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Safety, immunogenicity and effect on viral rebound of HTI vaccines combined with a TLR7 agonist in early-treated HIV-1 infection: a randomized, placebo-controlled phase 2a trial

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Building on results from the AELIX-002 trial with HIVACAT T-cell immunogen (HTI)-based vaccines, the AELIX-003 (NCT04364035) trial tested the safety of the combination of ChAdOx1.HTI (C) and MVA.HTI (M), with the TLR7 agonist vesatolimod (VES), in a double-blind, placebo-controlled, randomized clinical trial in 50 virally suppressed early-treated men with HIV-1 infection. Secondary objectives included immunogenicity and effects on viral rebound kinetics during a 24-week antiretroviral treatment interruption (ATI). The most common treatment-related adverse events were mild-to-moderate injection-site pain, influenza-like illness, headache, and fatigue. Strong, broad, and HTI-focused T-cell responses were induced by vaccination. All participants experienced viral rebound in ATI; 33.3% and 23.5% ($P = 0.4494$) of CCMM + VES and placebo recipients, respectively, remained off antiretroviral therapy for 24 weeks. Post hoc analysis confirmed a correlation between levels of HTI-specific T cells and prolonged time off antiretroviral therapy. The combination of HTI vaccines and VES was safe and elicited robust T-cell responses.

The lifelong requirement for antiretroviral therapy (ART) in people with HIV (PWH) remains a global challenge, particularly in resource-limited settings[1]. Despite effective ART suppression, ongoing HIV transcription from intact and defective proviruses in the viral reservoir contributes to chronic inflammation and immune activation, contributing to non-AIDS-associated diseases. Achieving an HIV cure or durable ART-free viral remission, thus, remains an unmet clinical need[2].

Therapeutic vaccines expressing the HIVACAT T-cell immunogen (HTI) were designed to elicit T-cell responses associated with spontaneous HIV control in the absence of ART. In the AELIX-002 (NCT03204617) randomized clinical trial[3] of early-treated PWH, a complex combination of HTI vaccines was found to be safe, highly immunogenic, and showed promise in improving virologic control, although the combination did not reduce the viral reservoir, prevent or delay viral rebound upon ART interruption, and/or induce sustained viral suppression to undetectable levels. Results from trials of other therapeutic vaccines given alone [4] or combined with latency-reversing agents such as vorinostat[5] or romidepsin[6,7] have also shown a lack of effect on the viral reservoir, possibly due to insufficient latency reversal and expression of HIV antigens for immune recognition[8], or caused by reservoir-cell compartmentalization in anatomical sites that are poorly accessible to immune effector cells[9], intrinsic resistance to cytotoxic T-lymphocyte-mediated killing[10] and/or insufficient antiviral activity.

Vesatolimod (VES) is an oral toll-like receptor 7 (TLR7) agonist in development as a potential HIV cure regimen component. VES in combination with a therapeutic vaccine and/or broadly neutralizing antibody (bNAb) showed efficacy in delaying viral rebound, decreasing

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viral setpoint, and inducing viral remission after ART cessation in a subset of early-treated SIV-infected rhesus macaques[11–13]. Data from human ex-vivo and in-vivo studies have shown that VES induces potent immune stimulation[14–16]. In a randomized, double-blind, placebo-controlled, phase 1b trial in ART-suppressed viremic HIV controllers, multiple-dose administration of VES up to 8 mg was safe and well-tolerated and promoted a modest delay in time to HIV rebound after cessation of ART[14].

Building on the non-human primate (NHP) studies, the phase 2a, double-blind, randomized, placebo-controlled, multicenter AELIX-003 trial (NCT04364035) was conducted in Spain to assess the safety, immunogenicity, and efficacy of ChAdOx1.HTI and MVA.HTI therapeutic HIV-1 vaccines given in combination with VES in ART-suppressed, early-treated PWH.

Results

Fifty cisgender men with confirmed HIV-1 infection who initiated ART within 6 months of the estimated HIV-1 acquisition date and maintained undetectable viral load for at least 1 year were recruited at nine clinical sites in Madrid and Barcelona, Spain. Participants were randomized 2:1 to receive a heterologous intramuscular vaccine regimen with ChAdOx1.HTI and MVA.HTI (referenced collectively as CCMM) and VES ($n = 33$) or matched placebo ($n = 17$) (Fig. 1a). While on ART, ChAdOx1.HTI or placebo were administered at weeks 0 and 12, followed by MVA.HTI or placebo at weeks 24 and 36. VES or placebo was administered once every other week as a 6-mg oral dose for a total of ten doses, with five doses between week 26 (2 weeks after the first MVA.HTI immunization) to week 34 and the other five doses between week 38 (2 weeks after the second MVA.HTI immunization) to week 46; the last VES dose was administered 2 weeks before entering a 24-week antiretroviral treatment interruption (ATI). The treatment regimen was completed by 30/33 active group (CCMM + VES) and 17/17 placebo group participants. Two (6.1%) participants voluntarily withdrew from the study and one (3.0%) was discontinued by investigator's decision due to transient elevated transaminases present before the first ChAdOx1.HTI dose. The remaining 47 participants received all four planned doses of vaccines or matching placebo, while 21 (63.6%) received all ten doses of VES or matching placebo. All 47 participants who entered the ATI phase received at least eight doses of VES or a matching placebo (Fig. 1b).

Demographics

Characteristics of randomized participants (intent-to-treat population) are shown in Table 1. ART was initiated after a median (range) of 61 (7–170) and 86 (16–167) days after the estimated HIV-1 acquisition date in CCMM + VES and placebo recipients, respectively. All but one participant, who was receiving tenofovir alafenamide/emtricitabine/rilpivirine (TAF/FTC/RPV), were on an integrase strand transfer inhibitor-based ART regimen at study entry. At enrollment, the median (range) time on ART was 41 (16–132) and 43 (17–116) months, and the median CD4 + T-cell counts (range) were 882 (451–1600) and 831 (534–1333) cells/μL in CCMM + VES and placebo recipients, respectively. Six CCMM + VES recipients (18.2%) and four placebo recipients (23.5%) had at least one human leukocyte antigen (HLA) class I allele associated with spontaneous control of HIV replication, including HLA-B27, HLA-B57, HLA-B58:01, HLA-B15:16, and/or HLA-B15:17[17].

Safety

Investigators reported a total of 598 treatment-emergent adverse events (TEAEs) in 32 (97.0%) and 17 (100%) participants in the CCMM + VES and placebo groups, respectively (Supplementary Table 1). Nearly all TEAEs (384/385 events) in CCMM + VES recipients were graded as mild to moderate; one serious event of acute cholangitis in the CCMM + VES group was considered not related to the study treatment (Supplementary Table 1).

Thirty (90.9%) CCMM + VES recipients and 11 (64.7%) placebo recipients had TEAEs related to study drugs (Supplementary Tables 2–5). Most of these treatment-related TEAEs (238/337) were reported in CCMM + VES recipients. The most common treatment-related TEAEs (>30%) in CCMM + VES recipients included injection-site pain (87.9%), influenza-like illness (36.4%), headache (33.3%), and fatigue (33.3%) (Supplementary Table 2). All TEAEs related to study drugs were mild or moderate, and most had an outcome of recovered/resolved or recovering/resolving. There were no serious TEAEs related to study drugs or TEAEs leading to study drug discontinuation (Supplementary Table 1).

In the CCMM + VES and placebo groups, 31 (93.9%) and 13 (76.5%) participants, respectively, recorded a total of 1533 solicited local or systemic reactions in the participants' diaries (Supplementary Table 1). In the CCMM + VES group, but not in the placebo group, five (15.2%) participants reported eight grade 3 or 4 solicited local reactions of pain/tenderness; one event was assessed by the investigator to be related to ChAdOx1.HTI and seven were related to MVA.HTI (Supplementary Table 6). Ten (30.3%) participants in the CCMM + VES group reported 40 grade 3 solicited systemic reactions and three (17.6%) participants in the placebo group reported 27 grade 3 or 4 solicited systemic reactions. In the CCMM + VES group, 27 out of 40 solicited grade 3 or 4 events were assessed by the investigator to be related to the study drugs (20 were related to ChAdOx1.HTI, five were related to MVA.HTI, and three were related to VES) (Supplementary Table 6). The most common solicited systemic symptoms at any dose of the study drug were fatigue/general malaise, muscle aches, and low appetite. All participant-reported solicited local or systemic reactions were investigator-assessed as mild or moderate, with an outcome of resolved (except for one solicited systemic reaction assessed as recovering or resolving).

Vaccine immunogenicity

The magnitude of total HTI-specific T-cell responses was compared between CCMM + VES and placebo participants before and 4 weeks after the first and second ChAdOx1.HTI vaccination (weeks 0, 4, 12, and 16) and the first and second MVA.HTI vaccination (weeks 24, 28, and 36) and at ATI start (week 48). Differences in HTI magnitude at each timepoint relative to baseline (week 0) were calculated. Peak immunogenicity timepoint was defined as the timepoint with the highest HTI magnitude among all timepoints evaluated for each participant.

Persistent, statistically significant differences in absolute magnitude and change in magnitude from baseline of the HTI-specific T-cell responses were observed from week 16 (4 weeks after the second ChAdOx1.HTI) up to ATI start ($P < 0.0001$) between CCMM + VES and placebo participants (Supplementary Table 7). The highest magnitude of HTI-specific T-cell responses was observed at week 36 (12 weeks after the first MVA.HTI vaccination and after five doses of VES), with a difference (95% CI) between CCMM + VES and placebo recipients of 915 (385 and 1645) HTI-specific spot-forming cells (SFCs)/10^6 peripheral blood mononuclear cells (PBMCs) ($P = 0.0002$). HTI responses were well-maintained during the second VES cycle (week 38–46) as well as HTI-specific magnitude at ATI start (week 48); the median (range) was 970 (115–3895) in CCMM + VES participants versus 165 (0–1155) SFCs/10^6 PBMCs in placebo participants ($P < 0.0001$) (Fig. 2a, b).

The focus of the HTI-specific T-cell response, as a percentage of magnitude of the total anti-HIV-1-specific T-cell responses directed against HTI-covered regions, was compared between treatment groups in terms of absolute focus and change from baseline. At ATI start (week 48), the median percentage (95% CI) focus had significantly increased from baseline in CCMM + VES recipients, with a difference of 37.2% (21-53) versus placebo ($P = 0.0002$). Median (range) focus of HTI responses over the total of HIV-1-specific T cells was 47.2% (24–100%) with CCMM + VES and 25.5% (0–82%) with placebo ($P = 0.0024$) (Fig. 2c).

