fig. 4a–g). These analyses were limited to participants from whom reservoir viral sequences could be obtained. The mean time to ART restart among the three individuals in the control group who exhibited autologous antibody neutralizing activity against reservoir viruses was 7.3 compared with 8.5 weeks to all participants (Fig. 4b). Thus, in this small sample we see no measurable effect of the autologous antibodies in the absence of bNAb therapy. In contrast, the mean time to ART restart in the six bNAb recipients who showed baseline autologous neutralizing activity was 108 weeks compared with 27.5 to the 13 who did not ($P = 0.008$; Fig. 4a). Consistent with this observation, there was a significant correlation between preinfusion autologous neutralizing antibody titers to reservoir viruses and time to ART restart for individuals in the bNAb arm ($n = 14$, $r = -0.69$, $P = 0.004$; Fig. 4c). Time to ART restart was also correlated with initial reservoir proviral sensitivity to 10-1074 ($r = -0.79$, $P < 0.001$), but there was a trend only for 3BNC117 ($r = -0.45$, $P = 0.06$; Fig. 4d,e). Although we were unable to obtain additional intact proviral reservoir sequences, we were able to obtain intact *env*s, which were used to construct pseudotype viruses that were tested for sensitivity ($n = 5$, arm A) (Supplementary Table 2). When combined with the data obtained from intact proviruses, the correlation with time to ART restart remained significant for 10-1074 and autologous antibodies and became significant for 3BNC117 (Extended Data Fig. 6). There were no consistent associations between baseline autologous antibodies and baseline HIV-specific CD4^+ or CD8^+ T cell responses⁵² (Extended Data Fig. 7). Although correlations were observed for individual assays (AIM CD4 response to Nef and CD8 CTV response to Nef), these were not consistent across assays and sample size was limited precluding robust comparisons (Extended Data Fig. 7).

Although potentially insufficient on their own, persistent low levels of the two long-acting antibodies could contribute to control. The mean concentration of 3BNC117-LS and 10-1074-LS at 96–123 weeks after bNAb infusion in seven arm A controllers was 0.70 and $0.96 \mu\text{g ml}^{-1}$, respectively (Supplementary Table 5). To evaluate the combined influence of endogenous and therapeutic antibody pressure on rebound in arm A participants, we performed time-to-event analyses, modeling time to ART restart as a function of baseline reservoir sensitivity to autologous antibodies, 10-1074 and 3BNC117 using IC80 values. In univariable analyses, resistance to both autologous antibodies and 10-1074 was associated with shorter time

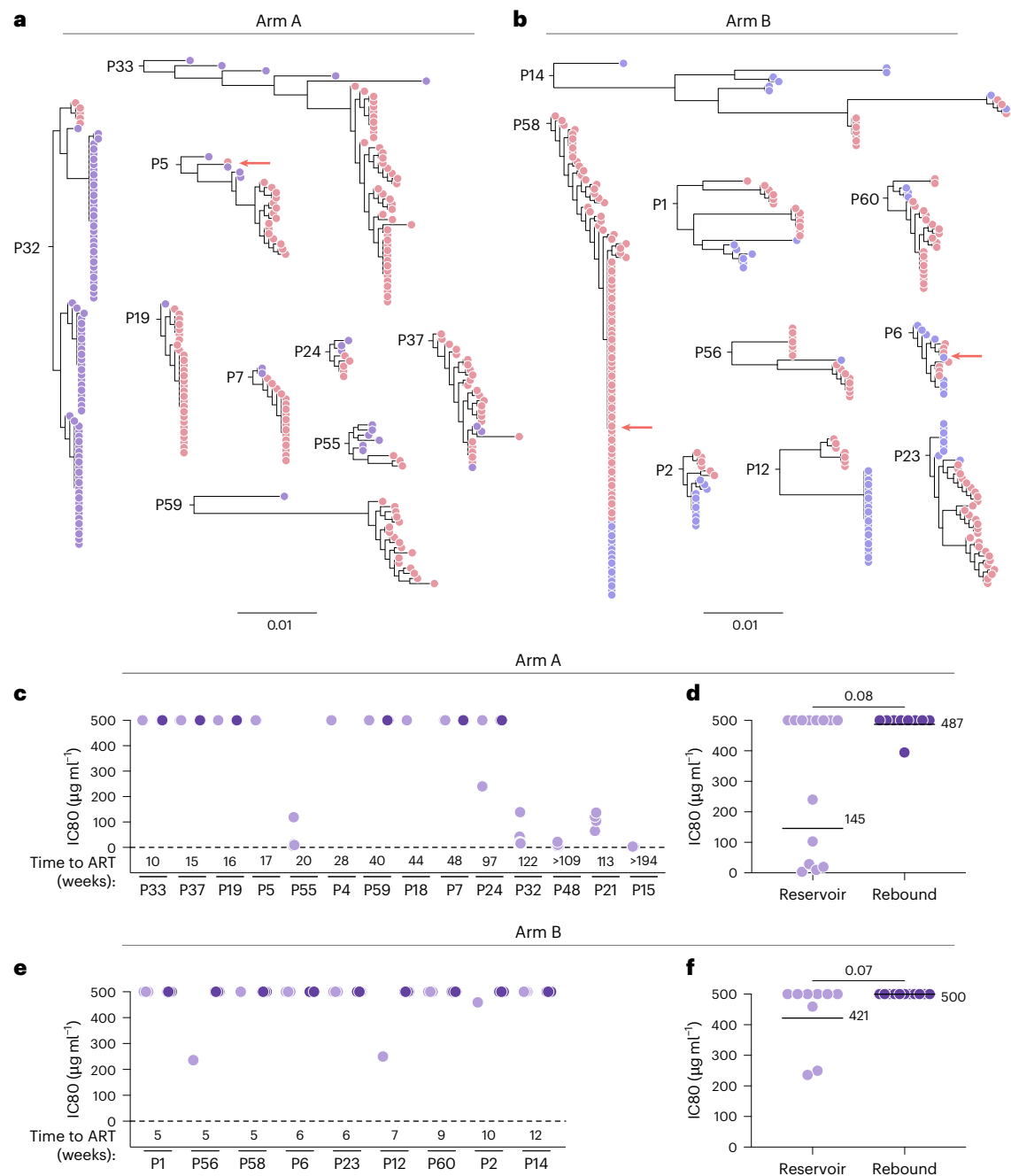


Fig. 2 | Relationship between intact proviral reservoir and rebound *env* nucleotide sequences from participants in both study arms. a,b, Phylogenetic trees were constructed using the GTR model for participants in arm A (a) and arm B (b). Intact reservoir proviruses are shown in purple and rebound in red. Red arrows indicate sequences shared between reservoir and rebound. c–f, Neutralization of reservoir and rebound pseudoviruses by autologous antibodies. Individual pseudovirus IC80s for participants from arm A (c)

and arm B (e). Numbers at the bottom indicate the time to ART restart in weeks. Each dot represents one participant (one biological replicate) from arm A (d) or arm B (f), using the geometric mean IC80 of all pseudoviruses from that participant. Arm A reservoir $n = 13$ and rebound $n = 9$. Arm B reservoir $n = 9$ and rebound $n = 11$. Lines and numbers indicate geometric mean for all participants. Group comparisons in d and f were performed using a two-sided Mann–Whitney test, with statistical significance defined as $P < 0.05$.

to ART restart. Because reservoir sensitivity to autologous antibodies tended to positively correlate with sensitivity to 10-1074 (and to a lesser extent 3BNC117), these variables were partially collinear and may reflect overlapping antiviral susceptibility profiles. Consistent with this, the associations were attenuated in exploratory multivariable models when included together (Supplementary Table 6). Notably, autologous antibodies and 10-1074 retained significance when adjusted for 3BNC117 alone.

There was an inverse correlation between 3BNC117 concentration at the time of rebound and the IC80 of the rebounding virus to the bNA

(Fig. 4f). In contrast, this relationship was not observed for 10-1074, which displayed a broad range of concentrations at rebound (Fig. 4g). These results are consistent with the idea that individuals who rebound do so when they develop resistance to 10-1074 and have subtherapeutic concentrations of 3BNC117 in circulation. Although the number of reservoir viruses analyzed was a small fraction of the total present in any given individual, the data indicate that the probability of escape from 10-1074 was directly related to the sensitivity of the intact proviruses in the initial reservoir (Fig. 4e). Furthermore, baseline reservoir size did not correlate with the presence or magnitude of autologous

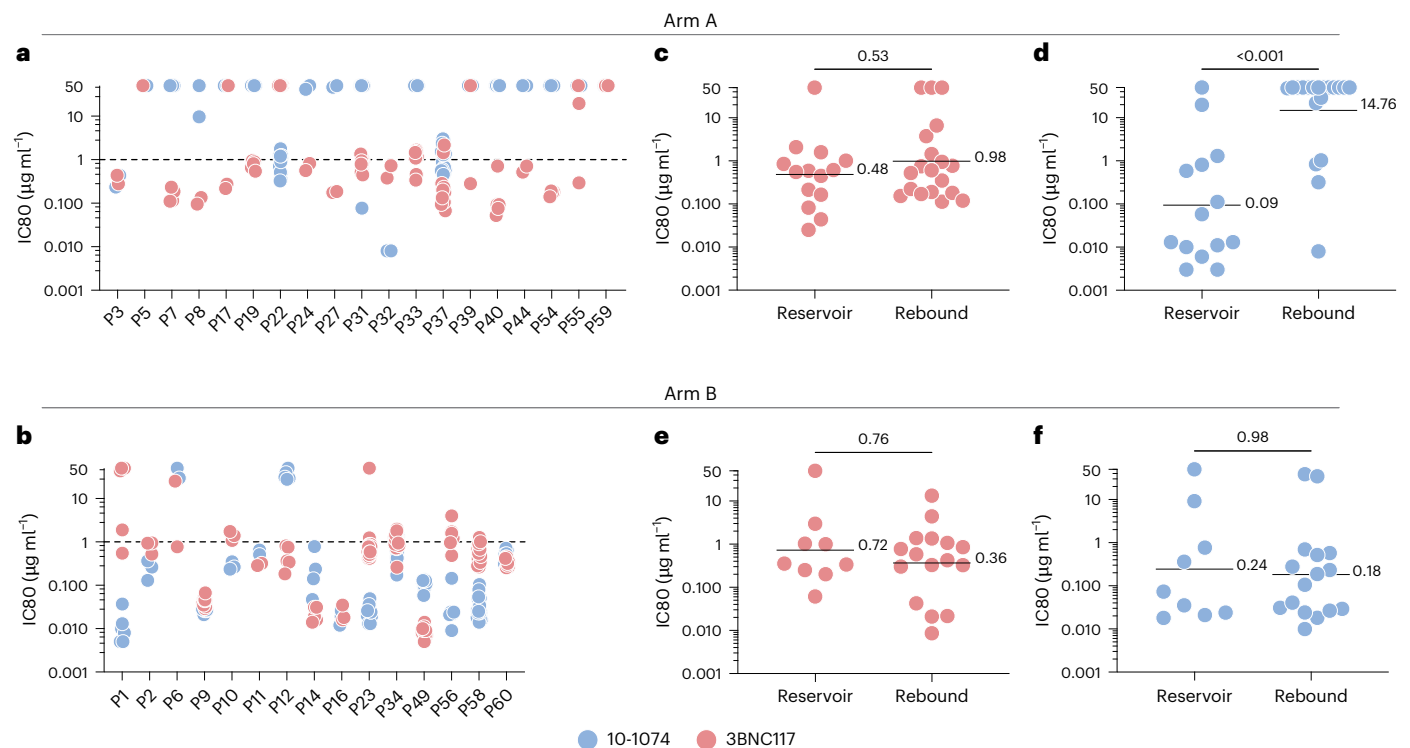


Fig. 3 | Pseudovirus neutralization. Pseudovirus neutralization by 3BNC117 (red) and 10-1074 (blue) for participants expressed as IC80. **a, b**, Each dot represents an individual rebound pseudovirus for arm A (**a**) or arm B (**b**). **c–f**, Each dot represents the geometric mean IC80 for reservoir and rebound pseudoviruses in one participant (one biological replicate). Arm A, 3BNC117 (**c**)

and 10-1074 (**d**) (reservoir $n = 14$, rebound $n = 19$); arm B, 3BNC117 (**e**) and 10-1074 (**f**) (reservoir $n = 9$, rebound $n = 16$). Line and numbers indicate geometric mean for all participants. Group comparisons in **c, d** and **e, f** were performed using a two-sided Mann–Whitney test, with statistical significance defined as $P < 0.05$.

neutralizing activity against reservoir viruses, indicating that delayed rebound in bNAb-treated participants was not explained simply by a smaller baseline reservoir (Supplementary Fig. 1).

Among the 29 arm A participants who remained in follow-up according to protocol, 13 went back on ART before meeting virological restart criteria (two consecutive VLs $\geq 10^5$ HIV-1 copies per ml or six consecutive VLs $\geq 10^3$ HIV-1 copies per ml; Extended Data Fig. 8). In a post hoc subgroup analysis, of the 16 remaining, 11 (68%) maintained fluctuating viremia and did not meet ART restart criteria for 16 to >58 weeks (Fig. 5a). In contrast, only two of the arm B control showed prolonged fluctuating viremia³⁸. We were able to obtain viruses from seven participants from time points associated with fluctuating viremia, and all were resistant to preinfusion autologous antibodies when present. In addition, five of these individuals showed viruses that were highly resistant to 10-1074 but retained sensitivity to 3BNC117 (Fig. 5c,d). These findings identify fluctuating viremia as a distinct post-bNAb virologic phenotype that others have recently associated with enhanced T cell immunity^{24,29,31}.

