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Effect of therapeutic intensification followed by HIV DNA prime and rAd5 boost vaccination on HIV-specific immunity and HIV reservoir (EraMune 02): a multicentre randomised clinical trial

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RLM, SGD, TJW, DC, CK, BA, VC, and RAK designed the EraMune 02 study. Patient recruitment, enrolment, and follow-up were done by CJA (Northwestern University), BB (Northwestern University), SGD (University of California San Francisco), and TJW (Cornell University). Virological assays were done by SL-N under the guidance of VC. Immunological assays, statistical analyses, and production of figures 4 and 5 were done at the National Institutes of Health Vaccine Research Center under the guidance of JPC and RAK. All statistical analyses, except immunology results from the National Institutes of Health Vaccine Research Center, and production of figures 1–3 were done by LA under the guidance of DC. The initial draft of the report was written by CJA, BB, and RLM. Throughout the study, all authors participated in discussions about the design, statistical analyses, and interpretation of findings. All authors were also involved in the review and editing process of initial and revised reports for submission.

EraMune 02 investigators and study team

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Declaration of interests

TJW has received grants and personal fees from GlaxoSmithKline and ViiV Healthcare outside the submitted work. DC has received travel grants, consultancy fees, honoraria, and study grants from various pharmaceutical companies including Gilead Sciences, Janssen-Cilag, Merck-Sharp & Dohme-Chibret, and ViiV Healthcare, outside the submitted work. All other authors declare no competing interests.

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Summary

Background—Achievement of a cure for HIV infection might need reactivation of latent virus and improvement of HIV-specific immunity. As an initial step, in this trial we assessed the effect of antiretroviral therapy intensification and immune modulation with a DNA prime and recombinant adenovirus 5 (rAd5) boost vaccine.

Methods—In this multicentre, randomised, open-label, non-comparative, phase 2 clinical trial, we enrolled eligible adults 18–70 years of age with chronic HIV-1 infection on suppressive antiretroviral therapy with current CD4 count of at least 350 cells per μL and HIV DNA between 10 and 1000 copies per 10^6 peripheral blood mononuclear cells. After an 8 week lead-in of antiretroviral intensification therapy (standard dose raltegravir and dose-adjusted maraviroc based on baseline antiretroviral therapy), patients were randomly assigned (1:1) to receive antiretroviral therapy intensification alone or intensification plus injections of HIV DNA prime vaccine (4 mg VRC-HIVDNA016-00-VP) at weeks 8, 12, and 16, followed by HIV rAd5 boost vaccine (10^{10} particle units of VRC-HIVADV014-00-VP) at week 32. Randomisation was computer generated in permuted blocks of six and was stratified by study site. The primary endpoint was a 0·5 $\log_{10}$ or greater decrease in HIV DNA in peripheral blood mononuclear cells at week 56. This study is registered with ClinicalTrials.gov, number NCT00976404.

Findings—Between Nov 29, 2010, and Oct 28, 2011, we enrolled 28 eligible patients from three academic HIV clinics in the USA. After the 8 week lead-in of antiretroviral intensification therapy, 14 patients were randomly assigned to continue antiretroviral therapy intensification alone and 14 to intensification plus vaccine. Enrolled participants had median CD4 count of 636 cells per μL, median HIV DNA 170 copies per 10^6 peripheral blood mononuclear cells, and duration of

antiretroviral therapy of 13 years. The median amount of HIV DNA did not change significantly between baseline and week 56 in the antiretroviral therapy intensification plus vaccine group. One participant in the antiretroviral therapy intensification alone group reached the primary endpoint, with 0·55 $\log_{10}$ decrease in HIV DNA in peripheral blood mononuclear cells. Both treatments were well tolerated. No severe or systemic reactions to vaccination occurred, and five serious adverse events were recorded during the study, most of which resolved spontaneously or were judged unrelated to study treatments.

Interpretation—Antiretroviral therapy intensification followed by DNA prime and rAd5 boost vaccine did not significantly increase HIV expression or reduce the latent HIV reservoir. A multifaceted approach that includes stronger activators of HIV expression and novel immune modulators will probably be needed to reduce the latent HIV reservoir and allow for long-term control in patients off antiretroviral therapy.

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Introduction

Eradication of HIV from an infected patient cannot be achieved with available antiretroviral therapies. Reduction and eventual eradication of the HIV reservoir potentially needs three steps: complete suppression of HIV replication in blood and tissues, reversal of HIV latency from quiescent immune cells, and restoration of HIV-specific host effector responses. We used these principles to design a first proof-of-concept study to assess an HIV eradication strategy in which antiretroviral therapy was first intensified (to reduce any residual cell-to-cell spread of HIV),[1,2] followed by the controlled administration of a vaccine with HIV DNA prime and recombinant adenovirus 5 (rAd5) boost, developed by the National Institutes of Health Vaccine Research Center (Bethesda, MD, USA). We postulated that this vaccine would reactivate latent virus (since HIV-1 pol and env antigens activate CD4 cells harbouring HIV DNA), induce replication, and improve the ability of the host immune system to clear virus-producing cells.[3–5]

Our overall aim was to establish whether or not treatment intensification followed by HIV DNA prime and rAd5 boost vaccine has merit as an eradication strategy. We used a non-comparative, proof-of-concept phase 2 trial, a design commonly used in oncology trials. In this type of clinical trial, failure of an intervention to achieve the primary outcome response leads to the decision not to pursue further studies.