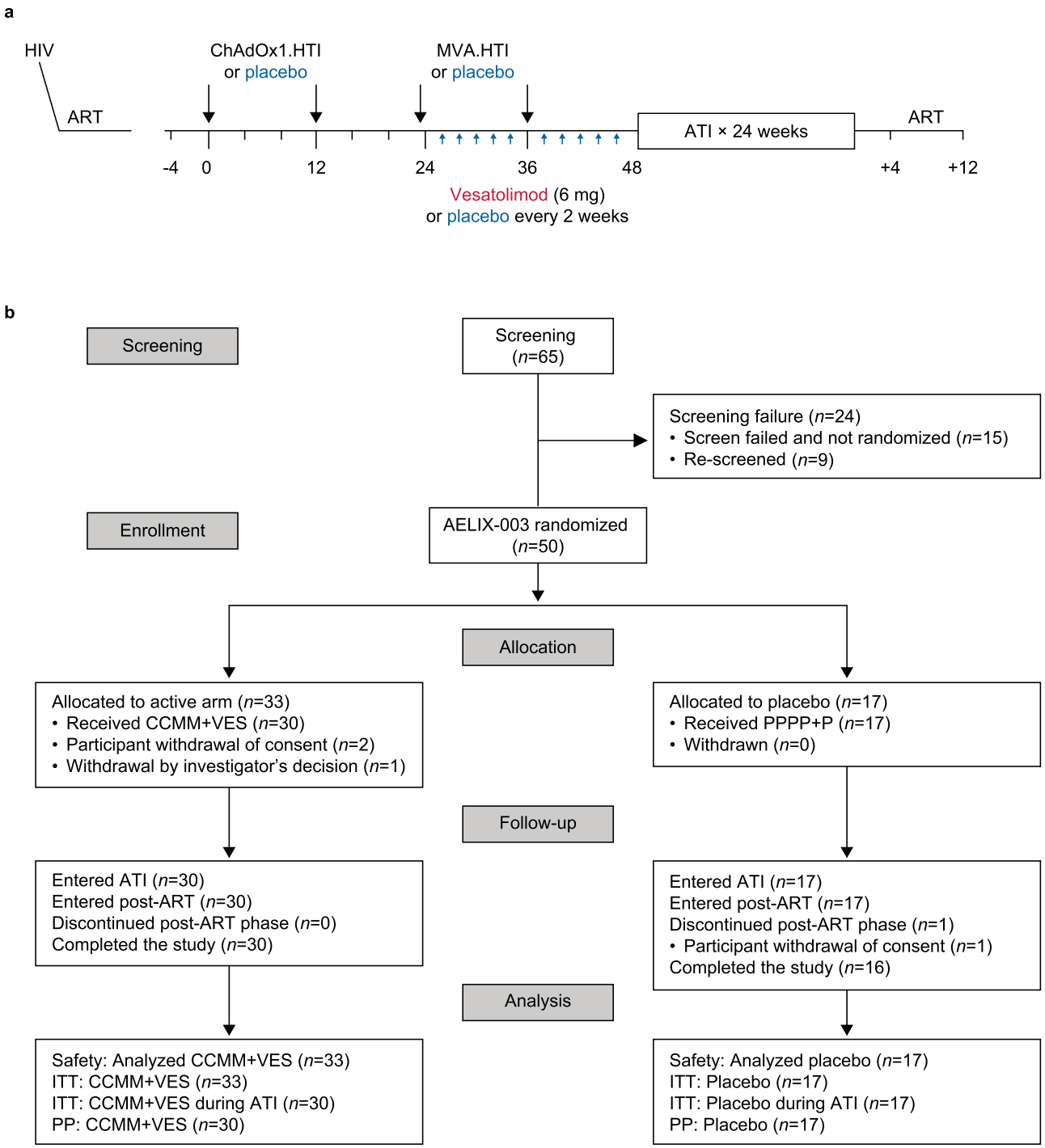

Fig. 1 | Trial design and participant disposition. a Trial schedule and study visits. While on ART, ChAdOx1.HTI or placebo were administered at week 0 and week 12, followed by MVA.HTI or placebo at weeks 24 and 36. VES was administered at a dose of 6 mg orally, once every other week, for a total of ten doses from weeks 26 to 34 and 38 to 46 of the study; the last dose was administered 2 weeks before interrupting ART for a maximum of 24 weeks. **b** Participant disposition (CONSORT flow diagram).

The breadth of the total HTI-specific T-cell response was defined as the number of positive-reactive peptide pools (out of the 10 HTI covering peptide pools used in the ELISpot assay) that exhibited a magnitude above a sample- and timepoint-specific cutoff. The highest breadth of HTI-specific T-cell response was observed in the CCMM + VES group after the first MVA.HTI administration (median of four reactive pools, range 1-8) that was sustained up to ATI start, with statistically significant differences versus placebo recipients, who had a median (range) of one (0–8) HTI-reactive pool at ATI start ($P = 0.0008$) (Fig. 2d). Distribution of T-cell responses within the different HIV subproteins covered by the HTI immunogen sequence showed a broad response toward HTI without revealing any specific pattern of immunodominance in vaccine recipients (Fig. 2e).

VES pharmacokinetics/pharmacodynamics

In the pharmacokinetic substudy population, mean VES concentrations in plasma peaked 2 h after administration and declined in a biphasic manner thereafter (Supplementary Fig. 1a), with a mean

Table 1 | Study population

Characteristics	CCMM + VES (*n* = 33)	Placebo (*n* = 17)	ITT population (*n* = 50)
Age, years	38 (24–55)	37 (21–59)	38 (21–59)
Sex at birth, male, *n* (%)	33 (100.0%)	17 (100.0%)	50 (100.0%)
BMI (kg m^{-2})	24.1 (16–44)	23.8 (19–33)	24.0 (16–44)
Days from estimated HIV acquisition to ART initiation	61 (7–170)	86 (16–167)	67 (7–170)
Fiebig stage at ART initiation, *n* (%)[a]			
Eclipse	0 (0.0%)	0 (0.0%)	0 (0.0%)
I	2 (6.1%)	0 (0.0%)	2 (4.0%)
II	3 (9.1%)	1 (5.9%)	4 (8.0%)
III	3 (9.1%)	0 (0%)	3 (6.0%)
IV	3 (9.1%)	3 (17.6%)	6 (12.0%)
V	12 (36.4%)	6 (35.3%)	18 (36.0%)
VI	7 (21.2%)	5 (29.4%)	12 (24.0%)
Missing	3 (9.1%)	2 (11.8%)	5 (10.0%)
ART regimen, *n* (%)			
INSTI-based	33 (100%)	16 (94.1%)	49 (98%)
Other, NNRTI-based	0 (0.0%)	1 (5.9%)	1 (2.0%)
Time on ART, months	40.6 (15.7–132.2)	43.46 (17–115.8)	42.03 (15.7–132.2)
Absolute CD4 (cells/mm^3)	882 (451–1600)	831 (534–1333)	872 (451–1600)
CD4 %	39 (24–49)	41 (34–53)	40 (24–53)
CD4/CD8 ratio	1.2 (1–2)	1.2 (1–2)	1.2 (1–2)
Beneficial HLA alleles, any[b]	6 (18.2%)	4 (23.5%)	10 (20.0%)

Baseline clinical and demographic characteristics of the study population (*n* = 50).
Characteristics related to demographics, clinical profiles, and treatment details of randomized study participants at study entry (*n* = 50).
Data presented as median (min–max) except where specified.
BMI body mass index, *INSTI* integrase strand transfer inhibitor, *ITT* intent-to-treat, *NNRTI* non-nucleoside reverse transcriptase inhibitor.
[a]According to Fiebig stage classification.
[b]Includes HLA-B27, HLA-B57, HLA-B58:01, HLA-B15:16, and HLA-B15:17.

half-life ($t_{1/2}$) of 13.3 h (standard deviation [SD] 3.40). Geometric mean (GCV%) VES maximum concentration (C_{max}), concentration at 24 h (C_{24}), and area under the concentration-time curve from time zero to 24 h ($AUC_{0\text{-}24}$) were 3520 (54.1%), 355 (65.4%) pg/ml and 22,400 (53.6%) pg·h/ml, respectively. VES concentrations collected at similar postdose timepoints were consistent across visits (Supplementary Fig. 1b).

VES pharmacodynamics (PD), cytokine levels, and immune-cell activation were evaluated before and 24 h after the first VES/placebo dose (week 26) and before and 24 h after the 10th planned VES/placebo dose (week 46). VES consistently increased plasma levels of interferon-γ (IFN-γ)-inducible protein-10 [IP-10], IFN-inducible T-cell-α chemoattractant [ITAC]), IFN-α, and interleukin-1 receptor antagonist [IL-1RA]) postdose in CCMM + VES recipients (Fig. 3).

Levels of activated (CD25+, HLA-DR+, and/or CD25+/HLA-DR+) CD4 T and CD8 T cells were similar between predose and postdose in both groups (Fig. 4a–f). A significantly higher frequency of activated (CD69+) natural killer (NK) cells was observed at both week 26 and week 46 postdose versus predose ($P = 0.0091$ and $P = 0.0039$, respectively), and versus baseline ($P = 0.0283$ and $P = 0.0034$, respectively) in CCMM + VES recipients but not in placebo recipients (Fig. 4g, h). However, the overall levels of NK activation were similar between groups.

We did not observe an effect of CCMM + VES on plasma viremia during the intervention phase. Six (20.0%) participants in the CCMM + VES group had a single transient increase ("blip") in HIV-1 RNA ≥50 copies/ml (range 52–350) detected during the VES administration period, specifically after doses four and five. Two (11.7%) participants in the placebo group had HIV-1 RNA to ≥50 copies/ml at least once during the intervention period, one of which showed several determinations suggestive of poor ART adherence.

Effects on viral rebound during ATI

Forty-seven participants (30 CCMM + VES recipients and 17 placebo recipients) entered the ATI period for a maximum of 24 weeks. Participants were monitored weekly for clinical symptoms and HIV-1 plasma viral load (pVL), and a safety assessment, including biochemistry and complete blood and CD4 T-cell counts, was conducted every 4 weeks. ART was resumed after a single HIV-1 pVL determination exceeding 100,000 copies/ml, 8 consecutive weeks with >10,000 copies/ml, two consecutive CD4 + T-cell counts below 350 cells/mm^3 and/or a reported grade 3 or higher-severity clinical symptom suggestive of acute retroviral syndrome. Time-to-event endpoints were adjudicated to be the first of at least two consecutive determinations of HIV-1 pVL >50 or >10,000 copies/ml.

HIV-1 rebound (pVL >50 copies/ml) was detected in all participants after ART discontinuation, at a median (95% CI) of 3.1 (2.3–4.0) and 3.0 (2.1–4.0) weeks in CCMM + VES and placebo recipients, respectively (log-rank test, $P = 0.1108$). HIV-1 pVL >10,000 copies/ml was reached in 37 participants (78.7%) after ART discontinuation at a median (95% CI) of 5.1 (4.1–8.0) and 5.0 weeks (3.1–10.9) in CCMM + VES and placebo recipients, respectively (log-rank test, $P = 0.5132$) (Fig. 5a–c). Rebound kinetics parameters, including peak pVL, time to peak pVL, and slope of pVL increase or AUC of viremia from ATI start until ART resumption were comparable between CCMM + VES and placebo recipients (Supplementary Table 8).

