Safety and immunogenicity of CD40.HIVRI.Env, a dendritic cell-based HIV vaccine, in healthy HIV-uninfected adults: a first-in-human randomized, placebo-controlled, dose-escalation study (ANRS VRI06)

Yves Levy,[a,b,c,*] Christiane Moog,[c,d,p] Aurélie Wiedemann,[a,c,p] Odile Launay,[e,f] Fabio Candotti,[g] Lucile Hardel,[c,h] Mélany Durand,[c,h] Véronique Rieux,[i] Alpha Diallo,[i] Christine Lacabaratz,[a,c] Sylvain Cardinaud,[a,c] Sandra Zurawski,[c,j] Gerard Zurawski,[c,j] Georgia D. Tomaras,[k] Song Ding,[l] Mireille Centlivre,[a,c] Rodolphe Thiebaut,[c,m,n,o] Giuseppe Pantaleo,[g] Jean-Daniel Lelièvre,[a,b,c,p] and Laura Richert,[c,m,n,o,p] the ANRS VRI06 Study Group[q]

[a]INSERM U955, IMRB, Univ. Paris Est Créteil, Créteil, France
[b]Groupe Henri-Mondor Albert-Chenevier, AP-HP, Créteil, France
[c]Vaccine Research Institute, France
[d]INSERM UMR_S1109, Université de Strasbourg, Strasbourg, France
[e]CIC 1417 F-CRIN I-REIVAC, INSERM, Hôpital Cochin, AP-HP, Paris, France
[f]Université Paris Descartes, Paris, France
[g]Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland
[h]Univ. Bordeaux, INSERM, MART, UMS 54, Bordeaux, France
[i]ANRS MIE, Paris, France
[j]Baylor Scott & White Research Institute, Dallas, TX, USA
[k]Duke University School of Medicine, Durham, NC, USA
[l]EuroVacc Foundation, Lausanne, Switzerland
[m]Univ. Bordeaux, INSERM, Bordeaux Population Health Research Center, UMR1219, Bordeaux, France
[n]Inria SISTM Team, Talence, France
[o]CHU de Bordeaux, Service d'Information Médicale, Bordeaux, France

Summary

Background Current HIV prophylactic vaccines evaluate HIV Env as purified proteins. CD40.HIVRI.Env is an innovative antigen delivery targeting gp140 Env from HIV Clade C 96ZM651 to CD40-expressing antigen-presenting cells, thus harnessing the intrinsic immune-stimulant properties. DNA-HIV-PT123 vaccine encodes 96ZM651 gp140/Gag and 97CN54 Pol/Nef.

Methods Seventy-two HIV-negative volunteers were enrolled between 05/2021 and 10/2022 in a phase 1 placebo-controlled trial conducted in France and Switzerland (N° EudraCT: 2020-001814-40; NCT04842682). Volunteers were randomized (5:1 active versus placebo) in groups receiving either 0.3, 1.0, or 3.0 mg CD40.HIVRI.Env (Hiltonol® adjuvanted) alone or co-administered with DNA-HIV-PT123 at weeks (W) 0, 4, and 24. Safety and immunogenicity were monitored until W48. The primary safety endpoint was the proportion of participants per dose cohort and randomized arm without any grade 3 or 4 biological (abnormal laboratory values), or clinical local or systemic solicited, or unsolicited adverse events between W0 and W48 considered to be related or possibly related to the investigational products.

Findings CD40.HIVRI.Env was well tolerated. Env-specific $CD4^+$ T-cells ($IL\text{-}2^+$ or $IFN\text{-}\gamma^+$ or TNF^+) were detected in all vaccinees from W6 to W26 and persisted until W48 without a dose–response signal or an effect of DNA-HIV-PT123 co-administration. At W26, IgG response rates (RR) against autologous and nine heterologous gp120/gp140 were 89–100% across all groups and 56–100% at W48. RR against 96ZM651gp70V1V2 were high (90–100%) at W6 and W26 in all groups. Tier1A MW965.26 neutralizing antibody (nAb) titres were detectable in 50–100% of vaccinated individuals at W26, with a dose–response signal, while one volunteer developed nAbs against five Tier2 viruses.

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*Corresponding author. Vaccine Research Institute, Université Paris-Est Créteil, INSERM U955, Team Levy, Hôpital Henri Mondor, 51 Av Marechal de Lattre de Tassigny, 94010, Créteil, France.
E-mail address: yves.levy@aphp.fr (Y. Levy).
[p]Contributed equally.
[q]See the list of the ANRS VRI06 Study group at the Supplementary Material.

Interpretation CD40.HIVRI.Env alone or administered with DNA-HIV-PT123 was safe and induced early, and sustained anti-Env cellular and V1V2 IgG responses, identified as correlates of protection in the RV144 trial. CD40 targeting Env-based vaccines may be instrumental for inducing protective vaccine responses in prime-boost strategies.

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Keywords: HIV vaccines; Immunogenicity; DNA vaccine; Dendritic cell targeting; First-in-human clinical trial

Research in context

Evidence before this study

The development of an HIV vaccine remains a goal for achieving or at least controlling the HIV epidemic. Several HIV vaccine candidates have been tested in efficacy trials but only one, the RV144 trial, evaluating a prime-boost strategy combining a recombinant canarypox vector expressing HIV Env, Gag, and protease (ALVAC-vCP1521) and a bivalent recombinant monomeric gp120 protein (AIDSVAX® B/E) has shown partial protection and allowed for the assessment of correlates of protection. Because of the pathogenic effects of HIV, impacting immune functions, and the diversity of the virus and its escape from immune responses, such vaccine development remains a major challenge and complex vaccine strategies are likely be required. Among those, approaches for improved antigen delivery to the immune cells and/or combinations of different vaccine platforms may be of interest. A novel vaccine technology directing the HIV Env antigen to CD40-expressing antigen-presenting cells has been developed (CD40.HIVRI.Env vaccine) and previously tested in animal studies. We searched PubMed for articles published in English anytime through April 1, 2024, and terms combining ((HIV vaccine[MeSH Terms]) AND (clinical trial[MeSH Terms])) AND (dendritic cell targeting).

Added value of this study

The CD40.HIVRI.Env is an innovative sub-unit vaccine consisting in a fusion protein composed of a fully humanized IgG4 monoclonal antibody to human CD40 fused to the C terminal region of the heavy chain with a gp140 Env monomer from Clade C 96ZM651. In this first-in-human phase I dose-escalation we assessed the safety and immunogenicity of the CD40.HIVRI.Env vaccine with a TRL3 agonist adjuvant (i.e., Poly-ICLC, Hiltonol®) in 72 healthy volunteers. Three different dose levels of this vaccine were tested (0.3, 1.0, or 3.0 mg) and administered in three injections (W0, W4 and W24). We also tested combination strategy of this CD40 targeting vaccine co-administered with the DNA-HIV-PT123 vaccine expressing autologous HIV-1 clade C 96ZM651gp140 plus 96ZM651Gag and CN54PolNef. CD40.HIVRI.Env alone or administered with DNA-HIV-PT123 was safe and induced early, potent, broad, and durable anti-Env cellular and V1V2 IgG responses, identified as correlates of protection in the RV144 trial. Tier1A MW965.26 neutralizing antibody titres were detected in 50–100% of vaccinated individuals after three injections, with a dose–response signal, while one volunteer developed neutralizing antibodies against five Tier2 viruses.

Implications of all the available evidence

The CD40.HIVRI.Env vaccine has shown promising safety and immunogenicity results in this first-in-human trial. Standardisation of assay methods have allowed for comparison with public data from other vaccine trials (e.g., HVTN 096, HVTN 100, HVTN 105, RV144). The present trial is, to our knowledge, the largest first-in-human study to test the DC-targeting of antigens through the CD40 receptor. The results open the way for further testing of this novel platform and new possibilities for HIV Env vaccine delivery aiming to induce protective vaccine responses.

Introduction

Over the last four decades, the AIDS epidemic has been a global public health issue and has resulted in ~40 million deaths. Despite the increasing availability of prevention measures, including pre-exposure prophylaxis, 1.5 million new infections occurred in 2021.[1] The development of an HIV vaccine, even partially effective, remains a goal for achieving or at least controlling the HIV epidemic.

