Effect of 3BNC117 and romidepsin on the HIV-1 reservoir in people taking suppressive antiretroviral therapy (ROADMAP): a randomised, open-label, phase 2A trial

Gruell, Henning; Gunst, Jesper D; Cohen, Yehuda Z; Pahus, Marie H; Malin, Jakob J; Platten, Martin; Millard, Katrina G; Tolstrup, Martin; Jones, R Brad; Conce Alberto, Winnifer D; Lorenzi, Julio C C; Oliveira, Thiago Y; Kümmerle, Tim; Suárez, Isabelle; Unson-O'Brien, Cecilia; Nogueira, Lilian; Olesen, Rikke; Østergaard, Lars; Nielsen, Henrik; Lehmann, Clara; Nussenzweig, Michel C; Fätkenheuer, Gerd; Klein, Florian; Caskey, Marina; Søgaard, Ole S

Published in:
The Lancet. Microbe

DOI (link to publication from Publisher):
10.1016/S2666-5247(21)00239-1

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

Effect of 3BNC117 and romidepsin on the HIV-1 reservoir in people taking suppressive antiretroviral therapy (ROADMAP): a randomised, open-label, phase 2A trial

Henning Gruell, Jesper D Gunst*, Yehuda Z Cohen*, Marie H Pahus, Jakob J Malin, Martin Platten, Katrina G Millard, Martin Tolstrup, R Brad Jones, Winnifer D Conce Alberto, Julio C C Lorenzi, Thiago Y Oliveira, Tim Kümmerle, Isabelle Suárez, Cecilia Unson-O'Brien, Lilian Nogueira, Rikke Olesen, Lars Østergaard, Henrik Nielsen, Clara Lehmann, Michel C Nussenzweig, Gerd Fätkenheuer, Florian Klein, Marina Caskey, Ole S Søgaard*

Summary

Background The administration of broadly neutralising anti-HIV-1 antibodies before latency reversal could facilitate elimination of HIV-1-infected CD4 T cells. We tested this concept by combining the broadly neutralising antibody 3BNC117 in combination with the latency-reversing agent romidepsin in people with HIV-1 who were taking suppressive antiretroviral therapy (ART).

Methods We did a randomised, open-label, phase 2A trial at three university hospital centres in Denmark, Germany, and the USA. Eligible participants were virologically suppressed adults aged 18–65 years who were infected with HIV-1 and on ART for at least 18 months, with plasma HIV-1 RNA concentrations of less than 50 copies per mL for at least 12 months, and a CD4 T-cell count of greater than 500 cells per µL. Participants were randomly assigned (1:1) to receive 3BNC117 plus romidepsin or romidepsin alone in two cycles. All participants received intravenous infusions of romidepsin (5 mg/m² given over 120 min) at weeks 0, 1, and 2 (treatment cycle 1) and weeks 8, 9, and 10 (treatment cycle 2). Those in the 3BNC117 plus romidepsin group received an intravenous infusion of 3BNC117 (30 mg/kg given over 60 min) 2 days before each treatment cycle. An analytic treatment interruption (ATI) of ART was done at week 24 in both groups. Our primary endpoint was time to viral rebound during analytic treatment interruption, which was assessed in all participants who completed both treatment cycles and ATI. We used a log-rank test to compare time to viral rebound during analytic treatment interruption between the two groups. This trial is registered with ClinicalTrials.gov, NCT02850016. It is closed to new participants, and all follow-up is complete.

Findings Between March 20, 2017, and Aug 14, 2018, 22 people were enrolled and randomly assigned, 11 to the 3BNC117 plus romidepsin group and 11 to the romidepsin group. 19 participants completed both treatment cycles and the ATI: 11 in the 3BNC117 plus romidepsin group and 8 in the romidepsin group. The median time to viral rebound during ATI was 18 days (IQR 14–28) in the 3BNC117 plus romidepsin group and 28 days (21–35) in the romidepsin group B (p=0·0016). Although this difference was significant, prolongation of time to viral rebound was not clinically meaningful in either group. All participants in both groups reported adverse events, but overall the combination of 3BNC117 and romidepsin was safe. Two severe adverse events were observed in the romidepsin group during 48 weeks of follow-up, one of which—increased direct bilirubin—was judged to be related to treatment.

Interpretation The combination of 3BNC117 and romidepsin was safe but did not delay viral rebound during analytic treatment interruptions in individuals on long-term ART. The results of our trial could serve as a benchmark for further optimisation of HIV-1 curative strategies among people with HIV-1 who are taking suppressive ART.

Funding amfAR, German Center for Infection Research.

Introduction

Durable viral control in the absence of antiretroviral therapy (ART) is the goal of strategies to cure HIV-1 infection.[1] Although ART effectively suppresses viral replication, proviral DNA integrated into immune cells allows HIV-1 to persist as latent infection. If ART is interrupted, viral replication can rapidly resume from the reservoir of infected cells, resulting in a rebound of viraemia within weeks. Thus life-long ART is necessary to prevent disease progression.

The primary barrier to eradication of HIV-1 is a pool of long-lived latently infected memory CD4 T cells. In their resting state, these cells do not produce viral particles and elude recognition by the immune system. Reversing latency could expose these cells to immune-mediated elimination in an approach termed shock and kill.[2] Latently infected cells can be induced to resume HIV-1 expression by different classes of latency-reversing agents, of which histone deacetylase inhibitors are the most extensively studied.[3–5]

Clinical proof-of-concept trials[3–6] showed that latency-reversing agents can transiently increase HIV-1 RNA transcription in people using ART. However, this

Lancet Microbe 2022; 3: e203–14

Published Online January 24, 2022 https://doi.org/10.1016/S2666-5247(21)00239-1

*These authors contributed equally

Laboratory of Experimental Immunology, Institute of Virology (H Gruell MD, Prof F Klein MD) **and Department I of Internal Medicine** (H Gruell, J J Malin MD, M Platten MD, T Kümmerle MD, I Suárez MD, C Lehmann MD, Prof G Fätkenheuer MD), **Faculty of Medicine and University Hospital Cologne, University of Cologne, Cologne, Germany; German Center for Infection Research (DZIF) Bonn-Cologne, Cologne, Germany** (H Gruell, I Suárez, C Lehmann, Prof G Fätkenheuer, Prof F Klein); **Department of Clinical Medicine, Aarhus University, Aarhus, Denmark** (J D Gunst MD, M H Pahus, M Tolstrup PhD, R Olesen PhD, Prof L Østergaard MD, Prof O S Søgaard MD); **Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark** (J D Gunst, M H Pahus, M Tolstrup, R Olesen, Prof L Østergaard, Prof O S Søgaard); **Laboratory of Molecular Immunology** (Y Z Cohen MD, K G Millard RN, J C C Lorenzi PhD, T Y Oliveira PhD, C Unson-O'Brien PhD, L Nogueira PhD, Prof M C Nussenzweig MD, Prof M Caskey MD) **and Howard Hughes Medical Institute** (Prof M C Nussenzweig), **The Rockefeller University, New York, NY, USA; Wisplinghoff Laboratories, Cologne, Germany** (M Platten); **Division of Infectious Diseases, Department of Medicine, Weill Cornell Medicine, New York, NY, USA** (R B Jones PhD,

W D Conce Alberto); **Praxis am Ebertplatz, Cologne, Germany** (T Kümmerle); **Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark** (Prof H Nielsen MD); **Center for Molecular Medicine Cologne, University of Cologne, Cologne, Germany** (C Lehmann, Prof F Klein)

Correspondence to: Prof Ole S Søgaard, Department of Infectious Diseases, Aarhus University Hospital, 8200 Aarhus N, Denmark
olesoega@rm.dk

Research in context

Evidence before this study

We searched PubMed with the terms "HIV remission", "HIV cure", "HIV eradication", "HIV reservoir", "HIV latency", "broadly anti-HIV neutralizing antibodies (bNAbs)", "latency-reversing agent (LRA)", and "histone deacetylase inhibitor (HDACi)" for clinical trials published in any language up to Aug 1, 2021. In phase 1 and 2 clinical trials, infusions of broadly neutralising anti-HIV-1 antibodies (bNAbs) transiently decrease plasma HIV-1 RNA concentrations in viraemic people infected with HIV-1. Multiple infusions of bNAbs alone or in combination prolonged HIV-1 control after interruption of antiretroviral therapy (ART). Latency reversal with histone deacetylase inhibitor (vorinostat, panobinostat, or romidepsin) monotherapy enhanced HIV-1 transcriptional activity as measured by increased concentrations of cellular or plasma HIV-1 RNA. However, neither of these interventions alone leads to significant decreases in the HIV-1 reservoir or HIV-1 remission. In pre-clinical animal models, combinations of latency-reversing agents and bNAbs have resulted in reductions of the HIV-1 reservoir.