Methods

Study design and participants

The EraMune 02 study was a prospective, multicentre, randomised, open-label, non-comparative, phase 2 pro-of-of-concept clinical trial of antiretroviral therapy intensification alone or combined with vaccination with DNA prime and rAd5 boost. After an 8 week lead-in of raltegravir–maraviroc intensification therapy, eligible participants were randomly allocated (1:1) in an open-label, unmasked manner to continue raltegravir and maraviroc intensification alone or raltegravir and maraviroc intensification plus DNA prime and rAd5 boost vaccination for a total of 56 weeks.

People 18–70 years of age with chronic HIV-1 infection, on suppressive antiretroviral therapy (plasma HIV RNA <500 copies per mL for at least 3 years and below the limit of detection within the past year) and with a current CD4 count of at least 350 cells per μL were eligible for inclusion. Participants also had cell-associated HIV DNA between 10 and 1000 copies per 10^6 peripheral blood mononuclear cells. Because of concerns that existing immunity to Ad5 might negatively affect the vaccine's efficacy,[6] we excluded people who had a serum Ad5 90% neutralisation antibody titre higher than 1/250. We also excluded individuals who were using an integrase inhibitor or CCR5 inhibitor, those who had received any immuno-therapeutic intervention within the past year, and those with active hepatitis B or C co-infection.

The study protocol was approved by the Investigational Review Board of Northwestern University (Chicago, IL, USA) and by institutional review boards at the two other participating study sites (University of California San Francisco [San Francisco, CA, USA] and Cornell University [New York, NY, USA]). All participants provided written informed consent.

Randomisation and masking

To ensure tolerability and optimise participant follow-up, randomisation was done after an initial 8 week lead-in of the raltegravir and maraviroc intensification. Participants were then randomly allocated (1:1) either to continue to receive raltegravir and maraviroc intensification alone or to receive raltegravir and maraviroc intensification plus DNA prime and rAd5 boost vaccine. Randomisation was computer generated in permuted blocks of six and stratified by study site. This study was open-label and did not have a placebo group. All investigators, site pharmacists, study nurses, and participants were unmasked and were aware of the treatment allocation throughout the study.

Procedures

After enrolment, all participants remained on their baseline antiretroviral therapy and received in addition oral raltegravir 400 mg twice daily (provided by Merck & Co, Kenilworth, NJ, USA) and oral maraviroc 150 mg, 300 mg, or 600 mg (depending on interaction with baseline antiretroviral therapy) twice daily (provided by Pfizer Inc [New York, NY, USA] and ViiV Healthcare [Brentford, UK]) for 56 weeks. Participants assigned to the vaccine group also received 4 mg VRC-HIVDNA016-00-VP priming (DNA prime) vaccinations at weeks 8, 12, and 16, followed by 10^{10} particle units of VRC-HIVADV014-00-VP (rAd5 boost; provided by National Institute of Allergy and Infectious Diseases Vaccine Research Center, Bethesda, MD, USA) as a boost vaccination at week 32. Both vaccines have been described previously.[7–9] All vaccines were given intramuscularly in a 1 mL volume. The Biojector 2000 injection system was used to give VRC-HIVDNA016-00-VP, whereas a needle and syringe were used to give VRC-HIVADV014-00-VP. Study supplies were manufactured under current good manufacturing practices. Safety evaluations were done monthly and included history, physical examination, and laboratory measures of electrolytes, amylase, creatinine kinase, creatinine, liver function tests, complete blood counts, CD4 and CD8 T-cell counts, and HIV RNA. Local (pain, swelling, or redness) and systemic (fever, malaise, myalgia, headache, chills, or nausea)

reactogenicity symptoms were recorded by the participants on 72 h diary cards after each vaccination. At week 56, raltegravir–maraviroc intensification was discontinued and participants remained on baseline antiretroviral therapies.

We did clinical investigations and collected samples at screening, baseline, and on a monthly basis thereafter to assess safety, cell-associated HIV DNA, plasma HIV RNA (using regular and single copy assays), routine biochemistry tests, and CD4 or CD8 cell counts. An additional clinical visit with blood sample collection and storage was done at week 80 when participants were off all study interventions, including antiretroviral therapy intensification. A subgroup of 16 participants volunteered to have sigmoidoscopy to collect rectal tissue at baseline and at week 56. Safety was assessed according to the National Institute of Allergy and Infectious Diseases Division of AIDS table for grading the severity of clinical and biological events (version 1.0 [December, 2004; clarification August, 2009]).

Blood samples were collected in EDTA tubes and peripheral blood mononuclear cells were cryopreserved as viable cells and non-viable cell pellets. Rectal biopsy pieces were flash frozen and stored at −80°C. Cell-associated HIV DNA was quantified in peripheral blood mononuclear cells and rectal tissue by ultrasensitive real-time PCR[10–12] (Generic HIV DNA Cell kit [Biocentric, Bandol, France]) at a central laboratory (Pitié-Salpêtrière Virology, Paris, France). Longitudinal frequencies of T-cell responses to vaccine antigens were measured with a validated ELISpot assay.[13] We used ELISpot to identify HIV-derived epitopes as previously described.[14] Flow cytometry and intracellular cytokine staining assays were used to further characterise CD4 and CD8 subset T-cell responses to the vaccine. Permeabilised cells were stained with anti-CD3, anti-CD4, anti-CD8, anti-interleukin 2, anti-interferon γ, and anti-tumour necrosis factor α as previously described.[15] The limit of detection was 0·02%. Data were collected on an LSR II flow cytometer (BD Immunocytometry Systems, San Jose, CA, USA). All assays used cryopreserved cells.