Ten of 30 CCMM + VES recipients (33.3%; 95% CI 17.3–52.8) remained off ART for 24 weeks versus four of 17 placebo recipients (23.5%; 95% CI 6.8–49.9) ($P = 0.4494$). Reasons for ART resumption before 24 weeks of ATI (Supplementary Table 9) included reaching an HIV-1 pVL of ≥100,000 copies/ml in 16 (53.3%) and eight (47.1%) participants in the CCMM + VES or placebo groups or having a sustained HIV-1 pVL of ≥10,000 copies/ml on two consecutive weekly tests that did not decrease to <10,000 copies/ml by 8 weeks after the first test in two (6.7%) and five (29.4%) CCMM + VES and placebo recipients, respectively. Two CCMM + VES recipients resumed ART for other reasons; one at ATI week 23 by scheduling mistake (i.e., without meeting ART resumption criteria, confirmed by detection of antiretroviral drugs in plasma at ATI week 24), the other at week 18 due to reactivation of occult hepatitis B co-infection. No participant resumed ART due to CD4 + T-cell decrease or a clinically significant acute retroviral syndrome per protocol criteria. At 12 and 24 weeks of ATI, HIV-1 pVL was <2000 copies/ml in eight (26.7%) and five (16.7%) CCMM + VES recipients versus three (17.6%) and two (11.8%) placebo recipients ($P = 0.3686$ and $P = 0.5872$, respectively).

All participants resumed effective ART at the end of the ATI. One CCMM + VES recipient had HIV-1 pVL >50 copies/ml at the end of the study (12 weeks after ART resumption). This participant resumed ART at ATI week 4 with an HIV-1 pVL >10,000,000 copies and achieved an expected decline in viral load by weeks 4 and 12 after ART resumption (HIV-1 pVL 4460 and 369 copies/ml, respectively).

Viral reservoir

HIV reservoir evaluation was conducted with PBMC samples collected at baseline (week 0) and ATI start (week 48) and included total and intact proviral HIV DNA. Paired determinations of total proviral DNA at baseline and at ATI start were available for 29 (96.6%) and 17 (100%) CCMM + VES and placebo recipients, respectively. Intact proviral DNA data were not available in three (10.0%) and seven (41.2%) participants

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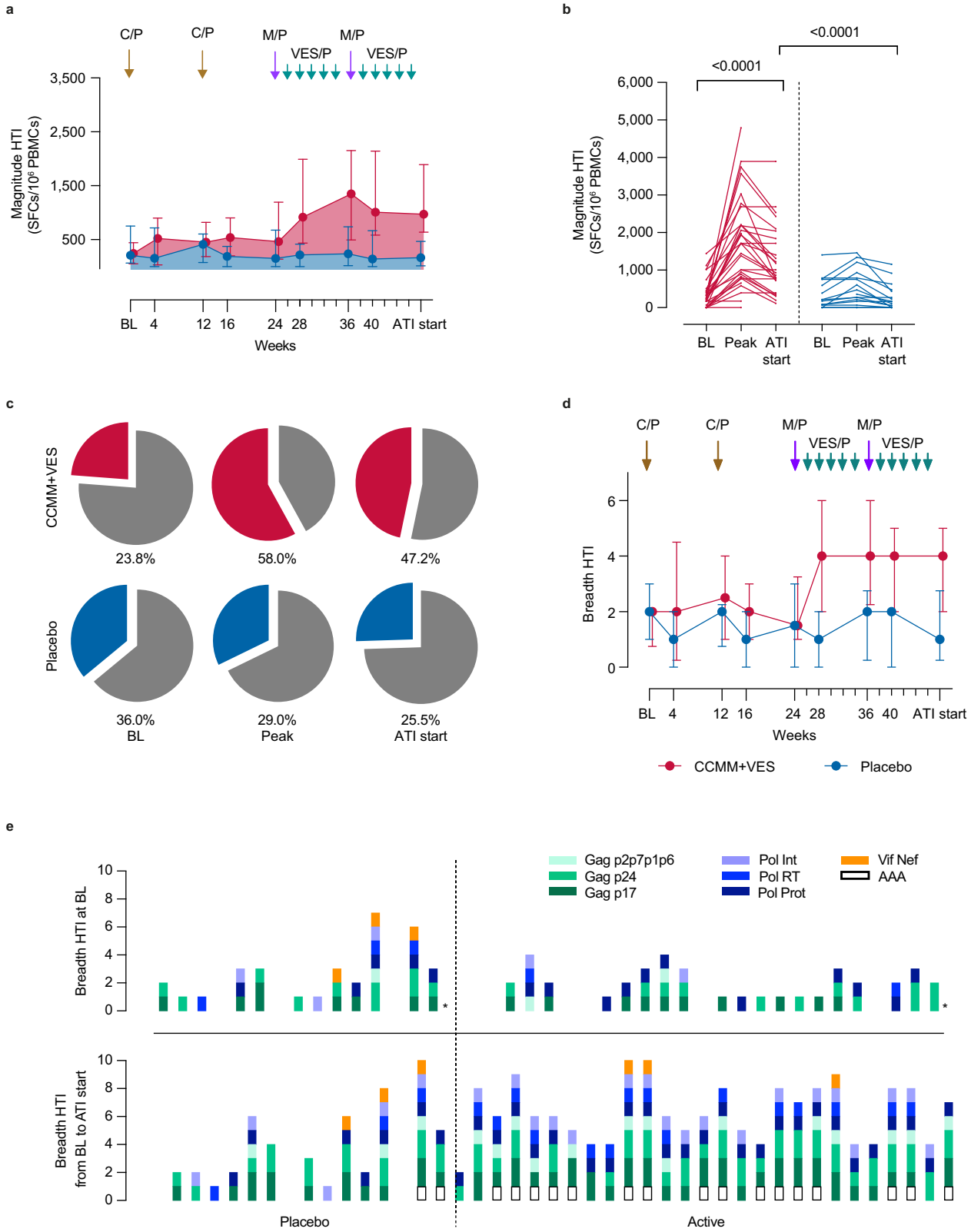

from the CCMM + VES and placebo groups who entered the ATI, respectively, due to Amplicon signal issues. At study entry, reservoir levels were numerically higher in CCMM + VES versus placebo recipients, both for total and intact HIV-1 proviral DNA (median [range], 268 (1.5–2579) total HIV-1 copies/10^6 CD4 + T cells in CCMM + VES recipients and 121 (1.5–842) copies/10^6 CD4 + T cells in placebo recipients). No significant changes from baseline to week 48 (ATI start) were detected in either treatment group ($P = 0.5529$ for total and $P = 0.7902$ for intact HIV-1 proviral DNA), consistent with a population with a median time on ART longer than 3 years at study entry[18]. Similarly, at ATI start, reservoir levels remained numerically higher in CCMM + VES recipients versus placebo recipients, both for total and intact HIV-1 proviral DNA. Median (range) total and intact HIV-1 proviral DNA were 210 (3.2–2432) and 55 (1–797) copies/10^6 CD4 + T cells

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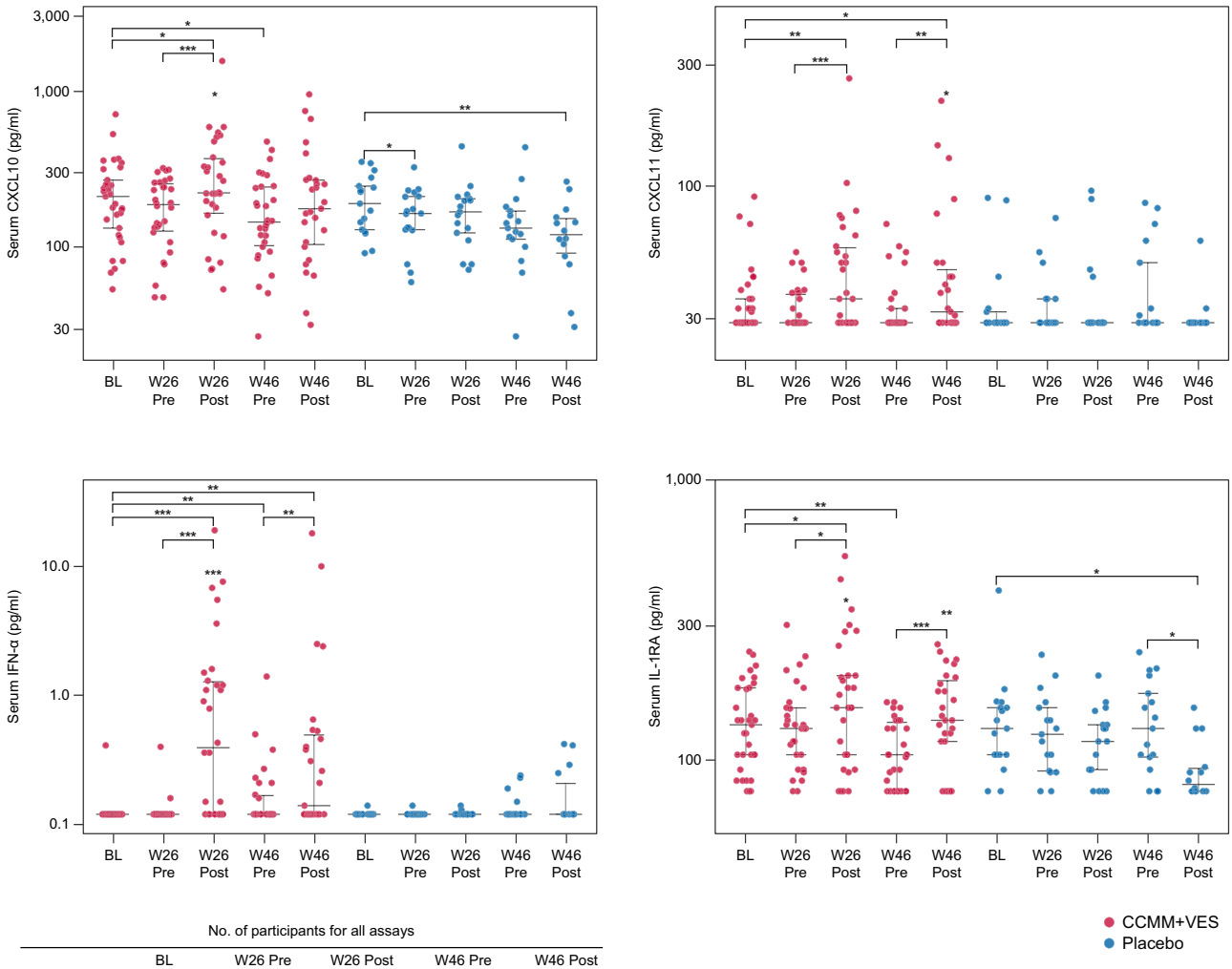
Fig. 2 | Vaccine immunogenicity. a Median and IQR magnitude of total HTI-specific T-cell response (sum of SFCs per 10^6 PBMCs for HTI pools P1–P10) over time in 33 CCMM + VES (shown in red) and 17 placebo (shown in blue) recipients from baseline to week 48. Statistics are derived from data from 24–30 (out of 33) CCMM + VES recipients and 13–16 (out of 17) placebo recipients at each timepoint, depending on sample availability and valid results after assay QC. Arrows indicate vaccination or VES/placebo administration dates. BL baseline, C ChAdOx1.HTI, M MVA.HTI, P placebo. **b** Individual magnitudes of HTI-specific response (sum of SFCs per 10^6 PBMCs for HTI pools P1–P10) in CCMM + VES (shown in red) and placebo (shown in blue) recipients, at study entry (BL), the timepoint between study entry and week 48 with the strongest observed total HTI-specific T-cell responses (peak) and at week 48 (ATI start). *P* value tested using a two-sided van Elteren test (*P* value <0.0001; unadjusted for multiple comparisons, with 5% error rate), with a stratification factor for the actual potential for superior viral control (yes/no). **c** Median contribution of HTI-specific T cells to total virus-specific responses, according to specificity. HTI-specific responses are shown in red for CCMM + VES and in blue for placebo recipients; non-HTI HIV-1-specific responses are shown in gray. **d** Median and IQR breadth of total HTI-specific T-cell response (number of reactive HTI pools P1–P10) over time in 33 CCMM + VES (red) and in 17 placebo (blue) recipients from baseline to week 48. Statistics are derived from data of 24–30 (out of 33) CCMM + VES recipients and 13–16 (out of 17) placebo recipients at each timepoint, depending on sample availability and valid results after assay QC. Arrows indicate vaccination or VES/placebo administration dates. **e** Distribution of HTI-specific responses within the different HIV-1 subproteins at study entry (BL) and accumulated up to the start of ATI for each placebo (P01–P17) and CCMM + VES (V01–V30) recipient.



