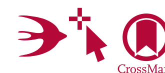
Combined effect of Vacc-4x, recombinant human granulocyte macrophage colony-stimulating factor vaccination, and romidepsin on the HIV-1 reservoir (REDUC): a single-arm, phase 1B/2A trial



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Summary

Background Immune priming before reversal of latency might be a component of a functional HIV cure. To assess this concept, we assessed if therapeutic HIV immunisation followed by latency reversal would affect measures of viral transcription, plasma viraemia, and reservoir size in patients with HIV on suppressive antiretroviral therapy.

Methods In this single-arm, phase 1B/2A trial, we recruited adults treated at the Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark (aged ≥18 years) with successfully treated HIV-1 with plasma RNA loads of less than 50 copies per mL for the previous year and CD4 counts of at least 500 cells per µL. Exclusion criteria included CD4 counts of less than 200 cells per µL within the past 2 years, active hepatitis B or C infections, and clinically significant cardiac disease, including QTc prolongation. Participants received six therapeutic intradermal HIV-1 immunisations with 12 mg/mL Vacc-4x and 0·6 mg/mL rhuGM-CSF over 12 weeks (at 0 weeks, 1 week, 2 weeks, 3 weeks, 11 weeks, and 12 weeks) before receiving 5 mg/m² intravenous romidepsin once a week for 3 weeks. This procedure was followed by analytical treatment interruption. Coprimary outcomes were changes in copies of HIV-1 DNA (total and integrated) per million CD4 T cells and infectious units per million (IUPM) resting memory CD4 T cells established by viral outgrowth, assessed in all patients receiving at least one dose of active treatment with assessable data. We assessed total HIV-1 DNA at screening, before romidepsin treatment, and 6 weeks after romidepsin treatment. We assessed integrated viral DNA at baseline, before romidepsin treatment, and 8 weeks after romidepsin treatment. We assessed IUPM at screening, 2 weeks before romidepsin treatment, and 6 weeks after romidepsin treatment. This trial is registered at ClinicalTrials.gov, number NCT02092116.

Findings Between May 19, 2014, and Oct 8, 2014, we enrolled 20 individuals, of whom 17 completed all Vacc-4x and rhuGM-CSF administrations and romidepsin infusions. 16 of 17 had assessable total HIV-1 DNA, 15 of 17 had assessable integrated HIV-1 DNA, and six of 17 had assessable IUPM at baseline and at one or more timepoints after study treatment. Total HIV-1 DNA declined from screening to 6 weeks after romidepsin treatment (mean reduction 39·7%, 95% CI –59·7 to –11·5; p=0·012). The decrease in integrated HIV-1 DNA from baseline to 8 weeks after romidepsin treatment was not significant (19·2%, –38·6 to 6·3; p=0·123). Among the six assessable participants, the mean reduction in IUPM from screening to 6 weeks after romidepsin treatment was 38·0% (95% CI –67·0 to –8·0; p=0·019). Of 141 adverse events, 134 (95%) were grade 1 and seven (5%) were grade 2–3.

Interpretation This in-vivo combinatorial approach provides the first evidence for the feasibility of a combined shock and kill strategy, but also emphasises that further optimisation of this strategy is needed to achieve a sizeable effect on the latent reservoir that will translate into clinically measurable benefits for people living with HIV-1.

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Introduction

Antiretroviral therapy (ART) leads to potent and durable suppression of HIV-1 replication, but does not cure infection. For this reason, lifelong treatment is needed to prevent virus rebound and disease progression. Furthermore, chronic HIV-1 infection and ART are associated with increased mortality and morbidity.[1] For these reasons, a curative therapy for HIV-1 remains an important goal.

Early in HIV-1 infection, a latent reservoir is established when activated CD4 T cells become infected before reverting into a resting memory state.[2,3] These long-lived cells containing proviral genomes persist in a latent transcriptionally silent state in which the proviruses remain non-susceptible to ART and unrecognisable to the immune system.[4,5] Although a large proportion of integrated HIV-1 DNA is defective, replication-competent proviruses persist in all individuals who receive ART and they drive rapid viral rebound after discontinuation of ART.[6–8] This latent reservoir is widely recognised as the primary barrier to eradication of infection.

Research in context

Evidence before this study

We searched PubMed and Scopus for articles published up to April 16, 2016, in any language, that reported clinical studies that aimed to reduce the size of the HIV reservoir through reversal of HIV latency with the search terms "HIV cure", "HIV reactivation", "HIV reservoir", "HIV latency", and "HIV eradication", and we reviewed the results for relevance. Investigators of human studies of the histone deacetylase inhibitor vorinostat and antialcoholic abuse agent disulfiram have successfully shown increased HIV-1 expression measured as increased cellular HIV-1 RNA levels in latently infected cells. Investigators of two clinical trials of the potent histone deacetylase inhibitor panobinostat and romidepsin, in addition to showing an increase in cellular HIV-1 expression, showed increased levels of viral HIV-1 particles in plasma during histone deacetylase inhibitor dosing with standard clinical assays. Investigators of none of these studies found a significant effect of the interventions on the size of the latent HIV-1 reservoir, measured as total DNA, integrated DNA, or quantitative viral outgrowth.