Reservoir dynamics

To determine how the bNAbs impact the size of the proviral reservoir in arm A, we compared the intact and defective proviral reservoirs at baseline with prerebound samples collected at a mean of 52 weeks after the initial infusion ($n = 22$; Fig. 6 and Supplementary Table 1). Reservoir size was also measured in arm B after a mean of 52 weeks directly before the second treatment interruption³⁸. We used ddPCR for these measurements because the relatively small reservoir size limited the number of samples from which we were able to obtain paired Q4PCR measurements (Supplementary Table 1). Although there was a great deal of variation between individuals in arm A over time, the intact reservoir but not the defective one was significantly smaller after bNAb therapy with an estimated half-life of 36 weeks ($n = 22$;

Fig. 6a and Extended Data Fig. 9). In contrast, there was no measurable change in reservoir size in arm B ($n = 20$; Fig. 6b). As expected from the relatively small overall change in the intact reservoir in arm A, compared with estimated reservoirs of millions of cells, there was no correlation between the change in reservoir size and time to ART restart (Supplementary Fig. 2).

Discussion

RIO is an ongoing double-blind, randomized placebo-controlled human trial in which participants who started ART during primary or early infection were randomly assigned to a group that received long-acting LS variants of 3BNC117 and 10-1074 (arm A) and a control group (arm B). Both groups subsequently discontinued ART³⁸. Upon rebound, participants in arm B received ART and the two bNAbs before undergoing a second treatment interruption³⁸. Despite similar preinfusion reservoir measurements, the groups differed significantly in time to rebound³⁸ and in the nature of the rebounding viruses. After 96 weeks, 25% and 6% of the individuals in the active and control arms, respectively, remained off ART. Viral rebound occurred across a wide temporal range in arm A encompassing periods of high circulating bNAb concentrations as well as later time points when antibody levels were low. Because participants received the LS versions of the bNAbs, antibody exposure extended over long periods of time during ATI. Prolonged control was associated with pre-existing autologous neutralizing antibodies and sensitivity to 10-1074. Paired preinfusion and prerebound intact reservoir measurements showed a decrease in intact, but not defective, proviruses in arm A that was not observed in arm B participants. Notably, participants in arm B experienced viral rebound and periods of detectable viremia between sampling time points, which may have contributed to increase of the circulating reservoir. In addition, differences in baseline intact reservoir between the two arms may further confound these comparisons. Therefore, these associations are correlative and based on

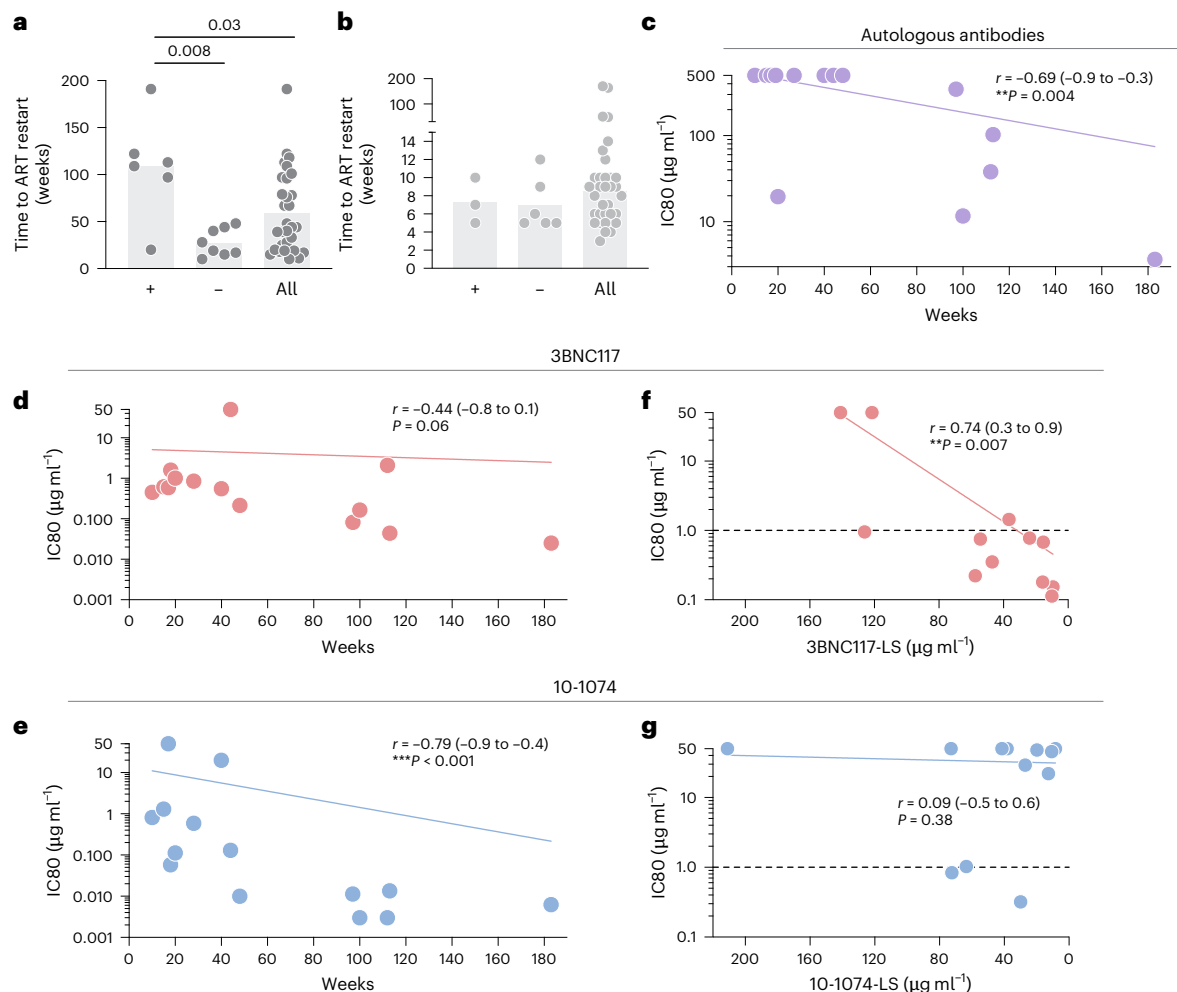


Fig. 4 | Viral suppression and reservoir sensitivity to autologous antibodies, 3BNC117 and 10-1074. a,b, Time to ART restart for individuals who did (+) or did not (-) display reservoir sensitivity to autologous antibodies or all participants; arm A (a) and arm B (b). Each dot represents the time to rebound for an individual participant (one biological replicate). Autologous antibody analyses included arm A: $n = 6$ (+) and $n = 8$ (-); arm B: $n = 3$ (+) and $n = 6$ (-). The 'all participants' groups include all randomized participants (arm A, $n = 30$; arm B, $n = 34$). The bars represent the geometric mean for each group. Two-sided Mann-Whitney group comparisons, with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ considered significant. **c-e,** Correlation between time to ART restart and baseline reservoir sensitivity to autologous antibodies (c), 3BNC117 (d) and 10-1074 (e). Each dot represents

the geometric mean IC80 of all reservoir-derived pseudoviruses from one participant (one biological replicate). Correlations were performed using one value per participant ($n = 14$). **f,g,** Correlation between IC80 from rebounding viruses for 3BNC117 (f) or 10-1074 (g) and the bNAb concentration at the time of rebound. Each dot represents one participant (one biological replicate), using the geometric mean IC80 (y axis) of all rebound-derived pseudoviruses from that participant and the corresponding serum bNAb concentration (x axis) at rebound ($n = 12$). Nonlinear regression was performed using a linear x axis and log-transformed y axis. Correlation coefficients were calculated using two-sided Spearman's r with 95% confidence intervals.

a limited number of individuals, but the observed reservoir decay is suggestive of altered reservoir dynamics in this cohort of PLWH undergoing ATI under LS-bNAb exposure.

As expected, based on their relatively early start on therapy, participants in RIO had small HIV-1 proviral reservoirs⁴¹⁻⁴³. Nevertheless, most of the intact reservoir in participants was clonal and did not contribute to rebound^{48,49}. Why reservoir proviruses are rarely found among rebound viruses is not entirely understood, but there is an inverse correlation between the size of an expanded clone and the probability that the sequence will be found among rebound viruses¹². The difference has been attributed to selection for proviral integration into silent sites in the genome^{18-20,53,54}, autologous antibody-mediated suppression^{35,50} and the possibility that rebound arises from tissue reservoirs not sampled in peripheral blood, as suggested by non-human primate studies^{55,56}. Notably, the disparity between reservoir and rebound viruses was evident in control-arm individuals who did not have measurable autologous antibodies or receive bNAb. Thus, the

discrepancy between reservoir proviruses in circulation and rebound viruses cannot entirely be explained by autologous antibody suppression of viremia in this cohort of early treated individuals.

HIV-1 infection is associated with the development of antibodies that neutralize the autologous virus and continually select for viruses that express resistant variants during the course of the infection^{33,34}. Moreover, autologous antibodies can sieve the outgrowth of reservoir viruses in vitro^{35,50}. But although autologous antibodies alter the viral swarm by selection of resistant variants, they fail to control viremia, leading to the suggestion that antibodies are ineffective against HIV-1. This idea was further supported by the observation that passively administered first-generation bNAb had little, if any, measurable effect on infection⁵⁷⁻⁵⁹. However, subsequent experiments in mice and macaques showed that administration of broader and more potent bNAb suppresses viremia and elicits CD8⁺ T cell responses that lead to prolonged control of infection^{21-23,60-62}. In addition, small interventional studies in which PLWH received second generation

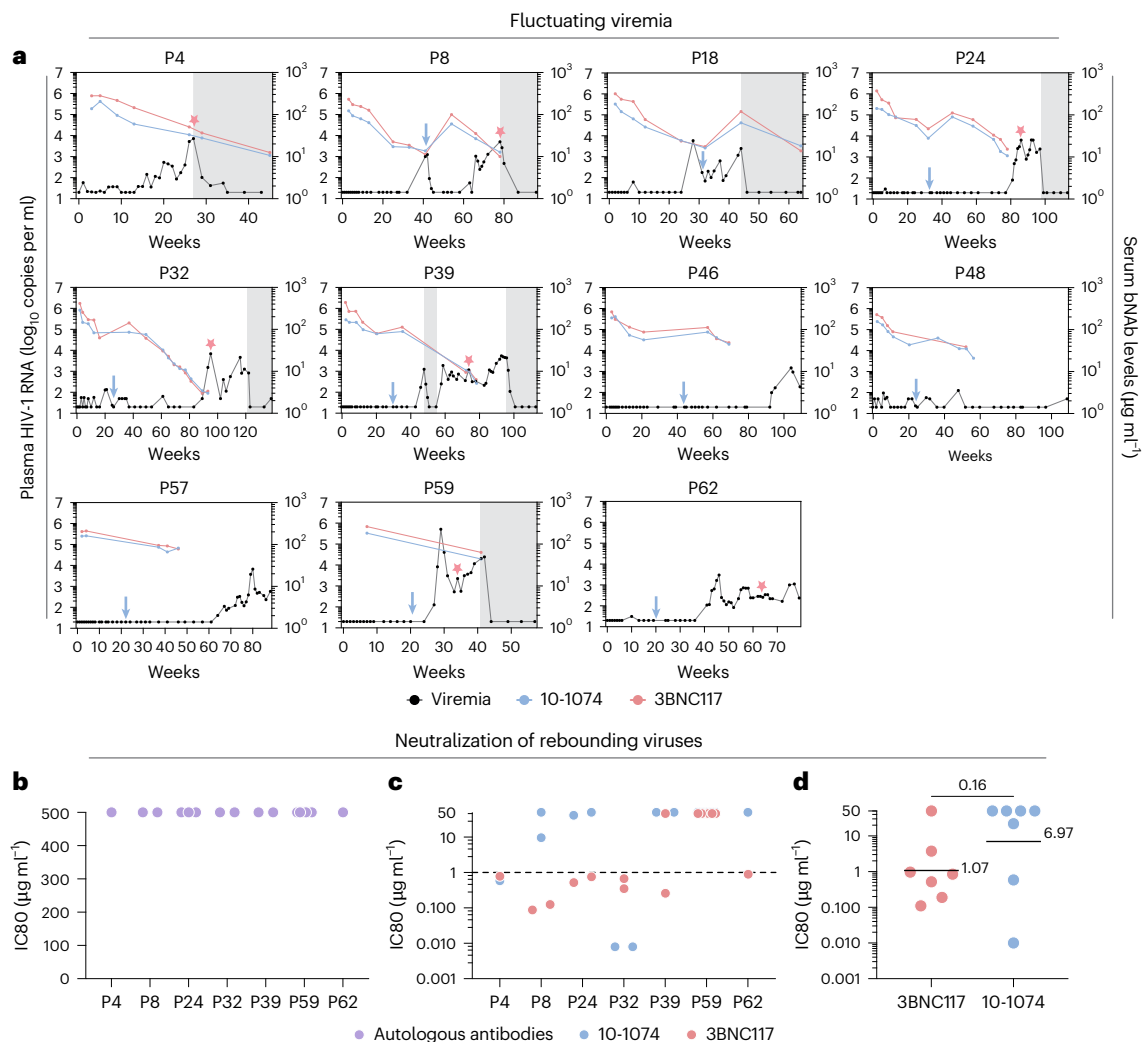


Fig. 5 | Virologic dynamics and antibody sensitivity during fluctuating viremia following bNAb therapy. a, Longitudinal plasma HIV-1 RNA levels (black) and circulating bNAb concentrations for 10-1074 (blue) and 3BNC117 (red) are shown for individual participants who maintained fluctuating viremia (defined as >16 weeks without sustained HIV-1 RNA >1,000 copies per ml). Blue arrows indicate administration of a second bNAb infusion. Gray shading denotes periods on ART. **b**, Neutralization sensitivity (IC80) of rebound-derived pseudoviruses to preinfusion autologous IgG for individual pseudoviruses.

c, Neutralization sensitivity (IC80) of rebound-derived pseudoviruses to 3BNC117 (red) and 10-1074 (blue), shown for individual pseudoviruses. **d**, Each dot represents one participant (one biological replicate), using the geometric mean IC80 of all rebound-derived pseudoviruses from that participant against 3BNC117 or 10-1074 ($n = 7$ participants). Lines and numbers indicate geometric means across participants. Group comparisons were performed using a two-sided Wilcoxon matched-pairs signed-rank, with statistical significance defined as $P < 0.05$.

bNAbs demonstrated that antibodies can be both safe and effective in reducing viremia in the absence of ART^{28,31,51,63–65}. Moreover, a fraction of individuals receiving bNAbs during treatment interruption appear to control the infection for an unexpectedly long time.