No. of participants for all assays

	BL	W26 Pre	W26 Post	W46 Pre	W46 Post
CCMM+VES	33	30	30	30	27
Placebo	17	17	17	17	14

Fig. 3 | VES PD responses in CCMM + VES and placebo recipients. Serum levels were measured by single-molecule array assay for IFNα and multiplex cytokine assay for IP-10 (CXCL10), ITAC (CXCL11), and IL-1RA at baseline (BL), before and 24 h after the first VES/placebo dose (week 26), and before and 24 h after the 10th (last) VES/placebo dose (week 46). Colored-coded dots represent data collected from individual participants (red color was used to mark participants receiving CCMM + VES and blue for those receiving placebo). Median and IQR are shown with black dots and lines. Wilcoxon test was used to compare data at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired; shown by brackets), reported using nominal *P* values. $^*P \le 0.0500$, $^{**}P \le 0.0100$, $^{***}P \le 0.0010$. CXCL C-X-C motif chemokine ligand.

in CCMM + VES recipients and 86 (4.3–718) and 18 (1.5–391) copies/10^6 CD4 + T cells in placebo recipients, respectively ($P = 0.1238$ and $P = 0.6858$) (Fig. 6a, b). Of interest, one CCMM + VES recipient and two placebo recipients had undetectable intact proviral HIV-1 DNA both at study entry and ATI start. The CCMM + VES recipient remained off ART for the entire ATI period and showed only five detectable determinations of HIV-1 pVL >50 copies/ml, the highest being 104 copies/ml at 21 weeks of ATI. On the contrary, the two placebo recipients with undetectable intact reservoir levels at ATI start showed a fast viral rebound of up to 4 log of HIV-1 RNA copies/ml in both cases and resumed treatment at 16 and 24 weeks of ATI with 19,500 and 4280 copies/ml, respectively.

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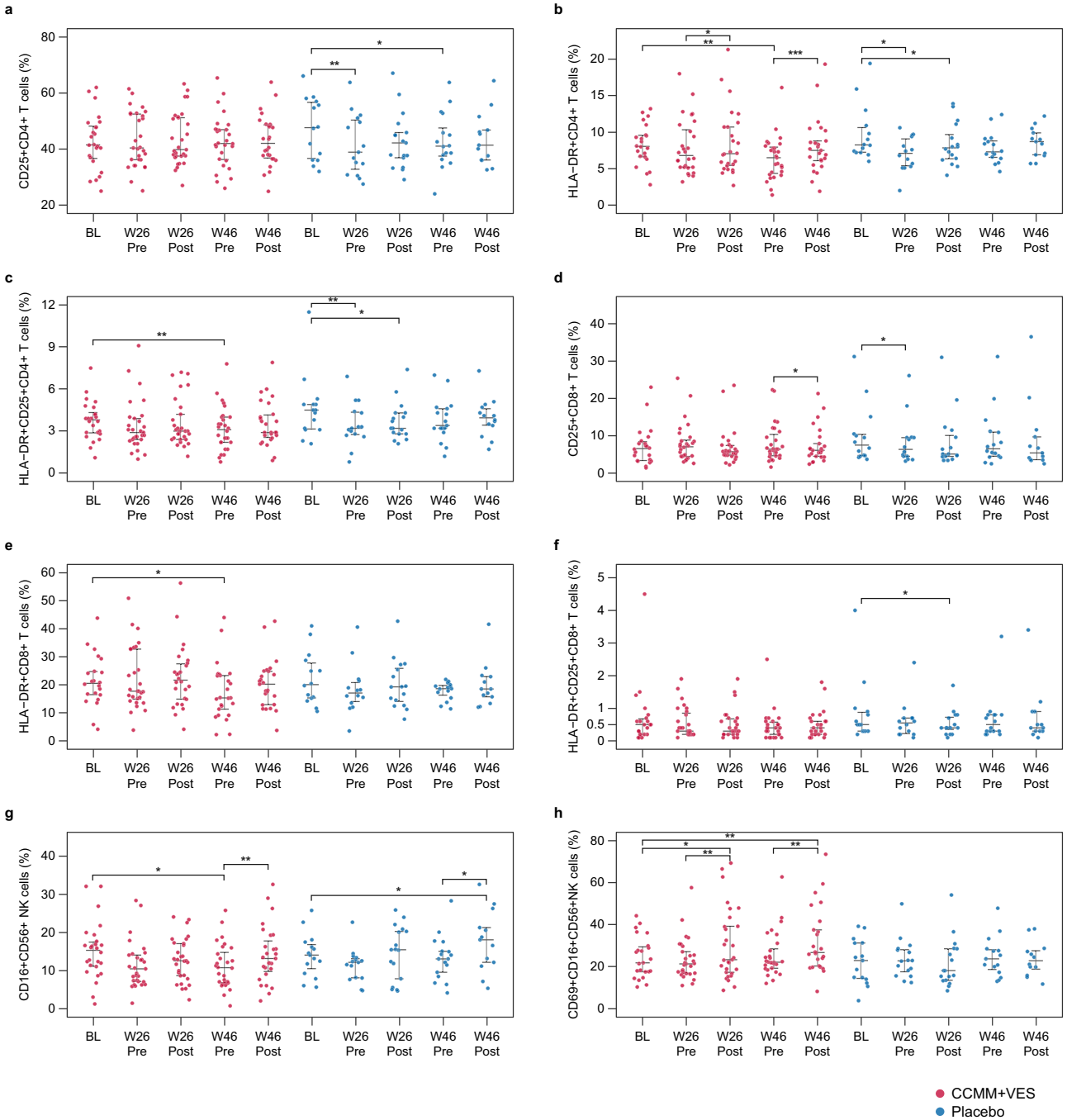

Fig. 4 | Immune-cell activation in CCMM + VES and placebo recipients. Whole blood specimens were collected to evaluate immune-cell activation (T cells, **a**–**f**) and natural killer (NK) cells (**g**, **h**) with flow cytometry at baseline (BL), before and 24 h after the first VES/placebo dose (week 26), and before and 24 h after the 10th planned (last) VES/placebo dose (week 46). Colored-coded dots represent data collected from individual participants (red color was used to mark participants receiving CCMM + VES, blue for those receiving placebo). Median and IQR are shown with black dots and lines. Wilcoxon test was used to compare data at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired; shown by brackets), reported using nominal *P* values. **P* ≤ 0.0500, ***P* ≤ 0.0100, ****P* ≤ 0.0010.

Post hoc correlation analysis

We evaluated demographic, reservoir, and immune parameters and other baseline characteristics for potential associations with ATI outcomes, both when considering CCMM + VES ($n = 30$) and placebo ($n = 17$) groups separately and when considering all participants who entered the ATI phase ($n = 47$). Spearman's ρ was used for individual correlations, unadjusted for multiple comparisons, and results are presented in the correlogram in Fig. 7a, b. In CCMM + VES recipients, lower pre-ART viremia and longer time on ART were associated with delayed viral rebound and lower HIV-1 pVL at the end of ATI (ρ = −0.4399, $P = 0.0150$ for time to HIV-1 pVL >50; ρ = −0.3325, $P = 0.0070$ for time to HIV-1 pVL >10,000 copies/ml; and ρ = 0.4301, $P = 0.0177$ for HIV-1 pVL at the end of ATI with pre-ART viremia, respectively; and ρ = 0.3908, $P = 0.0327$ for time to HIV-1 pVL >50; ρ = 0.4648, $P = 0.0097$ for time to HIV-1 pVL >10,000 copies/ml; and ρ = −0.3805, $P = 0.0380$ for HIV-1 pVL at the end of ATI with time on ART, respectively). As for reservoir parameters, lower total and intact levels of HIV-1 proviral DNA at ATI start were associated with longer