Added value of this study

Our study is, to our knowledge, the first randomised clinical trial of the combination of a potent bNAb (3BNC117) with a latency-reversing agent (romidepsin) as a novel approach to reduce the viral reservoir in HIV-1-infected individuals on suppressive ART. Assessments of the cell-associated viral reservoir and the time to viral rebound after analytic treatment interruption of ART suggested that the combination did not substantially affect the HIV-1 reservoir. Our results show that the combination of a bNAb with a latency-reversing agent was safe and well tolerated, but highlight the challenges in achieving HIV-1 clearance in chronically infected people on ART.

Implications of all the available evidence

The combination of the bNAb 3BNC117 and the latency-reversing agent romidepsin did not significantly affect the latent HIV-1 reservoir. Eradication of the HIV-1 reservoir in individuals on long-term ART with a shock-and-kill strategy might be very difficult to achieve.

increased transcription produced no or only slight reductions in the size of the HIV-1 reservoir, possibly as a result of insufficient stimulation of immune-mediated clearance of infected cells when latency-reversing agents are given alone.[7] When the histone deacetylase inhibitor romidepsin was combined with an experimental HIV-1 peptide vaccine (Vacc-4x), the mean total HIV-1 DNA reservoir was reduced by 40%.[8] However, HIV-1-specific T-cell activity after Vacc-4x immunisation was not significantly increased, and time to viral rebound during subsequent analytic treatment interruption (ATI) of ART did not increase.[8,9]

A proposed alternative approach to enhance elimination of latently infected cells is to combine latency-reversing agents with broadly neutralising anti-HIV-1 antibodies (bNAbs) that target the HIV-1 envelope protein (Env).[10–12] In clinical trials, bNAbs suppressed viraemia and delayed viral rebound during ATI.[13] In addition to potently neutralising HIV-1, bNAbs can engage different components of the host immune system. These interactions are mediated by the antibodies' Fc regions and can result in accelerated viral clearance, induction of antibody-dependent cellular cytotoxicity, and enhanced antigen presentation.[10,11,14] In humanised mouse and non-human primate models of HIV-1 infection, a combination of bNAbs and latency-reversing agents resulted in a significant delay in time to viral rebound in the absence of ART compared with bNAbs given alone.[12,15]

We aimed to investigate this concept in humans with a combination of the bNAb 3BNC117 and the latency-reversing agent romidepsin. 3BNC117 targets the CD4 binding site on the HIV-1 Env and has high antiviral activity in clinical studies.[16,17] Romidepsin is a pan-histone deacetylase inhibitor approved for treatment of peripheral and cutaneous T-cell lymphoma. It is one of the most potent and extensively clinically tested latency-reversing agents,[5,8,18] but did not result in detectable latency reversal in individuals on ART in a dose-escalation trial[19] published in 2020. To assess the effect of the combination of 3BNC117 and romidepsin in individuals on ART compared with romidepsin alone, we quantitatively assessed the HIV-1 reservoir and measured the time to viral rebound during ATI in a clinical trial.

See Online for appendix

Methods

Study design and participants

ROADMAP was a randomised, open-label, parallel-group, phase 2A trial done at three university centres in Denmark, Germany, and the USA. Eligible participants were virologically suppressed adults aged 18–65 years who were infected with HIV-1 and on ART for at least 18 months, with plasma HIV-1 RNA concentrations of less than 50 copies per mL for at least 12 months (one blip <500 copies per mL was allowed), and a CD4 T-cell count of greater than 500 cells per µL. Exclusion criteria included a CD4 T-cell nadir of fewer than 200 cells per µL within the past 5 years, concomitant hepatitis B or C virus infection, receipt of any anti-HIV-1 monoclonal antibody or therapeutic HIV-1 vaccine in the past, receipt of any histone deacetylase inhibitor in the past 2 years, and prolongation of corrected QT interval. A full list of inclusion and exclusion criteria is provided in the trial protocol, which is available in the appendix (MCA-0896, version 1.8, Oct 10, 2017).

The study was done in accordance with Good Clinical Practice and is reported in accordance with the

CONSORT 2010 statement.[20] The protocol was approved by the Paul-Ehrlich-Institute (#2944/01), the US Food and Drug Administration (IND 118229), the Danish Medicine Authorities (#2016080161), the Institutional Review Boards at the University of Cologne (#16-452) and the Rockefeller University, and the National Committee on Health Research Ethics in Denmark (#1-10-72-355-15). All participants provided written informed consent.

Randomisation

Participants were recruited by study physicians and then randomly assigned (1:1) to receive 3BNC117 plus romidepsin or romidepsin alone. Randomisation was done using randomly permuted blocks of two or four per site and was stratified by study site. The Institute of Medical Statistics, Informatics and Epidemiology (University of Cologne, Cologne, Germany) oversaw and implemented randomisation and patient allocation. The trial was open label, with neither investigators nor participants masked to group assignment. Because of the demanding visit schedule, we wanted participants to know what intervention they were receiving.

Procedures

Screening visits occurred up to 8 weeks before the first administration of study medications. Participants were switched to an integrase inhibitor-based regimen (raltegravir or dolutegravir) before enrolment if their ART regimen included non-nucleoside reverse transcriptase inhibitors (due to their long half-life), or cobicistat or protease inhibitors (due to the potential for interactions with romidepsin). All participants underwent leukapheresis at baseline (week –2), which was 2 weeks before study treatment began, and then again in week 22 (pre-ATI). All participants received intravenous infusions of romidepsin (5 mg/m² given over 120 min) at weeks 0, 1, and 2 (treatment cycle 1) and weeks 8, 9, and 10 (treatment cycle 2). Participants in the 3BNC117 plus romidepsin group were given 3BNC117 as an intravenous infusion (30 mg/kg given over 60 min) 2 days before each treatment cycle. Dosing of 3BNC117 was based on previously observed antiviral efficacy,[16] and the interval of 6 weeks between the two romidepsin treatment cycles was based on clinical findings related to the administration of only one treatment cycle.[5]

At week 24, participants initiated an ATI. This timepoint was chosen to ensure that 3BNC117 serum concentrations were sub-therapeutic (<10 µg/mL) in the 3BNC117 plus romidepsin group (the serum half-life of 3BNC117 is approximately 2 weeks).[16,21] During ATI, plasma HIV-1 RNA concentrations were monitored weekly and CD4 T-cell counts were assessed every second week. Viral rebound was defined as the date of the first of two consecutive plasma HIV-1 RNA measurements of at least 200 copies per mL. ART was resumed in cases of viral rebound or when a participant's CD4 T-cell count was confirmed to have fallen to fewer than 350 cells per µL. After resumption of ART, plasma HIV-1 RNA concentrations were measured every second week until undetectable (ie, <20 copies per mL) on two consecutive measurements. Thereafter, participants were followed up every 8 weeks until the final visit at week 48.

All blood samples were processed within 4 h of collection. Serum and plasma samples were stored at –80°C. Peripheral blood mononuclear cells were isolated by density gradient centrifugation and cryopreserved in fetal bovine serum with 10% dimethyl sulfoxide. Plasma HIV-1 RNA concentrations were measured with standardised clinical assays at every visit. CD4 counts were measured on days –56, –14, 0, 2, 9, 16, 30, 56, 58, 65, 72, and 82. Safety markers were measured at every visit except for those on day 12 and day 68. We used droplet digital PCR to assess cell-associated unspliced HIV-1 RNA (CA usHIV-1 RNA) concentrations on days –14, 0 or 2, 3, 9, 10, 16, 17, 23, 56 or 58, 59, 65, 66, 72, 73, and 82. HIV-1 reservoir size and T-cell immunity were assessed at leukapheresis visits before and after trial treatment. We used droplet digital PCR to assess HIV-1 reservoir size and flow cytometry to assess HIV-1-specific immunity (by cytokine secretion after peptide stimulation) and T-cell function (specifically, we looked for the activation markers HLA-DR, CD38, and CD69, and the exhaustion marker PD1). Total HIV-1 DNA concentrations, which included both defective and replication-competent proviruses, were also measured between the two treatment cycles at week 3.