Challenges for the development of an HIV vaccine include the genetic diversity of HIV clades, especially via variability of the HIV envelope protein, the rapid establishment of a viral reservoir, and the lack of clear correlates of protection. A prime-boost strategy combining a recombinant canarypox vector expressing HIV Env, Gag, and protease (ALVAC-vCP1521) and a bivalent recombinant monomeric gp120 protein (AIDSVAX® B/E) was tested in the RV144 trial and showed partial protection, with a 31% reduction in HIV infection risk at 3.5 years.[2] Subsequent prophylactic HIV vaccine development focused on optimizing prime-boost strategies, combinations of DNA/viral vectors

with protein formulations. Subsequent efficacy trials have not reproduced RV144 results.[3] These disappointing results could be explained by differences between vaccine trials in terms of regimen, adjuvants, immunogens, vaccine delivery, indicating that better immunogen design and/or changes in the immunisation regimens must be explored for the development of efficacious HIV vaccines. Correlates of protection in the RV144 trial highlighted the importance of an antibody response that recognizes the V1V2 variable region of the HIV envelope glycoprotein, antibodies mediating antibody dependent cellular cytotoxicity,[4–6] and Env-specific $CD4^+$ T-cell responses.[7] Although the phase 2b/3 trials that followed the RV144 trial strategy were unsuccessful, they do not rule out the role of anti-env gp70 V1V2 antibodies as a marker of reduced risk of infection.[5,8] The highest rate of protection in the RV144 trial was observed at one year (60% risk of infection) post-prime and waned during the follow-up, associated with a drop in the levels of anti-Env V1V2-binding antibodies,[2] underscoring the need to improve the durability of such protective responses.[9]

Targeting vaccine antigens to dendritic cells (DCs), key professional antigen-presenting cells (APCs), via surface receptors is an appealing strategy to improve subunit-vaccine efficacy.[10] DCs orchestrate immune responses, directing cellular and humoral Ag-specific T- and B-cell responses to the pathogen. Direct antigen delivery, capable of activating cell receptors, may trigger a danger signal, stimulating an immune response.[11] Among the various DC receptors tested, we have reported the superiority of targeting viral antigens to CD40-expressing APCs in several animal models.[12–15] CD40 is a potent activating TNFR superfamily member expressed on APCs and B cells, and is intricately involved in promulgating effective antigen-specific T and B cell responses.

The CD40.HIVRI.Env vaccine is a fusion protein composed of a fully humanized IgG4 monoclonal antibody to human CD40 fused to the C terminal region of the heavy chain with a gp140 Env monomer from Clade C 96ZM651. Here, we report the results of a first-in-human dose-escalation study to assess the safety and immunogenicity of the CD40.HIVRI.Env vaccine with a TRL3 agonist adjuvant (i.e., Poly-ICLC, Hiltonol®)[16] in healthy volunteers. Building on studies showing improved immunogenicity with combined DNA vaccines and adjuvanted protein regimens,[17–19] we also tested a novel immunisation strategy combining a CD40 targeting vaccine co-administered with the DNA-HIV-PT123 vaccine expressing autologous HIV-1 clade C 96ZM651gp140 plus 96ZM651Gag and CN54PolNef.

Methods

Study design and participants

This trial (N° EudraCT: 2020-001814-40; NCT04842682) is a placebo-controlled, multicentre dose-escalation trial. Seventy-two HIV-negative volunteers, aged 18–65 years and at low risk of infection, were enrolled. CD40.HIVRI.Env vaccine (0.3, 1.0, or 3.0 mg) adjuvanted with TLR3 agonist Poly-ICLC (Hiltonol®) (1.0 mg) was either administered alone or co-administered with DNA-HIV-PT123 vaccine (4.0 mg) at weeks (W) 0, 4, and 24. Twelve volunteers were randomized (5:1) per cohort to the vaccination or placebo arms. Detailed eligibility criteria, sequential enrolment, pausing rules for safety, safety assessments throughout the follow-up, and full details of the protocol are provided in the appendix (Supplementary Methods). Blood samples for immunogenicity assessments were collected at each visit and frozen until use (Fig. 1).

The trial was approved by the French and Swiss National Competent Authorities (ANSM and Swissmedic) and Ethics Committees (CPP Sud-Ouest et Outre-Mer 4 for French sites, and CER-VD for the Swiss site). All trial participants provided written informed consent.

Randomisation and masking

The randomized and blinded control group was mainly implemented for unbiased adverse event reporting in this trial, i.e., by blinding participants and clinicians to receipt of active vaccine versus placebo, and did not aim at achieving specific statistical operating characteristics in the placebo arm. The ratio of 5:1 was defined in order to minimize cost of the trial by keeping the number of placebo participants low; and to have pooled placebo sample size roughly similar to each active vaccine group for the description of immune responses.

The randomisation sequence, using stratification by cohort and a block size of six, was computer generated using SAS software (version 9.4) by an unblinded statistician and implemented in a validated web-based randomisation tool (Ennov Clinical software). Participants were randomised at their first vaccination visit (W0). Upon randomisation, the Ennov Clinical software allocated the blinded treatment number via the electronic case report form (eCRF). The correspondence list was made available at the pharmacy of each site. Unblinded pharmacists prepared and dispensed the masked vaccine syringes. All other site staff and participants were blinded to the treatment assignment.

Vaccine candidates

CD40.HIVRI.Env was manufactured by Novasep-Henogen SA (Seneffe, Belgium) on behalf of VRI and ANRS MIE. The adjuvant Poly-ICLC (Hiltonol®), manufactured by Dalton Pharma Services (Toronto, Canada) on behalf of and supplied by Oncovir Inc. (Washington DC, USA), is a highly stable synthetic double-stranded RNA known for its potent interferon-inducing properties. It has a long history of safe clinical use. Administered at a dose of 1.0 mg, it was mixed

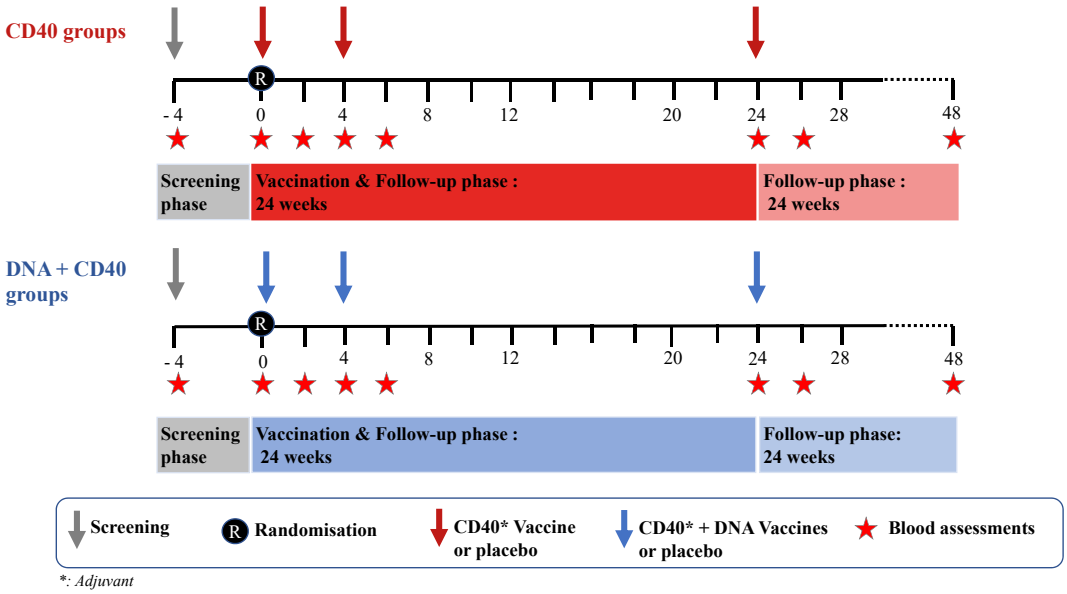


Fig. 1: **Overview of the ANRS VRI06 trial design**.

with CD40.HIVRI.Env immediately before subcutaneous injection into the right arm. CD40.HIVRI.Env was diluted using commercial 0.9% NaCl, which also served as the placebo. DNA-HIV-PT123, manufactured by Ajinomoto Bio-Pharma Services (San Diego, USA) on behalf of and supplied by the EuroVacc Foundation (Lausanne, Switzerland), is an equi-mass mixture of three recombinant plasmids expressing clade C 96ZM651gp140, 96ZM651Gag, and 97CN54Pol-Nef. It was intramuscularly administered at 4.0 mg into the left arm.

Immune response assays

All assays were performed in centralized laboratories on cryopreserved peripheral blood mononuclear cells (PBMCs) and serum collected at baseline (W0), W6, W26 (2 weeks after the second and third injections), and W48.

HIV-specific T-cell responses

To evaluate antigen-specific T-cell responses, an intracellular cytokine staining (ICS) assay was conducted on cryopreserved peripheral blood mononuclear cells (PBMCs). The PBMCs were rested for 3 h before being stimulated overnight (37 °C, 5% CO2) with a panel of overlapping HIV peptides (15-mers with an 11 amino-acid overlap, total n = 532). This stimulation was carried out in the presence of anti-CD28 and anti-CD49d antibodies (1 µg/mL each) and Golgi Plug (10 µg/mL) (BD Biosciences, Le Pont de Claix, France). Unstimulated cells served as the negative control. Post-stimulation, cells were stained for dead cells using an amine-reactive dye (LIVE/DEAD Aqua, Invitrogen, Life Technologies, Saint Aubin, France) for 20 min at room temperature. As an exploratory post-hoc experiment, we performed an enrichment technique of HIV-specific T cells using PBMC collected at W0 and W6 in the CD40 0.3 mg group (n = 12) using an eight-day amplification period using the same pool of peptides as previously described.[20,21] The flow cytometry panel included a viability marker, CD3, CD4, and CD8 for T-cell lineage determination and antibodies against IFN-γ, IL-2, and TNF. For the eight-day amplification experiments, we used the same flow cytometry panel, with the addition of the MIP-1b cytokine. Data were acquired on an LSR Fortessa 4-laser (488, 640, 561 and 405 nm) cytometer (BD Biosciences) and analysed using FlowJo software version 9.9.6 (Tree Star Inc.)