Enhanced CD8⁺ T cell responses were associated with bNAb therapy in some but not all reported clinical studies, and therefore other mechanisms are thought to contribute to enhanced control^{24,30,31}. The RIO trial is a blinded placebo-controlled trial in which the data indicate that individuals treated early who have pre-existing autologous neutralizing antibodies are more likely to control for longer periods of time. The association between autologous antibodies and suppression is correlative and does not establish a direct mechanistic role for autologous antibodies in viral suppression. Persistent bNAbs may also contribute to prolonged control. Rather than acting independently, autologous antibodies may function as an adjunct to the infused antibodies, making viral escape from combined antibody pressure more difficult⁶⁶. Notably, sensitivity to autologous antibodies correlated with sensitivity to 10-1074, suggesting partially overlapping aspects of viral neutralization susceptibility^{67,68}. Several mechanisms by which

antibodies can contribute to viral control have previously been documented, including direct neutralization of circulating viruses⁶⁹, clearance of infected cells in vivo^{61,70} and antibody-mediated enhancement of antigen presentation to elicit cellular immune responses^{71–73}. These mechanisms represent a plausible framework by which autologous antibodies could contribute to the delayed rebound observed in a subset of participants.

A substantial fraction of the participants in the bNAb arm of the RIO trial maintained fluctuating viremia for 16 to >58 weeks in the face of complete or partial bNAb resistance (11 of 29, or 38% of arm A). Although it did not reach significance, enhanced post-bNAb control has also been recently reported in the TITAN trial and in a single-arm study of ten individuals who received bNAbs, a Toll-like receptor agonist and a vaccine^{24,29}. Similar results were also observed in bNAb-treated macaques^{21–23}. Together, these observations point to fluctuating viremia as a reproducible post-bNAb virologic phenotype. The precise mechanistic explanation for these observations is not well defined, but the role of residual antibody as one contributing component cannot be dismissed.

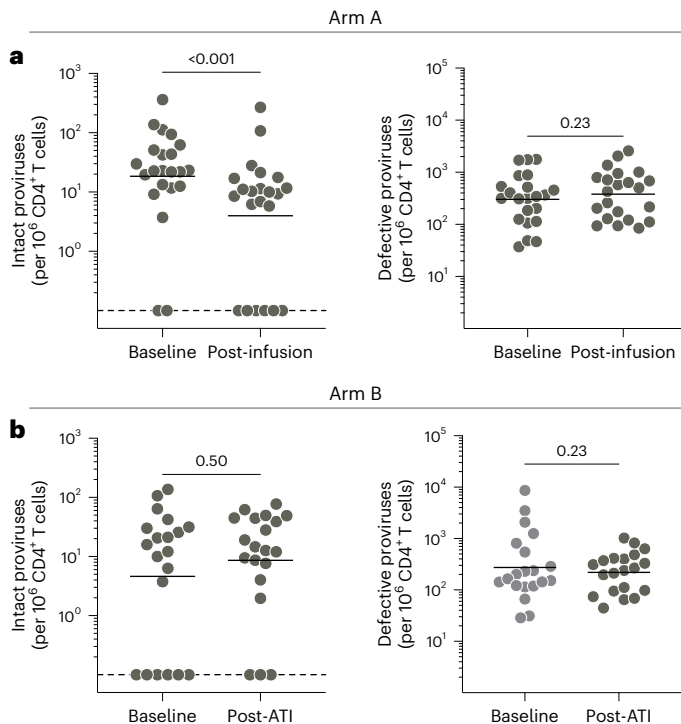


Fig. 6 | Intact and defective proviral reservoirs measured by ddPCR.

a, b. Quantification of intact and defective proviral reservoirs by ddPCR at baseline and at follow-up time points in participants from arm A ($n = 22$) (**a**) and arm B ($n = 20$) (**b**). Each paired data point represents one participant (one biological replicate). Changes over time within each group were evaluated using a two-sided Wilcoxon matched-pairs signed-rank test ($P < 0.05$).

Antibody monotherapy therapy like small-molecule therapy results in selection for resistant variants. Combination therapy durably suppresses viremia because the probability of developing escape mutations at a combination of non-overlapping different sites is lower. Consistent with this idea, rebound typically occurred in the RIO trial under functional monotherapy. Rebound viruses were most frequently resistant to 10-1074 and pre-existing autologous antibodies. In contrast, selection against 3BNC117 was far more limited, with no significant difference between initial reservoir and rebound sensitivity to the antibody, suggesting that the antibody was subtherapeutic at rebound. The median estimated 3BNC117-LS concentration at rebound was $52.4 \mu\text{g ml}^{-1}$, which is similar to the median 3BNC117IC80 for these participants at the time of rebound ($41.8 \mu\text{g ml}^{-1}$; Fig. 4f) and higher than the estimates in previous small clinical trials^{25,27}. These findings further support the interpretation that fluctuating viremia and delayed rebound may not be explained by antibody effects alone.

RIO trial participants who received bNAbs demonstrated accelerated intact reservoir decay: 0.75 years compared with prior estimates of 4–7 years^{14–17}. However, the accelerated intact reservoir decay was not uniform across the cohort, with 85% of the cohort showing a decrease. In contrast, there was no measurable change in reservoir in arm B between the first and second treatment interruption. The difference could be due to reservoir changes in response to viremia in arm B or the addition of ART to bNAb therapy in arm B or both. Accelerated reservoir decay was also a feature of a small study in which participants received multiple doses of a short-acting native version of 3BNC117 and 10-1074²⁵, but not in other small studies^{24,29}. The difference may be due to inherent variability and the small numbers of participants assayed, who were treated during chronic infection as opposed to early therapy participants in the RIO trial, differences in the antibodies used and their half-lives and finally substantial variability in the nature of the reservoir between individuals. Accordingly, we interpret intact reservoir decay in

RIO as suggestive of altered reservoir dynamics under the specific conditions of early treatment and long-acting bNAb exposure. Why some participants in this trial showed only small or no change in reservoir size remains to be determined but may be due to heterogeneous and/or variable levels of proviral transcription among CD4^+ T cells and even among members of a clone of CD4^+ T cells^{74–76}. Moreover, the observed changes in reservoir size cannot account for and do not correlate with prolonged antibody-mediated suppression (Supplementary Fig. 2). Far greater changes in reservoir would be required to alter the time to rebound in most individuals.

In conclusion, the data establish that a substantial fraction of PLWH who receive bNAb therapy can partially control viremia for prolonged periods of time. Correlates of prolonged control of HIV-1 after bNAb therapy include pre-existing autologous antibodies and pre-existing or enhanced CD8^+ T cell responses^{29,36,52}, suggesting that vaccination to elicit or enhance pre-existing host immunity may further increase the number of individuals exhibiting post-bNAb control.

Study limitations

Although 68 individuals were recruited into the RIO study, we were unable to obtain sequence information on the HIV-1 reservoir in 33 of the participants, thereby limiting the number of participant samples available for analysis. This constraint primarily reflects technical limitations of single-genome amplification in individuals treated early, who often harbor small circulating reservoirs, as well as issues related to sample availability and non-B clade viruses. Analyses of autologous neutralizing activity were restricted to participants with detectable reservoir-derived viral sequences. Moreover, sampling was limited to peripheral blood and may not entirely reflect tissue reservoirs. This problem was further accentuated when considering the relationship between autologous antibodies and virologic outcomes because early ART interferes with the development of autologous neutralizing antibody responses. Associations between autologous antibodies and delayed rebound are correlative, and reservoir decay estimates are limited by participants with values below assay detection. Finally, the timing of sample acquisition was predetermined and did not always correspond to time points of interest, limiting analysis of rebound viremia.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41591-026-04644-8>.

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Methods

Ethics statement

This study complied with all relevant ethical regulations. The RIO trial was approved by the London–Westminster Research Ethics Committee (REC reference 19/LO/1669; IRAS ID 266322), the UK Health Research Authority and the UK Medicines and Healthcare products Regulatory Agency (MHRA reference 19174/0413/001) and the Danish Medicines Agency. The trial was registered under the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database (2019-002129-31) and the European Union Clinical Trials Regulation (EU CT number 2024-514564-13-00) and registered at ClinicalTrials.gov (NCT04319367). The trial was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All participants provided written informed consent before enrollment. Trial oversight included independent Data Monitoring and Trial Steering Committees that regularly reviewed participant safety and trial conduct.

Study participants

The RIO trial is a randomized, placebo-controlled, double-blinded phase 2 study³⁷. Participants were recruited across multiple HIV clinical research sites in the United Kingdom and one site in Denmark. Participants were randomly assigned in a 1:1 ratio using a centralized web-based randomization system with variable block sizes of two or four and no stratification. Participants, treating clinicians, investigators, study personnel and outcome assessors remained blinded to treatment allocation throughout the randomized phase of the trial.

This study enrolled individuals who initiated ART during confirmed primary HIV infection or during early HIV infection and remained on suppressive ART for at least 1 year. Primary infection was defined according to standard clinical and laboratory criteria at the time of diagnosis, as detailed in the RIO clinical protocol. Specifically, the estimated time of primary infection was determined by one of six criteria: (1) positive HIV-1 serology within 24 weeks of a documented negative HIV-1 serology result, with the estimated infection date defined as the midpoint between the negative and positive tests; (2) the date of a positive p24 antigen result, with or without a negative HIV antibody test, depending on local laboratory reporting; (3) the date of a negative HIV antibody test with detectable HIV RNA or proviral DNA; (4) a Public Health England Recent Infection Testing Algorithm result reported as ‘Incident,’ indicating antibody avidity consistent with infection within the preceding 16 weeks, with the estimated date of infection defined as 2 months before the incident test result, with the Asanté HIV-1 Rapid Recency Assay also permitted for recency testing; (5) the date of a weakly reactive or equivocal fourth-generation HIV antibody/antigen test; or (6) the date of an equivocal or reactive antibody test with fewer than four bands on western blot. Early-stage infection was operationally defined as ART initiation before substantial immune compromise, using a nadir CD4⁺ T cell count >500 cells per μ l as a pragmatic surrogate for early disease stage.

Additional inclusion criteria included age 18–60 years, ability to provide informed consent including consent to long-term follow-up; willingness to comply with study visits and blood sampling; initiation of ART within 6 months of estimated primary HIV infection or during early HIV infection; virological suppression on ART for at least 1 year, with a single HIV-1 RNA measurement >50 but <500 copies per ml permitted during this period; no evidence of viral insensitivity to either 10-1074-LS or 3BNC117-LS; current CD4⁺ T cell count >500 cells per ml or CD4:CD8 ratio >1.0; nadir CD4⁺ T cell count >250 cells per μ l for participants diagnosed with confirmed primary HIV infection; receipt of an integrase inhibitor (INSTI)- or boosted protease inhibitor (PI)-based ART regimen at randomization, with participants receiving a non-nucleoside reverse transcriptase inhibitor required to have switched to an INSTI- or boosted PI-based regimen at least 4 weeks before randomization; adequate hemoglobin (≥ 12 g dl⁻¹ for males or ≥ 11 g dl⁻¹ for females), body weight ≥ 50 kg; hepatitis B and C screening consistent with protocol

eligibility (hepatitis B virus surface antigen or hepatitis B virus DNA negative and hepatitis C virus antigen or hepatitis C virus RNA negative or anticore antibody negative); absence of significant comorbidities; and SARS-CoV-2 vaccination at least 4 weeks before enrollment. Females capable of becoming pregnant were additionally required to use protocol-defined effective contraception or complete abstinence from at least 2 weeks before the first bNAb/placebo infusion until 20 months after the last bNAb infusion.