To more precisely quantify changes in the reservoir of replication-competent proviruses, we used the quantitative droplet digital PCR-based intact proviral DNA assay (IPDA), which distinguishes defective from intact proviral sequences.[22,23] Additional analyses included assessments of viral 3BNC117 sensitivity by *env* sequencing of proviral DNA at baseline and with pseudovirus TZM-bl cell neutralisation assays of plasma single genome amplification-derived viruses at rebound, and the establishment of serum 3BNC117 concentrations by TZM-bl cell assay (done only in the 3BNC117 plus romidepsin group). Proviral 3BNC117 resistance was based on a predefined threshold of 30% of resistant sequences.

Safety data are reported up to the end of study. Solicited adverse events were recorded for 2 weeks after infusions. Unsolicited adverse events were recorded at all visits. The Common Terminology Criteria for Adverse Events (CTCAE) scale (version 4.03) was used to grade infusion-related adverse events, and the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (version 2.0) was used to grade non-infusion-related adverse events. Safety assessments included directed physical examinations, vital sign measurements, and clinical laboratory tests. Study participants were on continuous cardiac monitoring during romidepsin infusions and electrocardiograms (ECGs) were repeatedly recorded throughout the treatment cycles. An independent safety monitoring committee regularly reviewed data to ensure trial participants' safety.

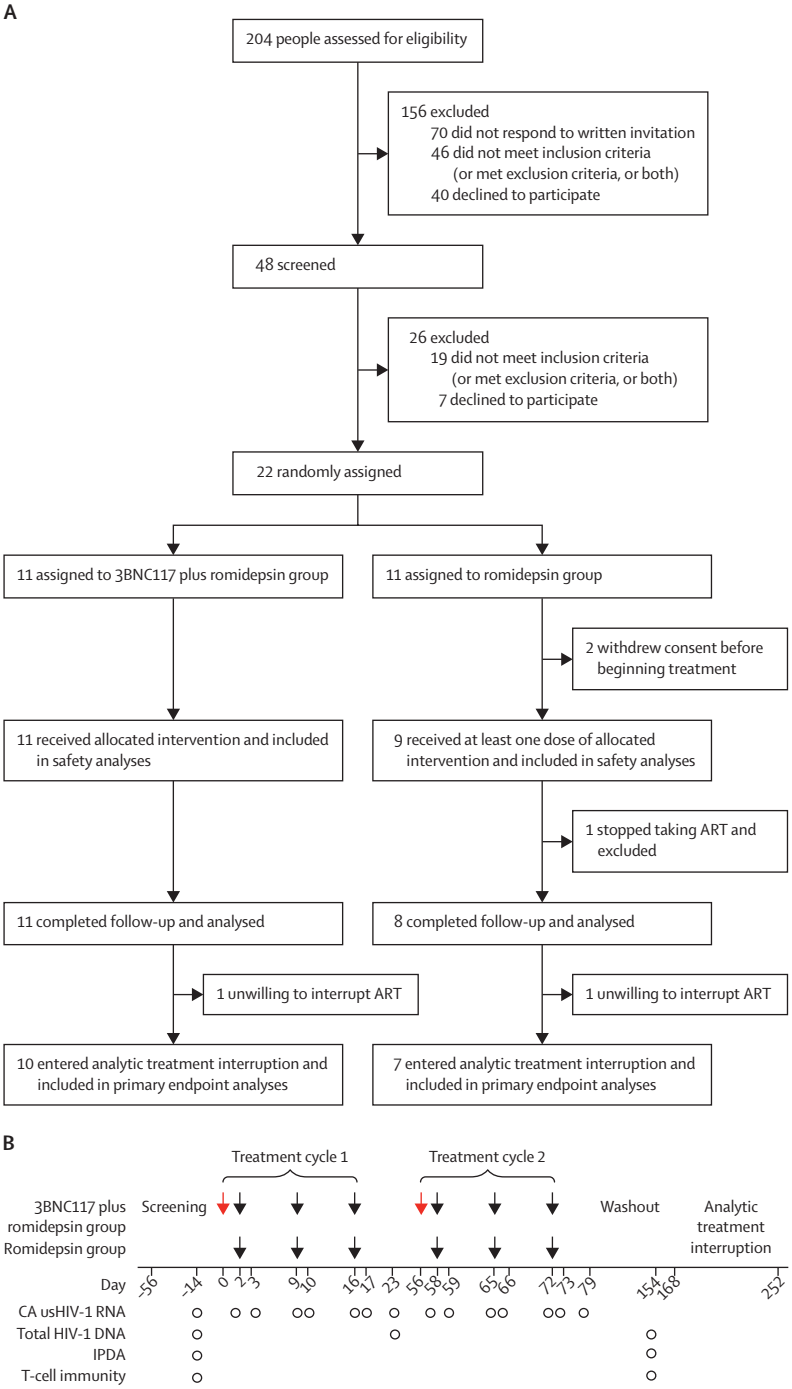


Figure 1: **Trial profile (A) and ROADMAP study design (B)**
Red arrows in (B) show when 3BNC117 was administered, whereas black arrows show when romidepsin was given; circles show assessment timepoints. ART=antiretroviral therapy. CA usHIV-1 RNA=cell-associated unspliced HIV-1 RNA. IPDA=intact proviral DNA assay.

	3BNC117 plus romidepsin group (n=11)	Romidepsin group (n=9)
Age, years	40 (33–51)	51 (38–62)
Sex		
Female	2 (18%)	1 (11%)
Male	9 (82%)	8 (89%)
Ethnicity		
African American	2 (18%)	3 (33%)
Caucasian	8 (73%)	6 (67%)
Hispanic	1 (9%)	0
Time since HIV-1 diagnosis, years	6 (2–11)	16 (6–31)
Time from HIV-1 diagnosis to ART initiation, years	0 (–10)	0 (–2)
Time on ART, years	5 (2–11)	10 (6–21)
HIV-1 subtype		
A	1 (9%)	1 (11%)
B	9 (82%)	5 (56%)
Not available	1 (9%)	3 (33%)
CD4 T cells per μL*	716 (450–1090)	600 (450–1150)
Cell-associated unspliced HIV-1 RNA*†	9 (1–111)	11 (5–84)
Total HIV-1 DNA*†	604 (57–3281)	686 (75–2071)
Intact proviral HIV-1 DNA*†	58 (27–482)	46 (0–270)

Data are n (%) or median (range). ART=antiretroviral therapy. *Analyses done at first leukapheresis timepoint (day –14). †Per million CD4 T cells.

Table 1: **Baseline characteristics**

Outcomes

The primary outcome was the time to viral rebound during ATI, which was defined as the time from treatment interruption to the first of two consecutive measurements of plasma HIV-1 RNA concentrations of at least 200 copies per mL. Secondary outcomes were safety, the size of the HIV-1 reservoir based on total HIV-1 DNA concentrations and intact proviral DNA concentrations per million unfractionated CD4 T cells, and plasma HIV-1 RNA concentrations. HIV-1 transcriptional activity was an exploratory outcome that was prespecified in the trial protocol. Additional analyses included assessments of viral 3BNC117 sensitivity and the determination of serum 3BNC117 concentrations.

Statistical analysis

The sample size calculation was based on the primary endpoint. Assuming an SD of 10 days, we calculated that a sample size of 12 individuals in each of the two groups would have 80% power to detect at least 13 days' difference in time to viral rebound between the two groups at a 5% significance level. A futility rule was implemented so that if ten participants in a single group had shown viral rebound within 2 weeks of initiating ATI, no additional participants from the affected group would have undergone ATI. To accommodate for potential dropouts, we aimed to enrol 30 participants (15 in each group), but because of slow enrolment, the study was terminated early (see Results).

The primary efficacy analysis population (of time to viral rebound) included all participants who completed

both treatment cycles and ATI. The safety analysis population included all participants who received any study drug. Reservoir and immunological assessments were done in all participants who completed both treatment cycles. Serum 3BNC117 concentrations were measured in all individuals who received 3BNC117 and antibody sensitivity assessments were attempted in all 3BNC117 recipients.