The ICS assay targeted a panel of overlapping HIV peptides (15-mers with an 11 amino-acid overlap, totalling n = 532). These peptides covered sequences from the Env gp140 Clade C 96ZM651 protein and Gag 96ZM651 and Pol-Nef 97 CN54 proteins. The peptides were organized into nine pools: Env1, Env2, Env3, Gag1, Gag2/Pol, Pol1, Pol2, Gag/Pol, and Nef. Total Env-specific responses were determined by summing the Env1, Env2, and Env3 responses and Gag/Pol and Pol responses by summing the Gag1, Gag2/Pol and Gag/Pol pool and Pol1 and Pol2 pool responses, respectively.

Binding antibody multiplex assay (BAMA)

Serum binding antibody reactivity to HIV-1 envelope gp120, gp140, and gp70-V1V2 breadth panel antigens was determined by BAMA as previously described.[22] Recombinant

envelope and V1V2 peptides and the providers are described in the appendix (Supplementary Table S1). Serum samples were diluted at 1/20 and 1/80 for IgG and at 1/20 for IgG3 and incubated with carboxylated fluorescent bead sets (Bio-Rad) covalently coupled to Env proteins. The BAMA assay and the definitions of responses are described in the appendix (Supplementary Methods).

Neutralizing antibodies

The conventional TZM-Bl neutralisation assay was used as previously described.[23] Information on the pseudoviruses and primary isolates used and the definitions of responses are available in the appendix (Supplementary Table S1 and Supplementary Methods).

Outcomes

The primary safety endpoint was the proportion of participants per dose cohort and randomized arm without any grade 3 or 4 biological (abnormal laboratory values), or clinical local or systemic solicited, or unsolicited adverse events (AEs) between W0 and W48 considered to be related or possibly related to the investigational products. AEs were graded using the FDA grading scale.[24] Secondary safety endpoints included the number and proportion of AEs and serious AEs at various time points after vaccination. The secondary objective was to assess the immunogenicity of the vaccines two weeks after the second and third injections (W6, W26), and at W48.

Statistical analysis

The fixed sample size of 10 per active group in this trial was determined based on the ability to detect AEs with a given power and sample size, and not on formal hypothesis testing of an expected effect size. This is a common choice in phase I dose-escalation vaccine trials, which usually have sample sizes between 10 and 15 per active group, and with little gain in statistical efficiency when increasing the sample size beyond 10.[25,26] Ten participants per active group at the final analysis has 90% power to observe at least one grade 3 or 4 biological, or clinical local or systemic solicited, or unsolicited adverse events AE related or possibly related to vaccination, assuming the true underlying event rate is at least 21%.

Statistical analyses were performed per dose cohort and randomized arm and for the pooled placebo arms. The trial was not powered for direct comparisons between groups.

The main analyses were conducted as treated, i.e., the participants having received at least one injection of a given vaccine strategy were included in the as-treated analyses according to the true vaccine strategy received, on available data. Given the absence of major protocol errors in this trial, this corresponds to analysing the participants according to their assigned group and randomized arm.

Proportions of participants with safety endpoints are described and detailed listings of relevant endpoints provided. The distribution of antibody titres, as well as the proportion of responders with their two-sided 95% confidence interval, are described.

The total cytokine response among $CD4^+$ T-cells assessed by ICS was compared between each post-vaccination visit and W0 using a bivariate linear regression model for intra-arm comparison with an alpha risk of 5%.[27] This was the only planned statistical significance test in the protocol and statistical analysis plan. Exploratory correlation analyses between various immune response markers at W26 were performed using Spearman correlations and false discovery rate (FDR) adjustment.

All analyses were performed using SAS software (version 9.4), R (version 4.2.2) and LabKey.

Role of the funding source

Inserm-ANRS MIE was the legal sponsor of the study and funder, in addition to the Vaccine Research Institute (with funds from ANR, reference ANR-10-LABX-77-01). EuroVacc provided the DNA-HIV-PT123 vaccine and Oncovir the Hiltonol®. VRI, EuroVacc, and ANRS MIE representatives were members of the Trial Steering Committee and, as such, were involved in the study design, overview of the study conduct and analyses, writing of the report, and the decision to submit the manuscript for publication.

Results

Seventy-two participants were enrolled between 05/2021 and 10/2022. Forty-three (59.7%) were assigned the male sex at birth. The median (IQR) age was 29.5 years (interquartile range 24; 40.3) (Supplementary Table S2). All volunteers received at least one vaccine or placebo injection and 67 received the three planned injections. Three volunteers did not complete the follow-up until W48 (Fig. 2A and B).

Overall, the vaccinations were safe and well tolerated. All volunteers assigned to the vaccine or placebo groups experienced at least one AE (Supplementary Table S3). Most AE were vaccine-related, mild to moderate in severity, and associated with systemic and local reactogenicity (Fig. 3). Twenty symptoms were classified as grade 3 related to vaccination (no grade 4 events were observed) in 13 volunteers from the different groups, with a duration of less than seven days for the majority of them (Supplementary Table S4). There was no evidence of a dose-effect or increasing reactogenicity with repeated injections. The severity of AEs did not vary between CD40.HIVRI.Env (CD40) only and co-administration groups. Local reactogenicity consisted of injection site reactions. The most frequently reported systemic reactogenicity symptoms were fatigue, headache, and myalgia. One reactogenicity event at the

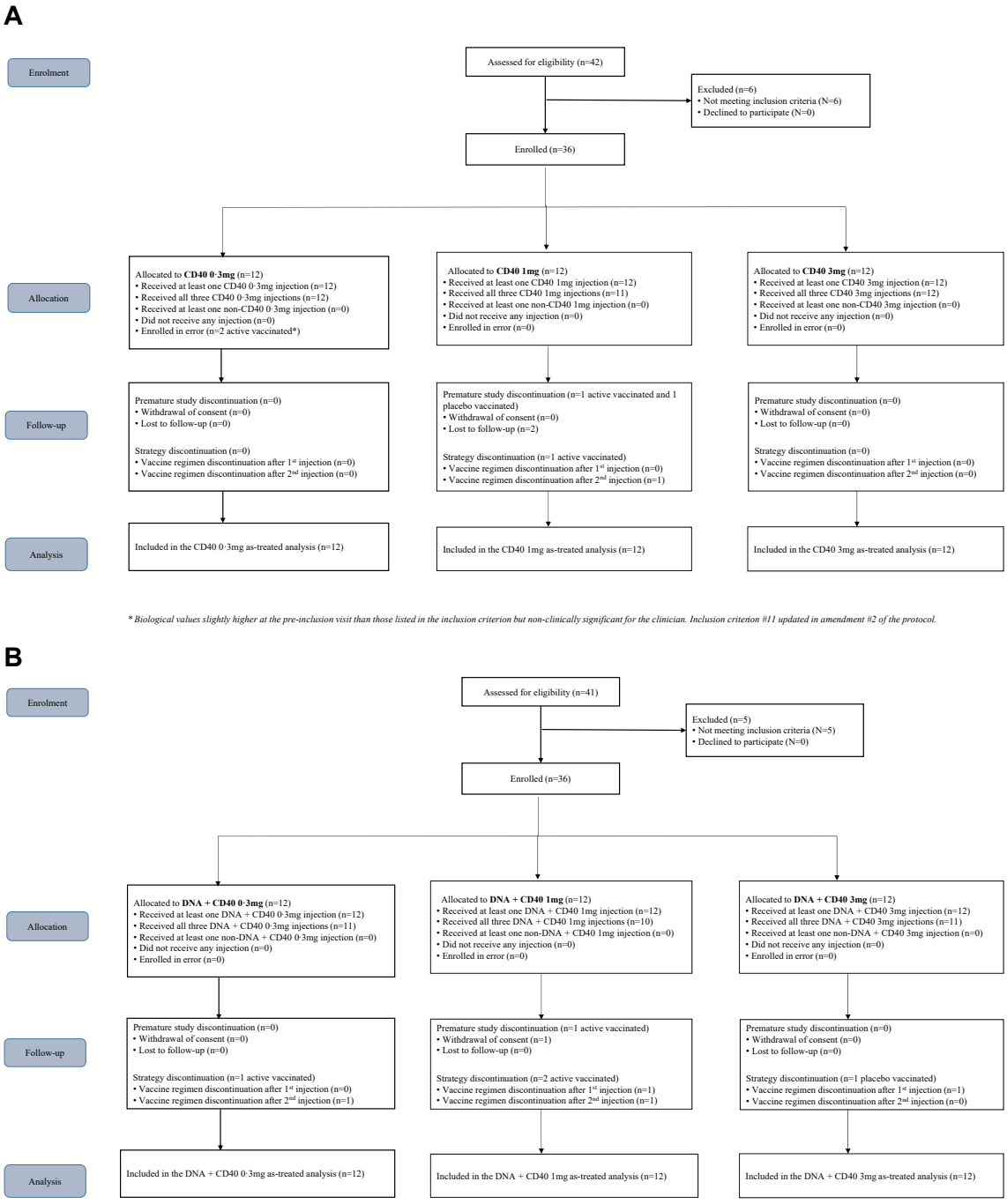
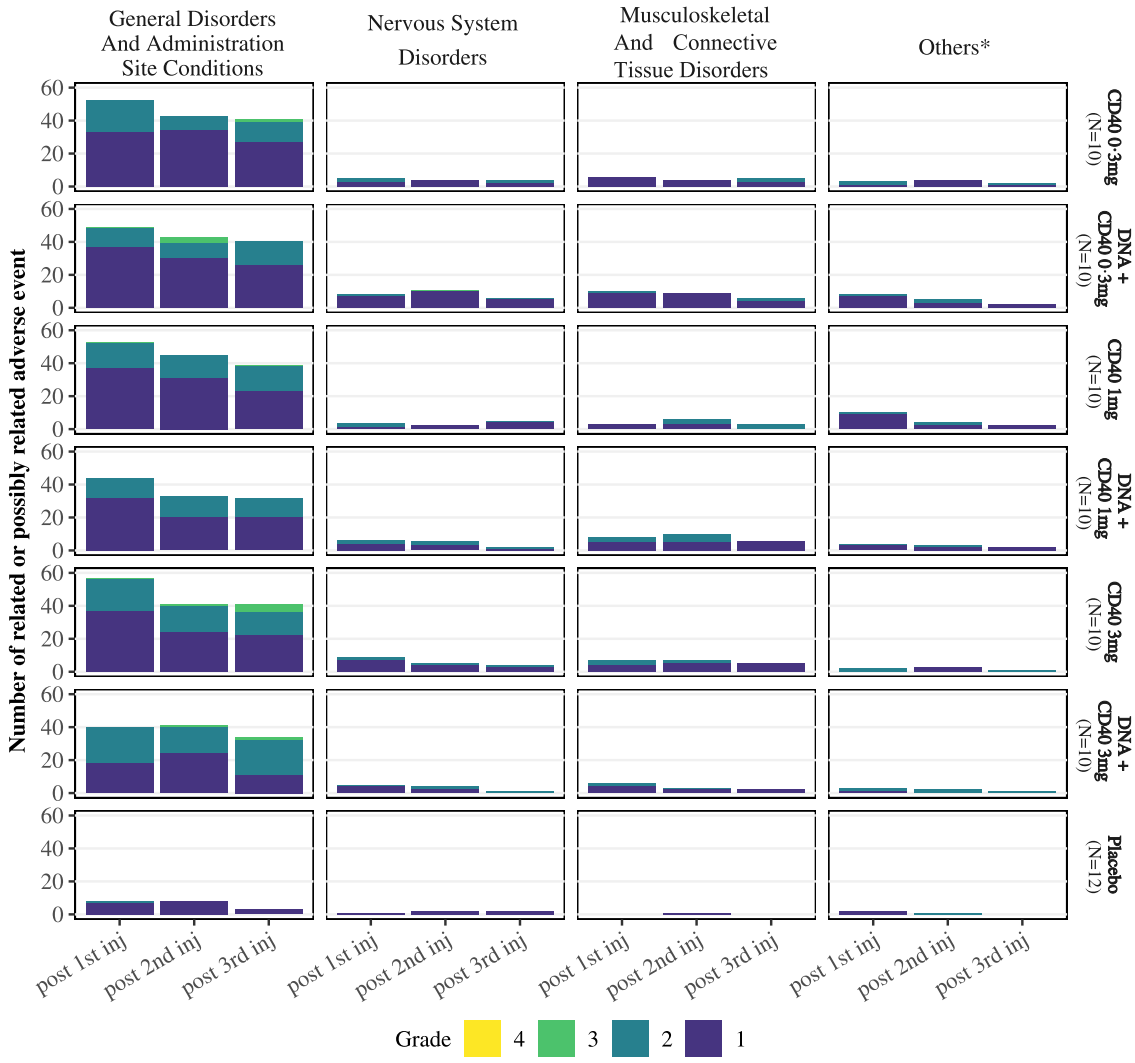