Exclusion criteria included significant cardiovascular disease, including previous ischemic heart disease (ST- or non-ST-elevation myocardial infarction, Q3-risk > 20, stable or unstable angina) or stroke; malignancy except squamous cell skin cancer; opportunistic infection or significant comorbidity or a comorbidity considered likely to occur during the trial, including malabsorption syndromes or autoimmune disease; contraindication to receipt of BHIVA-recommended combination ART; HTLV-1 co-infection; SARS-CoV-2 infection confirmed by RT-PCR up to 72 h before randomization/dosing where applicable according to local guidance; high risk of severe COVID-19 as determined according to applicable guidance and the participant’s physician; current or planned systemic immunosuppressive therapy (with inhaled and topical corticosteroids permitted); participation in another interventional trial of an experimental agent or a non-interventional study requiring additional blood draws, with observational studies permitted and non-clinical trials of investigational medicinal product studies involving minimal blood sampling considered on a case-by-case basis; history of severe reaction to antibody infusion or hypersensitivity to 3BNC117-LS, 10-1074-LS or their constituents or excipients; treatment with IV immunoglobulin or other monoclonal antibodies during the trial; clinically significant abnormal laboratory results including significant hepatic impairment with advanced fibrosis or cirrhosis with decompensation; alanine aminotransferase >5 $\times$ the upper limit of normal; estimated glomerular filtration rate <60 ml per min per 1.73 m²; urine protein-to-creatinine ratio >30 mg mmol⁻¹ or international normalized ratio >1.5; evidence of organ dysfunction or any clinically significant abnormality on physical examination or vital signs considered by the investigator to preclude enrollment; active alcohol or substance use likely to impair adherence; insufficient venous access; pregnancy or breastfeeding; or concern regarding risk-reduction precautions during ATI.

A total of 68 participants were enrolled between May 2021 and July 2024 as part of a two-stage clinical design. Clinical data and biospecimens used in this study were collected prospectively throughout trial follow-up between May 2021 and September 2025. Although the protocol permitted enrollment of both sexes, all participants enrolled in the trial were men, predominantly white. Participant disposition, including completion of follow-up, withdrawals and exclusions from specific analyses, is summarized in Extended Data Fig. 1. The RIO trial was powered for the primary clinical endpoint as described in the clinical paper³⁸. Briefly, the study was designed to detect a reduction in the rebound rate at 36 weeks from 90% in arm B to 55% in arm A (hazard ratio 0.35), with 90% power at a 5% significance level. No formal power calculations were performed for the exploratory mechanistic analyses reported here. Sex and gender information were collected by self-report at study enrollment. Race and ethnicity information were self-reported according to local clinical site procedures. Because all enrolled participants were men, sex- and gender-based analyses were not performed. Participants received financial compensation for each visit. Blood samples were collected at baseline and at multiple time points following 3BNC117-LS and 10-1074-LS or placebo infusions. Samples were processed briefly after collection, with serum and plasma stored at -80°C . PBMCs were isolated by density gradient centrifugation. The PBMC number was determined either manually or using an automated cell counter (Vi-Cell XR; Beckman Coulter), and cells were cryopreserved in liquid nitrogen in fetal bovine serum supplemented with 10% dimethyl sulfoxide.

Definitions of virological control and ART-free status

Virological control was defined as sustained plasma HIV-1 RNA <1,000 copies per ml during treatment interruption. ART restart criteria were defined per protocol as either (1) two consecutive plasma HIV-1 RNA measurements >10⁵ copies per ml or (2) six consecutive plasma HIV-1 RNA measurements >10³ copies per ml. In addition to these virological criteria, ART could be restarted for other predefined clinical or participant-driven endpoints not meeting the above thresholds, including participant preference or clinician judgment. All ART restart events and their classification were adjudicated by an independent Endpoint Adjudication Committee. The distribution of ART restart outcomes by study arm is summarized in Extended Data Table 1.

Intact proviral DNA analysis

The frequency of CD4⁺ T cells harboring intact, 5'-deleted and 3'-deleted proviruses was determined using a ddPCR assay modified from the Intact Proviral DNA Assay¹⁰. Briefly, CD4⁺ T cells were enriched from PBMCs using a negative immunomagnetic selection kit (Miltenyi Biotec). Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. DNA concentrations were quantified using the Qubit 3.0 Fluorometer with the Qubit dsDNA BR Assay Kit (Thermo Fisher). Samples were from the preinfusion time point and obtained from an aviremic time from a minimum of 6 weeks before rebound, when HIV-1 RNA was <20 copies per ml.

For ddPCR, two sets of primers and probes specific to the HIV *gag* and *env* regions, along with two fragments of the housekeeping gene RPP30, were utilized in separate reactions⁷⁷. HIV quantification was performed using 750 ng of genomic DNA per sample with the following primers and probes for the HIV *gag* region: forward (5'-GACTAGCG GAGGCTAGAAGGAGAGA-3'), reverse (5'-CTAATTCTCCCCGCTTAAT AYTGACG-3') and probe (5'-6FAM-ATGGGTGCGAGA-IABkFQ-3'). For the HIV *env* region: forward (5'-AGTGGTGCAGAGAGAAAAA GAGC-3'), reverse (5'-GTCTGGCCTGTACCGTCAGC-3') and probes (5'-VIC-CCTTGGGTCTTGGGA-MGB-3' and an unlabeled hypermutated probe 5'-CCTTAGGTTCTTAGGAGC-MGB-3'). An alternative primer/probe set targeting the PS region was used as a backup: forward (5'-CAGGACTCGGCTTGCTGAAG-3'), reverse (5'-GCACC CATCTCTCTCTTCTAGC-3') and probe (5'-6FAM-TTTTGGCGTACT CACCACT-IABkFQ-3')¹⁰. To measure input cell numbers and correct for DNA shearing, 7.5 ng of DNA was used with RPP30 primers and probes (RPP30-1: forward (5'-GATTTGGACCTGCGAGCG-3'), reverse (5'-GCGGCTGTCTCCACAAGT-3'), probe (5'-6FAM-TTCTGACCTGAA GGCTCTGCGC-IABkFQ-3'); RPP30-2: forward (5'-GTGTGAGTCAAT CACTAGACAGAA-3'), reverse (5'-AACTGCAACAACATCATAGAGC-3'), probe (5'-HEX-AGAGAGCAACTTCTCAAGGGCCC-IABkFQ-3')). Four technical replicates were performed per sample. Positive and negative controls were included in each reaction.

The ddPCR assays were conducted using the Bio-Rad QX200 AutoDG system with the ddPCR Supermix for Probes (no dUTPs) (Bio-Rad). Thermal cycling conditions included an initial denaturation at 95 °C for 10 min, followed by 45 cycles of 94 °C for 30 s and 59 °C for 1 min, with a 2 °C s⁻¹ ramp rate. A final extension was performed at 98 °C for 10 min, followed by a hold at 12 °C. The results were adjusted for DNA shearing using the ratio of double-positive RPP30 partitions and normalized to 10⁶ CD4⁺ T cells.

Q4PCR

The Q4PCR assay was conducted to characterize the composition of the reservoir, as previously described¹¹. Briefly, total CD4⁺ T cells were enriched from cryopreserved PBMCs using a negative immunomagnetic selection kit (Miltenyi Biotec). Genomic DNA was extracted from CD4⁺ T cells using the Gentra Puregene Cell Kit (Qiagen), and DNA concentration was measured using the Qubit dsDNA BR Assay Kit (Thermo Fisher).

A total range of 1 to 10 × 10⁶ CD4 T cells were screened per participant. An initial outer PCR (near full length, step 1 (NFL1)) was performed

on genomic DNA at a single-copy dilution using the outer primers BLOuterF (5'-AAATCTCTAGCAGTGGCGCCGGAACAG-3') and BLOuterR (5'-TGAGGGATCTCTAGTTACCAGAGTC-3')⁷⁸. A 1-μl aliquot of the undiluted NFL1 PCR product was then subjected to a Q4PCR reaction, utilizing four primer–probe sets targeting conserved regions of the HIV-1 genome: PS: forward (5'-TCTCTCGACGAGGACTC-3'), reverse (5'-TCTAGCCTCCGCTAGTCAAA-3'), probe (5'-Cy5/TTTGCGTA/TAO/CTCACCAGTCGCC-3'/IABRQSp); *env*: forward (5'-AGTGGTGCAGAGAGAAAAAGAGC-3'), reverse (5'-GTCTGGCC TGTACCGTCAGC-3'), probe (5'-VIC/CCTTGGGTCTTGGGA-3'/MGB); *gag*: forward (5'-ATGTTTTCAGCATTATCAGAAGGA-3'), reverse (5'-TGCTTGATGTCCCCCACT-3'), probe (5'-6-FAM/CCACCCAC/ZEN/AAGATTTAAACACCATGCTAA-3'/IABkFQ); and *pol*: forward (5'-GCACTTTAAATTTCCCATAGTCCTA-3'), reverse (5'-CAAATTC TACTAATGCTTTTATTTTTC-3'), probe (5'-NED/AAGCCAGG AATGGATGGCC-3'/MGB). Quantitative PCR was performed under the following thermal cycling conditions: an initial denaturation at 94 °C for 10 min, followed by 40 cycles of 94 °C for 15 s and 60 °C for 60 s. Quantitative PCR assays were conducted in a 384-well plate format using the Applied Biosystem QuantStudio 6 or 7 Flex real-time PCR system. Data analysis was performed using Thermo Fisher Design and Analysis Software 2.4.3. Samples that exhibited reactivity with two or more of the four probes were selected for a nested PCR (NFL2).

The NFL2 reaction was performed on undiluted 1-μl aliquots of the NFL1 PCR product. Each reaction was conducted in a 20-μl volume using Platinum Taq high-fidelity polymerase (Thermo Fisher) and the primers 3LTri (5'-TCAAGGCAAGCTTTATTGAGGCTTAA-3') and U5-638F (5'-GCGCCGAACAGGGACYTGAAARCGAAAG-3')¹⁹. The thermocycler conditions for NFL2 were the same as those used for the NFL1 PCR. Library preparation and sequencing were conducted as previously described¹¹.

HIV-1 sequence assembly and annotation

HIV-1 genome reconstruction was performed using an in-house pipeline, Defective and Intact HIV Genome Assembler, designed for assembling raw sequencing reads into annotated HIV genomes²⁵. The pipeline includes rigorous quality control steps to enhance accuracy and reliability. First, quality control checks were conducted to remove PCR-amplified reads and correct sequencing errors using clumpify.sh from the BBtools package v38.72 (<http://sourceforge.net/projects/bbmap>). Next, adapter sequences and low-quality bases were trimmed, and potential contaminant reads were removed using the Trim Galore package v0.6.4 (<https://github.com/FelixKrueger/TrimGalore>). HIV-1 sequences were assembled using SPAdes v3.13.0, and the longest assembled contig was aligned to the HXB2 HIV-1 reference genome via BLAST⁷⁹. Sequences exhibiting double peaks—regions indicating the presence of two or more viral variants within a sample (defined by a consensus identity cut-off of <70% for any residue)—or samples with an insufficient number of sequencing reads (≤500 reads) were excluded from downstream analyses.

Sequences that did not meet the double-peak criteria (consensus identity for any residue <70%) were further classified as either intact or defective proviruses. Only intact HIV-1 *env* sequences were considered for subsequent analyses.

Single-genome amplification of plasma rebound virus *env* genes

Sequencing of HIV-1 plasma rebound *env* genes was performed as previously described⁸⁰. In brief, HIV-1 RNA was extracted from viremic plasma samples using the MinElute Virus Spin kit (Qiagen) according to the manufacturer's recommendation. First-strand cDNA synthesis was carried out using SuperScript III reverse transcriptase (Invitrogen) and an antisense primer, envB3out (5'-TTGCTACTTGTGATTGCTC CATGT-3') for subtype B or OFM19 (5'-GCACTCAAGGCAAGCTTTATTGAG GCTTA-3') for subtype C. To ensure single-genome amplification, cDNA

was endpoint diluted according to Poisson distribution, achieving <30% of wells yielding a PCR product. The envelope gene was then PCR-amplified using subtype B primers envB3out and envB5out (5'-TAGGCATCTCCTATGGCAGGAAGAAG-3') or subtype C primers OFM19 and Vi1 (5'-GGTTTATTACAGGGACAGCAGAG-3'). A second round of PCR was performed using 1 µl of the first-round PCR product as a template, with subtype B primers envB3in (5'-GTCTCGAGATACTGCTCCACCC-3') and envB5in (5'-TAGGCATCTCCTATGGCAGGAAGAAG-3') or subtype C primers env A (5'-GGCTTAGGCATCTCCTATGGCAGGAAGA-3') and env N (5'-CTGCCAATCAGGAAGTAGCCTTGTGT-3'). PCR amplifications were conducted using High Fidelity Platinum Taq (Invitrogen) under the following conditions: an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 15 s, 55 °C for 30 s and 68 °C for 4 min, with a final extension at 68 °C for 10 min. PCR products of the expected size were subjected to library preparation and sequencing using the Illumina MiSeq platform.

Sequence and phylogenetic analysis

Nucleotide alignments of intact *env* sequences were performed using Muscle v5.1 with the PPP algorithm⁸¹. Sequences containing premature stop codons, truncations or frameshift mutations were excluded from further analyses. Maximum-likelihood phylogenetic trees were generated from these alignments using FastTree 2.1.11 with the GTR model and 1,000 bootstrap replicates⁸².