Added value of this study

We report the first clinical trial of a combinatorial approach in which latency reversal is preceded by immunotherapy for improved targeting of the HIV-1 reservoir. In this phase 1B/2A trial, therapeutic HIV-1 immunisation with Vacc-4x and rhuGM-CSF combined with the histone deacetylase inhibitor romidepsin resulted in about a 40% reduction in the size of the HIV-1 reservoir from baseline to follow-up, a statistically significant reduction of not only the proviral HIV-1 reservoir on a cohort level, but also the replication-competent HIV-1 reservoir in a subset of individuals. Additionally, despite marked increases in histone acetylation and cell-associated unspliced HIV-1 RNA shortly after each romidepsin infusion, only four of 17 individuals pretreated by immunisation with Vacc-4x and rhuGM-CSF had quantifiable plasma HIV-1 RNA immediately after one of the romidepsin infusions, raising the possibility that priming of the immune system before latency reversal induced an immune pressure on virus-producing cells leading to their killing and therefore limited extracellular release of viral particles during romidepsin treatment. However, although these findings indicate that the size of the viral reservoir was diminished, the combined so-called shock and kill intervention did not seem to prolong median time to virus rebound during ART interruption.

Implications of all the available evidence

A combined approach to a shock and kill strategy for HIV cure is feasible, but optimisation of this strategy is needed to achieve a sizeable effect on the latent reservoir that will translate into clinically measurable benefits for people living with HIV-1.

One strategy to cure HIV-1 aims to purge the reservoir by reactivating virus transcription with a latency-reversing agent, in turn allowing elimination of infected cells by viral cytopathic or immune effector mechanisms.[2] Findings from clinical trials[9–14] with latency-reversing agents show that viral reactivation is indeed possible. However, latency reversal has not produced measurable reductions in the size of the reservoir, possibly because of insufficient immune-mediated killing of infected cells (eg, inadequate CD8 cytotoxic T-lymphocyte or natural killer cell responses).[15] Hence, great interest exists in interventions to improve antiviral immune responses in combination with latency reversal; the ultimate goal is eradication of latently infected CD4 T cells to sustain long-term drug-free remission of HIV-1.

We present the results of part B of the REDUC trial. Part A, showing latency reversal by the histone deacetylase inhibitor romidepsin alone, has been published.[10] The trial's overall objective was to assess the effect on the size of the latent HIV-1 reservoir of the combination of a peptide-based therapeutic HIV-1 vaccine (Vacc-4x; a synthetic p24 gag peptide vaccine) and recombinant human granulocyte macrophage colony-stimulating factor (rhuGM-CSF) as local adjuvant followed by treatment with the latency-reversing agent romidepsin.

Methods

Study design and participants

In this single-arm, phase 1B/2A trial, we enrolled, by invitation letter, adults (aged ≥18 years) with HIV-1 infections and on ART with plasma viral RNA loads of less than 50 copies per mL for the previous year (with a minimum of two viral load measurements taken) and a CD4 count of at least 500 cells per μL treated at the Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark. Exclusion criteria included a CD4 count of less than 200 cells per μL within the past 2 years, active hepatitis B or hepatitis C infection, and clinically significant cardiac disease, including QTc prolongation. The study was approved by the National Committee on Health Research Ethics (#M-2013–364–13) in accordance with the principles of the Helsinki Declaration. Each patient provided written informed consent before any study procedures.

Procedures

We scheduled eligible individuals to receive a series of six intradermal immunisations over 12 weeks (at 0 weeks, 1 week, 2 weeks, 3 weeks, 11 weeks, and 12 weeks) with 0·1 mL of 12 mg/mL Vacc-4x (Bionor Pharma, Oslo, Norway) and 0·1 mL of 0·6 mg/mL rhuGM-CSF (Genzyme, Cambridge, MA, USA) as adjuvant to ensure optimal exposure of the immunogen to antigen-presenting

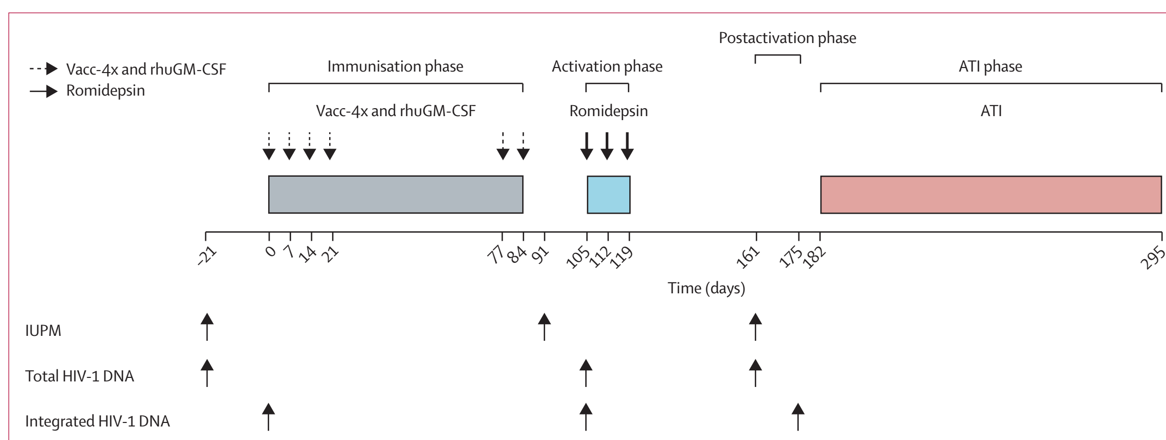

Figure 1: **Study design**
The screening timepoint is shown at day –21, six immunisations with Vacc-4x and rhuGM-CSF between days 0 and 84 are indicated by small dashed arrows above the timeline, three infusions with romidepsin between days 105 and 119 are indicated by large solid arrows above the timeline, and the ATI is shown from days 182 to 295. Sampling timepoints for measurements of the reservoir size are indicated by small solid arrows below the timeline. ATI=analytical treatment interruption. IUPM=infectious units per million. rhuGM-CSF=recombinant human granulocyte macrophage colony-stimulating factor.