Fig. 2: **Flowchart diagram of participant enrolment and follow up for the CD40 groups (A) and DNA + CD40 groups (B).**

injection site of CD40 led to discontinuation of the vaccine injections after the second injection in the DNA + CD40 0.3 mg group; all other discontinuations were related to other reasons (Fig. 2A and B). Two SAEs were reported for participants enrolled in the CD40 groups, both judged to be unrelated to the vaccination. No SAEs related to the vaccines were reported. Several AEs with serum lipase or CPK increases were thoroughly investigated and considered to be unrelated to vaccination.



* *Others : Skin and subcutaneous tissue disorders, respiratory thoracic and mediastinal disorders, reproductive system and breast disorders, cardiac disorders, metabolism and nutrition disorders, eye disorders, blood and lymphatic system disorders, gastrointestinal disorders, psychiatric disorders, investigations.*

***Fig. 3*: Adverse events related to vaccination by grade, group, and sequence of injection.**

Cellular immunogenicity

The median (IQR) frequencies of Env-specific $CD4^+$ T-cell responses (cells producing up to three cytokines (IFN-γ ± IL-2 ± TNF)) were 0.22 (0.16; 0.38), 0.33 (0.21; 0.38), and 0.5 (0.3; 0.57)% for the CD40 0.3, 1.0, and 3.0 mg groups, respectively at W6. Similarly, the frequencies were 0.32 (0.26; 0.65), 0.36 (0.23; 0.54), and 0.31 (0.17; 0.47)% for the DNA + CD40 0.3, 1.0, and 3.0 mg groups, respectively. At W26, the frequencies of specific $CD4^+$ T-cell responses remained consistent with those observed at W6 for all groups (Fig. 4A).

The magnitude of Env-specific $CD4^+$ T-cell responses increased from W0 to W6 in all vaccinated individuals ($P \leq 0.005$ for all W6/W0 comparisons for the CD40 groups and P = 0.24, 0.002, and <0.0001 for the DNA + CD40 0.3, 1.0, and 3.0 mg groups, respectively). At W26 as well, the frequency of Env-specific $CD4^+$ T-cells showed a significant difference compared to baseline (P = 0.03, <0.0001, and <0.0001 for the CD40 0.3, 1.0, and 3.0 mg groups, respectively, and P = 0.02, 0.002, and <0.0001 for the DNA + CD40 0.3, 1.0, and 3.0 mg groups, respectively). Analyses at W48, six months following the third injection, indicated sustained and statistically significant $CD4^+$ T-cell responses (for the W0/W48 comparisons) in all groups, except DNA + CD40 0.3 mg (Supplementary Figure S1). Analysis of the polyfunctionality of Env-specific $CD4^+$ T-cells revealed a high frequency of cells producing three cytokines (i.e., IFN-γ/IL-2, and TNF) in all six groups