Outcomes

The primary endpoint was change in cell-associated HIV DNA at week 56. We defined success in meeting this endpoint as a decrease in HIV DNA of greater than or equal to 0·5 $\log_{10}$ copies per 10^6 peripheral blood mononuclear cells from week 0 to week 56. We selected this size of change and 56 week timepoint on the basis of HIV DNA assay variability and the 6 month half-life of central memory CD4 T cells, which account for most of the HIV reservoir.

Secondary endpoints were changes in cell-associated HIV DNA copies per 10^6 CD4 T cells (ie, HIV DNA copies per 10^6 peripheral blood mononuclear cells × 90% × %CD4 cells) or per mL of whole blood ([HIV DNA copies per 10^6 peripheral blood mononuclear cells] × [lymphocytes per μL+monocytes per μL]/1000); changes in rectal mucosa tissue associated HIV DNA copies per mL; changes in CD4 and CD8 T-cell counts; changes in CD4 to CD8 T-cell count ratio; changes in HIV-specific T-cell immunity according to both ELISPOT and intracellular cytokine staining assays; changes in proportion of participants with more than 1 copy of plasma HIV RNA per mL; and occurrence of serious adverse events.

Statistical analysis

We powered our study based on the frequency of peripheral blood mononuclear cells containing integrated DNA, postulating that some of these cells produce virus proteins and that an effective vaccine will enhance the immune system's ability to clear these cells. With a phase 2 oncology clinical trial design, we established that a sample size of 14 evaluable participants in each group was needed such that if no patient in the vaccine group reached the predetermined primary endpoint (at least 0·5 log copies per 10^6 peripheral blood mononuclear cells decline in HIV DNA), this indicated a 95% likelihood that the success rate was lower than 20% and the strategy was not worth further study in larger clinical trials. The probability of type I error (α) for this study was 0·05.

We described categorical variables as number and percentage, and continuous variables as median (IQR). In our primary and secondary non-comparative endpoint analyses, we used a paired Wilcoxon signed-rank test to compare changes in total HIV DNA level in blood compartments and rectal tissue between baseline (week 0) and key study timepoints (weeks 8, 20, 32, 36, 56, and 80) in each group. In secondary immunology analyses, we used an unpaired Wilcoxon rank-sum test to compare background adjusted ELISpot and intracellular cytokine staining responses between the study groups at weeks 32, 36, and 56. We used IBM SPSS 22, STATA SE 13, SAS 9.3, and GraphPad Prism 6.0 for our analyses and to generate figures.

This study is registered with ClinicalTrials.gov, number NCT00976404.

Role of the funding source

The funder of the study 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 data in the study and had final responsibility for the decision to submit for publication.

Results

Between July 6, 2010, and Sept 26, 2011, we screened 86 individuals and enrolled 28 eligible patients on See Online for appendix suppressive antiretroviral therapy with no detectable antibodies against rAd5 (figure 1, table 1). After the 8 week lead-in, 14 participants were randomly assigned to receive antiretroviral therapy intensification alone and 14 to receive antiretroviral therapy intensification plus DNA prime and rAd5 boost vaccine. All participants were men, 20 (71%) of 28 were white, and their median age was 50 years (IQR 46–55). They had been on any antiretroviral therapy for a median of 13 years (IQR 8–19) with median time of undetectable HIV RNA (<50 copies per mL) of at least 2·6 years (IQR 2·2–3·0). Their median nadir CD4 count was 202 cells per μL (IQR 88–280), baseline CD4 count was 636 cells per μL (485–790), and baseline total cell-associated HIV DNA was 170 copies (60–361) per 10^6 peripheral blood mononuclear cells. Baseline characteristics were well balanced between groups (table 1).

From baseline to week 56, we found no significant change in total HIV DNA in the peripheral blood mononuclear cells in either study group (figure 2, table 2). We also recorded no significant change in total HIV DNA in the whole blood, number of CD4 T

cells, or rectal tissue in either group (table 2). At week 56, one patient in the intensification alone group reached the primary endpoint, with a decrease in cell-associated HIV DNA of 0·55 $\log_{10}$ copies per 10^6 peripheral blood mononuclear cells. Cell-associated HIV DNA in this patient decreased from 156 to 44 copies per 10^6 peripheral blood mononuclear cells between week 0 and week 56. The proportion of patients with HIV RNA single copy assay higher than 1 copy per mL decreased from five (36%) of 14 at baseline to one (7%) of 14 at week 56 in the intensification alone group (p=0–13) and increased from five (36%) of 14 at baseline to eight (57%) of 14 at week 36 (1 month after rAd5 boost) in the vaccine group (p=0–45; figure 3).