Pseudotyped-virus production

Selected single-genome sequences from CD4⁺ T cell reservoir or plasma rebound samples were used as templates to produce pseudoviruses from a cytomegalovirus (CMV) promoter as described⁸³. The CMV promoter was amplified by PCR from pcDNA 3.1 (Life Technologies) using the primers CMVenv (5'-AGTAATCAATTACGGGGTCATTAGTTCAT-3') and CMVenv1A (5'-CATAGGAGATGCCTAAGCCGGTGGAGCTCTGCTTATATAGACCTC-3'). Thermocycling conditions were 94 °C for 2 min, followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 68 °C for 4 min. A 1-µl aliquot of the second-round PCR product from NFL amplification (reservoir) or single-genome amplification (rebound) was used as a template for *env* to which we added CMV overhanging regions using the forward primer ENVfwd (5'-CACCGGCTTAGGCATCTCCTATGGCAGGAAGAA-3') and the reverse primer envB3in or ENV N, depending on the subtype. The CMV promoter amplicon was fused to individual *env* genes via overlapping PCR with 10 ng of *env* and 0.5 ng of CMV using CMVenv primer and envB3in or ENV N as the reverse primer. Thermocycling conditions were 94 °C for 2 min followed by 20 cycles of 94 °C for 30 s, 55 °C for 30 s, 68 °C for 4 min, followed by a final extension at 68 °C for 10 min. All PCR reactions were carried out using Platinum Taq HiFi polymerase. Resulting amplicons were analyzed by gel electrophoresis, purified using the Macherey-Nagel gel and PCR purification kit and cotransfected with the pSG3Δ*env* vector (National Institutes of Health AIDS Reagent Program) into 293T cells to produce pseudoviruses as previously described.

Neutralization assays

Viruses were tested against bNAbs and purified autologous IgGs using the TZM-bl cell neutralization assay with cells obtained through the National Institutes of Health HIV Reagent Program, as previously described^{47,84}. Briefly, pseudoviruses were incubated with eight fivefold serial dilutions of antibodies for 1 h at 37 °C in 96-well plates before addition of 10,000 TZM-bl cells per well in growth medium containing DEAE-dextran. Cells were incubated for 48 h at 37 °C in 5% CO₂, after which infection was quantified by measuring luciferase activity using Bright-Glo reagent (Promega). Neutralization was calculated relative to virus-only control wells, and IC values were determined by nonlinear regression. Assays were performed in duplicate under Good Clinical Laboratory Practice quality assurance standards. For bNAb testing, the starting antibody concentrations were 50 µg ml⁻¹ for 3BNC117

and 10-1074, and purified autologous IgG was tested at an initial concentration of 500 µg ml⁻¹ followed by eight fivefold serial dilutions. Pseudovirus clones derived from both the reservoir and rebound were tested. For autologous antibody testing, baseline IgG was purified using Protein G Sepharose 4 Fast Flow (GE Life Sciences). Neutralization breadth was evaluated using a panel of ten HIV-1 *env* pseudoviruses selected based on reported resistance to both 3BNC117 and 10-1074 (<https://www.hiv.lanl.gov/catnap>). The panel included the following *env* clones: 700010040.C9.4520, H030.7, H086.8, THRO4156.18, H035.18, H704_1109_140_RE_cs, 191955_A11, 89-F1_2_25, 6322.V4.C1 and 3637.V5.C3.

Depletion of residual bNAbs from longitudinal IgG samples

To remove residual bNAbs from longitudinal IgG samples, anti-3BNC117 and anti-10-1074 antibodies engineered with a FLAG epitope on the light chain were used as capture reagents. Following IgG purification from plasma, samples were incubated with the FLAG-tagged anti-bNAb-idiotype reagents at a twofold molar ratio of the anti-idiotype to the estimated bNAb concentration for 2 h at 4 °C to allow immune complex formation. Samples were then incubated overnight at 4 °C with anti-FLAG M2 magnetic beads (Sigma-Aldrich, catalogue number M8823). Beads were removed magnetically, and the supernatant (depleted of infused bNAbs) was collected, tested for residual bNAbs by ELISA and, if below the limit of detection, used for downstream autologous neutralization assays. Depletion was repeated if residual bNAb was detected until it no longer was.

Time-to-event analyses

To evaluate the combined influence of endogenous and therapeutic antibody pressure on time to ART restart, time-to-event analyses were performed using Cox proportional hazards models with time to ART restart as the outcome. Cox proportional hazards analyses incorporated observed ART restart events occurring beyond 96 weeks when available. Baseline reservoir sensitivity to autologous antibodies, 10-1074 and 3BNC117 was quantified using IC80 values, which were log₁₀-transformed and standardized before modeling. Univariable, pairwise (models including two resistance variables simultaneously) and multivariable models were fit to assess the individual and combined contributions of each resistance dimension to rebound timing. Hazard ratios reflect the effect of a one-standard-deviation increase in resistance. Analyses were performed using Cox proportional hazards regression in GraphPad Prism and R (survival package version 3.8-6).

Statistics and reproducibility

Statistical analyses were performed using GraphPad Prism version 10.6.1 (GraphPad Software) and R version 4.5.0 (R Foundation for Statistical Computing), as described for each analysis. All statistical tests were two-sided unless otherwise specified. Exact statistical tests, sample sizes, measures of central tendency and definitions of biological replicates are provided in the corresponding figure legends. No statistical method was used to predetermine sample size for laboratory analysis because sample size was determined by participant enrollment and sample availability within the RIO clinical trial. No data were excluded from the analyses unless explicitly stated. Statistical analyses of prespecified secondary and exploratory outcomes were conducted according to the trial Statistical Analysis Plan. Analyses not prespecified in the Statistical Analysis Plan are explicitly identified in 'Results' as non-prespecified exploratory or post hoc analyses. Participants were randomized as part of the RIO clinical trial according to the study protocol. Laboratory investigators performing the mechanistic analyses were initially blinded to treatment allocation. Blinding was maintained until participants met protocol-defined criteria for unblinding (for example, following viral rebound), after which treatment allocation became known for subsequent analyses. Each participant was considered one biological replicate unless otherwise

indicated, and technical replicates were not treated as independent observations. Correlation analyses were performed using two-sided Spearman rank correlation coefficients with 95% confidence intervals. Group comparisons were performed using two-sided Mann–Whitney tests or Wilcoxon matched-pairs signed-rank tests, as appropriate. Time-to-event analyses were evaluated using Cox proportional hazards regression. Statistical significance was assessed using exact two-sided *P* values, which are reported in the figures or text.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Source data underlying the figures and statistical analyses presented in this study are provided in Supplementary Tables. Specifically, participant-level reservoir quantification data are provided in Supplementary Table 1, baseline intact reservoir-derived proviral sequences and neutralization data in Supplementary Table 2, rebound virus *env* sequences and neutralization data in Supplementary Table 3, participant-level geometric mean neutralization sensitivities in Supplementary Table 4, serum concentrations of 3BNC117-LS and 10-1074-LS of long-term controllers in Supplementary Table 5, and Cox proportional hazards model outputs in Supplementary Table 6. HIV-1 sequence data generated for all reservoir-derived proviral genomes in this study have been deposited in GenBank under accession numbers [PZ799384–PZ799601](#) and [PZ799607–PZ802566](#). Newly generated research materials, including participant-derived HIV-1 *env* constructs, pseudovirus reagents and other unique materials generated in this study, will be made available to qualified researchers upon reasonable request to the corresponding author, subject to applicable institutional approvals, material transfer agreements, participant consent restrictions and the continued availability of the requested materials. Deidentified participant-level clinical and laboratory data from the RIO trial, including relevant data dictionaries and supporting study documentation, will be available through a controlled-access process following completion of the trial and database lock, in accordance with participant consent and institutional ethical requirements. Clinical data requests for access should be directed to S. Fidler (s.fidler@imperial.ac.uk), University of Oxford, who serves as chief investigator of the RIO trial. Requests will be acknowledged within ten working days and reviewed by the Trial Management Group, Independent Data Monitoring Committee and Trial Steering Committee. A decision regarding data access will normally be communicated within 8 weeks of receipt of a complete request. Approved investigators will be required to sign a data access agreement before receiving access to the requested datasets. The RIO clinical protocol and Statistical Analysis Plan are available as supplementary documents accompanying the primary clinical publication³⁸.

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Author contributions

M.J.F., M.C. and M.C.N. conceived the study and designed the experiments. J. Frater, S.F., M.C.N. and M.C. designed the clinical protocols. S.F., J. Frater and M.C. coordinated the study, collected clinical data and recruited participants. T.E., M.L., J. Fox, S.P., A.C., A.U., O.S., R.S., M.B., S.K.-L. and C.O. are clinical investigators responsible for recruitment and participant care. E.F. and L.-R.C. conducted trial statistical analysis. P.Z. conducted bNAb screening for study eligibility. H. Brown, N.R. and H. Box were responsible for management and operational conduct of the trial. S.C. is the community representative for PLWH. M.J.F., A.K., M.A., Á.L.-R., M.S.S., B.J.H. and A.G. performed the experiments. C.L., S.M.E. and M.S.S. performed the TZM-bl neutralization assays. M.J.F. and C.B. coordinated and processed clinical samples. M.J.F., T.O., M.C. and M.C.N. analyzed and interpreted the data. M.J.F. had full access to the raw data reported in this study. M.J.F., M.J., M.C. and M.C.N. wrote the paper with input from all authors. All authors discussed the results, provided critical feedback and approved the final version of the paper.

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Competing interests

Patents on 3BNC117 (PCT/US2012/038400) and 10-1074 (PCT/US2013/065696) list M.C.N. as an inventor. These antibodies are licensed to Gilead Sciences by The Rockefeller University, from which M.C.N. has received payments. M.C.N. serves on the Scientific Advisory Board of Celldex. M.C.N. had no control over the conduct, analysis or reporting of the clinical aspects of this research while holding these financial interests. All potential conflicts of interest were reviewed

and are managed by The Rockefeller University, University of Oxford and Imperial College in accordance with their institutional conflict of interest policies. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at <https://doi.org/10.1038/s41591-026-04644-8>.

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41591-026-04644-8>.

Correspondence and requests for materials should be addressed to Michel C. Nussenzweig.

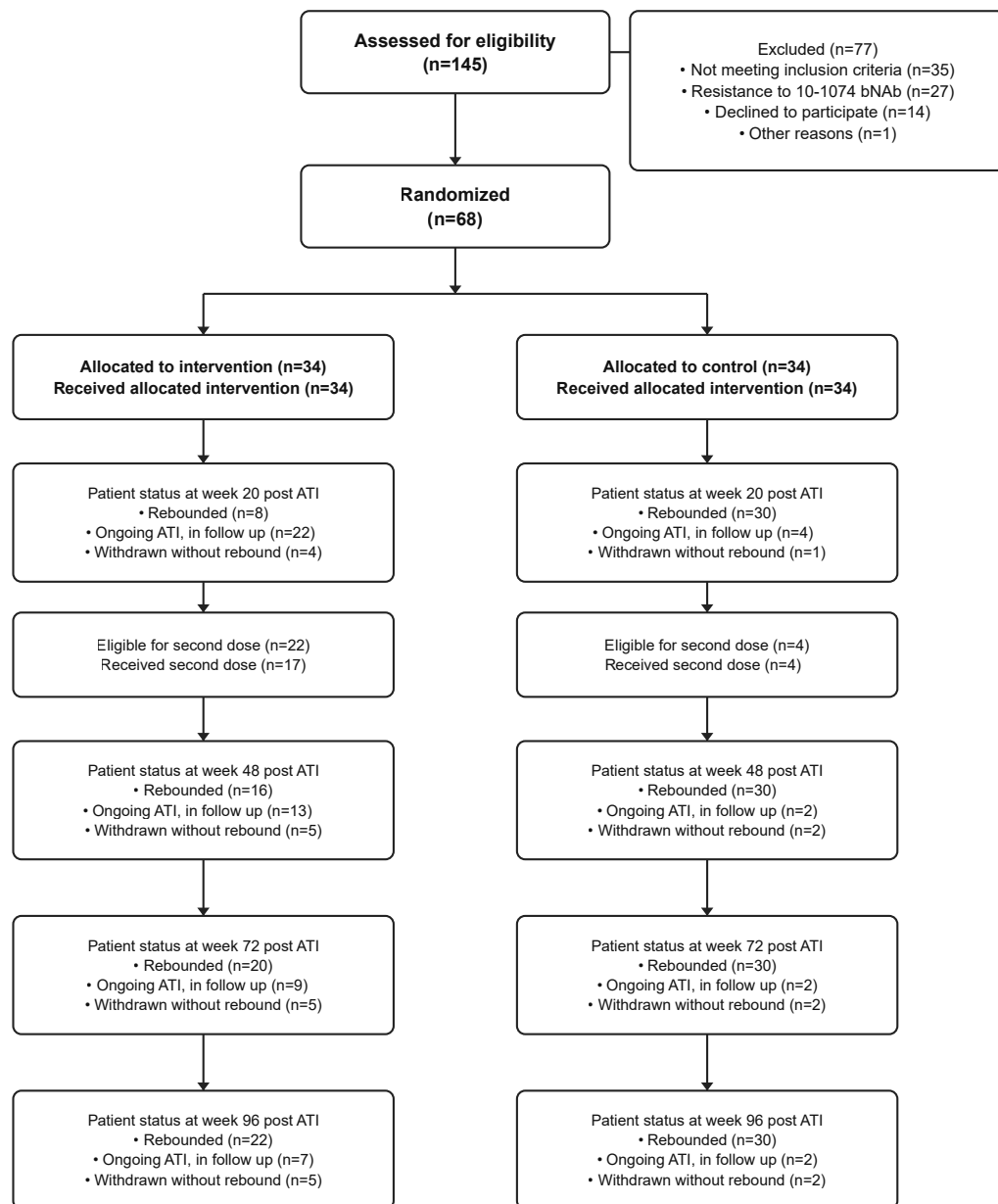
Peer review information *Nature Medicine* thanks Yunda Huang and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available. Primary Handling Editor: Lia Parkin, in collaboration with the *Nature Medicine* team.