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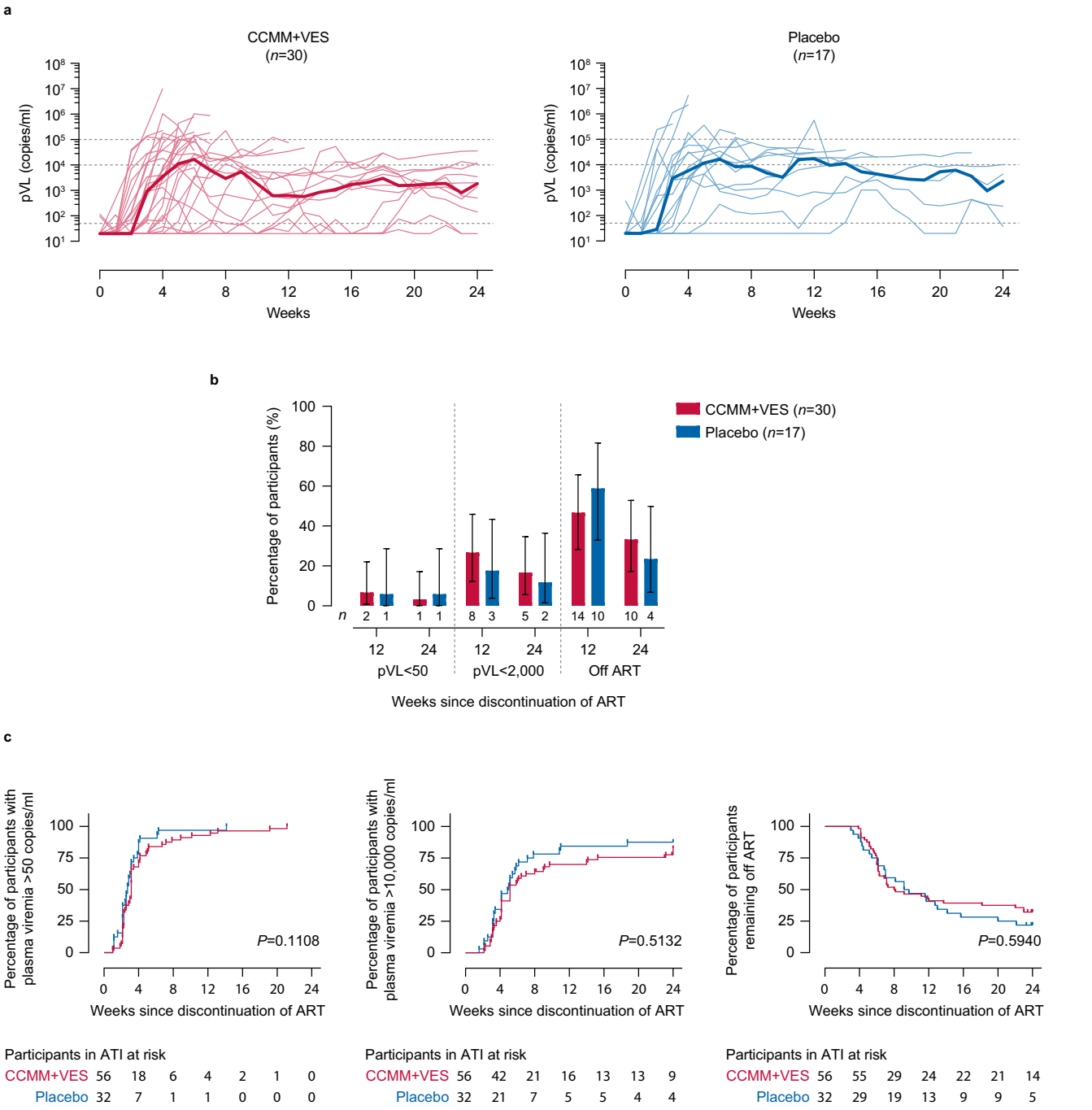

Fig. 5 | HIV-1 RNA pVL during the ATI period. a Individual and mean (thick line) values of HIV-1 plasma viral load (pVL) during the 24 weeks of ATI are represented in CCMM + VES (red) and placebo (blue) recipients. Lines are interrupted at the time of ART resumption. **b** Proportion of participants with HIV-1 pVL <50, <2000 copies/ml and remaining off ART at 12 and 24 weeks of ATI. Data shown for CCMM + VES (red) and placebo (blue) recipients. Error bars represent 95% confidence intervals estimated using the exact (Clopper–Pearson) method. Statistical comparison between treatment groups was performed using a two-sided Cochran–Mantel–Haenszel test, stratified by the potential for superior viral control (*P* values ≤0.05). **c** Time to HIV-1 pVL ≥50, ≥10,000, and percentage of participants remaining off ART during the ATI in CCMM + VES (red) and placebo (blue) recipients. Time to pVL ≥50 copies/ml was adjudicated as the first viral load assessment among the first occurrence of two consecutive visits with pVL ≥50 copies/ml during ATI. Median time to pVL ≥50 and 10,000 copies/ml was estimated in each treatment group using the Brookmeyer and Crowley method and compared between treatment groups using the stratified log-rank test adjusting for stratification factor potential for superior viral control.

time to HIV-1 pVL >10,000 copies/ml after ART interruption (ρ = −0.3910, *P* = 0.0326 and ρ = −0.4374, *P* = 0.0199, respectively). Of importance, several immune parameters were significantly correlated with ATI outcomes in CCMM + VES recipients. Among these, a higher magnitude of HTI-specific responses, in particular at the start of ATI, was significantly associated with longer time to viral rebound (HIV-1 pVL >50 copies/ml; ρ = 0.4076, *P* = 0.0388), slower time to viral rebound (HIV-1 pVL >10,000 copies/ml; ρ = 0.4823, *P* = 0.0126) and longer time off ART during ATI (ρ = 0.4905, *P* = 0.0110).

We then used univariate Cox proportional-hazard and logistic regression models to identify factors that could influence time to ART resumption, considering all participants (CCMM + VES and placebo)

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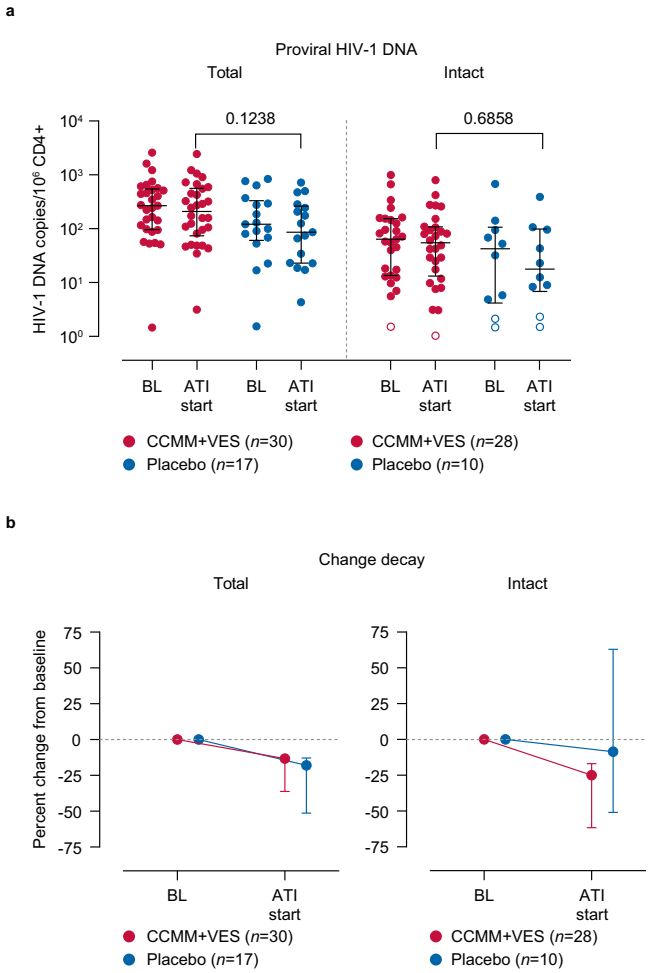

Fig. 6 | HIV reservoir. a Comparison between levels of total and intact proviral HIV-1 DNA at study entry (baseline; BL) and at week 48 (ATI start) in CCMM + VES (red) and placebo (blue) recipients. Participants with undetectable reservoirs are shown in open circles. Median and IQR values are shown. *P* values correspond to the van Elteren test stratifying on the factor for the potential for superior viral control. **b** Median and 95% CI percent change at ATI from baseline is shown for all the participants with a baseline and a post-baseline value at both visits. *P* values correspond to the van Elteren test stratifying on the factor for the potential for superior viral control.

together ($n = 47$). Cox proportional-hazard models treated time to ART resumption as a continuous variable. Logistic regression analyses modeled the proportion of participants with time to ART resumption >12 weeks ($n = 22$) or ≤12 weeks ($n = 25$) as a function of different demographic, reservoir, and immunogenicity parameters. In Cox proportional-hazard models, higher HTI magnitude (HR 0.94, $P = 0.0236$) and lower levels of total HIV-1 proviral DNA (HR 1.12, $P = 0.0047$) at ATI start were significantly associated with longer time to ART resumption (Supplementary Table 10). When using logistic regression models, in addition to the pre-ART HIV-1 pVL, HTI magnitude at study entry and at ATI start significantly increased the odds of remaining off ART >12 weeks during the ATI with odds ratios of 1.34 (95% CI 1.07-1.82; $P = 0.0260$) and 1.09 (95% CI 1.01-1.22; $P = 0.0522$) for each 100 SFC/10^6 PBMC specific for HTI, respectively (Fig. 8).

Discussion

AELIX-003, a phase 2a, double-blind, randomized, placebo-controlled study, confirmed the safety, tolerability, and immunogenicity of HTI-based vaccines (ChAdOx1.HTI plus MVA.HTI; CCMM) when combined with the TLR7 agonist VES in a multi-site trial involving early-treated individuals with HIV. Oral VES 6 mg was generally safe and well-tolerated. In agreement with previous NHP studies combining therapeutic vaccines with VES[13], all participants showed detectable viremia upon ART interruption. However, we did not observe a delay in viral rebound and/or a significant increase in posttreatment control rates, in contrast to previous reports in acutely treated SIV-infected macaques after VES administration in combination with vaccines and/or bNAbs[12]. Post hoc analyses showed that lower pre-ART viremia, smaller reservoir size, and stronger responses to HTI targets correlated with prolonged time to ART resumption. These findings align with and reinforce conclusions from the AELIX-002 trial[3], where an extended HTI vaccine regimen administered alone in a comparable population exhibited similar efficacy. Altogether, our results support further development of HTI vaccines as T-cell targeting backbone components in combination with HIV cure regimens.

Compared with the AELIX-002 trial[3], which tested a more complex and lengthy vaccine regimen comprising a total of eight sequential vaccinations with DNA.HTI, MVA.HTI, and ChAdOx1.HTI (DDDMM followed by CCM), AELIX-003 employed a shorter CCMM regimen. Using this simpler vaccine regimen, AELIX-003 demonstrated sustained strong and broad HTI-specific responses after the first MVA.HTI administration, during all VES dosing periods and upon the second MVA.HTI booster vaccination, and thereafter up to ATI start, potentially supported by the CCMM regimen or the combination with VES. VES PD cytokines were consistently induced over multiple VES dosing, as seen in previous studies in PWH[14,15,19]. However, there was high variability in immune-cell activation in this cohort, and the changes in cellular activation after VES dosing were negligible. This, along with previous data, suggests that a VES dose higher than 6 mg may be required to induce significant T-cell and NK-cell activation in PWH[14,15,19].