cells (figure 1). The immunisation phase was followed by 5 mg/m² intravenous romidepsin (Celgene, Summit, NJ, USA) infusions once weekly for 3 weeks while maintaining ART. An analytical treatment interruption (ATI) followed the immunisation and activation phase. We planned eight visits for the immunisation phase (days 0–84), four visits for the activation phase with romidepsin (days 105–19), and two follow-up visits for the postactivation phase (days 161–75). During the ATI phase (day 182 to end of study), we monitored patients for plasma HIV-1 viral load (COBAS TaqMan; Roche, Basel, Switzerland; limit of quantification of 20 copies per mL) twice weekly for 4 weeks followed by once-weekly sampling thereafter. We monitored CD4 T-cell counts every other week. Two consecutive viral load measurements of more than 1000 copies per mL or CD4 T-cell counts of less than 350 cells per µL prompted resumption of ART and end of study (appendix pp 1–2).

We chose the dosing concentration and frequency of Vacc-4x and rhuGM-CSF administration on the basis of previous experience.[16] Before each immunisation, we stored blood for endpoint analyses. 10 min after each rhuGM-CSF injection, we injected Vacc-4x immediately next to the rhuGM-CSF injection site. We based the rationale for dosing romidepsin at 5 mg/m², corresponding to about 36% of the recommended cancer dosing (14 mg/m²), on the potent induction of HIV-1 transcription observed with this dosing in our pilot trial.[10] We checked haematological and clinical chemistry before infusion and stored additional blood sample material for endpoint analyses. We administered ondansetron (8 mg) orally before infusion as prophylactic antiemetic treatment. Patients received romidepsin intravenously over a 4 h period, which was followed by a postinfusion blood draw. All patients were on cardiac monitoring before, during, and 30 min after romidepsin infusion. Before ATI, all patients had a cardiac stress test of pedalling a stationary exercise bicycle ergometer to assess the potential presence of ischaemic heart disease. Criteria for engagement in ATI were CD4 cell counts of more than 350 cells per µL and no clinically significant findings in the cardiac stress test. After reinitiation of ART, we monitored all individuals outside of the trial protocol with frequent plasma HIV-1 RNA measurements until resuppression was achieved (<20 copies per mL).

Outcomes

Coprimary outcomes were changes in total and integrated HIV-1 DNA (copies per million unfractionated CD4 T cells) and in frequency of infectious units per million (IUPM) resting memory CD4 T cells, assessed by a quantitative viral outgrowth assay (qVOA).[17] Secondary outcomes were safety; histone H3 acetylation in lymphocytes; HIV-1 transcription (cell-associated unspliced HIV-1 RNA copies per million CD4 T cells); plasma HIV-1 RNA load during immunisation, activation, and ATI phases (with COBAS TaqMan, limit of quantification of 20 copies per mL, and a qualitative transcription-mediated amplification-based method with Procleix Ultrio Plus [Genprobe, Marlborough, MA, USA]; 50% sensitivity at 3·8 copies per mL and 95% sensitivity at 12 copies per mL); time to reach a plasma HIV-1 RNA load of more than 50 copies per mL and more than 1000 copies per mL; time to reinitiation of ART; HIV-specific T-cell responses; T-cell count and phenotype; and antibody titre to Vacc-4x peptides and p24. Analyses of some secondary endpoints are ongoing and will be reported separately. We assessed total HIV-1 DNA at screening, before romidepsin treatment, and 6 weeks after romidepsin treatment. We assessed integrated HIV-1 DNA at baseline, before romidepsin treatment, and 8 weeks after romidepsin treatment. We assessed

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IUPM at screening, 2 weeks before romidepsin treatment, and 6 weeks after romidepsin treatment. We actively assessed safety at each visit by registering adverse events (AEs) and grading them according to the Common Terminology Criteria for Adverse Events (version 4.0).[18] For each AE, we assessed the relationship to investigational drugs.

Statistical analysis

On the basis of a previous study,[19] we estimated the individual variation in IUPM to be 0·64 $\log_{10}$, with SD estimated to be 0·35 $\log_{10}$. With these assumptions, a paired t test, and a two-sided type I error of 5%, 13 fully assessable study participants would be required to detect a 50% or higher change from baseline in IUPM, with a power of 90%. To allow for dropouts and advanced analyses, we chose a sample size of 20. Furthermore, we tested overall changes between timepoints with an analysis of covariance (ANCOVA; we log transformed positively skewed data before analysis and back transformed results to original scale) or a Wilcoxon matched-pairs signed-rank test, as appropriate. We considered a two-sided α value of less than 0·05 significant. We used the full analysis set, comprising all patients receiving at least one dose of active treatment with assessable data, for the efficacy and safety analyses. We used Stata version 13.0, SAS version 9.4, and Prism version 6.0h for statistical analyses. We generated graphical data depictions using Prism. This trial is registered with ClinicalTrials.gov, number NCT02092116.