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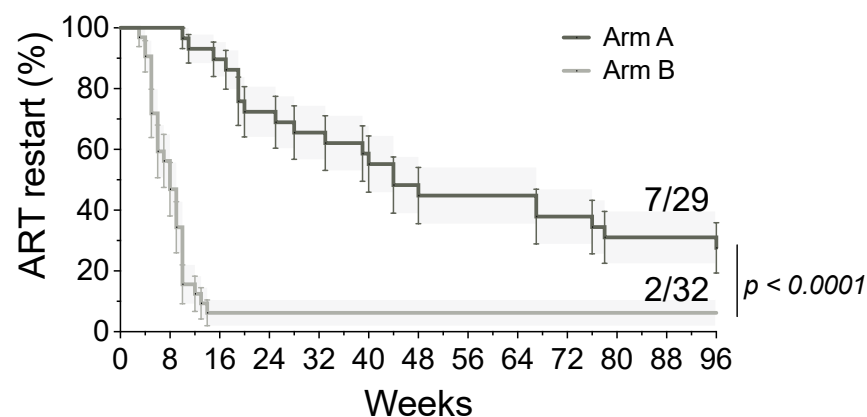
Extended Data Table 1 | Baseline demographic and clinical characteristics of RIO participants

Characteristic	Arm A: bNAbs (n=34)	Arm B: Placebo (n=34)	All (n=68)
Age, years (Median, IQR)	36 (31, 44)	42 (33, 51)	40 (32, 46)
Male sex assigned at birth, n (%)	34 (100.0%)	34 (100.0%)	68 (100.0%)
Asian or Asian British, n (%)	3 (8.8%)	0 (0.0%)	3 (4.4%)
Black or Black British, n (%)	1 (2.9%)	2 (5.9%)	3 (4.4%)
White ethnicity, n (%)	28 (82.4%)	30 (88.2%)	58 (85.3%)
Other ethnicity, n (%)	2 (5.9%)	2 (5.9%)	4 (5.9%)
Baseline CD4 count, cells per µl (Median, IQR)	742 (657, 937)	810 (596, 986)	776 (626, 963)
HIV clade B, n (%)	19 (76.0%)	21 (84.0%)	40 (80.0%)
Non-B clade, n (%)	6 (24.0%)	4 (16.0%)	10 (20.0%)
Clade information unavailable, n	9	9	18
Duration on ART before enrolment, years (Median, IQR)	5 (3–6)	5 (4–7)	5 (4–7)
Primary HIV infection, n (%)	32 (94%)	30 (88%)	62 (91%)
Early HIV infection, n (%)	2 (6%)	4 (12%)	6 (9%)

a



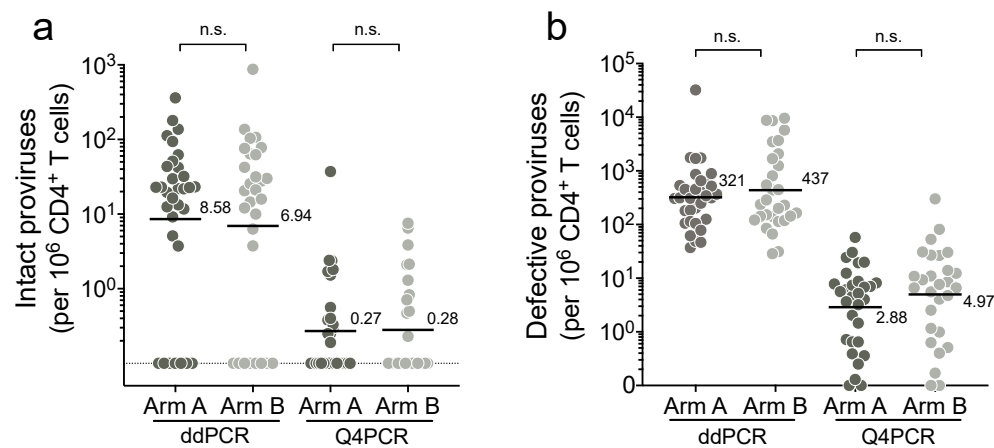
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Extended Data Fig. 1 | See next page for caption.

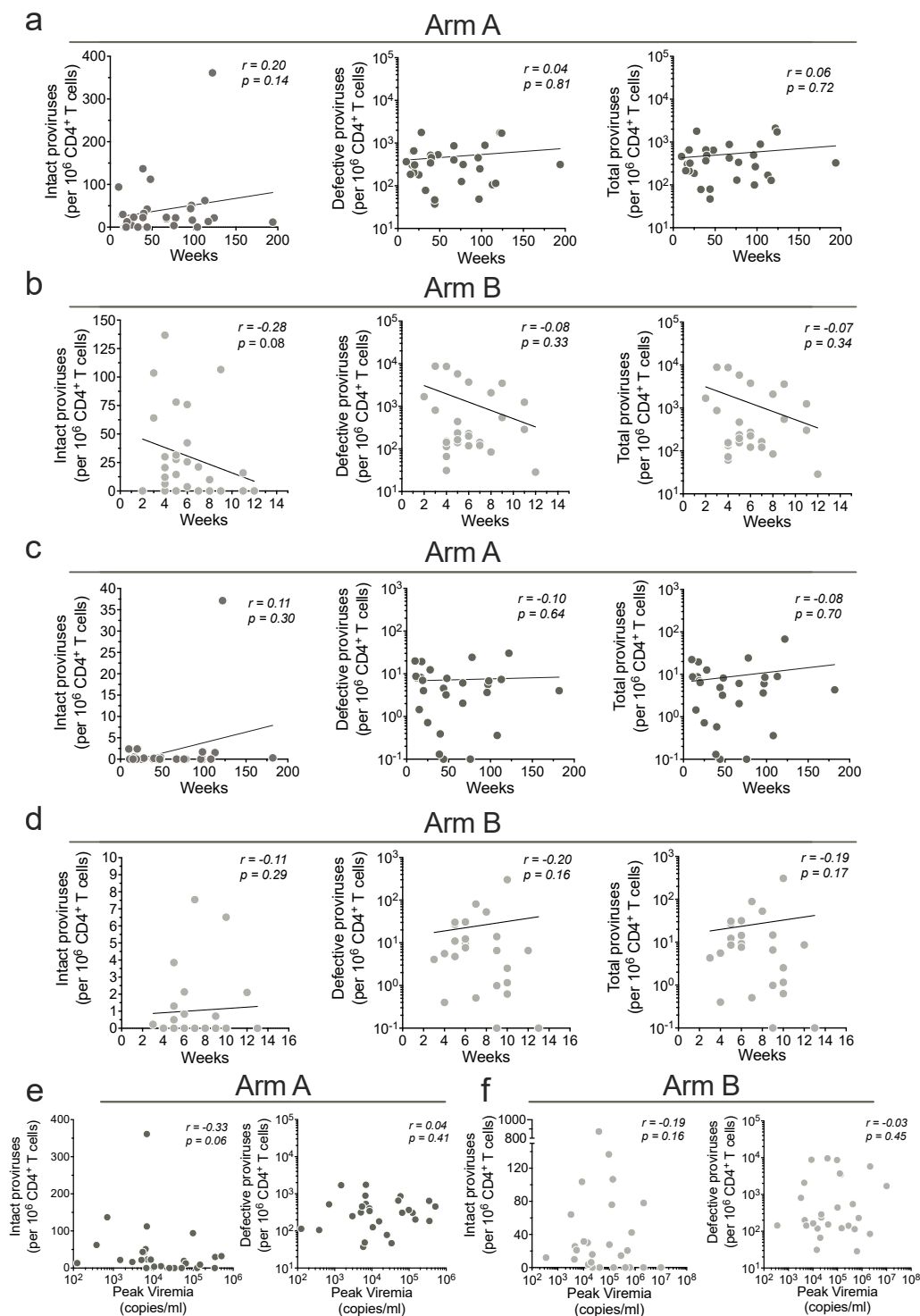
Extended Data Fig. 1 | Trial participant selection and time to ART restart after ATL. **a**, CONSORT flow diagram showing participant enrollment, randomization, allocation to Arm A or Arm B, follow-up, ART restart categories, and inclusion in virologic analyses. **b**, Kaplan–Meier curves showing time to ART restart (up to 96 weeks) in participants from Arm A (bNAbs; $n = 29$) and Arm B (placebo; $n = 32$).

Shaded areas indicate the standard error (SE) of the survival estimate. Two-sided Gehan–Breslow–Wilcoxon test, $\chi^2 = 30.09$, $P < 0.0001$. Numbers at the end of each curve indicate the number of participants remaining off ART at week 96 relative to the total in each arm.



Extended Data Fig. 2 | Baseline HIV reservoir in circulating CD4⁺ T cells measured by ddPCR and Q4PCR. Graphs show the numbers of (a) intact and (b) defective proviral genomes per million CD4⁺ T cells. Each dot represents the value for one participant. Arm A participants black and Arm B grey.

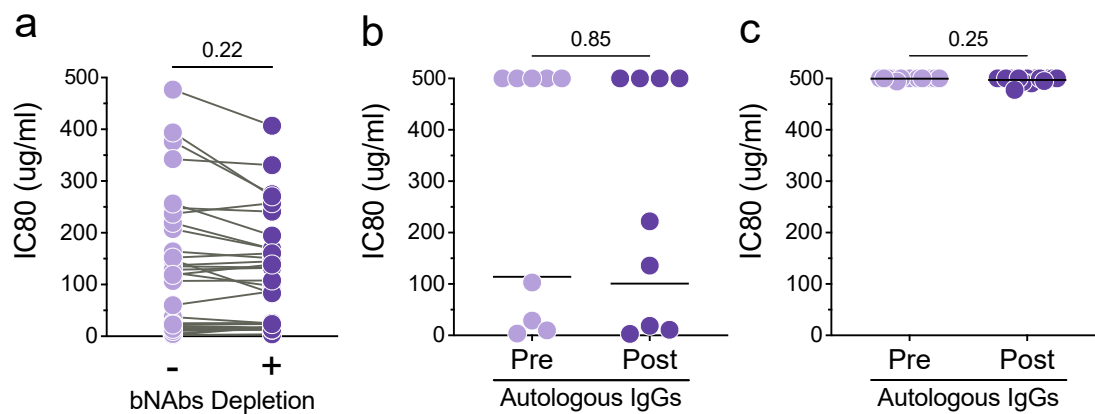
Sample size: ddPCR – Arm A, n = 32; Arm B, n = 29; Q4PCR – Arm A, n = 30; Arm B, n = 27. The black line and numbers indicate the geometric mean. Group comparisons were performed using a two-sided Mann–Whitney test, with statistical significance defined as $P < 0.05$.



Extended Data Fig. 3 | Correlation between baseline intact, defective and total reservoir size, peak viremia and time to ART restart in weeks.

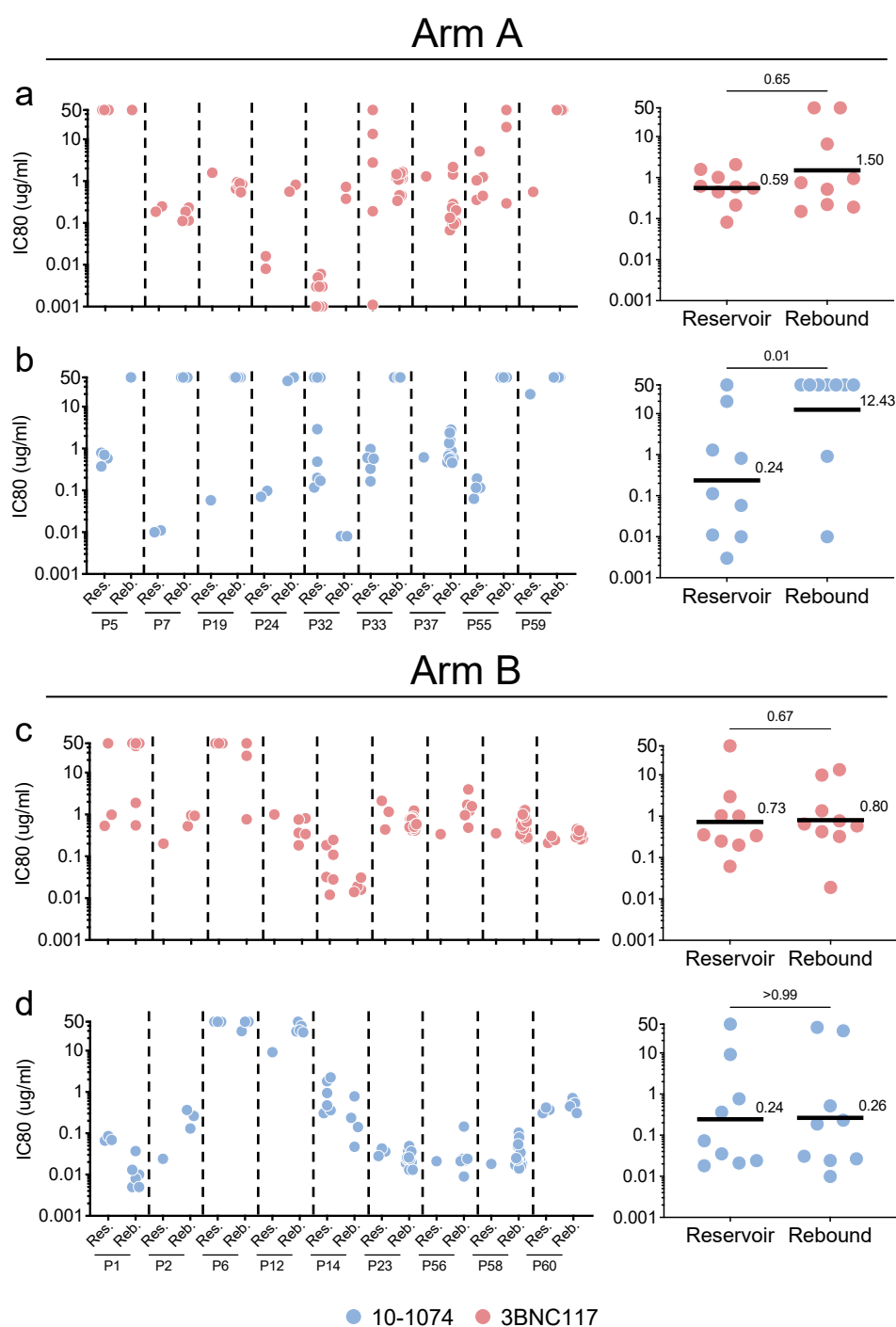
(a–d) Correlation between baseline intact, defective, and total proviral reservoir size measured by ddPCR (a–b, e–f) or Q4PCR (c, d) and time to ART restart in weeks in Arm A (a, c) and Arm B (b, d). Each dot represents one participant. For ddPCR measurements, $n = 29$ for Arm A and $n = 27$ for Arm B. For Q4PCR measurements, $n = 26$ for Arm A and $n = 25$ for Arm B. (e, f) Relationship between baseline