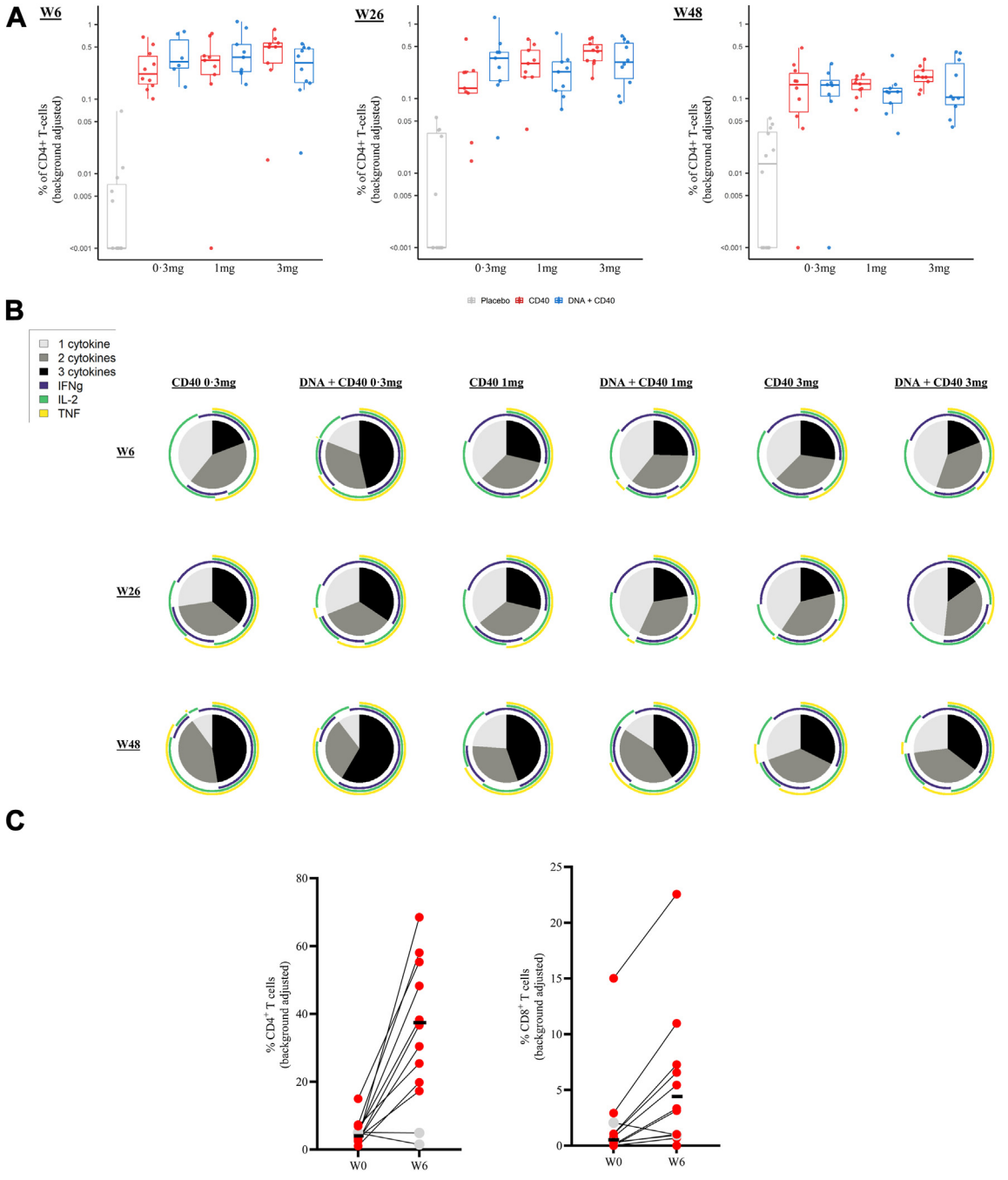


***Fig. 4:* Env-specific CD4⁺ T-cell response**. **(A)** Percentage of CD4⁺ T-cells expressing IL-2 and/or IFN-γ and/or TNF at W6, W26, and W48 for the CD40 groups (in red), DNA + CD40 groups (in blue), and Placebo (in grey). Boxplots with the median, interquartile range (IQR) and 1.5 times IQR. **(B)** Functional composition of CD4⁺ T-cell responses at W6, W26, and W48 for the CD40 and DNA + CD40 groups. Responses are color-coded according to the combination of cytokines produced. The arcs identify cytokine-producing subsets (IFN-γ, IL-2, and TNF) within the CD4⁺ T-cell population. **(C)** Percentage of CD4+ (left) and CD8+ (right) T-cells expressing IL-2 and/or IFN-γ and/or TNF at W0 and W6 for the CD40 0.3 mg group (active vaccinees in red, n = 10; and placebo in grey, n = 2) following in vitro stimulation at D0 and re-stimulation at D8 with Env peptide pools (exploratory post-hoc experiment). Each dot represents a participant and the median is shown as a solid line.

from W6 to W26, and this pattern persisted at W48 (Fig. 4B et Supplementary Figure S2).

There were no measurable ex vivo CD8$^+$ T-cell responses observed among the participants. In addition, individuals enrolled in the DNA + CD40 groups did not exhibit any responses (CD4 or CD8) against the Gag, Pol, or Nef peptide pools. As an exploratory post-hoc experiment, we assessed the detectability of Env-specific memory T cells after in vitro expansion of antigen-specific T cells at baseline (W0) and week 6 (W6) in the participants (n = 12) from the CD40 0.3 mg group. We detected the expansion of Env-specific CD4+ T cells with median frequencies of 4% (2.6; 7) at W0 and 37.5% (24; 56) at W6 (Fig. 4C, left). Using this enrichment technique, we revealed the expansion of Env-specific CD8+ T cells at W6, with a frequency of 4.4% (0.8; 8.2) compared to 0.5% (0.07; 1.5) at W0 (Fig. 4C, right).

Humoral immunogenicity

We observed high IgG binding response rates (RRs) and magnitudes to autologous gp140 Env antigens (96ZM651gp140) and heterologous clades B, C, BC, AE, consensus AE, and M for all vaccine groups (Fig. 5, Supplementary Figure S3, and Supplementary Table S5), with 80–100% responders at W6 and W26, regardless of the dose. The highest Mean Fluorescence Intensity (MFI) values were observed at W26 for all antigens tested and were consistent across the CD40 and DNA + CD40 groups (Fig. 5). For example, median MFI values for the CD40 and DNA + CD40 3 mg groups were >20,000 for 96ZM651gp140, CON-Sgp140 (consensus M), CN54gp140 (clade B/C), and 1086Cgp140 (clade C).

Although we observed a decrease in MFI values for all antigens tested at W48, they remained high (median MFI >4500 for 96ZM651gp140, with a rate of responders of 89–100%). Similarly, 70–100% responders to heterologous Env antigens were observed, regardless of the dose or regimen tested, except for the DNA + CD40 1.0 mg group, for which the frequency of responders was <70% for some heterologous gp120/gp140 antigens (Supplementary Table S5 and Supplementary Figure S3).

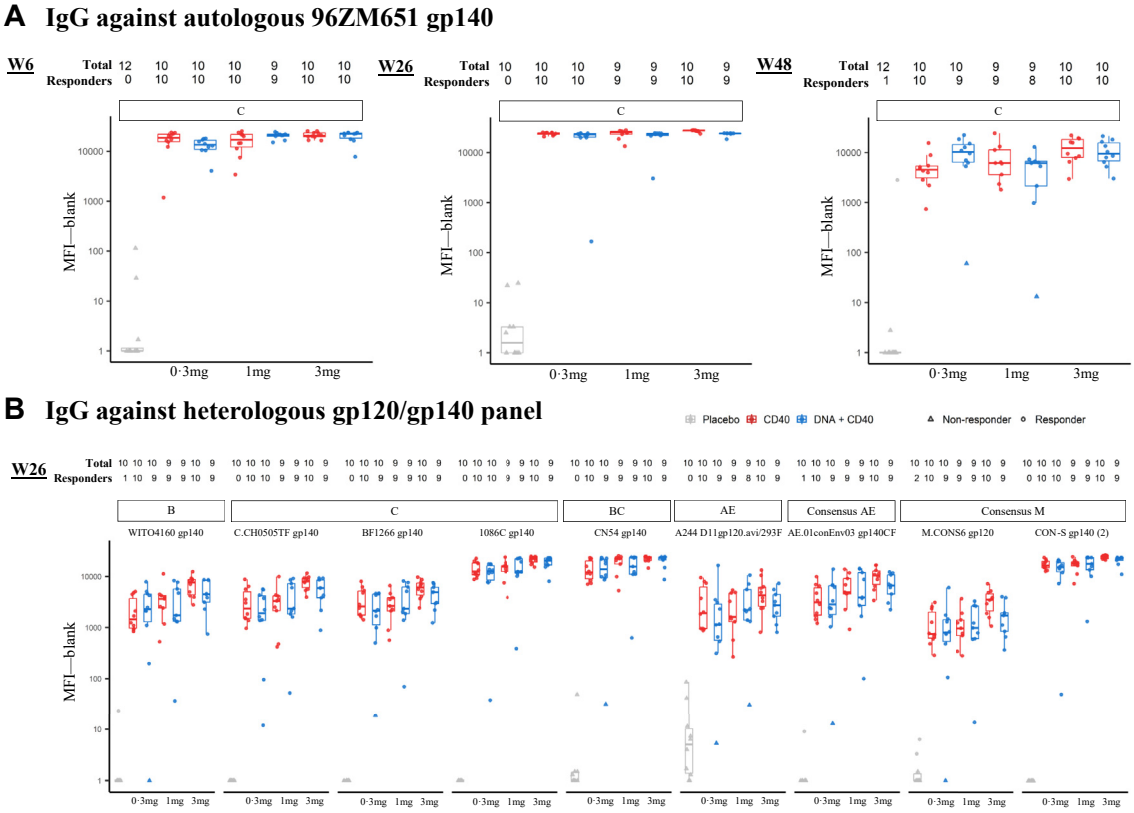


Fig. 5: **gp120/gp140 antibody response**. **(A)** IgG responses as the background subtracted mean fluorescence intensity (MFI—blank) for CD40 groups (in red), DNA + CD40 groups (in blue), and Placebo (in grey) are described with boxplots against autologous gp140 antigens at W6, W26 and W48. **(B)** and a heterologous gp120/gp140 panel at W26. Response rates and relevant clades are summarized above each plot. Non-responders, defined by a post-vaccination MFI-blank < antigen/isotype specific MFI-blank threshold based on the 95th percentile of the MFI-blank at W0 or MFI-blank ≤3 × MFI-blank at W0, are shown as triangles. Boxplots are shown with the median, interquartile range (IQR), and 1.5 times the IQR.

IgG responses and RRs against autologous 96ZM651gp70V1V2 were high at W6 and W26 in the CD40 groups, regardless of the dose and co-administration of the DNA vaccine (Fig. 6A). The median MFI at different time points are reported in Supplementary Table S6. At W26, the RR was 90–100% for all vaccine groups. The RR against heterologous V1V2 ranged from 50 to 100% (92TH02gp70V1V2 and CaseA2gp70V1V2HIS6) and 70–100% (CE1086-B2-V1V2gp70), with overall MFI values 1–2 $logs_{10}$ lower than for autologous 96ZM651gp70V1V2 (Supplementary Figure S4A and Supplementary Table S6). By W48, IgG responses against autologous and heterologous V1V2 antigens decreased markedly for all vaccine groups, with a maximum RR of 60% against 96ZM651gp70V1V2 (Fig. 6A, Supplementary Figure S4A, and Supplementary Table S6).