Similar to the AELIX-002 trial[3], in which HTI vaccines were administered alone, the combination of HTI vaccines with VES did not affect viral reservoir levels as measured by total or intact HIV-1 proviral DNA. A decrease in HIV-1 intact proviral DNA has been observed in ART-treated HIV controllers treated with VES at doses of 4 to 8 mg[14], but the immune effects of VES on adaptive and innate immune cells were also higher in that setting. Thus, this difference could be attributed to the unique characteristics of the studied HIV controllers, and generalization into wider PWH populations may be limited. Further exploration is warranted into whether the lower dose of VES administered in our study and/or lower resistance to cytotoxic T-lymphocyte-mediated killing in ART-suppressed HIV controllers compared with the AELIX-003 population (which had been ART-treated for a longer period of time), could contribute to this difference. We did not observe consistent increases in HIV-1 RNA measurements that could be related to vaccination and/or VES administration. This is consistent with previous human data, where no viral blips were observed after VES administration[15].

Confirming AELIX-002 findings[3], we observed a positive correlation between the magnitude of HTI T-cell responses and enhanced control over HIV-1 replication after ART interruption. Overall, the total magnitude of HTI-specific responses was associated with a delayed and slower viral rebound, and an extended duration of ART. Interestingly, plasma HIV-1 viremia levels before ART initiation during the acute/recent HIV infection phase were also associated with beneficial outcomes. This observation aligns with findings from NHP studies[11] and AELIX-002[3], and may reflect a lower replicative fitness due to pre-existing, HTI-specific T-cell responses. Also consistent with VES monotherapy studies, lower reservoir levels were correlated with improved ATI outcomes. In AELIX-003, longer periods on suppressive ART, which may contribute to lower reservoir levels, were also correlated with better ATI outcomes, but its role as a potential correlate of control was less clear in univariate Cox

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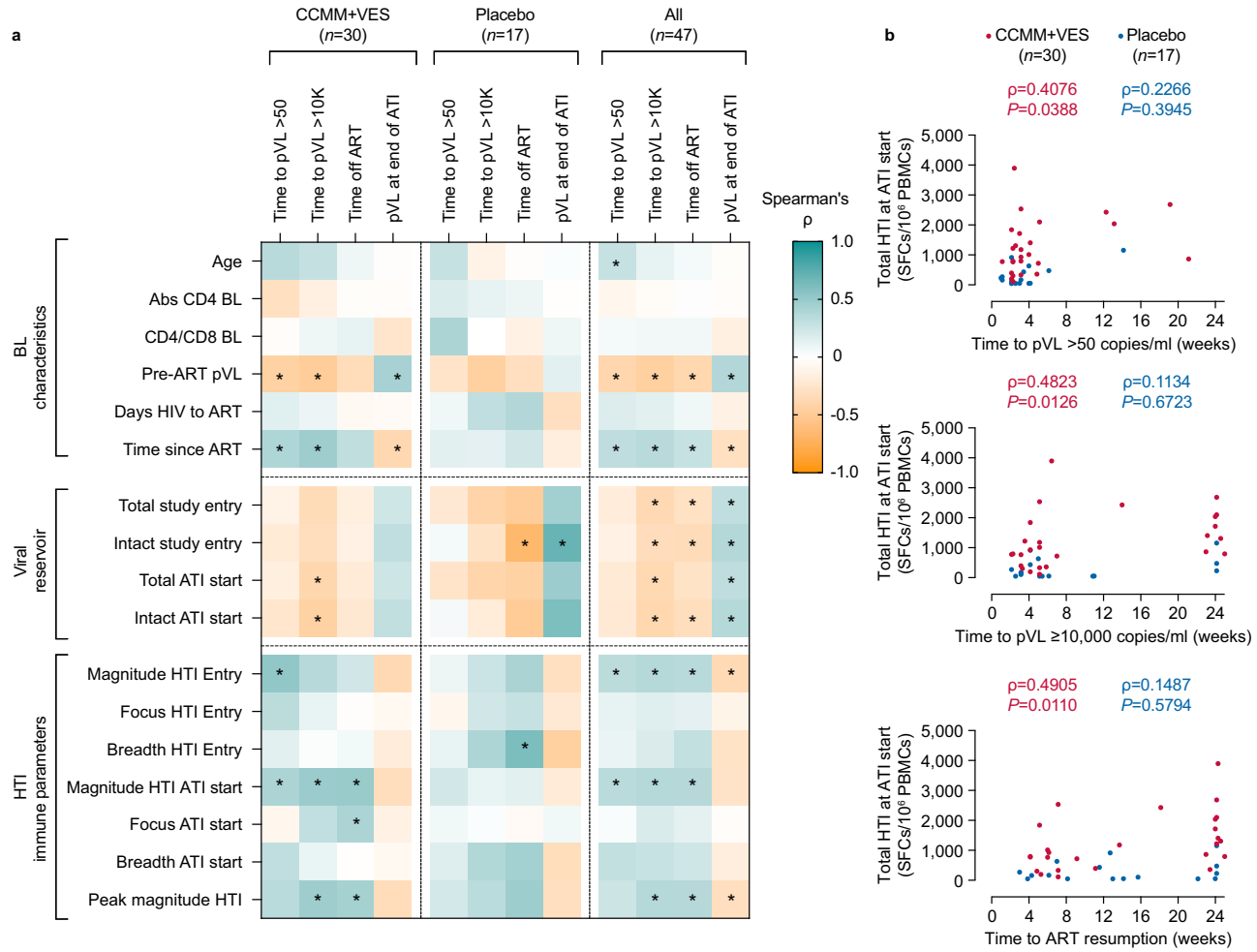

Fig. 7 | Clinical, virological, and immunological correlates of ATI outcomes. **a** are shown in a correlogram for CCMM + VES (left), placebo recipients (middle), and for all (right) participants who entered into the ATI phase ($n = 47$). Spearman's ρ is used for correlations. All tests are two-sided, unadjusted for multiple comparisons, with a 5% error rate. Significant correlations are shown by * when $P < 0.0500$. **b** Individual correlation is shown for HTI magnitude at ATI start and time to either pVL >50, >10,000 copies/ml, or to ART resumption during the ATI in CCMM + VES (red) and placebo (blue) recipients. Spearman's ρ and P values are shown for each treatment group.

proportional-hazard models or when using logistic regression. These findings emphasize the complex interplay of vaccine-induced responses and pre-existing viral characteristics in determining the efficacy of the intervention during ATI.

Limitations of our study include the lack of a single-agent group (receiving only VES or HTI vaccines) to define the VES contribution to CCMM-driven outcomes or to attribute the cytokine changes to VES, vaccination alone, or the combination of both. Based on existing information from NHP studies and AELIX-002[3] with HTI vaccines administered alone in early-treated PWH, the AELIX-003 trial design prioritized including a higher number of participants in the CCMM + VES group over a trial design with multiple single-agent arms to avoid diluting the power to detect meaningful efficacy results between the CCMM + VES and placebo groups. We did not perform additional immune analyses beyond the IFN-γ ELISpot assay in AELIX-003, as the prior AELIX-002 study, using the same HTI immunogen in a comparable trial population, already included a comprehensive characterization of vaccine-induced T cells, including polyfunctionality in both CD4 and CD8 T cells[3]. Notably, in both studies, the magnitude of HTI-specific responses demonstrated similar associations with clinical outcomes, suggesting that any contribution of VES at the tested doses to a distinct functional profile of vaccine-induced responses is likely minimal. Similar to AELIX-002[3] and other contemporaneous HIV cure trials, we were unable to recruit a more heterogeneous and diverse population; thus, extrapolation of our results is limited to those treated early during acute/recent HIV infection, in which both cisgender and transgender women are usually underrepresented. An early-treated population was selected for this translational study because the combination of early ART initiation and length of ART have been found to be beneficial to the preservation of long-lived HIV-specific CD8 + T cells, reducing inflammation, and decreasing HIV reservoir[20,21].

Compared with AELIX-002[3], no clear benefit of CCMM + VES over HTI alone in terms of viral control was observed. However, VES might have contributed to the maintenance of strong HTI responses using a less complex vaccination regimen than was evaluated in AELIX-002[3]. Two ongoing clinical trials (NCT05281510 and NCT06071767) are evaluating VES as an immunomodulator in combination with other modalities such as bNAbs and therapeutic vaccine for HIV remission[22,23]. Our study validates the HTI design and supports the idea that the induction of HIV-specific T cells to vulnerable sites of the virus is a key factor in improving post-rebound viral suppression during an ATI. Avoiding viral rebound, limiting fast viral rebound and/or improving post-rebound control of viremia, and combining therapeutic vaccines with B-cell immunogens or bNAbs (which may also enhance the suppressive capacity of vaccine-induced responses through a vaccinal effect)[24], are of great interest. This is being explored

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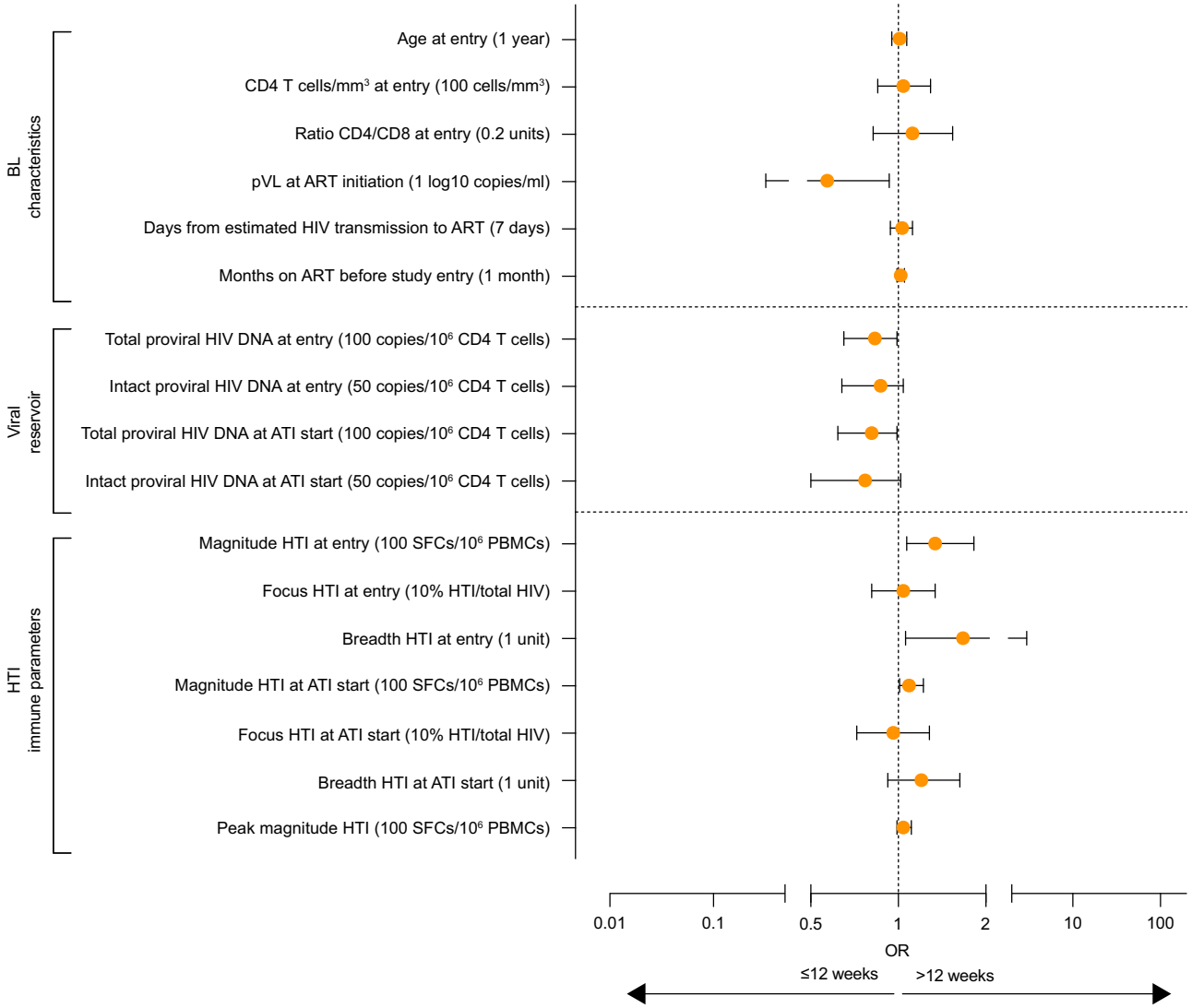