We used the log-rank test to compare time to viral rebound during ATI between the two treatment groups. The Kaplan-Meier estimator was used in a secondary analysis to assess the magnitude of the difference between the study groups. The Wilcoxon-Mann-Whitney or paired *t* test were used to analyse secondary outcomes. A two-sided α of less than 0·05 was considered significant. Statistical analyses were done in Stata (version 16.0) and Prism (version 7.0). The trial is registered at ClinicalTrials.gov, NCT02850016.

Role of the funding source

amfAR reviewed the study design and outcomes, but had no role in data collection, data analysis, data interpretation, or writing of the report. The other funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. Celgene (now Bristol Myers Squibb) provided romidepsin free of charge.

Results

22 individuals were enrolled in the study between March 20, 2017, and Aug 14, 2018. The final follow-up visit occurred on July 22, 2019. Enrolment was terminated early in February, 2019, because of difficulties in recruitment, with many potential participants citing the demanding visit schedule as the main reason to decline entering the study. Of the 22 enrolled participants, 11 were randomly assigned to each group. Two people assigned to the romidepsin group withdrew from the trial before any study drug administration (one because of scheduling problems, and one because of moving out of the study area; figure 1A; appendix pp 14–16). Thus, 20 participants remained in the trial and received study treatment, 11 in the 3BNC117 plus romidepsin group and nine in the romidepsin group (figure 1A). One participant (02-12-B)

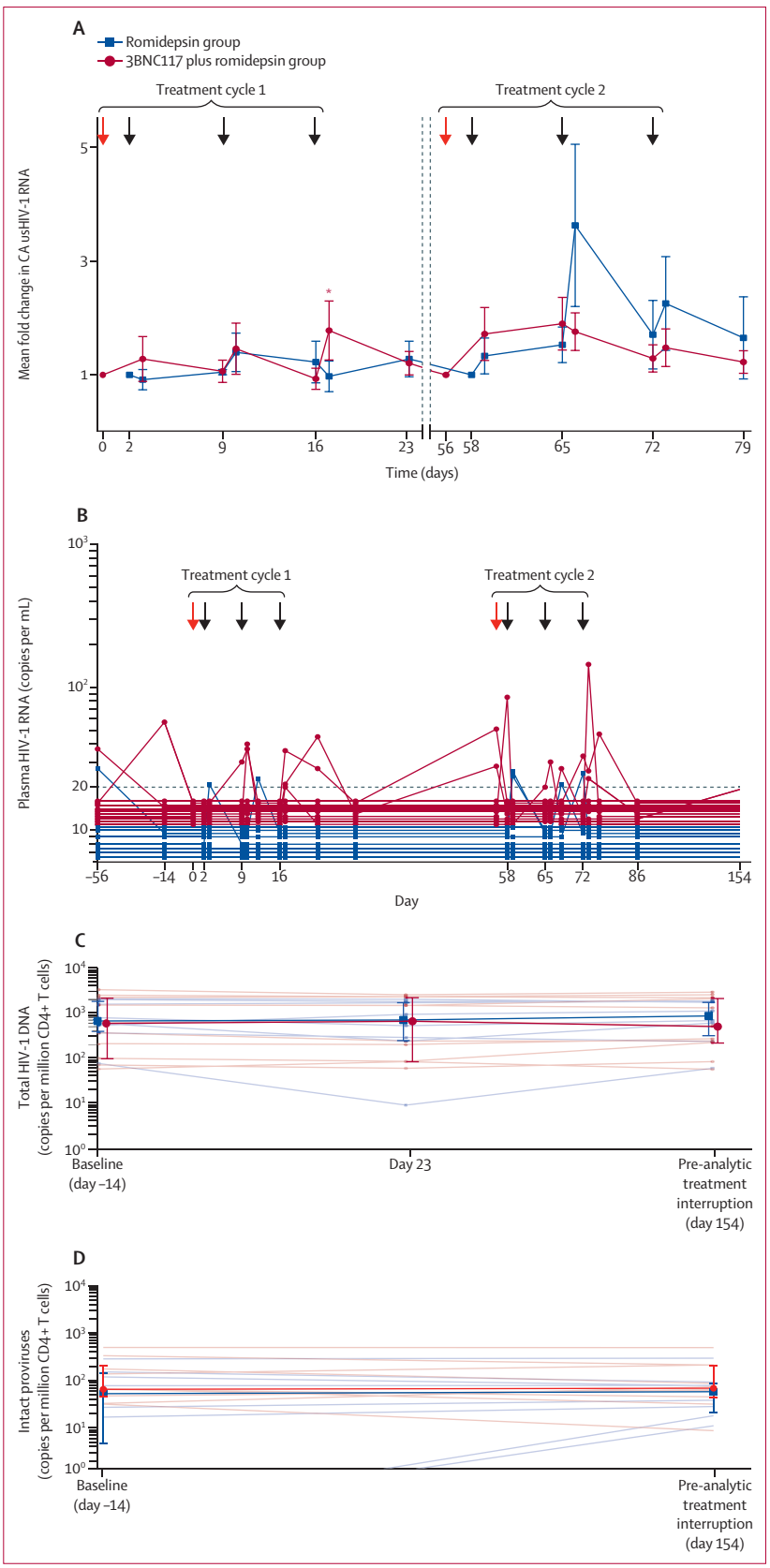
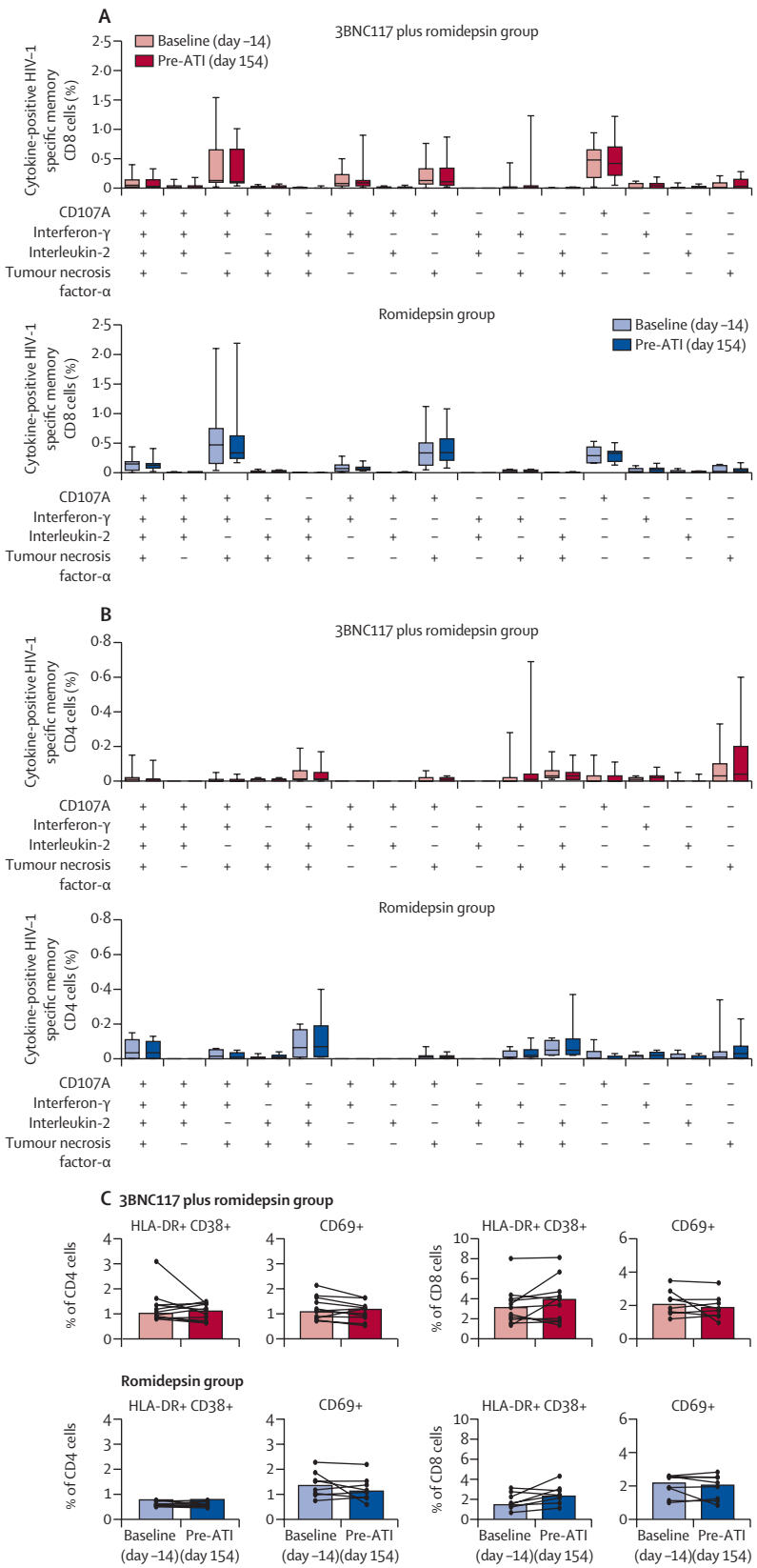