IgG3 responses were shown to be associated with a reduced risk of HIV infection in the RV144 trial.[9] As expected, the magnitude of IgG3 responses was lower than that of total IgG responses against all V1V2 antigens tested (Fig. 6B, Supplementary Figure S4B, and Supplementary Table S6). The RR to autologous 96ZM651gp70V1V2 was the highest at W6 (80–100%) and consistent for both vaccine regimens, regardless of the dose. The median MFI and RR were lower at W26 and did not persist at W48. Concerning heterologous V1V2 antigens, both the magnitude and rate of IgG3 responders were generally low and showed a decrease over time. The RRs were similar for the CD40 and DNA + CD40 groups. In the 3.0 mg group, the RRs were 40–60% (CaseA2gp70V1V2HIS6), 50–60% (CE1086-B2-V1V2gp70), and 60–80% (92TH02gp70V1V2) at W6. The frequency of responders diminished after the third injection and no responders were observed at W48 (Supplementary Figure S4B). Serum HIV-1 Env-specific IgA responses, linked to an increased risk of HIV acquisition in the RV144 trial,[5] were low against autologous 96ZM651 Env, with a mean MFI 10-fold lower than that for specific IgG binding antibodies at W26 for CD40 and CD40+DNA 3.0 mg groups (Supplementary Figure S5).

Neutralisation ID50 titres against Env-pseudotyped viruses that exhibited a Tier1A neutralisation phenotype (clade C: MW965.26) were detectable in approximately 50% of volunteers in the CD40 and DNA + CD40 1.0 and 3.0 mg groups at W6 (Supplementary Figure S6A). The RRs and magnitudes (median ID50)

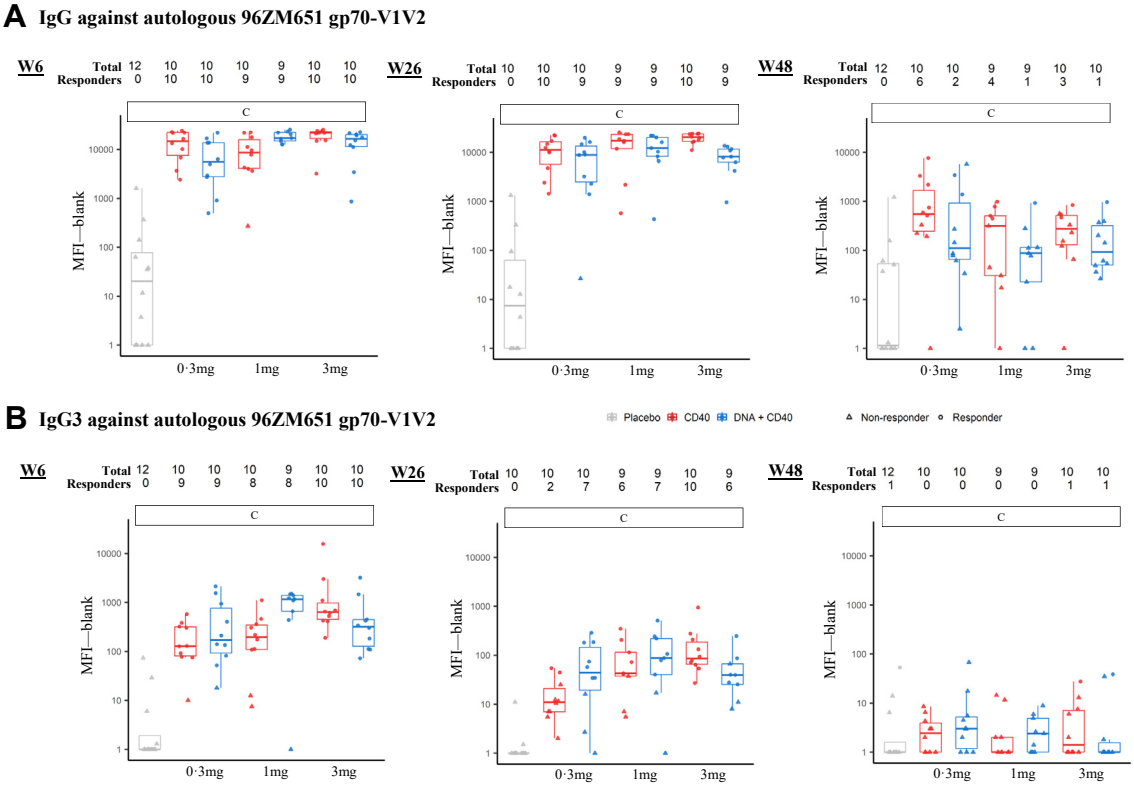


Fig. 6: **V1V2 antibody response**. **(A)** IgG responses as the background subtracted mean fluorescence intensity (MFI—blank) for CD40 groups (in red), DNA + CD40 groups (in blue), and Placebo (in grey) are described with boxplots against autologous gp70 antigens at W6, W26, and W48. **(B)** and the same with IgG3. Response rates and relevant clades are summarized above each plot. Non-responders, defined by a post-vaccination MFI-blank < antigen/isotype specific MFI-blank threshold based on the 95th percentile of the MFI-blank at W0 or MFI-blank ≤3 × MFI-blank at W0, are shown as triangles. Boxplots are shown with the median, interquartile range (IQR), and 1.5 times the IQR.

peaked at W26 for both vaccine regimens (Supplementary Table S7 and Fig. 7A) with RRs for Tier1A (clade C: MW965.26) of 50% (67), 100% (315), and 100% (516) for the 0.3, 1.0, and 3.0 mg CD40 groups, respectively. The RRs for the DNA + CD40 groups were 90% (123), 89% (201), and 100% (313) in the 0.3, 1.0, and 3.0 mg groups, respectively. At this time point, neutralisation against the subtype B virus SF162 was also detected, although with a lower intensity and only in 1.0 mg and 3.0 mg groups (Fig. 7B). At W48, the frequency of responders was ≥50% in the CD40 (3.0 mg) and DNA + CD40 (0.3 and 3.0 mg) groups for Tier1A MW965.26 (Supplementary Figure S6A and Supplementary Table S7). No nAb responses were detectable in the placebo recipients (Fig. 7AB, Supplementary Figure S6 and Supplementary Table S7). We did not detect neutralizing activity against the autologous 96ZM651.2 Tier2 strain at any time points for any group (data not shown).

We further screened for the induction of additional neutralizing activity at W26 in the CD40 and DNA + CD40 3.0 mg groups, which demonstrated the highest neutralizing activity against the two Tier1A strains tested. We detected high neutralizing titres (>60) against another Tier1A virus, sC22 in 5 and 10 CD40 3.0 mg and DNA + CD40 3.0 mg vaccinees, respectively (Supplementary Figure S7 and Supplementary Table S8).

We also tested the response against Tier1B and Tier2 pseudoviruses from subtypes B, C, and CRF07 and primary isolates from subtypes C, D, and E (Supplementary Table S1). We observed weak neutralizing response against seven (two Tier1B and five Tier2) of the 14 viruses tested for one volunteer (volunteer F9) of the DNA + CD40 3.0 mg group at W26 (Supplementary Table S8). No activity was detected against the control MLV pseudovirus (Supplementary Table S8). Neutralizing activity was absent at baseline and W6. However, activity detected at W26 persisted until W48 for two of three tested viruses, suggesting that the activity was induced by repeated vaccine injections (Supplementary Figure S8).

We tested the response against the mutated virus sC22 KIKO (Tier1A pseudovirus with glycan site KIKO mutations), which increases the accessibility to CD4 binding site (bs), and observed an increase in overall neutralizing activity, indicating that antibodies bound to the CD4 bs conformational epitope on Tier1A strains (Supplementary Figure S7 and Supplementary Table S8). Of note, for volunteer F9, who showed Tier2 neutralizing activity, the potency of neutralisation was only slightly increased with the mutated sC22 KIKO version and Tier2 mutated viruses (426c TM4 mutated at position S278R.G471S.N460D.N463D) (Supplementary Table S8), further suggesting that the neutralizing activity observed against these viruses was, at least partially, attributable to antibodies directed against another conserved epitopes.[28]

Comparative analyses with other HIV vaccine prophylactic trials

A comparison of the humoral responses to vaccination in this trial with those of other recent HIV vaccine trials

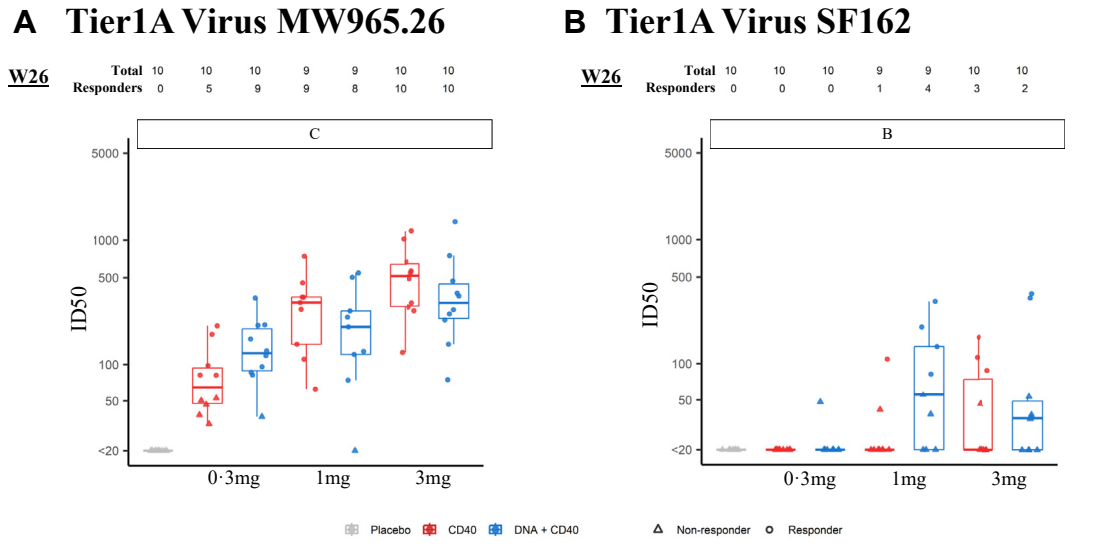


Fig. 7: **Neutralizing antibody activity. (A)** Neutralizing antibody response as the ID50 for the CD40 groups (in red), DNA + CD40 groups (in blue), and Placebo (in grey) are described with boxplots for Tier1A virus MW965.26. **(B)** and Tier1A virus SF162 at W26. Response rates and relevant clades are summarized above each plot. Non-responders, defined as those with an ID50 < 60, are shown as triangles. Boxplots are shown with the median, interquartile range (IQR), and 1.5 times the IQR.