Fig. 8 | Univariate correlate analysis for time off ART. Data were shown for time to ART resumption >12 weeks in univariate logistic regression models in all AELIX-003 participants who entered the ATI phase ($n = 47$). Data were presented as odds ratios (orange-filled circles), and error bars represent 95% confidence intervals. In parentheses, for each variable, the unit of increment is shown for interpretation of the odds ratio. Univariate analyses were not adjusted for multiple comparisons. Abs CD4, absolute CD4 T cells/μl.

in the recently completed BCN03 trial (NCT05208125)[25], which might provide insights into the interaction of humoral responses induced by ConM SOSIP.v7 gp140 vaccines when combined with HTI vaccines.

In conclusion, the combination of ChAdOx1.HTI and MVA.HTI with the TLR7 agonist VES was safe and immunogenic in early ART-treated individuals. This study also validated correlates of improved post-rebound viral control previously identified in AELIX-002[3], which had demonstrated for the first time an enhanced viral control in PWH who mount strong vaccine responses with an extended vaccination regimen. HTI vaccines and VES represent promising components of potential HIV cure regimens, which will need further validation in large clinical trials. Their potential benefits, when combined with other immunomodulators, B-cell vaccines, bNAbs, or alternative vectors to increase HTI vaccine efficacy, warrant continued investigation.

Methods

Study design

AELIX-003 (NCT04364035) was a phase 2a, double-blind, randomized, placebo-controlled, multicenter trial to evaluate the safety, immunogenicity, and efficacy of a heterologous prime-boost HIV-1 therapeutic vaccine regimen (ChAdOx1.HTI and MVA.HTI; CCMM) in combination with a TLR7 agonist (vesatolimod, VES) in 50 early-treated PWH. Participants were enrolled at nine sites in Spain; the first study visit was conducted on May 20, 2019. Study recruitment was interrupted temporarily during the first amendment development and in 2020 due to the disruptions caused by the COVID-19 pandemic. The last participant's last visit was December 16, 2022.

Participants aged 18–61 years old at screening with an HIV-1 confirmed infection treated within 6 months after acquisition (early treatment) with a triple-drug ART regimen were included. Early treatment initiation was confirmed using an in-house-developed algorithm based on Fiebig classification[18,26]. Participants had to be on a suppressive therapy that included ≥3 antiretroviral drugs at study entry, but historical temporary use of a two-drug ART regimen between ART was permitted. Viral suppression (HIV-1 pVL <50 copies/ml) for at least 1 year was required at screening with CD4 T-cell counts ≥450 cells/mm^3 for the past 6 months. Full inclusion/exclusion criteria are available in the protocol as Supplementary Information.

The study was approved by the institutional Ethical Review Committee of Hospital Universitari Germans Trias i Pujol in Badalona, Barcelona, Spain. The study was conducted in compliance with the ethical principles of the Declaration of Helsinki, ICH harmonized

tripartite guideline E6(R2): GCP, and all applicable Spanish regulatory (AEMPS) requirements. Written informed consent (including separate consent for the intensive pharmacokinetic substudy if applicable, including potential updated or new information related to COVID-19, which could impact the study risk-benefit assessment) in compliance with regulatory authority regulations was obtained from each participant before the participant entered the study. All participants received financial compensation for their involvement in the study.

At the screening visit, and after obtaining informed consent, inclusion/exclusion criteria were validated, and blood was drawn for high-resolution HLA typing by next-generation sequencing at Versiti Blood Center of Wisconsin, meeting the American Society for Histocompatibility and Immunogenetics (ASHI) recommendations for resolution of common and well-documented (CWD) alleles. At baseline, participants were randomly assigned in a 2:1 ratio using interactive response technology to receive either CCMM + VES or a placebo. Randomization was stratified by the presence of any HLA class I allele associated with improved natural viral control (any subtype of HLA-B27 and HLA-B57, HLA-B58:01, HLA-B15:16, HLA-B15:17). A subset of participants consented to undergo an intensive sampling for a VES pharmacokinetic substudy. Vaccinations were administered intramuscularly on the non-dominant arm. ChAdOx1.HTI or placebo was given at weeks 0 and 12 weeks followed by MVA.HTI or placebo at 24 and 36 weeks. A total of 10 doses of 6 mg of VES were administered orally, every 2 weeks from weeks 26–34 and from weeks 38–46.

Criteria to enter ATI and resume ART

Two weeks after the last dose of VES or placebo (week 48), participants entered into the ATI period for a maximum of 24 weeks provided that, at week 46, HIV-1 pVL <50 copies/ml and CD4 count >400 cells/mm^3 was confirmed and active infections (hepatitis B, hepatitis C, syphilis, or SARS-CoV-2) were ruled out. Additionally, participants were required to have received at least three doses of the planned CCMM or placebo and at least seven of ten doses of VES or placebo. During ATI visits, symptoms suggestive of acute retroviral syndrome and HIV-1 pVL were monitored weekly, and CD4 T-cell counts on a monthly basis. Criteria for resuming ART included (1) a single HIV-1 pVL ≥100,000 copies/ml, (2) eight consecutive HIV-1 pVL ≥10,000 copies/ml, (3) two consecutive determinations of CD4 count <350 cells/mm^3, and (4) at the investigator's discretion based on clinically significant adverse events, such as grade ≥3 acute retroviral syndromes and/or COVID-19 infection, whichever appeared first. In addition to the abovementioned ART resumption criteria, during the ATI period, intensive efforts were made to monitor for sexually transmitted infections and to implement HIV transmission risk-mitigation strategies. Among those, pre-exposure prophylaxis was provided to HIV-seronegative sexual partners. In cases where the investigator identified activities considered as potentially increasing the risk of HIV transmission, participants were recommended to resume ART per the investigator's criteria. Participants reaching 24 weeks of ATI off ART were then required to resume ART. Time off ART was derived by ART resumption calendar date minus ATI start (week 48) date + 1. Adherence to ART, side effects, viral re-suppression, and CD4 T-cell counts were monitored for 4 and 12 weeks before the study ended.

Study vaccines

The HTI immunogen, comprising a chimeric protein sequence spanning a total length of 529 amino acids covering 26 regions derived from HIV-1 Gag (45%), Pol (44%), Vif (8%), and Nef (3%) proteins, was identified in previous analyses[27] as being (1) preferentially targeted by PWH with low viral loads, (2) having a higher degree of conservation versus other parts of the proteome, and (3) inducing responses characterized by higher functional avidity and broader variant cross-reactivity than responses to other regions[28]. The HTI immunogen is expressed in two viral vectors, ChAdOx1—a replication-defective recombinant chimpanzee adenovirus (ChAd) vector originating from a chimpanzee adenoviral isolate Y25[29]—and the live, attenuated recombinant vaccinia virus, MVA.HTI vaccine, denoted as M (modified vaccinia virus Ankara)[30]. Good manufacturing practice lots for the AELIX-003 trial were produced by ReiThera/Advent/Advaxia (Italy) and IDT Biologika (Dessau, Germany), respectively. ChAdOx1.HTI was dosed at 5×10^{10} viral particles in 0.5 ml and MVA.HTI at 2×10^8 plaque-forming units in 0.5 ml for injection. The matched vaccine placebo included a commercially available 0.9% NaCl solution, delivered as one 0.5-ml intramuscular injection. Syringes were filled and masked by the local unblinded pharmacists and/or study nurses and dispensed to the blinded study personnel for administration. All preparation of the study vaccines/placebos was performed in sterile conditions and following standard procedures.

Vesatolimod

VES was provided by Gilead Sciences, Inc., as unit-dose, 2 mg, round, biconvex, plain-faced, white, film-coated tablets. Placebo tablets matched to the VES tablets were provided as round, plain-faced, white film-coated tablets. Matched placebo tablets for VES were provided as 2 mg tablets of a formulation of lactose anhydrous, microcrystalline cellulose, croscarmellose sodium, magnesium stearate, polyethylene glycol, polyvinyl alcohol, talc, and titanium dioxide. Participants received a total of ten doses of their assigned study treatment (6 mg VES or matched placebo) orally once every 14 days while on ART, with five doses between week 26 and week 34 and the other five doses between week 38 to week 46.

Objectives

The primary objective was to evaluate the safety and tolerability of the heterologous regimen of ChAdOx1.HTI and MVA.HTI administered intramuscularly in combination of the oral TLR7 agonist VES in early-treated PWH. Secondary objectives included (1) evaluating the safety of the ATI period and ART resumption phase; (2) evaluating whether CCMM + VES was able to prevent or delay viral rebound, induce post-rebound viral control, and/or prevent or delay the need for resumption of ART during an ATI period of maximally 24 weeks; and (3) evaluating the immunogenicity of CCMM + VES. Exploratory analyses included VES pharmacokinetic/pharmacodynamic responses and changes in the viral reservoir. Post hoc analyses were performed to explore correlates of ATI outcomes.