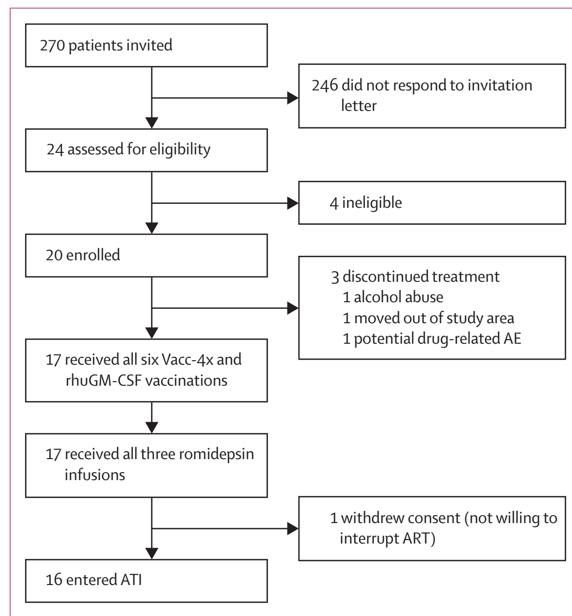

Figure 2: **Trial profile**
AE=adverse event. ART=antiretroviral therapy. ATI=analytical treatment interruption. rhuGM-CSF=recombinant human granulocyte macrophage colony-stimulating factor.

Role of the funding source

Bionor Pharma had a role in study design, data collection, data analysis, data interpretation, and writing of the report. The Research Council of Norway and SkatteFUNN had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all of the data in the study and had final responsibility for the decision to submit for publication.

Results

Between May 19, 2014, and Oct 8, 2014, we enrolled 20 individuals with HIV-1 infections (figure 2, table 1). Three individuals discontinued the trial during the immunisation phase: one because of potential Vacc-4x-related AEs, one moved out of the study area, and one relapsed into alcohol abuse. 17 individuals completed the assigned investigational drugs. 16 of 17 had assessable total HIV-1 DNA, 15 of 17 had assessable integrated HIV-1 DNA, and six of 17 had assessable IUPM at baseline and at one or more timepoints after study treatment. Before the scheduled ATI, one individual withdrew consent

	Study participants (n=20)
Sex	
Female	3
Male	17
Ethnic origin	
Caucasian	17
African Danish	1
Hispanic	1
Asian	1
Age (years)	49 (32–63)
Time since HIV-1 diagnosis (months)	115 (31–333)
Time from HIV-1 diagnosis to ART initiation (months)	24 (1–116)
ART regimen	
PI based	12
NNRTI based	5
INI based	2
NRTI based	1
Time on ART (months)	75 (28–217)
Time with HIV-1 RNA of <50 copies per mL (months)	51 (24–183)
Nadir CD4 count (cells per µL)	280 (60–710)
Baseline CD4 count (cells per µL)	730 (593–898)
$\log_{10}$ pre-ART viral load (copies per mL)	4·83 (3·57–7·00)
Baseline total HIV-1 DNA (copies per million CD4 T cells)	1082 (10–5738)
Baseline cell-associated HIV-1 RNA (copies per million CD4 T cells)	7·8 (1·0–26·3)

Data are n (%) or median (range). ART=antiretroviral therapy. INI=integrase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. NRTI=nucleoside reverse transcriptase inhibitor. PI=protease inhibitor.