is shown in Fig. 8. At W26, ANRS VRI06 exhibited homogeneous IgG responses to ConS gp140 (Fig. 8B) and autologous gp120/gp140 (Fig. 8A), and V1V2 (Fig. 8C) sequences, at least similar or above those from previous HIV prophylactic trials HVTN 096,[18] HVTN 105,[19] HVTN 100,[28] and RV144.[9] A similar profile of neutralizing antibody titres against MW965.26 was observed in the ANRS VRI06 trial, with regards to previous trials, in particular HVTN 100 (Fig. 8D).

Correlations between immune response markers

Exploratory correlation analyses at W26, combining all groups, showed significant positive correlations not only within specific assay types and immune response branches but also between $CD4^+$ T-cell responses, Tier1A neutralizing responses, and binding antibodies (Fig. 9).

Discussion

The ANRS VRI06 trial evaluated the safety and immunogenicity of the first-in-human administration of CD40.HIVRI.Env an adjuvanted DC-targeting HIV vaccine. The vaccine was safe and elicited robust and durable humoral and cellular immunogenicity, regardless of the tested dose, whether administered alone or in combination with the DNA-HIV-PT123 vaccine.

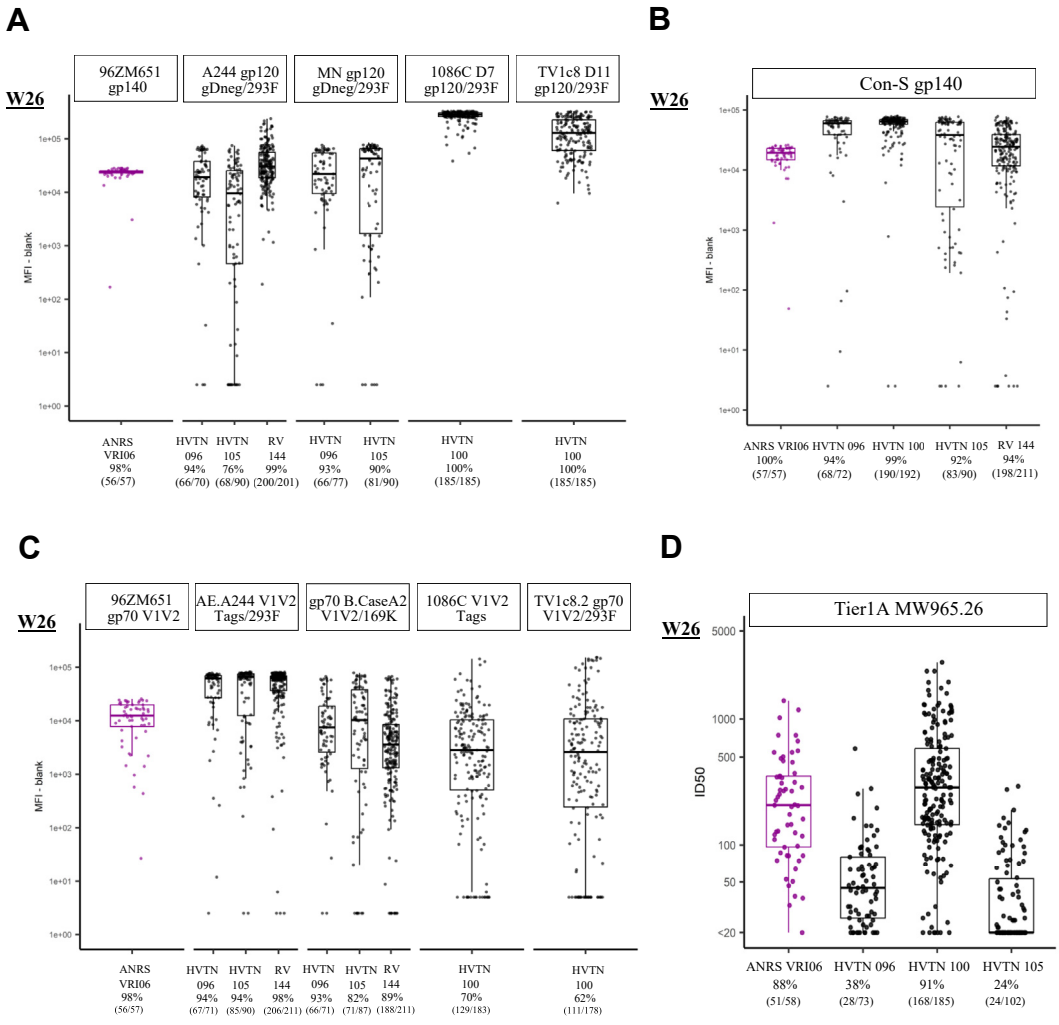


Fig. 8: **Cross-trial comparison of antibody responses**. **(A)** W26 IgG responses against autologous ENV, **(B)** heterologous ENV, **(C)** and autologous V1V2 antigens for the HVTN 096, HVTN 100, HVTN 105, and RV144 trials are shown to compare the ANRS VRI06 results with those of contemporary HIV vaccine trials. All data from other studies were modified by cross-multiplication of dilution factors to the 1:20 dilution factor used in the ANRS VRI06 trial to ensure comparability. **(D)** The neutralizing antibody response against Tier1A virus MW965.26 at W26 for the HVTN 096, HVTN 100, and HVTN 105 trials is provided to compare the ANRS VRI06 results with those of contemporary HIV vaccine trials. Response rates are summarised below each plot. Boxplots with median, interquartile range (IQR) and 1.5 times IQR.

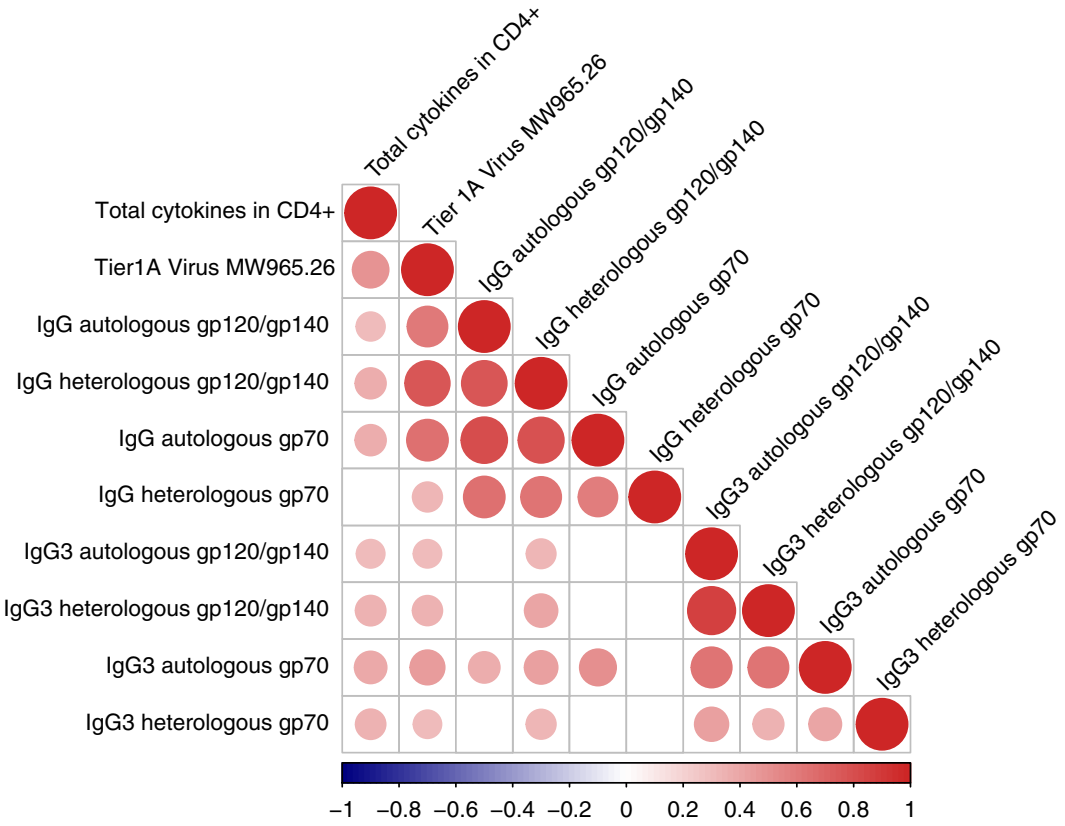


***Fig. 9:* Correlations between immune response markers at W26.** Multiparametric matrix correlation plot of immune responses between assays at W26 in the CD40 and DNA + CD40 groups (pooled). Spearman's correlation coefficients are shown by colour intensity. For heterologous multiple sequences, the mean response across sequences was used in this analysis. Only statistically significant correlations, after FDR-adjustment for test multiplicity, are shown.