reservoir size measured by ddPCR and peak plasma viremia in Arm A (e) and Arm B (f). Each dot represents one participant. $n = 28$ for Arm A and $n = 27$ for Arm B. Y axes indicate the number of intact or defective proviruses, and x axes indicate plasma viremia at peak rebound. Correlation coefficients were calculated using a two-sided Spearman's rank correlation with 95% confidence intervals. Nonlinear curve fitting was performed with x as a linear function, and y as a linear or logarithmic function for intact and defective genomes, respectively.



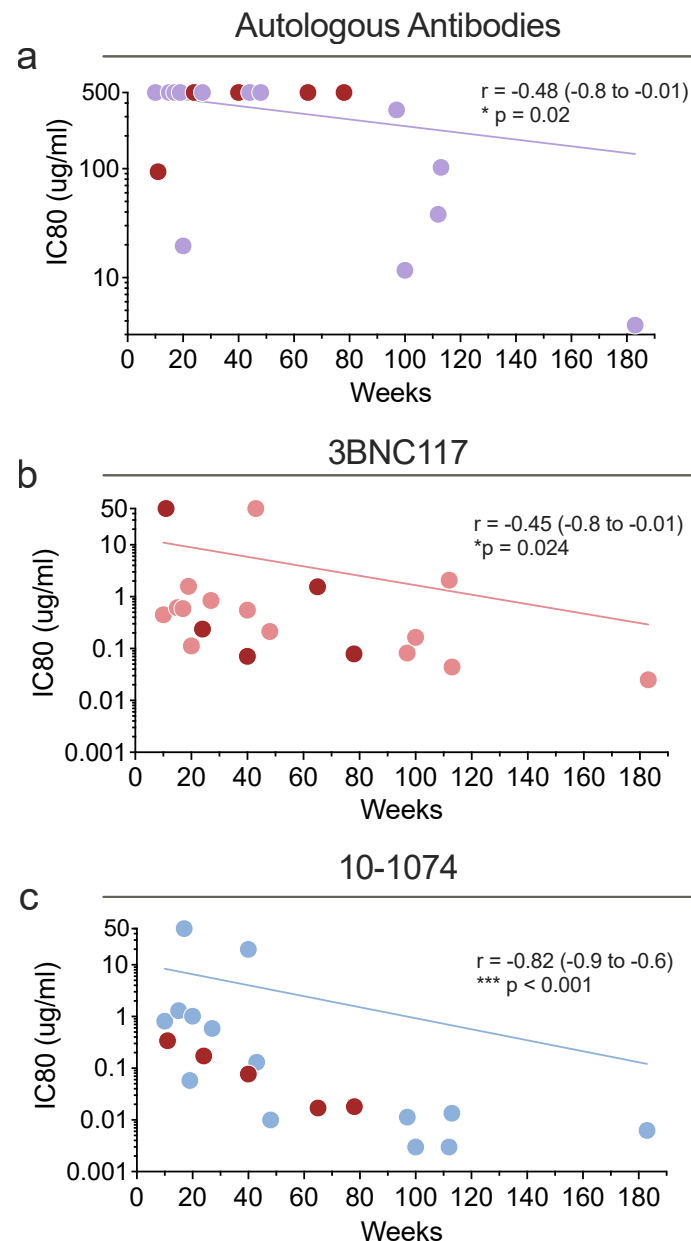
Extended Data Fig. 4 | Autologous neutralization activity and breadth post-bNAb therapy in Arm A participants. (a) Control depletion experiments performed in six individuals comparing depleted and non-depleted IgG preparations against participant-matched autologous viruses demonstrated that the depletion procedure preserved endogenous autologous neutralizing activity. (b) Autologous neutralization sensitivity (IC80) of baseline reservoir-derived pseudoviruses tested against IgG isolated before bNAb infusion and at later post-infusion time points after depletion of residual infused bNAbs. Each circle represents the geometric mean IC80 for one participant (one biological replicate, n = 9). Post-infusion IgG samples were collected at the following time

points, with corresponding residual plasma concentration ($\mu\text{g ml}^{-1}$) of 3BNC117 and 10-1074, respectively: P4, week 39 (12.5 and 10.7); P7, week 64 (4.1 and 4.7); P15, week 134 (<1 and <1); P18, week 64 (13.8 and 18.1); P19, week 36 (25.9 and 30.0); P21, week 119 (2.2 and 3.3); P32, week 123 (<3 and <3); P33, week 27 (338 and 32.5); and P48, week 56 (22.0 and 20.8). (c) Neutralization breadth of autologous IgG isolated before and after bNAb therapy was evaluated in a panel of 10 HIV-1 strains resistant to both 3BNC117 and 10-1074. Analyses included 15 participants, with post-infusion IgG samples collected at a mean of 65.7 weeks after infusion. Group comparisons were performed using a two-sided Wilcoxon matched-pairs signed-rank test ($P < 0.05$).



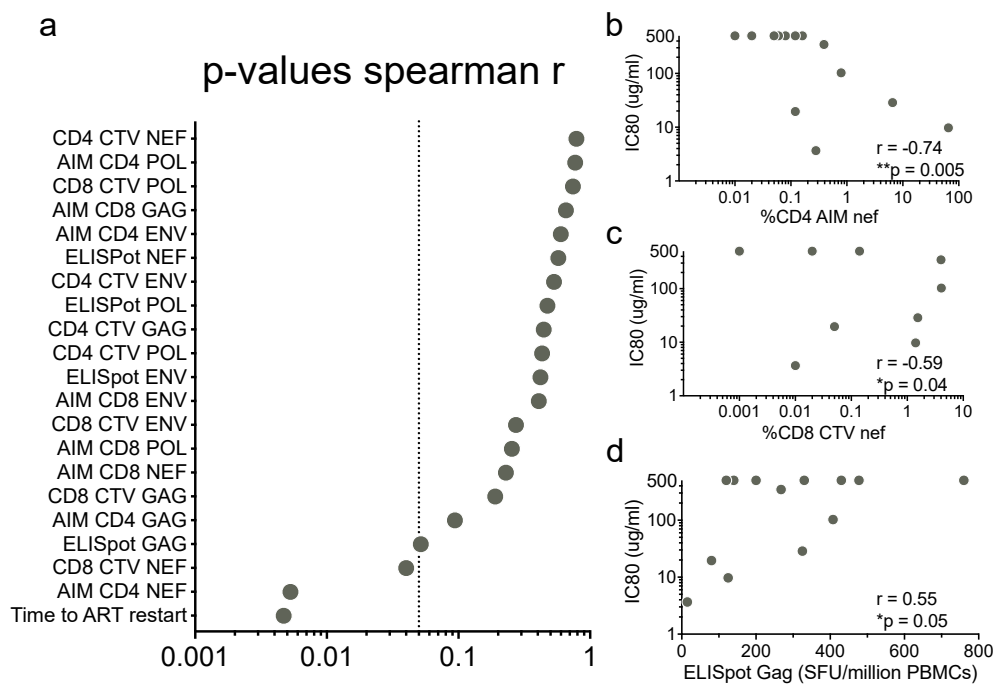
Extended Data Fig. 5 | Per-participant matched reservoir- and rebound-derived pseudovirus neutralization sensitivities to 3BNC117 and 10-1074 in both study arms, expressed as IC80 values. a-d. Each dot represents a reservoir- or rebound-provirus from each participant in (a, b) Arm A and (c, d) Arm B. N = 27 participants for each Arm. Members of expanded clones are represented

only once, irrespective of the size of the clone. Geometric mean IC80 values of all reservoir- and rebound-derived clones per participant are indicated alongside each graph and were used for statistical comparison. Group comparisons were performed using a two-sided Mann-Whitney test, with statistical significance defined as $P < 0.05$. Black lines indicate the geometric mean.



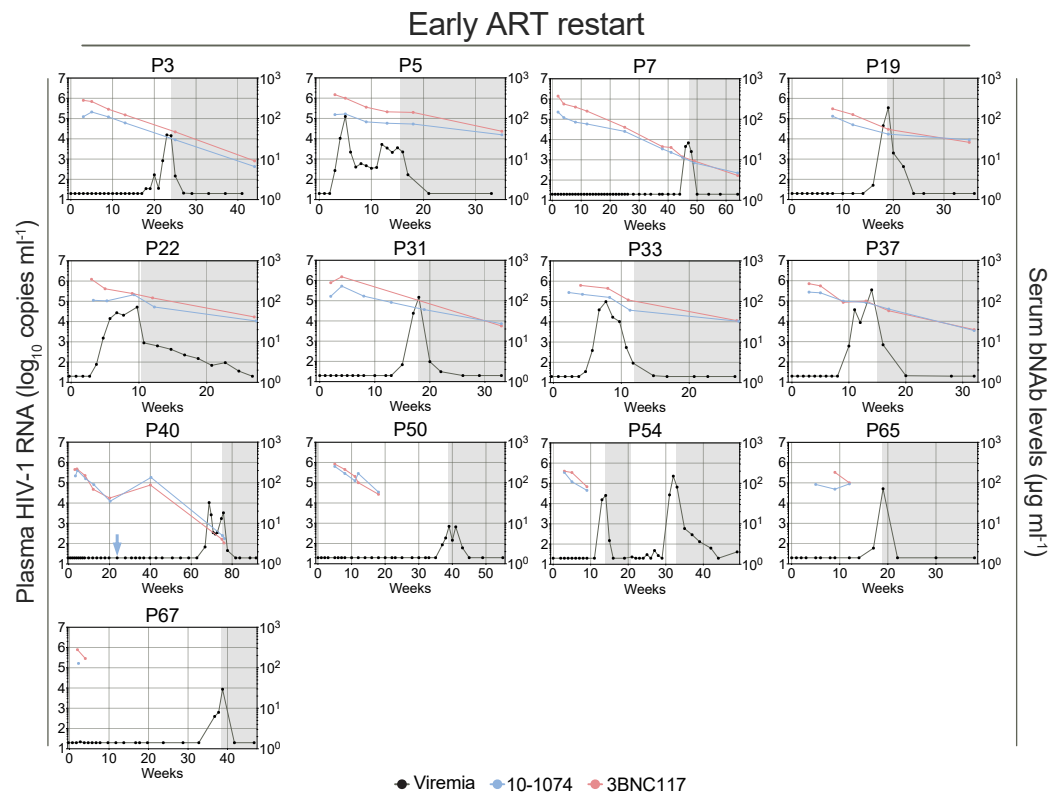
Extended Data Fig. 6 | Reservoir sensitivity to autologous antibodies, 3BNC117 and 10-1074 including participants with intact *env* but not confirmed intact proviral genomes. a-c, Correlation between time to ART restart and baseline reservoir sensitivity to (a) autologous antibodies, (b) 3BNC117, and (c) 10-1074. Each dot represents one participant (one biological replicate), using the geometric mean IC80 (Y axis) and time to rebound in weeks (X axis) for

one participant. Red dots represent participants that we were able to detect intact *env*s but not confirmed intact provirus. Nonlinear curve fitting was performed with x as a linear function of time and y as a logarithmic function of IC80 values. Correlation coefficients were calculated using a two-sided Spearman's r with 95% confidence intervals.