Figure 2: **Change in HIV-1 transcription, plasma HIV-1 RNA, total HIV-1 DNA, and intact proviruses**

(A) Mean fold change in CA usHIV-1 RNA concentrations from baseline. Red arrows show 3BNC117 administration, whereas black arrows show romidepsin administration. Error bars represent the standard error of the mean. (B) Individual plasma HIV-1 RNA concentrations. The dotted line represents the limit of quantification (20 copies per mL). Red arrows show 3BNC117 administration, whereas black arrows show romidepsin administration. (C) Individual and median concentrations of total HIV-1 DNA. Error bars represent IQRs. (D) Individual and median concentrations of intact proviruses. Two individuals in the romidepsin group had no intact proviruses per million CD4 T cells at baseline. Error bars represent IQRs. CA usHIV-1 RNA=cell-associated unspliced HIV-1 RNA. *p <0·05.



in the romidepsin group erroneously stopped ART at the start of the first treatment cycle and was prematurely discontinued from the trial and excluded from efficacy analyses. The remaining 19 participants completed both treatment cycles (11 assigned to the 3BNC117 plus romidepsin group and eight assigned to the romidepsin group). One participant in each group opted out of the subsequent ATI for personal reasons, and therefore 17 participants underwent ATI (ten in the 3BNC117 plus romidepsin group and seven in the romidepsin group).

Demographic characteristics were similar in the two groups, although participants in the romidepsin group had a higher median age (table 1). HIV-1 reservoir size and transcription were also similar between the two groups, although the median time since diagnosis and the median duration of ART were longer in the romidepsin group than in the 3BNC117 plus romidepsin group (table 1). Most participants had started ART in the calendar year of HIV-1 diagnosis (eight in the 3BNC117 plus romidepsin group and five in the romidempsin group; appendix p 9), but we did not select for individuals who had initiated ART during primary HIV-1 infection and the timepoint of infection was generally not known.

Individual romidepsin infusions resulted in modest and diverse effects on concentrations of CD4 T CA usHIV-1 RNA 1 day post-infusion compared with pre-infusion concentrations (appendix p 10). The median fold-change in CA usHIV-1 RNA concentrations after romidepsin administration across all infusions was 1·14 (IQR 0·71–1·95; p=0·0029) and was similar in both groups (appendix p 17). During the first treatment cycle, CA usHIV-1 RNA concentrations increased significantly compared with baseline concentrations after the third romidepsin infusion in the 3BNC117 plus romidepsin group (p=0·039; figure 2A). Four participants in each group had quantifiable plasma HIV-1 RNA (range 20–144 copies per mL) during one or both treatment cycles (appendix p 11). Collectively, 16 participants (nine in the 3BNC117 plus romidepsin group and seven in the romidepsin group) had detectable but not quantifiable plasma HIV-1 RNA during the treatment cycles.

We noted no significant changes in the concentrations of HIV-1 DNA from baseline to midway between the treatment cycles, or to the pre-ATI timepoint after completion of both treatment cycles in either group (figure 2C). Median concentrations of intact proviral

Figure 3: **Changes in T-cell immunity**
(A) Individual box-and-whisker plot of the proportion of polyfunctional HIV-1-specific memory CD8 T cells. The line represents the median, the box shows the IQR, and the whiskers show the minimum and maximum values. (B) Individual box-and-whisker plot of the proportion of polyfunctional HIV-1-specific memory CD4 T cells. The line represents the median, the box shows the IQR, and the whiskers show the minimum and maximum values. (C) Individual and median expression of T-cell activation markers. Bars show the median. ATI=analytic treatment interruption.

HIV-1 DNA did not change significantly from baseline to before the ATI in either group; the between group difference was also not significant (figure 2D). Thus, neither romidepsin alone nor in combination with 3BNC117 affected the size of the HIV-1 reservoir.

No significant changes in HIV-1-specific CD4 and CD8 T-cell responses were noted from baseline to the pre-ATI timepoint in either group (figure 3A, 3B). Additionally, the expression of activation (figure 3C; appendix pp 18–19) and exhaustion (appendix p 20) markers on CD4 and CD8 T cells remained unchanged after the two treatment cycles in both groups.

Serum 3BNC117 concentrations had dropped to sub-therapeutic concentrations (mean concentration 1·3 µg/mL [range <0·2–5·2]) by the time of ATI initiation in all individuals who received the antibody (appendix p 12). Viral rebound occurred by day 35 of the ATI in 16 of 17 individuals (figure 4A); one participant in the romidepsin group (02-11-B) had fewer than 200 copies of HIV-1 RNA per mL until day 84 (appendix p 11). The median time to viral rebound was 18 days (IQR 14–28) in the 3BNC117 plus romidepsin group compared with 28 days (21–35) in the romidepsin group (p=0·016; figure 4B). However, this difference of 10 days in time to viral rebound was significant but not clinically meaningful. All participants achieved viral re-suppression upon re-initiation of ART (median 28 days [IQR 14–42]). The appendix (p 21) shows data for the relation between time to viral rebound and HIV-1 reservoir size at different timepoints. The participant who maintained low plasma HIV-1 RNA concentrations until day 84 had a small HIV-1 reservoir before the ATI (59 HIV-1 DNA copies and nine intact proviruses per million CD4 T cells; appendix p 19), although other participants who rebounded earlier had similar reservoir sizes.

We obtained 317 proviral *env* sequences from seven of the 11 individuals in the 3BNC117 plus romidepsin group (no proviral *env* DNA could be amplified from the remaining four individuals despite our using two different primer sets; appendix p 13). 15 (5%) of these 317 sequences were predicted to be associated with resistance to 3BNC117 (appendix p 13). The 15 sequences were obtained from two individuals (three of the 27 sequences taken from one participant, and 12 of the 38 sequences obtained from a second participant, were predicted to be resistant). The reservoir of one of these participants (02-15-A) was assessed as 3BNC117-resistant, although single genome amplification-derived pseudoviruses obtained from plasma after viral rebound remained moderately sensitive to 3BNC117 (appendix p 13). Overall, 28 single genome amplification-derived rebound viruses were obtained from six participants who received 3BNC117. All 28 showed moderate-to-high sensitivity to 3BNC117 (appendix p 13).

Both treatment cycles were completed without interruption by all participants who began the study treatments, (except for participant 02-12-B, who was excluded from the study after erroneous early ART interruption).