Table 1: **Baseline characteristics**

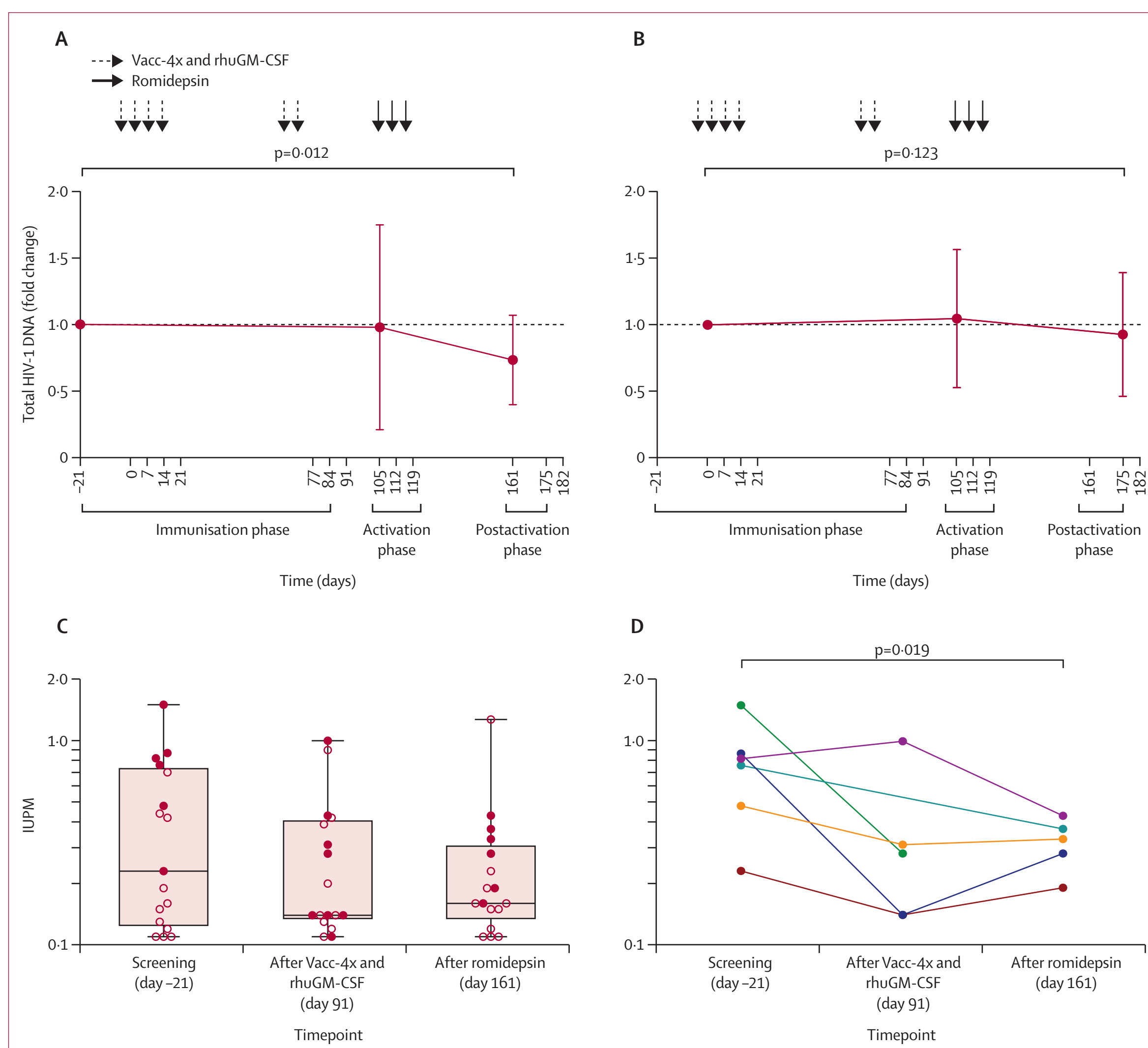

Figure 3: **HIV DNA measurements**

Mean fold change in (A) total and (B) integrated HIV-1 DNA. Error bars are SDs. (C) Results of the quantitative viral outgrowth assay, which was used to assess the frequency of resting memory CD4 T cells carrying inducible replication-competent proviruses at baseline, after immunisation, and after activation on a cohort level; boxes represent the 25th to 75th percentiles, the middle line represents the median, and the whiskers indicate the range; open circles indicate estimated maximum IUPM and closed circles indicate calculated IUPM estimates. (D) The six individuals with a positive (calculated minimum value) quantitative viral outgrowth assay at baseline and at least one positive timepoint after study treatment. IUPM=infectious units per million. rhuGM-CSF=recombinant human granulocyte macrophage colony-stimulating factor.

because of personal reasons, therefore 16 (80%) individuals entered the ATI and were followed up until resumption of ART. 14 of 16 individuals had reached the stable plateau phase of year 4 of ART for total HIV-1 DNA and all six had reached it for IUPM.

We observed a significant mean reduction of 39·7% (95% CI –59·7 to –11·5) in total HIV-1 DNA from screening to 6 weeks after romidepsin treatment (day 161; p=0·012; figure 3, appendix p 3). 14 of 16 assessable individuals had a decrease in total HIV-1 DNA from screening to day 161 (appendix p 3). Integrated HIV-1 DNA decreased from baseline to 8 weeks after romidepsin treatment, but this change did not reach statistical significance (mean reduction 19·2%, 95% CI –38·6 to 6·3; p=0·123; figure 3, appendix p 3).

IUPM, which estimates the frequency of resting memory CD4 T cells carrying inducible replication-competent proviruses, was under the limit of detection in 31 (61%) of 51 measurements. Thus, we estimated the lower limit of detection values on the basis of the number of cells used for estimation (appendix p 5). qVOA allowed establishment of IUPM at baseline and at least at one timepoint after study treatment in six of 17 participants. In these six assessable participants, mean IUPM decreased by 38·0% (95% CI –67·0 to –8·0;