Compared with antiretroviral intensification therapy alone, vaccination resulted in significantly stronger HIV-specific T-cell responses to env (clades A, B, and C), gag, and pol by ELISpot (figure 4A, appendix p 1). The frequency of these responses all increased significantly 1 month after rAd5 boosting, but only the env (clades A, B, and C) response remained significantly higher than baseline at week 56 (appendix p 1). When the response was measured by CD8 T-cell interferon γ intracellular cytokine staining and was separated into env, gag, pol, and nef-specific gene products, env (clades A, B, and C) and gag peptide-specific responses were significantly increased 1 month after boosting (figure 4B, appendix p 2). When the response was measured by CD4 T cell interleukin 2 intracellular cytokine staining and separated into env, gag, pol, and nef-specific gene products, env (clades A, B, and C) and pol peptide-specific responses were significantly increased 1 month after boosting (figure 5 and appendix pp 3–4).

We recorded no significant change in median CD4 or CD8 counts from baseline to week 56 in either study group (CD4 T cells: −1 cell per μL [IQR −144 to 151; p=0·89] in the intensification alone group, and +23 cells per μL [−100 to 114; p=0·97] in the vaccine group; CD8 −14 cells per μL [IQR −210 to 179; p=0·89] in the intensification alone group, and +8 cells per μL [IQR −139 to 89; p=0·60] in the vaccine group.

One patient in the intensification alone group and three in the vaccine group had transient low detectable HIV RNA by standard commercial assays (all <200 copies RNA per mL) during the study. No severe or systemic post-vaccine reactions occurred to either the DNA prime or rAd5 boost components of the vaccine product. Only mild to moderate localised reactions were reported within 72 h after vaccination, including tenderness, redness, and swelling at the injection site. General symptoms of fatigue, malaise, and myalgias were noted after ten (24%) of 42 DNA prime injections and three (21%) of 14 rAd5 boost injections.

Five serious adverse events occurred in three participants during the study follow-up. One patient in the intensification alone group had a grade 4 increase in creatine kinase that resolved spontaneously without complications. One patient in the vaccine group, who had a history of coronary artery disease, had an acute coronary syndrome characterised by grade 3 left arm, neck, and chest pain before receipt of the vaccine product; he eventually recovered and completed the study. A second participant in the same group had acute renal failure with grade 4 increases in creatinine roughly 5 weeks after receiving the rAd5 boost component of the vaccine. His illness was preceded by 1 week of fatigue, low-grade fever, abdominal

distention, and bilateral lower leg and foot oedema. Renal biopsy showed several pathological patterns, including acute tubular necrosis, tubular interstitial disease, and a mild proliferative form of glomerulonephritis. His antiretroviral therapy regimen, including all study drugs, was stopped for several weeks and eventually restarted after close clinical follow-up with a new regimen (darunavir boosted with low-dose ritonavir, maraviroc, and raltegravir). After careful review, we established that the renal failure was unlikely to be related to study drugs or vaccine. After the renal failure event, the patient was admitted to hospital two additional times for haemodialysis catheter-associated infection and deep vein thrombosis.

Discussion

In this EraMune 02 proof-of-concept clinical trial, antiretroviral therapy intensification (raltegravir and maraviroc) followed by DNA prime and rAd5 boost vaccination did not significantly reduce the total cell-associated HIV DNA in either peripheral blood or rectal tissue in chronically infected patients with long-term HIV RNA suppression. Only one patient, who was in the antiretroviral therapy intensification only group, reached the predefined primary endpoint of a decrease in HIV DNA by at least 0·5 $\log_{10}$ copies per 10^6 peripheral blood mononuclear cells. Therefore, antiretroviral therapy intensification with or without this vaccine will probably not be curative. Other approaches will need to be pursued.

EraMune is a programme that focuses on eradication of HIV from reservoirs. A parallel trial (EraMune 01), with the same design, measured the effect of interleukin 7 on the HIV DNA reservoir. This non-comparative phase 2 oncology clinical trial design allowed us to assess HIV reservoir changes between patients and maximise power with a small sample size. Other studies have used antiretroviral therapy interruption and subsequent viral rebound or time off therapy as surrogates for HIV reservoir reduction.[16–19] Our approach was to measure total cell-associated HIV DNA and consider antiretroviral therapy interruption for patients who achieved undetectable levels of HIV DNA. However, ultimately, no patient in either EraMune 01 or EraMune 02 had reductions in total HIV DNA to levels that justified interruption of antiretroviral therapy. Several different approaches to targeting the HIV reservoir have been investigated in clinical studies (panel).

Compared with other antiretroviral therapy intensification trials,[1,31–48] EraMune 02 was unique in that we intensified treatment with both raltegravir and maraviroc for longer than a year (56 weeks). Overall, we did not record a significant decrease in total cell-associated HIV DNA in blood or rectal tissue with this treatment strategy, but one patient did reach our predefined primary endpoint. At the time when the study was designed, this finding might have warranted further study in a larger clinical trial; however, recent raltegravir and maraviroc intensification studies have not replicated this result.[31–33,35,43,45] We did not measure episomal DNA (two long terminal repeat circles), which has been shown in randomised controlled trials to be responsive to raltegravir intensification.[1,2] Therefore, we cannot conclude that our approach did not suppress residual replication, but our data do contribute to mounting evidence that the mere addition of therapies with similar mechanisms of action and pharmacokinetic profiles as existing antiretroviral drugs will not enable HIV eradication.

