Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

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 (n) 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

Only common tests should be described solely by name; describe more complex techniques in the Methods section.

☒ ☐ A description of all covariates tested

☒ ☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons

☒ ☐ A full description of the 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. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted Give P values as exact values whenever suitable.

☒ ☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings

☒ ☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes

☒ ☒ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

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
DIVA 8.0.1 (cytometer), Immunospot 5.0 (ELISpot), MiSeq Control Software 2.6 2.1 (sequencing)

Data analysis
Flow cytometry data were analyzed with Flowjo v10 (Treestar) and statistical analyses were performed with Prism v8 for Mac (Graphpad Software Inc). Amino acid alignments of intact gag sequences were obtained by using ClustalW v.2.1 under the BLOSUM cost matrix. Sequences with premature stop codons were excluded from all analyses. Maximum likelihood phylogenetic trees were then generated from these alignments using RAxML v.8.2.9 under the GTRGAMMA model with 1,000 bootstraps. To analyze changes between reservoir and rebound viruses, gag sequences were aligned at the amino acid level to a HXB2 reference using ClustalW v.2.1.

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

Data

Policy information about availability of data

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

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

Sequences from all isolated viruses are available in GenBank, accession numbers MN750027 - MN750174. Additional datasets that support the findings of this study are available from the corresponding authors on reasonable request.
Field-specific reporting

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

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

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

| Sample size | No sample size calculation was performed. Results obtained from the bNAb+ATI clinical trial (NCT02825797) have been previously published in Mendoza et al., Nature 561, 479–484 (2018). Nine of the 15 study participants who fulfilled the study eligibility criteria harbored latent reservoir that was sensitive to both bNAbs (10-1074 and 3BNC117) and maintained viral suppression for 15 to >30 weeks after ART discontinuation. We analyzed all available study participants (n=9) with prolonged viral suppression for this manuscript. In addition, two study participants who harbored latent reservoir that was resistant to one of the two bNAbs and who rebounded early after ATI (week 5 or 7) were analyzed (Extended Data Figure 5). The number of individuals included in the historical comparison group of people on continuous ART (n=13) was chosen to approximately match the number of participants enrolled into the Phase Ib trial. |
| Data exclusions | No participant fulfilling the criteria mentioned above was excluded from the analyses. |
| Replication | Samples analyzed in this study were obtained from participants of a clinical trial (bNAb+ATI) or an observational study (ART) and samples were analyzed on individual study participants. Experiments did not include replicates as all participants and data points are unique. |
| Randomization | The bNAb+ATI clinical trial was single arm. |
| Blinding | The bNAb+ATI clinical trial was open label. |

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 | | |
| Animals and other organisms | | |
| Human research participants | | |
| Clinical data | | |

| Methods | n/a | Involved in the study |
|---------|-----|-----------------------|
| ChIP-seq | | |
| Flow cytometry | | |
| MRI-based neuroimaging | | |

Antibodies

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

All antibodies used for flow cytometry are listed in supplementary tables 1, 2 and 5, which describe the specific panels used.

1. CD3 BUV395, UCHT1, BD Biosciences 563548, lot #6343984, 3 μl/test
2. CD4 BUV496, SK3, BD Biosciences 564651, lot #9080989, 4 μl/test
3. CD8 APC-Fire750 SK1, Biolegend 344745, lot#637278, 0.5 μl/test
4. CD14 BV510 M5E2, Biolegend 301842, lot#B237278, 3 μl/test
5. CD19 BV510, M1B19, Biolegend 302242, lot#B239285, 3 μl/test
6. CD40L BV786, H4A3, BD Biosciences 563869, lot#8144866, 5 μl/test
7. CD56 BUV737, NCM16.2, BD Biosciences 564448, lot#2888818, 1 μl/test
8. CD69 PerCP-eFluor710, FN50, eBioscience 46-0699-42, lot#1920361, 4 μl/test
9. CD107A BV786, H4A3, BD Biosciences 563869, lot#2888818, 0.5 μl/test
10. IFNγ PE-Cy7, B27, BD Biosciences 557643, lot#7202642, 4 μl/test
11. IL-2 PE-Dazzle594, MQ1-17H12, Biolegend 500344, lot#5245312, 3.5 μl/test
12. MIP1β PE, D21-1351, BD Biosciences 550078, lot#8176503, 1 μl/test
Recruitment Participants of the bNAb+ATI trial were pre-screened for sensitivity of latent proviruses against 3BNC117 and 10-1074 antibodies by bulk PBMC viral outgrowth. Sensitivity was defined as an IC50 < 2 μl/test for both antibodies against outgrowth virus. Participants harboring sensitive viruses were invited for screening and were enrolled in the study sequentially. Participants were enrolled at the two clinical sites at the Rockefeller University (New York, USA) and Cologne University Hospital (Germany).

Validation

3BNC117 and 10-1074 that were administered to the participants were manufactured by Celldex Therapeutics under Good Manufacturing Practice and have been fully characterized in terms of biophysical properties and potency (INDs 118225 and 123713). Both drug products are under long term stability monitoring.

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

All antibodies used for flow cytometry were commercially available. Clones and companies are listed in the supplementary tables 1, 2 and 5.
No potential self-selection bias or other bias are known.

Ethics oversight
The clinical trial protocol was approved by the Federal Drug Administration in the USA, the Paul-Ehrlich-Institute in Germany, and the Institutional Review Boards (IRBs) at the Rockefeller University and the University of Cologne. The protocol for collection of samples from ART-suppressed participants was approved by the Rockefeller University IRB.

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

Clinical data

Policy information about clinical studies
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
NCT02825797

Study protocol
https://clinicaltrials.gov/ct2/show/NCT02825797

Data collection
Results concerning this clinical trial have been previously published in Mendoza et al., Nature, 561, 479–484 (2018). For this study, we used PBMC samples collected at Rockefeller University or University of Cologne from enrolled HIV-infected trial participants at week -2, 6/7, 12 and 18 (see Extended Data Figures 1 and 5) for analysis.

Outcomes
All primary and secondary outcomes of the trial are described under https://clinicaltrials.gov/ct2/show/NCT02825797. For the immunological exploratory substudy presented here, we pre-selected participants with a specific outcome (maintained viral suppression for >12 weeks after analytical treatment interruption).

Flow Cytometry

Plots
Confirm that:
- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation
Cryopreserved PBMCs were thawed, rested, stimulated, fixed, permeabilized and stained according to the demands on each experiment. All details are mentioned in the Methods section.

Instrument
LSRII (BD Biosciences) and LSR Fortessa (BD) for standard flow cytometry.

Software
Flow cytometry data were collected by DIVA 8.0.1 and analyzed with Flowjo v10 (Treestar).

Cell population abundance
For Flow Cytometry, we collected 0.1-15M events depending on the experiment. FMO controls and DMSO-treated controls were used as controls.

Gating strategy
The generic gating strategy is explained in supplementary figures 1&2 and extended data figures 6& 9.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.