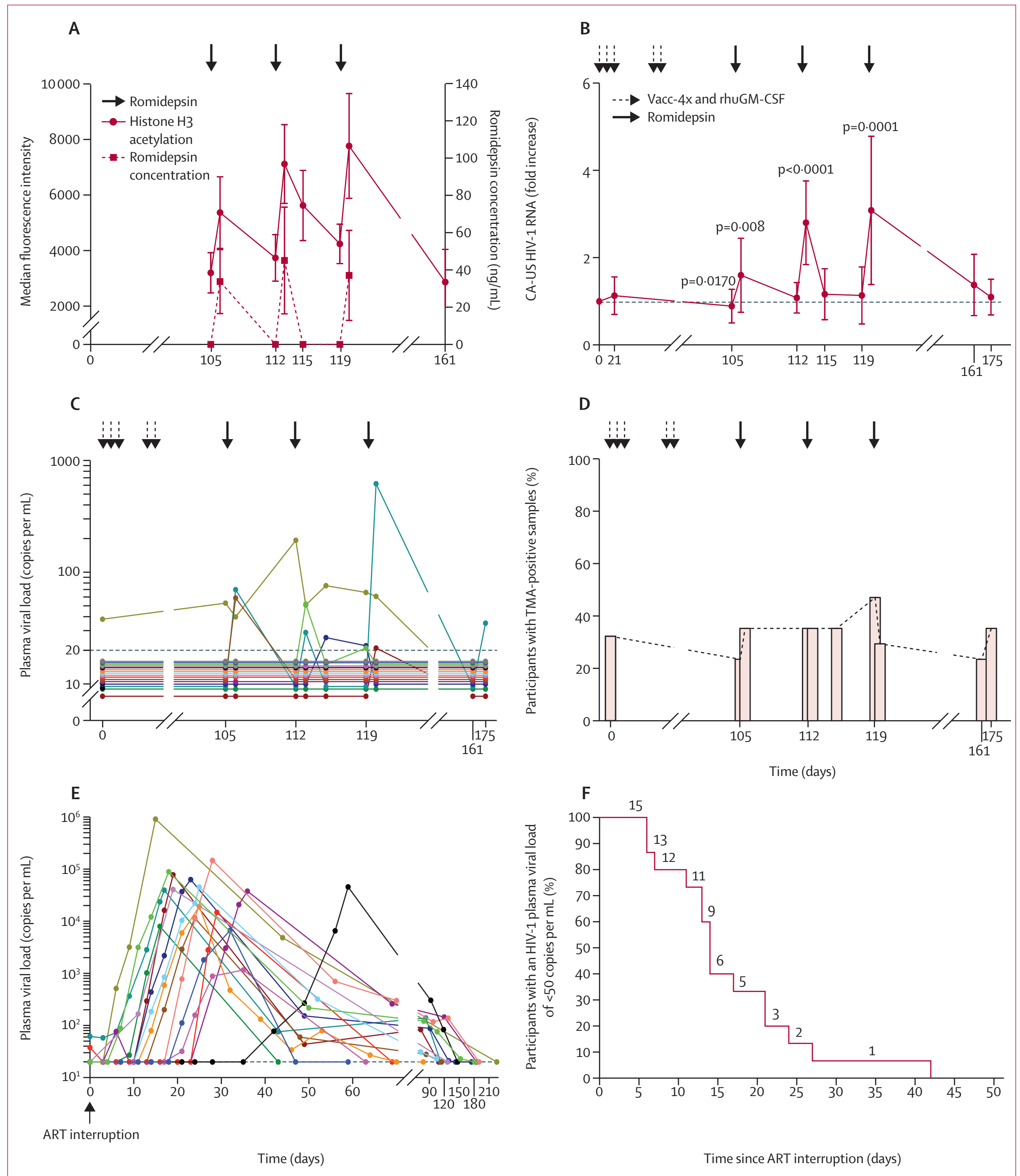

Figure 4: **Transcriptional activity and time to viral rebound**

(A) Cyclic pattern of mean histone H3 acetylation and romidepsin drug concentrations. Error bars are SDs. (B) Mean fold change in CA-US HIV-1 RNA during immunisation and activation phases, with peak values during romidepsin treatment approximately 30 min after infusion. Error bars are SDs. (C) Individual plasma viral loads during romidepsin infusions. The dotted line represents the limit of quantification (20 copies per mL). (D) The proportion of participants with detectable viral RNA at selected timepoints with use of a TMA assay. (E) Individual viral rebound after interruption of antiretroviral therapy; the dotted line represents the limit of quantification (20 copies per mL). (F) Proportion of individuals with plasma viral loads of less than 50 copies per mL after interruption of antiretroviral therapy. ART=antiretroviral therapy. CA-US=cell-associated unspliced. TMA=transcription-mediated amplification.

p=0·019) from screening to 6 weeks after romidepsin treatment (day 161; figure 3, appendix p 5). Collectively, these data suggest that sequential administration of Vacc-4x and rhuGM-CSF followed by romidepsin results in a measurable reduction of the HIV-1 reservoir.

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To investigate the direct effect of romidepsin on chromatin further, we used flow cytometry to measure histone H3 acetylation in lymphocytes.[9,10,20] In all 17 individuals, H3 histone acetylation increased rapidly within hours of each romidepsin administration and then decreased between 3 days and 7 days after infusion; this finding supports our previously published data (figure 4).[10] We observed a significant decrease in viral transcriptional activity (cell-associated unspliced HIV-1 RNA) from baseline to day 105 after Vacc-4x and rhuGM-CSF immunisation (figure 4). Furthermore, we observed significant increases in cell-associated unspliced HIV-1 RNA about 30 min after each romidepsin infusion (3·1-fold mean increase after third infusion compared with baseline), exceeding the natural variation.[21]

In one individual, plasma HIV-1 RNA remained in the low detectable range throughout the intervention phases (range 53–193 copies per mL). Quantifiable plasma HIV-1 RNA increased in seven of the 16 individuals going into the activation with romidepsin phase (table 2). Of these 16 individuals, two had quantifiable plasma HIV-1 RNA after the first infusion (range 59–70 copies per mL), three did after the second infusion (range 29–53 copies per mL), and two did after the third infusion (range 21–619 copies per mL; figure 4); however, only in four individuals was this detected immediately after one of the romidepsin infusions. In addition to the quantitative assay, qualitative transcription-mediated amplification results showed that between four (24%) and eight (47%) of 17 patients had detectable plasma HIV-1 RNA throughout the course of the study (figure 4). Collectively, these findings mirror previous results showing a reproducible effect of romidepsin on H3 acetylation and HIV-1 transcriptional activity. However, we observed a lower proportion of participants with quantifiable plasma HIV-1 RNA than that previously observed.[10]

The ultimate test of interventions targeting the HIV-1 reservoir is to withdraw ART under close monitoring.[22] Median time from stopping ART to having a plasma HIV-1 RNA of more than 50 copies per mL was 14 days (IQR 7–21; figure 4, appendix p 6). Median time to reinitiation of ART was 25 days (17–29). All individuals achieved viral resuppression after a median of 91 days (41–142). We observed a minor statistically significant, but clinically insignificant, change in CD4 T-cell count from baseline to day 175 (appendix pp 4, 6). CD4 to CD8 ratios generally remained within normal ranges, except for the last timepoint during the ATI, which showed a significant reduction from baseline. Finally, we observed no major changes in the CD4 T-cell compartment during romidepsin infusions (data not shown).