CD40.HIVRI.Env vaccine and adjuvant were mixed prior to administration, which made it impossible to distinguish the reactogenicity of each product. However, the immunisations were globally safe and well tolerated, with most AEs classified as mild or moderate, without indication of a an effect due to dose or repetition of the CD40.HIVRI.Env injection or co-administration with DNA-HIV-PT123. This is an important step for the future clinical development of CD40-targeting vaccines against other pathogens. The anti-CD40 12E12 mAb clone selected as the common backbone for the anti-CD40 vaccine platform differs significantly from other therapeutic anti-CD40 mAbs developed in cancer-adjuvant settings by several aspects. The 12E12 clone is fully humanized at the VH/VL regions and acts as a partial agonist of CD40, thus preventing bystander immune activation.

Here, we tested the hypothesis of targeting the HIV Env antigen directly to DCs for the induction of a robust immune response. This hypothesis was driven by the results of previous preclinical studies showing advantages of targeting antigens, with stronger induction of both T- and B-cell responses than with non-targeted antigens[12–15] and protective anti-viral efficacy in various preclinical models.[13,29] Here, the lowest dose of the CD40 vaccine (equivalent to less than 0.15 mg of Env protein) was immunogenic. A dose–response signal was apparent in terms of neutralizing antibody responses. Although the trial was not designed for a direct comparison between regimens, the co-administration DNA + CD40 did not appear to confer higher immunogenicity than CD40 targeting alone, thus supporting the robust immunogenicity of the DC-targeting approach.

The DNA + CD40 regimen tested was designed based on previous vaccine trials performed by our group and others showing that vaccine induction of immune responses can be influenced by the sequential or co-administration of immunogens.[17–19] In particular, in the HVTN 105 and 111 studies, the co-administration of DNA-HIV-PT123 with Env proteins was considered to be superior to the prime-boost regimen in terms of HIV Env gp120 binding IgG responses.[19,30] Moreover, the HVTN 096 study demonstrated the earlier induction of antibody responses in a co-administration regimen with DNA-HIV-PT123 plus protein. The reason for the

absence of an improvement of immune responses for DNA + CD40 recipients versus those for CD40 alone is not clear. The CD40.HIVRI.Env vaccine was combined with Hiltonol®, a TLR3 agonist.[16] This approach was based on previous preclinical and clinical studies demonstrating the adjuvant's good safety profile and its ability to strongly induce T-cell and humoral responses when administered alone or with vaccines, including CD40-targeting vaccines.[12,14–17,29,31] In contrast to these preclinical studies, we detected Env-specific $CD8^+$ T-cells only after in vitro expansion of PBMC from volunteers enrolled in the CD40 0.3 mg group. This result contrasts with observations from the HVTN 096 trial, in which DNA-HIV-PT123 was co-administered with AIDSVAX B/E.[18] In this study, Env-specific $CD8^+$ T-cells were observed at a low magnitude and in a limited number of vaccinees (26% and 16% after the third and fourth administration of the combined immunogens, respectively). An extension of the ANRS VRI06 study is ongoing to test whether a fourth injection of the CD40 vaccine could improve the breadth and durability of vaccine-induced immunity.

Prior preclinical studies have shown that the CD40 12E12 mAb fused to various HIV antigens (Env or T-cell epitopes) can elicit robust HIV-specific T- and B-cell responses either when administered alone, when combined with adjuvants (TLR3, TLR4 or TLR9 agonists), or in a prime-boost regimen in combination with HIV DNA, MVA, or NYVAC vectors autologous or heterologous for HIV antigens.[12,15,29,32,33] We have expanded upon these findings, showing that CD40.HIVRI.Env elicits high rates and magnitudes of binding antibody responses to both autologous vaccine gp140 clade C antigens and a heterologous panel of gp120/gp140 antigens. We also show the induction of IgG and IgG3 responses against autologous and heterologous (at a lower magnitude) gp70-V1V2 antigens across vaccine groups. Finally, polyfunctional (secreting up to three cytokines) Env-specific $CD4^+$ T-cell responses were observed for all vaccinees from W6 onwards, without a clear booster effect after the third injection at W26. By contrast, Tier1 nAbs against MW.965.26 peaked at W26. Interestingly, 50% of Tier1 responders were observed for the lowest dose (CD40 0.3 mg), whereas 100% of volunteers were responders in all other vaccine groups, suggesting a dose effect. Of note, responses remained detectable six months after the last injection.

These immunological read outs are highly reminiscent of immune correlates associated with a reduced risk of HIV infection in the RV144 phase 3 trial, in particular binding IgG antibodies targeting conserved regions of the V1/V2 loop.[4–7] Although the HVTN 702 HIV vaccine trial failed to demonstrate protection, the combination of high levels of IgG binding responses against vaccine-matched A244 V1V2 and vaccine-matched $CD4^+$ T-cell responses were also shown to correlate with a lower risk of HIV acquisition.[5,8] Overall, recipients of the CD40 or DNA + CD40 vaccines in the ANRS VRI06 trial exhibited these markers. By using similar assays and protein antigens as in previous vaccine trials, we show that a new delivery method of HIV Env, using a less complex design and vaccine regimen, elicits comparable, or at least more homogenous, IgG and IgG3 binding responses, as well as nAb titres, as previous HIV prophylactic trials.[9,18,19,28] To date, vaccines under clinical development have not been able to induce Tier2 virus nAbs or only in a few volunteers following a DNA prime–protein boost HIV-1 vaccine formulation.[34] Here, one volunteer from the DNA + CD40 group developed nAb responses against several Tier2 viruses. This observation suggests that CD40-targeting Env-based vaccines could be a promising platform for Env protein-based vaccines. They could serve as a delivery method for more tailored Env conformational immunogens aimed at eliciting neutralizing antibodies. The combined safety and immunological properties of the CD40 targeting vaccine also render this platform suitable for evaluation in multiple sequential immunization strategies to induce the maturation of the B cell and antibody response.

The trial has limitations inherent to phase I dose escalation trials. Due to the small sample sizes and to the non-randomized enrolment into the consecutive dose groups, balanced demographic characteristics are hard to achieve in this type of trial. Although some sex differences in responses to vaccines have been previously described in the literature, the effect size of sex is usually relatively small compared to the overall vaccine effect itself. Given that the age range in our trial was restricted (inclusion criterion 18–65 years), a major age effect in this young to middle-aged population is unlikely.[35–37] For these reasons, but also due to the fact that no inter-group comparisons are performed in this trial and that the sample size does not reasonably allow for statistical modelling, we did not explore the effect of age and sex in this trial.

In conclusion, since the seminal proposal from R. Steinman and J. Banchereau[11] to target DCs for manipulating and harnessing the immune system, the present trial is, to our knowledge, the largest first-in-human study to test the DC-targeting of antigens through the CD40 receptor. Globally, these results lend credibility to the use of this innovative approach to activate the immune system, and create new possibilities for addressing adapted HIV Env antigens suitable for inducing protective vaccine responses.

Contributors

Conceptualisation: LR, JDL, RT, YL, SC, SZ, GZ, and GP.
Data access and verification: LH, MDu, AW, CM, CL, AD, LR, JDL, and YL.
Data curation: LH, MDu, AW, CM, CL, and AD.
Formal analysis: LR, and MDu.
Funding acquisition: YL.
Investigation: JDL, OL, FC, CL, AW, and CM.
Methodology: LR, and RT.
Project administration: MC, LR, JDL, LH, VR, and YL.
Resources: JDL, OL, FC, SD, GP, MC, YL, and GT.

Supervision: LR, JDL, RT, and YL.
Validation: LH, AD, AW, CM, and CL.
Visualisation: MDu.
Writing original draft: LR, YL, CM, and AW.
Writing review and editing: all.

Data sharing statement

The data will be available beginning 6 months following article publication. The data include the individual participant data that underlie the results reported in this article, after de-identification (text, tables, figures and appendices). The study protocol, statistical analysis plan, informed consent form will also be available. The data will be shared with researchers who provide a methodologically sound proposal, that needs to be approved by the relevant ANRS or trial committee, and for analyses to achieve aims in the approved proposal. Proposals should be directed to yves.levy@aphp.fr; to gain access, data requestors will need to sign a data access agreement and comply with GDPR.

Declaration of interests

The authors S.Z., G.Z., and Y.L., are named inventors on patent applications based on this work held by Inserm Transfert. J-D.L. declares having received remuneration by the Global HIV Vaccine Enterprise. O.L. declares receipt of grants, consulting fees and support for attending meetings and/or travel from several vaccine industries. She also participated in Data Safety Monitoring or Advisory Boards of Sanofi and MSS. The remaining authors declare no competing interests. Inserm Transfert provided a license for CD40 targeting vaccines to the biotech company LinKinVax.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2024.102845.

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