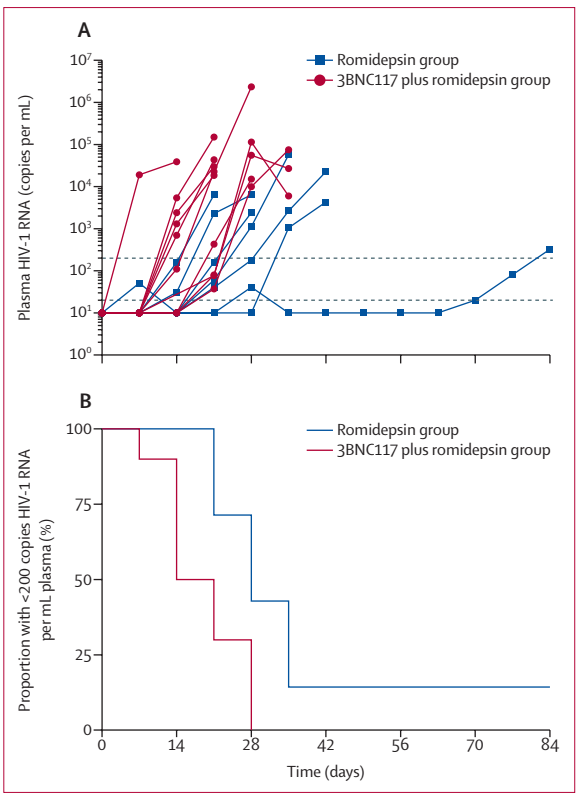


Figure 4: **Time to viral rebound during analytic treatment interruption**
(A) Individual plasma HIV-1 RNA concentration after interruption of antiretroviral therapy. The dotted lines represent the upper (200 copies per mL) and lower (20 copies per mL) limits of quantification. (B) Proportion of individuals with fewer than 200 copies of HIV-1 RNA per mL of plasma after interruption of antiretroviral therapy.

Adverse events were reported by all participants in both groups (table 2). Most adverse events were mild to moderate and similar between the groups. Overall, 267 adverse events were reported: 205 grade 1 adverse events, 60 grade 2 adverse events, and two grade 3 adverse events. 106 of the adverse events were solicited and 156 were unsolicited, including 39 drug-related adverse events. 159 adverse events were considered at least possibly related to study medications (appendix pp 14–15). Two severe adverse events were reported in the romidepsin group (concussion after a traffic incident that required hospital admission, which was judged unrelated to study medication, and increased direct bilirubin, which was judged related to romidepsin; appendix pp 14–16). Both of these events resolved without intervention. The most common solicited adverse events related to romidepsin were nausea, headache, chills, and vomiting (table 2). Romidepsin infusions were associated with more drug-related adverse events than 3BNC117 infusions (table 2). One participant in the 3BNC117 plus romidepsin group and two in the romidepsin group experienced transient corrected QT interval prolongations (>450 ms) 1 day after romidepsin infusions, and seven participants (nine infusions; two patients had reactions after two

	3BNC117 plus romidepsin group (n=11)					Romidepsin group (n=9)				
	Participants	Events (overall)	Mild events	Moderate events	Severe events	Participants	Events (overall)	Mild events	Moderate events	Severe events
Related to romidepsin										
Nausea*	11 (100%)	29	21	8	0	5 (56%)	9	8	1	0
Headache*	4 (36%)	13	10	3	0	3 (33%)	10	10	0	0
Fatigue	5 (45%)	12	12	0	0	4 (44%)	4	4	0	0
Chills*	3 (27%)	8	7	1	0	22 (22%)	2	2	0	0
Vomiting*	4* (36%)	8	5	3	0	22 (22%)	2	1	1	0
Decreased phosphorous	1 (9%)	5	0	5	0	22 (22%)	5	4	1	0
Malaise*	3 (27%)	5	3	2	0	22 (22%)	6	6	0	0
Arthralgia*	3 (27%)	4	3	1	0	1 (11%)	1	1	0	0
Constipation	1 (9%)	3	1	2	0	0	0	0	0	0
Increased creatinine	2 (18%)	3	3	0	0	0	0	0	0	0
Xerostomia	3 (27%)	3	3	0	0	1 (11%)	1	1	0	0
Heartburn	2 (18%)	2	1	1	0	1 (11%)	1	1	0	0
Myalgia*	1 (9%)	2	2	0	0	1 (11%)	3	3	0	0
Neutropenia	2 (18%)	2	1	1	0	0	0	0	0	0
Thrombocytopenia	2 (18%)	2	0	2	0	22 (22%)	2	2	0	0
Abdominal discomfort	1 (9%)	1	1	0	0	0	0	0	0	0
Borborygmi	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Change in body odour	1 (9%)	1	1	0	0	0	0	0	0	0
Diarrhoea*	1 (9%)	1	1	0	0	0	0	0	0	0
Dizziness	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Hyperhidrosis	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Increased lacrimation	1 (9%)	1	1	0	0	0	0	0	0	0
Leukopenia	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Prolongation of corrected QT interval	1 (9%)	1	1	0	0	22 (22%)	2	2	0	0
Secondary ammenorhoea	1 (9%)	1	1	0	0	0	0	0	0	0
Blurred vision	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Decreased appetite	0	0	0	0	0	1 (11%)	2	2	0	0
Ageusia	0	0	0	0	0	1 (11%)	1	1	0	0
Circulatory insufficiency	0	0	0	0	0	1 (11%)	1	0	1	0
Cold intolerance	0	0	0	0	0	1 (11%)	1	1	0	0
Conjunctival erythema*	0	0	0	0	0	1 (11%)	1	1	0	0
Dysesthesia	0	0	0	0	0	1 (11%)	1	1	0	0
Dyspepsia	0	0	0	0	0	1 (11%)	1	1	0	0
Dry skin	0	0	0	0	0	1 (11%)	1	1	0	0
Eosinophilia	0	0	0	0	0	1 (11%)	1	1	0	0
Decreased libido	0	0	0	0	0	1 (11%)	1	1	0	0
Haematoma	0	0	0	0	0	1 (11%)	1	1	0	0
Increased direct bilirubin	0	0	0	0	0	1 (11%)	1	0	0	1
Related to 3BNC117										
Change in body odour	1 (9%)	1	1	0	0	..	..	..	..	..
Decreased phosphorous	1 (9%)	1	0	1	0	..	..	..	..	..
Heartburn	1 (9%)	1	1	0	0	..	..	..	..	..
Xerostomia	1 (9%)	1	1	0	0	..	..	..	..	..

(Table 2 continues on next page)

separate infusions) had an increased corrected QT interval of more than 10 ms post-infusion compared with their pre-infusion ECG, but none of these changes were associated with clinical symptoms. No other clinically significant ECG changes were noted among the other 107 romidepsin infusions. Additionally, CD4 T-cell counts were unaffected between and after the treatment cycles in both groups (appendix p 22).

	3BNC117 plus romidepsin group (n=11)					Romidepsin group (n=9)				
	Participants	Events (overall)	Mild events	Moderate events	Severe events	Participants	Events (overall)	Mild events	Moderate events	Severe events
(Continued from previous page)										
Unrelated										
Upper respiratory tract infection (including common cold)	4 (36%)	11	4	7	0	5 (56%)	5	4	1	0
Constipation	4 (36%)	5	2	3	0	1 (11%)	1	0	1	0
Headache	3 (27%)	4	4	0	0	1 (11%)	1	1	0	0
Decreased phosphorous	1 (9%)	3	0	3	0	1 (11%)	7	7	0	0
Epistaxis	1 (9%)	2	2	0	0	0	0	0	0	0
Herpes labialis	2 (18%)	2	2	0	0	0	0	0	0	0
Musculoskeletal pain	2 (18%)	2	2	0	0	0	0	0	0	0
Thrombocytopenia	2 (18%)	2	2	0	0	22 (22%)	5	5	0	0
Myalgia*	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Accommodation disorder	1 (9%)	1	1	0	0	0	0	0	0	0
Acute stress disorder	1 (9%)	1	1	0	0	0	0	0	0	0
Back pain	1 (9%)	1	0	1	0	0	0	0	0	0
Increased diastolic blood pressure	1 (9%)	1	1	0	0	0	0	0	0	0
Chills*	1 (9%)	1	1	0	0	0	0	0	0	0
Increased creatinine	1 (9%)	1	1	0	0	1 (11%)	1	1	0	0
Cystitis	1 (9%)	1	1	0	0	0	0	0	0	0
Diarrhoea	1 (9%)	1	0	1	0	0	0	0	0	0
Fatigue	1 (9%)	1	1	0	0	0	0	0	0	0
Feverishness	1 (9%)	1	1	0	0	0	0	0	0	0
Fractured nose	1 (9%)	1	1	0	0	0	0	0	0	0
Gonorrhoea	1 (9%)	1	0	1	0	0	0	0	0	0
Hordeolum	1 (9%)	1	0	1	0	0	0	0	0	0
Hot flush	1 (9%)	1	1	0	0	0	0	0	0	0
Hypotension	1 (9%)	1	0	1	0	0	0	0	0	0
Lumboischialgia	1 (9%)	1	0	1	0	0	0	0	0	0
Mastodynia	1 (9%)	1	1	0	0	0	0	0	0	0
Rash	1 (9%)	1	1	0	0	0	0	0	0	0
Skin injury	1 (9%)	1	0	1	0	0	0	0	0	0
Urethritis	1 (9%)	1	0	1	0	0	0	0	0	0
Vomiting	1 (9%)	1	1	0	0	0	0	0	0	0
Decreased potassium	0	0	0	0	0	1 (11%)	2	2	0	0
Arthralgia*	0	0	0	0	0	1 (11%)	1	1	0	0
Concussion	0	0	0	0	0	1 (11%)	1	0	0	1
Epileptic seizure	0	0	0	0	0	1 (11%)	1	0	1	0
Haematuria	0	0	0	0	0	1 (11%)	1	1	0	0
Hoarseness	0	0	0	0	0	1 (11%)	1	1	0	0
Leg cramps	0	0	0	0	0	1 (11%)	1	1	0	0
Palpitations	0	0	0	0	0	1 (11%)	1	1	0	0
Stomach ache	0	0	0	0	0	1 (11%)	1	0	1	0
Toothache	0	0	0	0	0	1 (11%)	1	0	1	0