Our data are broadly consistent with another clinical trial assessing virological and immunological responses to DNA prime and rAd5 boost vaccination among individuals with HIV infection on suppressive antiretroviral therapy (the VRC 101 study).[9] In this study of 17 adults on non-intensified antiretroviral therapy, DNA prime and rAd5 boost did not reduce HIV RNA by standard or single copy assays and did not reduce the frequency of latently infected resting CD4 T cells measured by viral outgrowth assay (HIV DNA levels were not measured). In EraMune 02, we recorded overall boosting of immune responses to gag, pol, and env after administration of the rAd5 vaccine. Detailed immunological data from the VRC 101 study showed that, with few exceptions, the HIV-specific CD8 T cells induced by DNA prime and rAd5 boost vaccination were expansions of pre-existing responses without major changes in maturational phenotype or clonotype.[9] These T cells presumably target HIV epitopes that had already escaped and thus are unable to clear virus or infected cells, which could explain the failure of this approach to reduce HIV reservoirs in EraMune 02. New vaccines that generate responses to novel epitopes without escape mutations might be needed.

Three potential reasons exist for why DNA prime and rAd5 boost vaccination did not affect the reservoir of total cell-associated HIV DNA. First, we chose to measure the HIV reservoir with an endpoint of reduction in total cell-associated HIV DNA because this assay is widely used and the most standardised method available for measurement of the HIV reservoir.[10,12] This assay does not distinguish most defective or non-inducible integrated proviruses.[49,50] We did not measure changes in inducible replication competent latent proviruses, but only used the total HIV DNA assay. Nevertheless, we could detect in-vitro virus inducibility after maximum T-cell activation in all patient samples tested from the companion study (EraMune 01).[51] However, if interventions can reduce total HIV DNA to extremely low or undetectable levels, then replication-competent virus should also be significantly reduced. Second, the DNA prime and rAd5 boost vaccine might not be a strong enough inducer of transcription and production of HIV antigens from latent reservoir cells. We recorded only a transient trend toward increased HIV production (by the RNA single-copy assay) coinciding with administration of the vaccine. Our findings were limited because we did not measure cell-associated HIV RNA, which is a sensitive marker of HIV reactivation.[16,20–22] Third, as noted previously, the HIV-specific CD8 T cells, which were expanded, might target epitopes that escaped before antiretroviral therapy, or could be dysfunctional.

In conclusion, an intervention of antiretroviral therapy intensification (raltegravir and maraviroc) with DNA prime and rAd5 boost vaccination did not reduce total cell-associated HIV DNA. Further research is needed to establish treatment strategies capable of inducing latent virus transcription, reducing the inducible replication competent fraction of proviruses, facilitating penetration of antiretroviral therapy intensification into lymphoid tissues, or enhancing a neutralising antibody response and a strong gag-specific cellular immune response. DNA prime and rAd5 boost (EraMune 02) was tested in parallel with interleukin 7 (EraMune 01) with the same study design and antiretroviral therapy intensification regimen with the ultimate goal of combining the three approaches (antiretroviral therapy intensification plus interleukin 7 and DNA prime and rAd5 boost) in a shock and kill strategy. In view of our study findings and those of VRC 101,[9] we would not

recommend moving forward larger clinical trials evaluating DNA prime and rAd5 boost vaccine alone as an HIV eradication strategy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Panel: **Research in context**

Systematic review

On Jan 12, 2015, we searched PubMed using the keywords “HIV reservoir”, “HIV DNA”, or “HIV latency” and “clinical trials” for studies published between Jan 1, 2000, and Jan 12, 2015, that assessed the effect of non-antiretroviral therapy interventions on the HIV reservoir in infected patients. We identified 14 publications of clinical trials assessing various therapeutic approaches aimed at reducing the latent HIV reservoir.[9,16,19,20–30] Interventions were in two categories: first, immune modulators (interleukin 7,[23,24] interleukin 2,[25] pegylated interferon alfa-2a,[26] Toll-like receptor 9 agonist,[27] DNA prime and recombinant adenovirus 5 (rAd5) boost HIV vaccine,[9] rAd5 HIV-1 gag vaccine,[19] recombinant poxvirus HIV vaccine,[28] and CCR5 modified autologous CD4 T-cell infusion[29]); and second, HIV expression activators (valproic acid,[30] vorinostat,[20,21] romidepsin,[22] and panobinostat[16]). These clinical trials were pilot studies limited by small sample sizes and often without control groups; therefore, definitive conclusions are difficult. In the case of immune modulators, despite HIV-specific immune responses, they did not appreciably or durably reduce the latent HIV reservoir. A subset of patients receiving pegylated interferon alfa-2a who achieved viral control after interruption of antiretroviral therapy had significant reductions in CD4 T-cell-associated HIV DNA.[26] Total HIV DNA decreased minimally by 12·6% in patients receiving a Toll-like receptor 9 agonist compared with a 6·7% increase in the placebo group (p=0·02).[27] Recombinant modified vaccinia Ankara and Fowlpox-based HIV vaccines given to young adults resulted in modest transient reductions in the HIV reservoir measured by viral outgrowth assay.[28] Romidepsin (ex vivo),[22] vorinostat,[20,21] and panobinostat (in vivo)[16] activate HIV transcription, but none of the HIV expression activators have significantly reduced HIV reservoir size.

Interpretation

In this context, the EraMune 02 clinical trial of antiretroviral therapy intensification followed by DNA prime and rAd5 boost vaccine did not significantly increase HIV expression or reduce the latent HIV reservoir, despite improvements in CD8 T-cell immune response to HIV env, gag, and pol. Our findings, and those of previous studies, suggest that HIV cure strategies need new therapeutic vaccines that induce cytotoxic T-cell responses to novel HIV epitopes without escape mutations. A multifaceted approach that includes stronger activators of HIV expression and novel immune modulators will probably be needed to reduce the latent HIV reservoir and allow for long-term control in patients off antiretroviral therapy.