141 AEs were registered (134 [95%] were grade 1 and seven [5%] were grade 2–3; table 3). Of these, we considered 42 (31%) grade 1 AEs and one grade 2 AE related to Vacc-4x and rhuGM-CSF treatment. Transient redness and itching sensations at the injection site were the most common AEs. Furthermore, we considered 57 (40%) grade 1 AEs related to romidepsin, with fatigue (14 individuals) and nausea (15 individuals) being the most frequently reported. One presumed romidepsin-related AE (hair loss, grade 1) was not resolved at completion of the study. The rest of the presumed romidepsin-related AEs resolved spontaneously, with fatigue being the most durable AE. During the ATI, six (4%) grade 1 AEs and three (43%) grade 2–3 AEs (serious adverse events) were registered, of which two (33%) grade 1 AEs were presumed not related to ATI. One individual was admitted three times for 24 h in the emergency room (grade 3, serious adverse event) by personal request for supportive treatment of alcohol-related withdrawal symptoms during the ATI, but these events were presumed unrelated to the study drugs.

	Before first infusion (day 105)	After first infusion (day 105)*	Before second infusion (day 112)	After second infusion (day 112)*	After second infusion (day 115)†	Before third infusion (day 119)	After third infusion (day 119)*
21	<20	<20	<20	<20	<20	<20	<20
22	<20	<20	<20	<20	<20	<20	<20
23	<20	<20	<20	53	<20	21	<20
24	<20	<20	<20	<20	<20	<20	<20
25	<20	<20	<20	<20	<20	<20	<20
26	<20	<20	<20	<20	<20	<20	<20
27	<20	<20	<20	<20	<20	33	<20
28	<20	<20	<20	<20	<20	<20	<20
29	<20	<20	<20	<20	<20	<20	<20
32	<20	<20	42	<20	<20	<20	<20
33	<20	59	<20	<20	<20	<20	<20
34	<20	<20	<20	<20	<20	<20	<20
36	<20	<20	<20	<20	26	22	<20
41	<20	70	<20	29	<20	<20	619
42	<20	<20	<20	<20	<20	<20	<20
43	53	40	193	51	76	66	61
44	<20	<20	<20	<20	<20	<20	21

Participant identification numbers are shown in the first column. *30 min after infusion. †3 days after infusion.

Table 2: **Plasma viraemia during romidepsin treatment**

Discussion

In this phase 1B/2A trial, therapeutic HIV-1 immunisation with Vacc-4x and rhuGM-CSF combined with the histone deacetylase inhibitor romidepsin resulted in three important findings. First, this dual intervention significantly reduced the HIV-1 reservoir as evidenced by a decrease in total HIV-1 DNA and replication-competent virus in patients for whom data were assessable, although the decrease in integrated HIV-1 DNA was not significant. Second, despite marked increases in histone acetylation and cell-associated unspliced HIV-1 RNA shortly after each romidepsin infusion, only four of 17 individuals pretreated by immunisation with Vacc-4x and rhuGM-CSF had quantifiable plasma HIV-1 RNA immediately after one of the romidepsin infusions. Third, although these findings indicate that the so-called shock and kill

	Grade 1	Grade 2	Grade 3	Any grade	Patients (n=20)
Vacc-4x and rhuGM-CSF					
Presumed related					
Redness at injection site	8	0	0	8	8
Itch at injection site	18	0	0	18	14
Soreness at injection site	3	0	0	3	3
Headache	2	0	0	2	2
Fatigue	7	1	0	8	7
Nausea	1	0	0	1	1
Photosensitivity	1	0	0	1	1
Change in sense of smell	1	0	0	1	1
Fever	1	0	0	1	1
Any related	42	1	0	43	38
Presumed not related					
Urinary tract infection	1	0	0	1	1
Dry irritative cough	1	0	0	1	1
Nausea		1	0	1	1
Tinnitus	1	0	0	1	1
Flu-like symptoms	2	0	0	2	2
Whitlow	1	0	0	1	1
Constipation	1	0	0	1	1
Pneumonia		1	0	1	1
Cold	1	0	0	1	1
Upper respiratory tract infection	1	0	0	1	1
Incised wound on right hand (accident)	0	1	0	1	1
Pollakisuria	1		0	1	1
Any non-related	10	3	0	13	13
Romidepsin					
Presumed related					
Fatigue	18	0	0	18	14
Anorexia	1	0	0	1	1
Nausea	24	0	0	24	15
Constipation	3	0	0	3	3
Headache	2	0	0	2	2
Vomiting	3	0	0	3	2
Stomach ache	1	0	0	1	1
Flu-like symptoms	2	0	0	2	2
Borborygmi	1	0	0	1	1
Hair loss	1	0	0	1	1
Diarrhoea	1	0	0	1	1
Any related	57	0	0	57	43
Presumed not related					
Worsening of known colitis ulcerosa	1	0	0	1	1
Feeling feverish	1	0	0	1	1
Fatigue	2	0	0	2	2
Low concentration of ferritin	1	0	0	1	1
Caput radii fissure (accident)	1	0	0	1	1
Extraction of wisdom tooth	1	0	0	1	1
Short-term absence epilepsy (known epilepsia)	1	0	0	1	1
Fungal infection of the scalp	2	0	0	2	2
Cough	1	0	0	1	1
Urethral discharge	1	0	0	1	1
Sinuitis	1	0	0	1	1
Reflux	1	0	0	1	1
Back pain	2	0	0	2	2
Fever	1	0	0	1	1
Anorexia	1	0	0	1	1
Diarrhoea	1	0	0	1	1
Any non-related	19	0	0	19	19
ATI					
Presumed related					
Feeling feverish	1	0	0	1	1
Influenza-like symptoms	2	0	0	2	2
Sore lymph nodes in both armpits	1	0	0	1	1
Any related	4	0	0	4	4
Presumed not related					
Alcohol withdrawal symptoms	0	0	3	3	1
Sore throat	1	0	0	1	1
Verified influenza	1	0	0	1	1
Any non-related	2	0	3	5	3

(Table 3 continues in next column)

(Continued from previous column)

Data are n (%). Patients could be included in more than one adverse event category. None of the adverse events were serious other than alcohol withdrawal symptoms. rhuGM-CSF=recombinant human granulocyte macrophage colony-stimulating factor. ATI=analytical treatment interruption.