Data are n for number of events, and n (%) for the number of affected participants. Events are classed as related if they were considered to be possibly, probably, or definitely related to treatment by the investigators. *Solicited adverse event.

Table 2: **Adverse events**

Discussion

In this phase 2A randomised trial, neither the combination of a potent bNAb, 3BNC117, with the latency-reversing agent romidepsin nor romidepsin alone meaningfully reduced the HIV-1 reservoir in individuals on long-term suppressive ART. The most important clinical outcome

measure in HIV-1 cure trials is time to viral rebound after stopping ART.[24] Time to viral rebound during ATI was significantly shorter in the 3BNC117 plus romidepsin group than in the romidepsin group, but this effect was not clinically meaningful. The safety of each intervention was similar to that reported in previous studies.

The goal of administering 3BNC117 before latency reversal was to enhance killing of reactivated latently infected cells (eg, through antibody-dependent cellular cytotoxicity). Although a 2020 study[19] did not detect increases in HIV-1 transcription after romidepsin infusions in people on ART, increased concentrations of CD4 CA usHIV-1 RNA, peaking within hours of romidepsin infusion, have previously been reported in several trials of participants on suppressive ART.[5,8,18] Despite multiple romidepsin infusions, we noted only modest latency reversal and inconsistent blips in plasma HIV-1 RNA concentrations in a subset of participants in both groups. However, we collected blood samples 1 day after romidepsin infusions and might therefore have missed early post-infusion changes in HIV-1 transcriptional activity.

Neither romidepsin alone nor in combination with 3BNC117 led to a significant reduction in the size of the HIV-1 reservoir. Furthermore, we observed no clinically meaningful delay in viral rebound during ATI in either of the groups. In fact, by contrast with our hypothesis, the median time to viral rebound was longer in individuals receiving romidepsin alone than in those receiving the combination of 3BNC117 plus romidepsin. Notably, the romidepsin group had been diagnosed with HIV-1 infection for a longer time than the 3BNC117 plus romidepsin group, but the size of the HIV-1 reservoir and HIV-1 transcriptional activity were similar in the two cohorts. An insufficient level of antigen expression on reactivated infected cells following latency reversal could have limited 3BNC117 binding and immune-mediated elimination of these cells during suppressive ART. Less-than-desirable latency reversal probably contributed to the lack of reduction in the latent reservoir size and time to viral rebound that has been reported in clinical trials[8,18,25] investigating combinations of latency-reversing agents and therapeutic vaccines aimed at enhancing autologous antiviral immunity. Furthermore, reactivated latently infected cells might be relatively resistant to elimination.[26]

Because accurate quantification of the replication-competent viral reservoir in humans is notoriously difficult and virological control in the absence of ART is hard to predict, the effect of HIV-1 cure interventions should ideally be measured by assessing HIV-1 control in the absence of ART during an ATI.[24] The criterion for ART resumption that we used was two consecutive plasma HIV-1 RNA measurements of at least 200 copies per mL, which might have been too strict a threshold to observe post-treatment control of HIV-1. In other studies,[1,24] peak viral loads of more than 100 000 copies per mL were reported in some post-treatment controllers before immunological control of viral replication was regained. CD8 T-cell immunity against HIV-1 seems crucial for achieving HIV-1 remission, but we recorded no enhancement of HIV-1-specific cellular immunity following the treatment cycles.[11,27,28] Importantly, romidepsin did not seem to have detrimental effects on T cell HIV-1-specific immunity in our study, which accords with the findings of previous studies.[5,9,29] In previous studies,[5,9,29] romidepsin infusions temporarily affected activation and exhaustion markers on T cells during the interventional period, but in our study the expression of these markers was similar before the ATI to baseline concentrations. Furthermore, another study suggested that histone deacetylase inhibitors do not inhibit natural killer cell function.[30]

Sensitivity of archived proviruses to bNAbs seems crucial to the success of these monoclonal antibodies. In a phase 1B study,[31] three infusions of a combination of two potent bNAbs (3BNC117 and 10-1074; both at a dose of 30 mg/kg) during an ATI (which began 2 days after the first infusion) led to durable HIV-1 control in the absence of ART among individuals with sensitive archived proviruses, whereas in individuals with bNAbs-resistant reservoirs time to viral rebound was not prolonged. Pre-screening for bNAbs-sensitivity remains a challenge and was not feasible at the start of this study. However, post-hoc reservoir sequencing analyses of baseline sensitivity and neutralisation assays at rebound suggested moderate antibody sensitivity in the 3BNC117 plus romidepsin group.

The lack of effect on the HIV-1 reservoir in either group in our trial might also be partly due to immune exhaustion among participants, all of whom were on suppressive ART for at least 5 years. As a result of persistent exposure to viral antigen during HIV-1 infection, expression of co-inhibitory molecules such as programmed death 1 (PD-1) might be high on cells such as CD8 T cells, which reduces their ability to eliminate infected cells. PD-1 expression on T cells is only partly restored by ART, and thus CD8 T-cell function is generally expected to remain impaired despite ART.

To our knowledge, ours is the first randomised study to assess the combination of a potent bNAb and a latency-reversing agent designed to target the HIV-1 reservoir. However, our study has some limitations. The study size was substantially smaller than planned due to difficulties in recruitment, which reduced statistical power to find differences between the two groups in terms of immunological and virological parameters. Additionally, we did not include a placebo group, which would have enabled us to distinguish the effect of the individual aspects of the interventions. Furthermore, the study might not be generalisable to all HIV-1-infected individuals because of our stringent inclusion and exclusion criteria.

On the basis of the findings of this study and other trials of the shock-and-kill approach, we conclude that

latency reversal with a single histone deacetylase inhibitor and modulation of autologous HIV-1-specific immunity by a single bNAb is not sufficient to achieve HIV-1 remission in people on long-term suppressive ART. There are several potential explanations for the lack of effect on the HIV-1 reservoir. Romidepsin might be of insufficient potency (but dose escalation is not an option due to toxicity),[32] and so-called anatomical reservoir sanctuaries—eg, the CNS—are poorly penetrated by histone deacetylase inhibitors. Alternative HIV-1 cure strategies based on overall similar concepts are under investigation. For example, some HIV-1 curative strategies are moving towards intervening immediately before or during an ATI, or both, rather than during suppressive ART, which could allow for controlled release of antigen from the HIV-1 reservoir and enhanced immunological responses. Other study groups are selecting study participants who initiated ART during primary infection, or trying to intervene at the time of ART initiation instead of years later.[33] These approaches are a departure from the classic shock-and-kill approach. Instead of using latency-reversing agents that seem to have limited capacity to induce HIV-1 transcription, evidence suggests that the use of immunomodulators, such as toll-like receptor agonists or interleukin-15 super-agonists, in combination with bNAbs can lead to sustained viral control in simian–human immunodeficiency virus-infected non-human primates.[15] Outcomes from such combinations in people infected with HIV-1 are greatly anticipated. The results of our trial could serve as a benchmark for further optimisation of HIV-1 curative strategies among people with HIV-1 on suppressive ART.