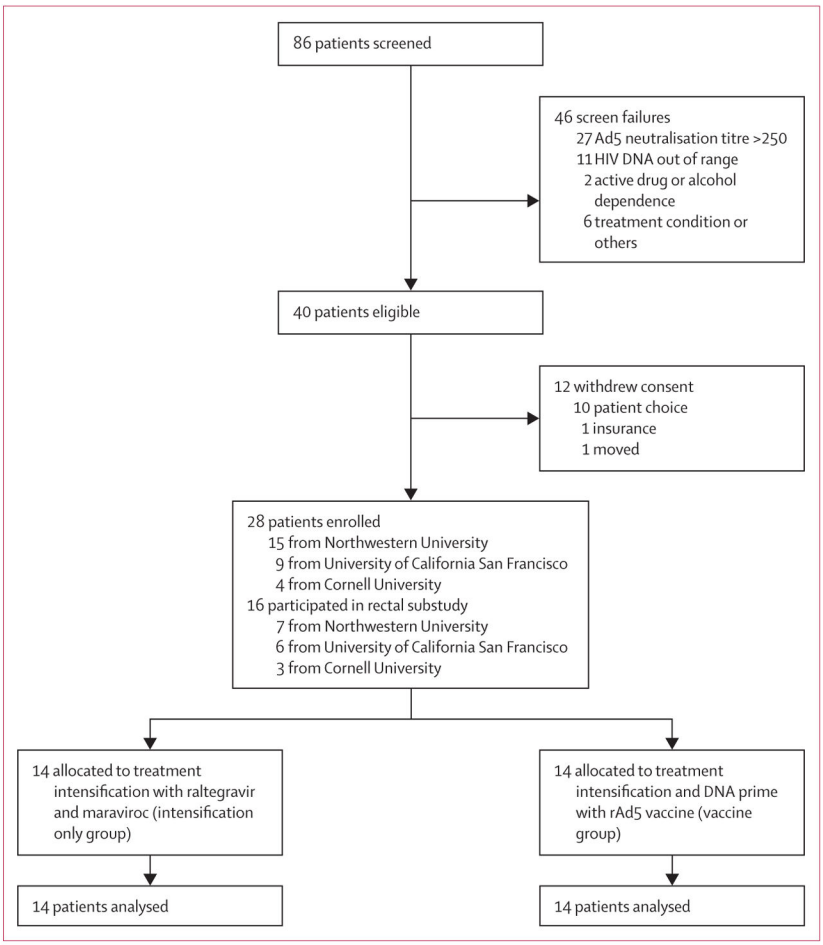

Figure 1: Trial profile

rAd5=recombinant adenovirus 5.

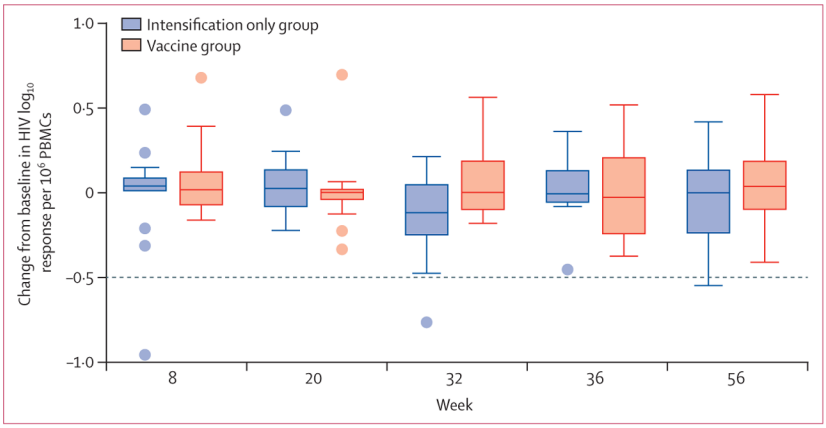

Figure 2: Changes from baseline to week 56 in total cell-associated HIV DNA in the two groups

PBMCs=peripheral blood mononuclear cells. Dashed line represents a 0·5 $\log_{10}$ copies per mL change threshold designated a priori as the primary endpoint. In the box-whisker plots, the top whisker is the smaller of the following two values: maximum or Q3 + 1·5 × IQR, and the bottom whisker is the larger of the following two values: minimum or Q1–1·5 × IQR. The plotted circles represent outliers.