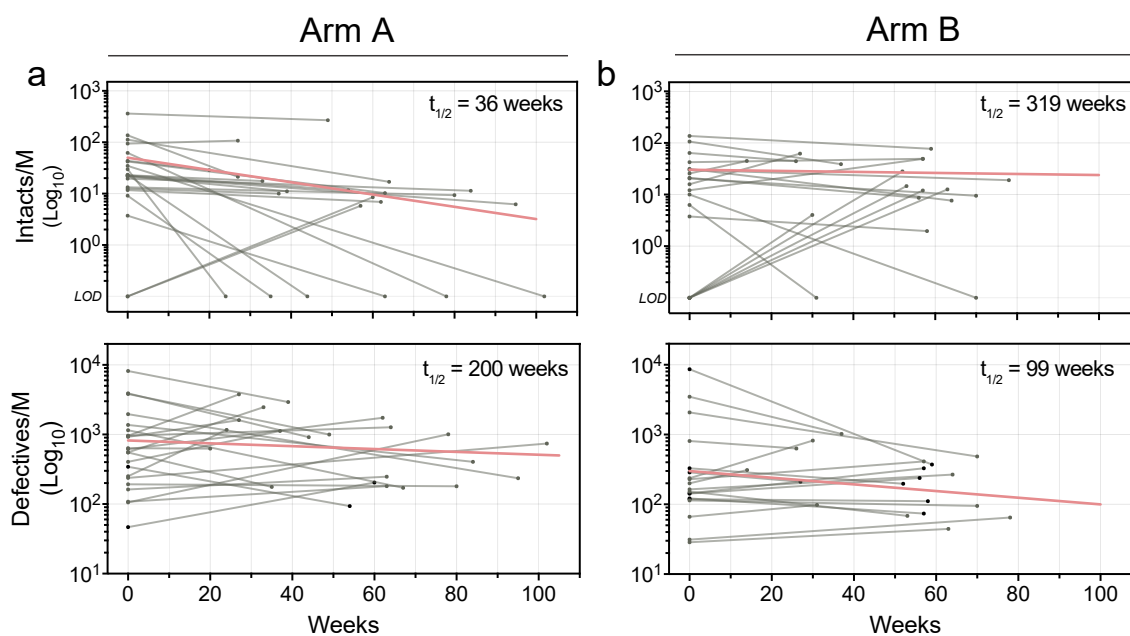
Extended Data Fig. 7 | Associations between baseline autologous neutralizing activity and cellular immune responses. (a) Summary of two-sided Spearman correlations between baseline autologous neutralizing activity against reservoir viruses and cellular immune responses measured by ELISpot, CTV, and AIM assays at week 0. P values are indicated for each assay; the dashed line marks

$P = 0.05$. (b–d), Scatter plots showing correlations between autologous antibody neutralization (IC_{80}) and (b) AIM CD4⁺ responses to Nef, (c) CD8⁺ CTV responses to Nef, and (d) ELISpot responses to Gag peptides. Each point represents one participant (one biological replicate).



Extended Data Fig. 8 | Longitudinal plasma viremia and circulating bNAb concentrations in participants with early ART restart. Longitudinal plasma HIV-1 RNA levels (black) and circulating bNAb concentrations for 10-1074 (blue) and 3BNC117 (red) are shown for participants who restarted ART early, defined

by protocol criteria. Time is shown in weeks on the x axis, with HIV-1 RNA copies per milliliter and bNAb concentrations in microgram per milliliter plotted on log10 scales on the y axes. Blue arrows indicate administration of a second bNAb infusion. Gray shading denotes periods on antiretroviral therapy.



Extended Data Fig. 9 | Decay of intact and defective proviral reservoirs measured by ddPCR. (a,b) Numbers of intact and defective proviruses quantified by ddPCR at baseline and follow-up time points in participants from Arm A ($n = 22$) and Arm B ($n = 20$). Time in weeks after the first infusion

or treatment interruption is shown on the x axis. Each grey line represents one participant (one biological replicate). Half-life estimates were derived using log-linear regression of $\log_{10}(x + 1)$ -transformed values; the red line indicates the fitted regression model.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Instrumentation & Associated Acquisition Software 1. Applied Biosystems QuantStudio 6 / 7 Flex Real-Time PCR System - (qPCR data acquisition) 2. Bio-Rad QX200 AutoDG System - (ddPCR droplet generation + data acquisition) 3. Illumina MiSeq Sequencing Platform - (next-generation sequencing data generation) 4. Beckman Coulter Vi-Cell XR Automated Cell Counter - (cell number acquisition) 5. FLUOstart Omega microplate reader - (Luminescence reading)
Data analysis	Commercial software Thermo Fisher Design and Analysis Software v2.4.3 (Thermo Fisher Scientific) – qPCR/ddPCR data analysis. GraphPad Prism v10.6.1 (GraphPad Software, LLC) – statistical analyses and graph generation. Adobe Illustrator (Adobe Inc.) – figure assembly and preparation. Interactive Tree of Life (iTOL) v6 – phylogenetic tree visualization and annotation. Open-source and bioinformatics software MUSCLE v5.1 – multiple sequence alignment. FastTree v2.1.11 – maximum-likelihood phylogenetic tree reconstruction. BBTools (clumpify.sh) v38.72 – read deduplication and error correction. Trim Galore v0.6.4 – adapter trimming and quality filtering. SPAdes v3.13.0 – genome assembly. BLAST+ (NCBI) – sequence alignment and similarity searches. R v4.5.0 (R Foundation for Statistical Computing) – statistical analyses.

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

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

Source data underlying all figures and statistical analyses are provided in the Supplementary Tables accompanying this manuscript. Participant-level reservoir quantification data are provided in Supplementary Table 1; baseline intact reservoir-derived proviral sequences and neutralization data in Supplementary Table 2; rebound virus env sequences and neutralization data in Supplementary Table 3; participant-level geometric mean neutralization sensitivities in Supplementary Table 4; serum concentrations of 3BNC117-LS and 10-1074-LS in long-term controllers in Supplementary Table 5; and Cox proportional hazards model outputs in Supplementary Table 6. HIV-1 sequence data generated for all reservoir-derived proviral genomes have been deposited in GenBank.

Because the study involves human participants enrolled in the RIO clinical trial, de-identified participant-level clinical and laboratory datasets are available through a controlled-access process following completion of the trial and database lock, in accordance with participant consent and institutional ethical requirements. Requests for clinical data should be directed to Professor Sarah Fidler (s.fidler@imperial.ac.uk) and will be reviewed according to the RIO trial data access policy. The RIO clinical protocol and Statistical Analysis Plan are available as supplementary documents accompanying the primary clinical publication (DOI: 10.1016/S2352-3018(26)00059-7). The generated research materials, including participant-derived HIV-1 env constructs, pseudovirus reagents, and other unique materials generated in this study, are available from the corresponding author upon reasonable request, subject to institutional approvals, material transfer agreements, participant consent restrictions, and material availability.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

All participants enrolled in the RIO trial were men. Sex was determined by self-report at enrollment. Gender identity was not collected separately. Because all enrolled participants were men, sex and gender were not considered as biological variables in the study design, and no sex- or gender-based analyses were performed.

Reporting on race, ethnicity, or other socially relevant groupings

Race and ethnicity were self-reported according to local clinical site procedures, they were collected for regulatory and safety reporting in the original RIO trial. These variables were not analyzed in the present study, did not inform inclusion/exclusion criteria, and were not used in any statistical analysis or interpretation. No conclusions were drawn based on race, ethnicity, or other socially constructed groupings.

Population characteristics

The population consisted of adults living with HIV-1 who initiated antiretroviral therapy (ART) during primary or early-stage infection and had maintained virologic suppression for ≥ 1 year prior to enrollment. Although the protocol permitted enrollment of both women and men, all enrolled participants were men. Baseline demographic characteristics are summarized in Supplementary Table 1. The majority had HIV clade B virus. Participants had well-preserved immunity (median baseline CD4 count 776 cells/ μ L) and had been on ART for a median of 5 years. These characteristics reflect the trial's focus on individuals with smaller and more homogeneous HIV reservoirs typical of early-treated infection.

Recruitment

Participants were recruited across nine sites in the UK and one in Denmark, as part of the RIO randomized controlled trial, using standard clinical research pathways within each participating National Health Service (NHS) or hospital system. Recruitment was restricted to individuals who met prespecified eligibility criteria (early or primary HIV infection, suppressed viral load, predicted sensitivity to bNAbs). Researchers involved in the present manuscript did not influence recruitment beyond the trial protocol, and only de-identified samples and data were used for the mechanistic analyses. Participants received financial compensation.

Ethics oversight

The RIO trial received ethics and regulatory approval in the United Kingdom from the London–Westminster Research Ethics Committee (REC reference 19/LO/1669; IRAS ID 266322), the UK Health Research Authority, and the Medicines and Healthcare products Regulatory Agency (MHRA reference 19174/0413/001). For the Danish study site, the trial received regulatory approval from the Danish Medicines Agency. The trial was registered under ClinicalTrials.gov (NCT04319367), the European Union Drug Regulating Authorities Clinical Trials (EudraCT) database (2019-002129-31), and the European Union Clinical Trials Regulation (EU CT number 2024-514564-13-00). All participants included in the present study were enrolled under these approved protocols and provided written informed consent before study participation. Trial conduct was overseen by the Imperial Clinical Trials Unit, with the Independent Data Monitoring Committee and Trial Steering Committee convening regularly to review participant safety and trial conduct. The analyses presented in this manuscript were performed using de-identified clinical samples and data collected under the approved study protocols.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

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Life sciences study design

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

Sample size	Sample sizes for all analyses were determined by the availability of clinical samples collected within the RIO trial. No statistical methods were used to predetermine sample size for this mechanistic study. The exact number of participants or samples used in each experiment is reported in the corresponding figure legends and Methods sections.
Data exclusions	No data or samples were excluded from the analyses unless they failed prespecified and objective quality-control criteria (e.g., insufficient sequencing depth, non-amplifiable templates, or samples with <500 reads in the DIHIVA pipeline). These criteria were applied uniformly and are described in the Methods. No post-hoc exclusions were made.
Replication	All experimental assays included appropriate technical replicates as described in the Methods. Where biological replication was possible, samples from independent participants were analyzed. All reported findings were reproducible within the available biological samples, and no findings failed replication.
Randomization	Randomization was not applicable to the laboratory analyses in this study. Participants were randomized as part of the RIO clinical trial, but the present study performed mechanistic laboratory analyses on de-identified specimens and was not involved in participant allocation.
Blinding	All laboratory experiments were performed on de-identified samples labeled only with participant codes, with investigators unaware of clinical arm assignment at the time of analysis. Laboratory investigators performing the mechanistic analyses were initially blinded to treatment allocation. Blinding was maintained until participants met protocol-defined criteria for unblinding (for example, following viral rebound), after which treatment allocation became known for subsequent analyses

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used	This study used purified broadly neutralizing antibodies (bNAbs) 3BNC117 and 10-1074 for neutralization assays and autologous plasma neutralization experiments. No commercial antibodies were used for cell staining or flow cytometry in this work
Validation	The bNAbs 3BNC117 and 10-1074 have been extensively validated in multiple peer-reviewed clinical studies for binding specificity and neutralization breadth. No new antibody validation was performed for the present study.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	TZM-bl cells were used for pseudovirus neutralization assays. Cells were obtained from the NIH AIDS Reagent Program.
Authentication	TZM-bl cells used for neutralization assays were obtained through the NIH HIV Reagent Program and are routinely monitored for expected assay performance through external quality assurance testing.

Mycoplasma contamination

TZM-bl Cell lines are routinely tested for mycoplasma contamination and were confirmed to be mycoplasma negative. No additional authentication (e.g., STR profiling or karyotyping) was performed.

Commonly misidentified lines
(See [ICLAC](#) register)

TZM-bl cells are not listed among commonly misidentified or cross-contaminated cell lines in the ICLAC database. No other eukaryotic cell lines were used.

Clinical data

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All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration

ClinicalTrials.gov: NCT04319367
European Union Drug Regulating Authorities Clinical Trials (EudraCT): 2019-002129-31
London–Westminster Research Ethics Committee (UK REC): 19/LO/1669
European Union Clinical Trials Regulation (EU CT): 2024-514564-13-00
Medicines and Healthcare products Regulatory Agency (MHRA): 19174/0413/001

Study protocol

The full clinical protocol and Statistical Analysis Plan are available as supplementary documents accompanying the primary clinical publication. (Lee MJ, et al. Time to HIV rebound after infusion of long-acting broadly neutralising antibodies 3BNC117-LS and 10-1074-LS and analytical treatment interruption (the RIO trial): a double-blind, randomised, placebo-controlled trial. *Lancet HIV*. 2026 Aug;13(8):e527-e537. doi: 10.1016/S2352-3018(26)00059-7. Epub 2026 May 27. PMID: 42202839.)

Data collection

Clinical data were collected prospectively between May 2021 and September 26, 2025, at participating HIV clinical research sites in the United Kingdom and Denmark following Good Clinical Practice (GCP) procedures. Data collection included viral load measurements, CD4 counts, pharmacokinetic sampling, and safety assessments. Samples for mechanistic assays (PBMCs, plasma, and serum) were collected at scheduled study visits, processed according to standardized procedures, and transferred as de-identified specimens for laboratory analyses.

Outcomes

The RIO trial prespecified time to viral rebound within 20 weeks after analytical treatment interruption as the primary clinical endpoint. Safety, delayed viral rebound, and prolonged ART-free viral control following analytical treatment interruption were pre-specified secondary in the trial protocol and reported previously. The present manuscript reports pre-specified secondary virologic outcomes, including reservoir quantification and baseline reservoir sensitivity to bNAbs, pre-specified exploratory analyses of viral sequencing, reservoir characterization, rebound virus evolution, and antibody correlates of viral control, as well as clearly identified post hoc analyses examining associations between reservoir characteristics and clinical outcomes. Clinical outcomes were prospectively assessed according to protocol-defined endpoint criteria using validated clinical and laboratory assays.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.