***Table 3:* Self-reported adverse events**

strategy might be a feasible approach to deplete the viral reservoir, the combined intervention did not prolong time to viral rebound during ART interruption. Further optimisation of this strategy is needed to achieve a sizeable effect on the latent reservoir that will translate into clinically measurable benefits for people living with HIV-1.

Use of epigenetic modifiers like histone deacetylase inhibitors to reverse HIV-1 latency has yielded promising results over the past few years. In part A of the REDUC study,[10] romidepsin increased H3 acetylation, induced significant bursts of transcription, and led to quantifiable plasma viraemia in five (83%) of six individuals with use of standard clinical assays. Reassuringly, the effect of romidepsin on HIV-1 transcription was reproduced in this part B, with nearly identical patterns of histone H3 acetylation and cell-associated unspliced HIV-1 RNA after romidepsin dosing. However, we observed quantifiable plasma viraemia in fewer individuals

receiving the combination of Vacc-4x and rhuGM-CSF and romidepsin in part B than in those receiving romidepsin monotherapy in part A. Although the number of patients in the study is small, this observation raises the possibility that priming of the immune system before latency reversal induced an immune pressure on virus-producing cells leading to their killing and, therefore, limited extracellular release of viral particles during romidepsin treatment. This hypothesis is supported by previous data showing that Vacc-4x and rhu-GM-CSF reduced the viral load setpoint during ATI in a placebo-controlled trial.[16] Data from this trial for the potential of Vacc-4x as a component of a HIV curative strategy are being analysed.

The combined intervention did not seem to affect time from interruption of ART to viral rebound. Median time from stopping of ART to an HIV-1 RNA of more than 50 copies per mL was 14 days, which does not differ from trials in which patients received no intervention.[6,23] Findings from mathematical modelling studies suggest that reductions of 3–4 $\log_{10}$ in the size of the latent reservoir might be needed to extend the time to viral rebound during ATI from a few weeks to several months or years.[24] Hence, although the observed reduction of about 40% in reservoir size among our participants is very encouraging, considerable optimisation of this shock and kill strategy is warranted. Such large reductions in the reservoir have so far only been achieved after stem-cell transplantation in patients with HIV-1 and haemopoietic malignant disease.[25,26]

Overall, the safety and tolerability of the investigational drugs were acceptable, with most drug-related AEs being transient Vacc-4x and rhuGM-CSF immunisation-site reactions and transient gastrointestinal-related symptoms and fatigue during romidepsin infusions. The observed AEs did not lead to dose de-escalations or other modifications of the study regimen. The ATI during which participants were monitored very closely was quite safe, with only minor ATI-related AEs reported (all grade 1). The rapid reinitiation of ART at viral rebound and subsequent viral suppression suggest that ATIs can be implemented in cure studies without compromising patient safety with use of the criteria for resumption of ART as used in this study.

Some limitations of the study should be acknowledged. First, the absence of a control group reduced our ability to differentiate between subtle effects on the size of the HIV-1 reservoir and longitudinal or stochastic variation. The decay rate in total HIV-1 DNA is highest in the first year of ART and then gradually slows down between year 1 and 4 (estimated decay rate 23% for years 1–4) before reaching a stable plateau by year 4.[27] In this study, median time on ART was 6·3 years, placing 14 of 16 individuals in the stable plateau phase for total HIV-1 DNA and all six in the stable plateau phase for IUPM. Additionally, the reduction from screening to day 161 (about half a year) in IUPM was 38% and 14 of 16 assessable individuals had a decrease in total HIV-1 DNA. Thus, the observed total HIV-1 DNA reduction seems highly unlikely to be caused by natural decay or stochastic variation.[21,27] Second, we were unable to assess changes in the replication-competent reservoir in eleven individuals, possibly because of the small dynamic range of qVOA and the fact that qVOA only reactivates one in about 60 replication-competent proviruses.[8] In the six assessable individuals, we found a small but significant reduction in the replication-competent reservoir, but as the analysis included fewer individuals than specified in the sample size calculation (because only six patients had assessable data), this finding should be interpreted with some caution. Our findings also suggest that qVOA might not be a very practical or predictive measure for sustained control of viraemia off ART in an ATI. Finally, most HIV-1 DNA is found within the effector and central memory CD4 T-cell compartment, so a potential bias in the observed decrease in total HIV-1 DNA could arise from homoeostatic changes or redistribution of the CD4 T-cell compartment.[28] However, we observed no major long-lasting changes in the composition of the CD4 T-cell compartments after romidepsin treatment.

Contributors

SL, OSS, MT, TAR, AM, MAS, and LØ developed the concept. SL, OSS, TAR, and MEG did the clinical studies. SL, SKN, JFH, MHS, SJ, ASK, RO, and PWD did the laboratory assays and experiments. SL, OSS, TAR, RO, MHS, JFH, MT, SKN, and PWD analysed data. SL, OSS, MT, and TAR drafted the manuscript. SL, OSS, MT, PWD, TAR, LØ, SJ, SKN, ASK, MHS, MEG, KK, MAS, AM, and RO critically revised the manuscript for important intellectual content.

Declaration of interests

AM, KK, and MAS are employed by Bionor Pharma. All other authors declare no competing interests.

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