Contributors

JDG, YZC, MT, JCCL, LØ, HN, MCN, GF, FK, and OSS developed the trial design. HG, JDG, YZC, JJM, MP, KGM, TK, IS, CU-O'B, HN, CL, GF, MC, and OSS did the clinical visits. HG, YZC, MHP, MT, WDCA, JCCL, LN, and RO did the laboratory assays. HG, JDG, YZC, RBJ, WDCA, TYO, FK, MC, and OSS, analysed the data. HG, JDG, YZC, and OSS drafted the Article, which all authors critically revised for important intellectual content. JDG and OSS had full access to all the data in the study, verified the data, and had final responsibility for the decision to submit for publication.

Declaration of interests

HG and FK are listed as inventors on a patent application for HIV-1 neutralising antibodies filed by the University of Cologne. MCN is listed as an inventor on patents for the antibody 3BNC117. All other authors declare no competing interests.

Data sharing

Data are not available for download due to privacy and ethical restrictions. Specific requests for access to the trial data can be sent to the corresponding author and access might be provided to a named individual in agreement with the rules and regulations of the national laws.

Acknowledgments

This study was funded by amfAR, The Foundation for AIDS Research, and the German Center for Infection Research. We thank all study participants, members of the Nussenzweig, Klein, and Søgaard groups for support and discussion; the clinical study teams for trial conduct, the Clinical Trials Center Cologne (ZKS Köln) for help with data management and monitoring, the Institute of Medical Statistics, Informatics, and Epidemiology Cologne for support with randomisation, and Bristol Myers Squibb for providing romidepsin.

References

1 Namazi G, Fajnzylber JM, Aga E, et al. The control of HIV after antiretroviral medication pause (CHAMP) study: post-treatment controllers identified from 14 clinical studies. *J Infect Dis* 2018; **218:** 1954–63.
2 Deeks SG. HIV: shock and kill. *Nature* 2012; **487:** 439–40.
3 Archin NM, Liberty AL, Kashuba AD, et al. Administration of vorinostat disrupts HIV-1 latency in patients on antiretroviral therapy. *Nature* 2012; **487:** 482–85.
4 Rasmussen TA, Tolstrup M, Brinkmann CR, et al. Panobinostat, a histone deacetylase inhibitor, for latent-virus reactivation in HIV-infected patients on suppressive antiretroviral therapy: a phase 1/2, single group, clinical trial. *Lancet HIV* 2014; **1:** e13–21.
5 Søgaard OS, Graversen ME, Leth S, et al. The depsipeptide romidepsin reverses HIV-1 latency in vivo. *PLoS Pathog* 2015; **11:** e1005142.
6 Elliott JH, McMahon JH, Chang CC, et al. Short-term administration of disulfiram for reversal of latent HIV infection: a phase 2 dose-escalation study. *Lancet HIV* 2015; **2:** e520–29.
7 Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. *Immunity* 2012; **36:** 491–501.
8 Leth S, Schleimann MH, Nissen SK, et al. Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial. *Lancet HIV* 2016; **3:** e463–72.
9 Tapia G, Højen JF, Ökvist M, et al. Sequential Vacc-4x and romidepsin during combination antiretroviral therapy (cART): immune responses to Vacc-4x regions on p24 and changes in HIV reservoirs. *J Infect* 2017; **75:** 555–71.
10 Lu C-L, Murakowski DK, Bournazos S, et al. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science* 2016; **352:** 1001–04.
11 Niessl J, Baxter AE, Mendoza P, et al. Combination anti-HIV-1 antibody therapy is associated with increased virus-specific T cell immunity. *Nat Med* 2020; **26:** 222–27.
12 Halper-Stromberg A, Lu C-LL, Klein F, et al. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. *Cell* 2014; **158:** 989–99.
13 Caskey M, Klein F, Nussenzweig MC. Broadly neutralizing anti-HIV-1 monoclonal antibodies in the clinic. *Nat Med* 2019; **25:** 547–53.
14 Bournazos S, Klein F, Pietzsch J, Seaman MS, Nussenzweig MC, Ravetch J V. Broadly neutralizing anti-HIV-1 antibodies require Fc effector functions for in vivo activity. *Cell* 2014; **158:** 1243–53.
15 Borducchi EN, Liu J, Nkolola JP, et al. Antibody and TLR7 agonist delay viral rebound in SHIV-infected monkeys. *Nature* 2018; **563:** 360–64.
16 Caskey M, Klein F, Lorenzi JCC, et al. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature* 2015; **522:** 487–91.
17 Scheid JF, Horwitz JA, Bar-On Y, et al. HIV-1 antibody 3BNC117 suppresses viral rebound in humans during treatment interruption. *Nature* 2016; **535:** 556–60.
18 Mothe B, Rosás-Umbert M, Coll P, et al. HIVconsv vaccines and romidepsin in early-treated HIV-1-infected individuals: safety, immunogenicity and effect on the viral reservoir (study BCN02). *Front Immunol* 2020; **11:** 823.
19 McMahon DK, Zheng L, Cyktor JC, et al. A phase I/II randomized, placebo-controlled trial of romidepsin in persons with HIV-1 on suppressive antiretroviral therapy to assess safety and activation of HIV-1 expression (A5315). *J Infect Dis* 2020; published online Dec 22. https://doi.org/10.1093/infdis/jiaa777.
20 Schulz KF, Altman DG, Moher D. CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *Trials.* 2010; **11:** 32.
21 Cohen YZ, Butler AL, Millard K, et al. Safety, pharmacokinetics, and immunogenicity of the combination of the broadly neutralizing anti-HIV-1 antibodies 3BNC117 and 10-1074 in healthy adults: a randomized, phase 1 study. *PLoS One* 2019; **14:** e0219142.
22 Bruner KM, Wang Z, Simonetti FR, et al. A quantitative approach for measuring the reservoir of latent HIV-1 proviruses. *Nature* 2019; **566:** 120–25.

23 Kinloch NN, Ren Y, Conce Alberto WD, et al. HIV-1 diversity considerations in the application of the Intact Proviral DNA Assay (IPDA). *Nat Commun* 2021; **12:** 165.

24 Julg B, Dee L, Ananworanich J, et al. Recommendations for analytical antiretroviral treatment interruptions in HIV research trials—report of a consensus meeting. *Lancet HIV* 2019; **6:** e259–68.

25 Fidler S, Stöhr W, Pace M, et al. Antiretroviral therapy alone versus antiretroviral therapy with a kick and kill approach, on measures of the HIV reservoir in participants with recent HIV infection (the RIVER trial): a phase 2, randomised trial. *Lancet* 2020; **395:** 888–98.

26 Ren Y, Huang SH, Patel S, et al. BCL-2 antagonism sensitizes cytotoxic T cell-resistant HIV reservoirs to elimination ex vivo. *J Clin Invest* 2020; **130:** 2542–59.

27 Nishimura Y, Gautam R, Chun T, et al. Early antibody therapy can induce long-lasting immunity to SHIV. *Nature* 2017; **543:** 559–63.

28 Lim S-Y, Osuna CE, Hraber PT, et al. TLR7 agonists induce transient viremia and reduce the viral reservoir in SIV-infected rhesus macaques on antiretroviral therapy. *Sci Transl Med* 2018; **10:** eaao4521.

29 Rosás-Umbert M, Ruiz-Riol M, Fernández MA, et al. In vivo effects of romidepsin on T-cell activation, apoptosis and function in the BCN02 HIV-1 Kick&Kill clinical trial. *Front Immunol* 2020; **11:** 418.

30 Garrido C, Tolstrup M, Søgaard OS, et al. In-vivo administration of histone deacetylase inhibitors does not impair natural killer cell function in HIV+ individuals. *AIDS* 2019; **33:** 605–13.

31 Mendoza P, Gruell H, Nogueira L, et al. Combination therapy with anti-HIV-1 antibodies maintains viral suppression. *Nature* 2018; **561:** 479–84.

32 Gunst JD, Tolstrup M, Rasmussen TA, Søgaard OS. The potential role for romidepsin as a component in early HIV-1 curative efforts. *Expert Rev Anti Infect Ther* 2016; **14:** 447–50.

33 Gunst JD, Tolstrup M, Søgaard OS. Beyond antiretroviral therapy: early interventions to control HIV-1 infection. *AIDS* 2017; **31:** 1665–67.