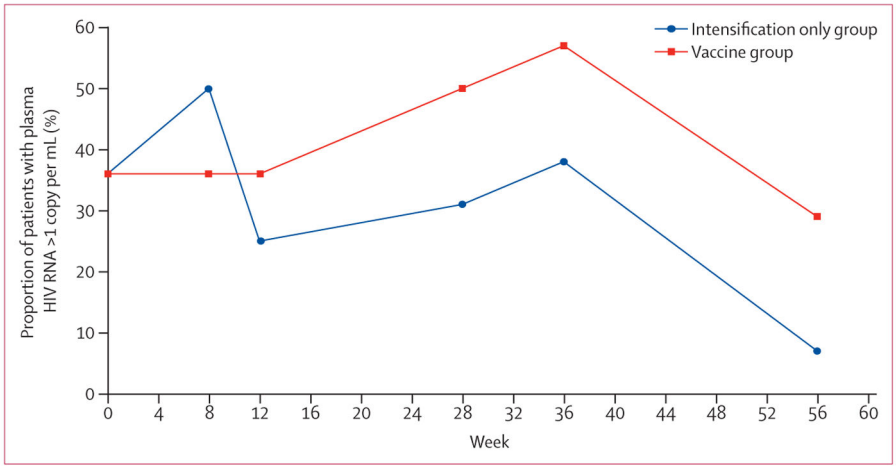

Figure 3:

Percentage of patients with HIV RNA single copy assay more than 1 copy per mL over time in the two groups

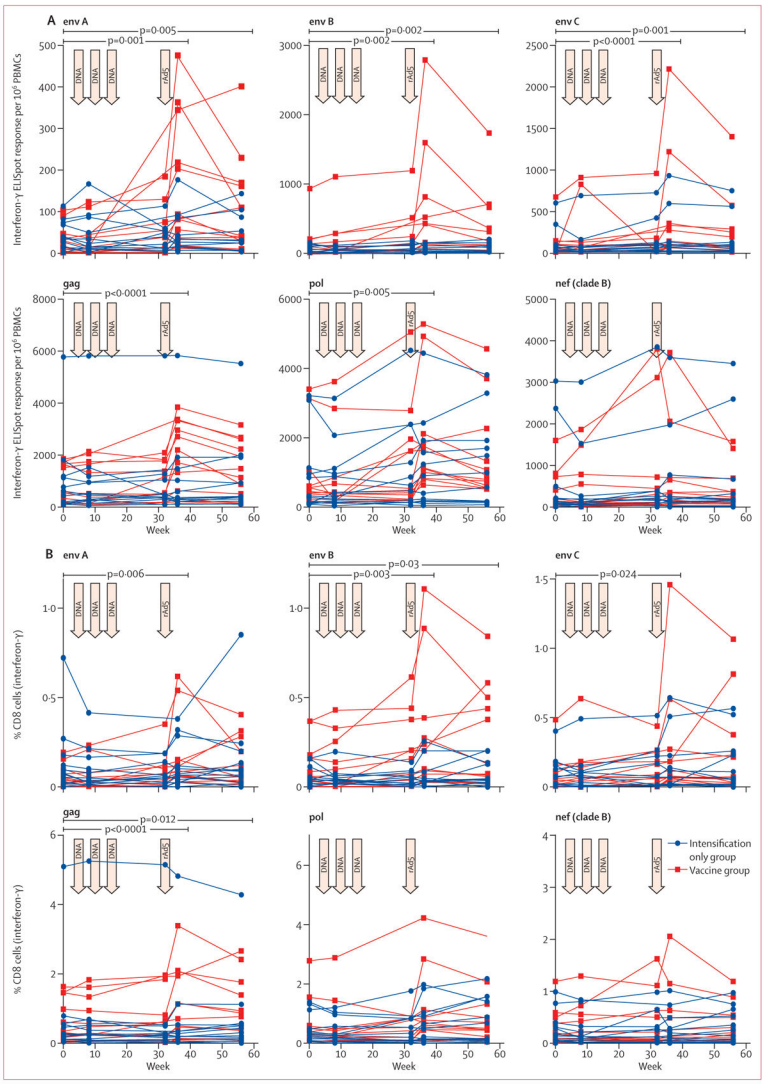

Figure 4: HIV-specific T-cell responses against env, gag, pol, and nef

(A) Total interferon γ ELISpot and (B) CD8 T-cell-specific interferon γ intracellular cytokine staining. The DNA vaccine was given at week 8 and the rAd5 boost vaccine was given at week 32. p values are for unpaired comparisons between the intensification only and vaccine study groups with respect to change from week 0. Only significant p values (p<0O5) are listed. rAd5=recombinant advenovirus 5.

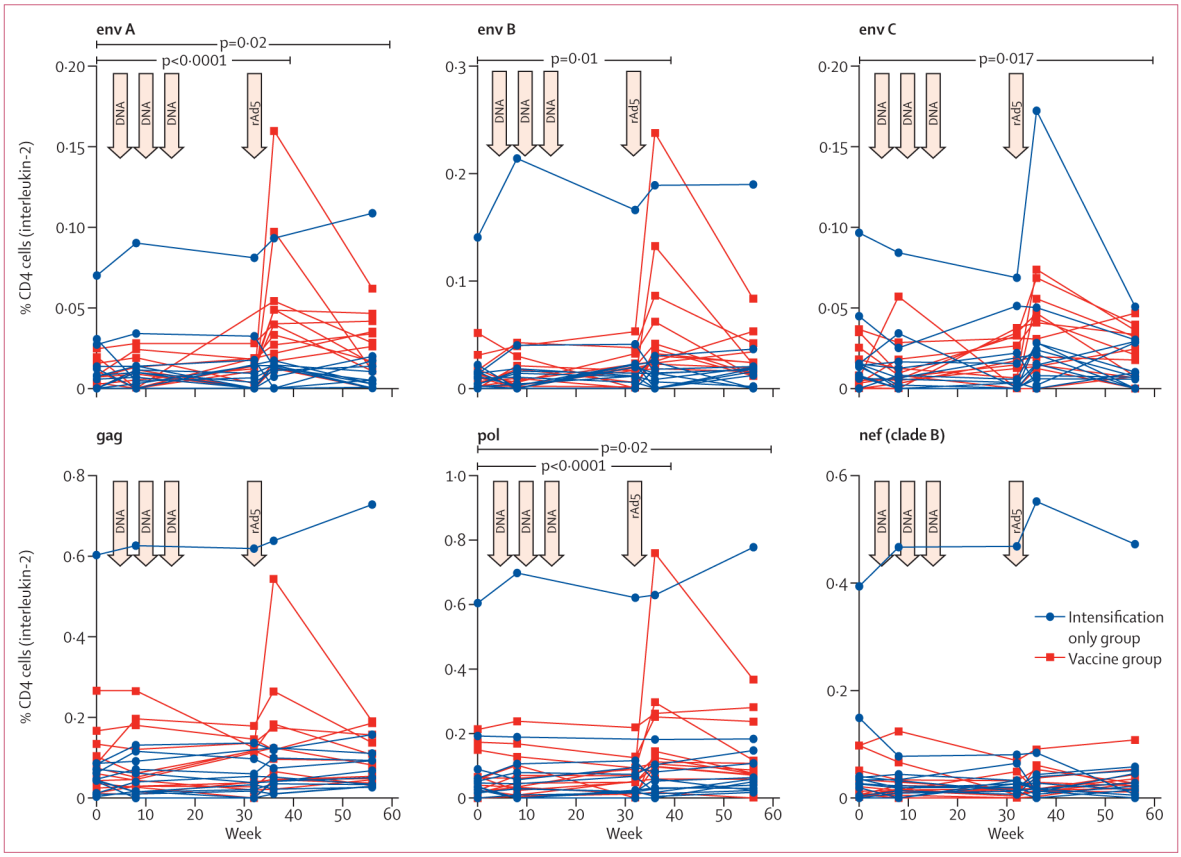

Figure 5: HIV-specific T-cell responses against env, gag, pol, and nef, according to CD4 T-cell-specific interleukin 2 intracellular cytokine staining

The DNA vaccine was given at week 8 and the rAd5 boost vaccine was given at week 32. p values are for unpaired comparisons between the intensification only and vaccine study groups with respect to change from week 0. Only significant p values (p<0·05) are listed. rAd5=recombinant advenovirus 5.

Table 1:

Baseline characteristics

	Intensification only group (n=14)	Vaccine group (n=14)
Men	14 (100%)	14 (100%)
White race	11 (79%)	9 (64%)
Age (years)	49 (46–55)	50 (46–55)
Time on antiretroviral therapy (years)	13 (8–19)	13 (6–19)
Participants with previous AIDS-defining events	3 (21%)	3 (21%)
Nadir CD4 cell count (cells per μL)	220 (146–419)	179 (50–219)
CD4 cell count (cells per μL)	686 (501–880)	563 (468–718)
CD8 cell count (cells per μL)	625 (475–925)	719 (535–796)
Ad5 90% neutralisation titre	18 (12–68)	12 (12–38)
Duration with HIV RNA <50 copies per mL (years)	2·4 (2·3–3·1)	2·7 (2·1–3·0)
HIV DNA (copies per 10^6 peripheral blood mononuclear cells)	97 (47–352)	228 (98–383)
HIV RNA single copy assay >1 copy per mL	5 (36%)	5 (36%)
Antiretroviral therapy regimen at baseline		
2 NRTIs plus PI/r	4 (39%)	7 (50%)
2 NRTIs plus NNRTI	10 (71%)	5 (36%)
NRTI and NNRTI plus PI/r	0	1 (7%)
NNRTI plus PI	0	1 (7%)

Data are n (%) or median (IQR). NRTI=nucleoside reverse transcriptase inhibitor. NNRTI=non-nucleoside reverse transcriptase inhibitor. PI/r=protease inhibitor boosted with low-dose ritonavir.

Table 2:

HIV DNA levels at baseline and at week 56 in blood compartments and rectal tissue in both groups

	Baseline	Week 56	p value*
Whole blood HIV DNA (log_{10} copies per mL)			
Intensification only group	2·68 (2·50–3·01)	2·72 (2·52–3·04)	1·00
Vaccine group	2·78 (2·40–3·16)	2·86 (2·14–3·24)	1·00
Total peripheral blood mononuclear cells HIV DNA (log_{10} copies per 10^6 cells)			
Intensification only group	2·32 (2·11–2·51)	2·33 (1·98–2·60)	0·73
Vaccine group	2·46 (2·17–2·94)	2·58 (2·09–2·97)	0·46
CD4 specific lymphocytes HIV DNA (log_{10} copies per 10^6 cells)			
Intensification only group	2·62 (2·43–2·87)	2·63 (2·39–3·04)	0·75
Vaccine group	2·84 (2·44–3·21)	2·87 (2·23–3·31)	0·94
Rectal tissue HIV DNA (log_{10} copies per 10^6 cells)			
Intensification only group (n=7)	2·11 (1·67–2·56)	2·21 (2·05–2·64)	0·18
Vaccine group (n=6)	2·46 (1·79–2·93)	2·41 (2·20–2·61)	0·92

Data are median (IQR) unless otherwise indicated.

* p values are for pairwise comparison between week 0 and week 56 for each study group.