Safety

All TEAEs, including serious adverse events, were recorded by the investigators from the time of participant signing the informed consent form until 30 days after the last dose of study treatment. Serious adverse events occurring beyond this period were reported only if considered study drug-related by the investigator. The severity of TEAEs was assessed using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected version 2.1 (March 2017). The National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE version 5.0) was used to grade cytokine release syndrome. MedDRA v23.0 was used to code all adverse events.

Solicited local or systemic reactions were recorded and graded by the participants for a minimum of 7 days after each treatment administration with the use of diary cards. The severity and relationship to each study drug for these participant-reported solicited events were assessed by the investigator per the DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, corrected v2.1, July 2017 (https://rsc.niaid.nih.gov/sites/default/files/daidsgradingcorrectedv21.pdf). Investigators recorded any solicited events noted as grade 3 or 4 by the participants or lasting >7 days after study drug administration as an adverse event.

Risk-mitigation strategies during COVID-19

After the 2020 global declaration of the SARS-CoV-2 pandemic by the World Health Organization, a risk-mitigation plan was implemented to reduce any potential COVID-19-related risks during vaccine or VES administrations. Informed consent was updated continually to provide participants with the latest information on potential COVID-19-related risks.

Individualized assessments were conducted for participants with comorbidities associated with higher risk of severe COVID-19 at study entry. SARS-CoV-2 testing was implemented before interventions and as clinically indicated. Positive test results led to intervention delay or omission, with participants receiving guidance for SARS-CoV-2 clinical care. All COVID-19 events, symptomatic or asymptomatic, were reported as non-related adverse events.

INF-γ ELISpot

HTI-specific and total HIV-1-specific T cells were assessed in cryopreserved and thawed PBMCs with an IFN-γ-detecting enzyme-linked immunoabsorbent spot assay (ELISpot IFN-γ Mabtech kit Cat#3420-2 A) by a central laboratory (Synexa Lab Sciences, London, UK)[3]. Briefly, 15-mer peptides overlapping by 11 amino acids were combined into ten pools spanning different HIV-1 proteins/subproteins of 7–22 peptides per pool corresponding to the HTI vaccine insert (P1–P10; total $n = 111$ peptides, Synpeptide) and eight pools of 62–105 peptides per pool spanning the rest of the HIV-1 viral protein sequences (OUT P1–P8; total $n = 637$ peptides, obtained through the NIH AIDS Reagent Program), tested in duplicate with a final concentration of individual peptide of 1.55 μg ml^{-1}. Medium only was used as no-peptide negative control in quadruplicate wells. Positive controls included two peptide pools covering lytic ($n = 16$) and latent ($n = 36$) Epstein-Barr viral proteins (1.55 μg ml^{-1}, Thermo Fisher), phytohemagglutinin (PHA; 50 μg ml^{-1}, Sigma), and a CEF peptide pool (2 μg ml^{-1}) consisting of 32 human CD8 + T-cell epitopes from cytomegalovirus, Epstein–Barr virus, and influenza virus (Pantec). Spots were counted using an automated Cellular Technology Limited ELISpot reader unit. The threshold for positive responses was set at ≥50 SFCs per 10^6 PBMCs (five spots per well), greater than the mean number of SFCs in negative control wells plus three standard deviations of the negative control wells, or more than three times the mean of negative control wells, whichever was higher.

VES pharmacokinetics

In 30 participants, one pharmacokinetic blood sample was collected within 24 h after vesatolimod dosing at each of weeks 26, 28, 30, 32, 38, 40, 42, and 46. Sixteen participants, with ten and six participants allocated to the CCMM + VES and placebo groups, respectively, were included in a pharmacokinetics substudy with intensive pharmacokinetic blood sampling over 24 h after the first VES dose (week 26). The first sample was collected within 30 min before the first VES dose, and the remaining samples were collected at the following timepoints after vesatolimod dosing: 30 min (±5 min); 1 h (±5 min); 2, 3, 4, 6, 8, and 10 h (all ±30 min); and 24 h (±2 h). The blood pharmacokinetic samples were collected for analysis of plasma VES concentrations using a fully validated high-performance liquid chromatography–tandem mass spectroscopy (LC-MS/MS) bioanalytical method[14]. For the pharmacokinetic substudy population, VES pharmacokinetic parameters were estimated by noncompartmental analysis using WinNonlin.

VES pharmacodynamic evaluation: cytokines and immune-cell phenotyping

The pharmacodynamics of VES were assessed through evaluation of circulating plasma biomarkers reflective of downstream effects of TLR7 stimulation[14], including several cytokines (IP-10, IL-1RA, and IFN-α) and markers of NK/T-cell activation (CD69, CD25, CD38, and HLA-DR) using serum or whole blood collected at baseline (week 0), predose, and 24 h after first (week 26, week 26 + 1 d) and last (tenth) VES dose (week 46, week 46 + 1 d). Serum concentrations of IFN-α were quantified using the ultrasensitive single-molecule array (Simoa), and IP-10, IL-1RA, and ITAC were evaluated with multiplexed immunoassays (Rules Based Medicine, Austin, TX). Whole blood immune-cell phenotyping was performed using a flow cytometry assay (Q^2 Solutions, Durham, NC). Three panels of antibody cocktails were used in the study; all were from BD Biosciences. Panel 1 included anti-CD56 (Cat#562751), anti-CD16 (Cat#563830), Lin3 (Cat#643510), anti-CD38 (Cat#342371), anti-HLA-DR (Cat#339216), anti-CD69 (Cat#340560) and anti-CD45 (Cat#641417); panel 2 included anti-CD4 (Cat#562970), anti-CD8 (Cat#562428), anti-CD3 (Cat#555332), anti-CD25 (Cat#341009), anti-CD45 (Cat#564105), and anti-HLA-DR (Cat#340549); and panel 3 included anti-CD19 (Cat# 562440), anti-CD4 (Cat# 562971), anti-CD3 (Cat# 345764), anti-CD16 (Cat# 332779), anti-CD56 (Cat# 345812), anti-CD45 (Cat# 332784), anti-CD8 (Cat# 345775), and anti-CD14 (Cat# 641394).

Total and intact HIV-1 DNA

Quantification of total and intact proviral HIV-1 DNA copies in CD4 + T cells, aiming to distinguish deleted and/or hypermutated proviruses from intact ones, was performed at screening and at ATI start visits by Accelevir Diagnostics on lysed CD4 + T-cell extracts using digital droplet PCR[31]. The DNA shearing index was computed, and values for intact and defective proviruses were standardized to copies per 10^6 input cells (determined by RPP30, the gene encoding ribonuclease P protein subunit p30) and adjusted for shearing using the DNA shearing index. The results were expressed as counts of HIV-1 DNA copies per 10^6 CD4 + T cells.

ART levels

To ensure ART adherence during the intervention and to rule out ART intake during the ATI, quantification of tenofovir (TFV), emtricitabine (FTC), lamivudine (3TC), and abacavir (ABV) in plasma samples was performed at several study timepoints by a validated method in ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) at Laboratory of Clinical Pharmacology and Pharmacogenetics from CoQua Lab s.r.l., Torino, Italy. Samples from study entry, week 48 (ATI start), and week 72 (end of ATI) were screened for all participants. Additional determination during the ATI period (up to eight determinations per participant) were included depending on the length of the participant's ATI.

Statistics

There were no power calculations for this descriptive study. The sample size was proposed to provide preliminary safety data on the CCMM + VES sequential treatment regimen (primary objective). In terms of the chances of observing an adverse event, 38 participants in the CCMM + VES group provided a high probability (85.8%) that this study would observe at least one event if the event occurred in the population with a true rate of 5%. For each of the primary safety endpoints, the number and percentage of participants were summarized for the safety set by the treatment group. Efficacy endpoints were performed on the intent-to-treat population that entered the ATI period: the number and proportion of the participants remaining off ART, with HIV-1 pVL <50 and <2000 copies/ml at 12 and 24 weeks of ATI were summarized by treatment group, with the associated 95% CI of the proportion using exact (Clopper–Pearson) and compared between groups using the Cochran–Mantel–Haenszel method, stratifying on the factor potential for superior viral control (yes/no) due to the presence of favorable HLA class I genotypes. For the time-to-event endpoints (time to HIV-1 pVL >50, >10,000 copies/ml, and time to ART resumption), the Kaplan–Meier method was used to

estimate the survival function. Median times and the associated 95% CIs estimated by using the Brookmeyer and Crowley method with log-log transformation were reported. Time-to-event endpoints were defined as the time interval (in weeks) between the start date of ATI and the event or censoring date, calculated as (the event or censoring date − the start date of ATI + 1)/7. HTI immunogenicity and reservoir analyses were performed on the intent-to-treat set using only observed values with no imputation in case of missing data. Descriptive statistics were provided for each group at each timepoint for each endpoint: number of participants, standard deviation, median and its 95% CI, min, max, Q1, and Q3. Differences in HTI magnitude at each timepoint, starting at week 4 relative to baseline (week 0), were calculated by Hodges–Lehmann estimates per treatment group, and between-group differences were tested using the van Elteren test with a stratification factor for beneficial HLA genetics. Between-group differences and location shifts are described with an exact CI at the 95% level. Spearman's ρ was used for correlations. All tests were two-sided, unadjusted for multiple comparisons, with a 5% error rate. Post hoc univariate Cox proportional-hazard models, univariate logistic regression models, and Spearman correlations were performed to analyze correlates of ATI outcomes. Non-parametric Wilcoxon tests were used to compare changes in serum/plasma cytokines, gene expression (including IFN-stimulated genes), and immune-cell phenotype/activation at given timepoints between groups (unpaired) or data from two different timepoints within the same group longitudinally (paired). Analyses were performed by PPD Biostatistical team, Gilead study team and AELIX Therapeutic subcontractor Fundació Lluita contra les Infeccions and Marie Pierre Malice of StatAdvice (Brussels) using SAS v9.3 or higher, R project v4.2.1 (https://www.r-project.org/) and GraphPad Prism v10.2.2 for Windows (GraphPad Software, https://www.graphpad.com). All performed analyses matched the pre-specified statistical analysis plan (AELIX-003 SAP, v2, November 2, 2022).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Gilead Sciences shares anonymized individual participant data such as demographics, lab values, etc., as well as related documents such as study protocols and statistical analysis plans, upon request or as required by law or regulation with qualified external researchers based on submitted curriculum vitae and reflecting non-conflict of interest. The request proposal must also include a statistician. Approval of such requests is dependent on the nature of the request, the merit of the research proposed, the availability of the data, and the intended use of the data. The data will be available for 1 year from the signing of Gilead's Data Sharing Agreement contract with an option of 3-month extensions at the deadline if further time is needed for research. Data requests should be sent to datarequest@gilead.com, and requestors can expect a response within 3 business days. For further information regarding Gilead's Data Sharing Policy, please visit GileadClinicalTrials.com.

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Author contributions

B.M., I.M., C.Brander., J.R.A., J.M., and D.S. conceived and designed the study. L.B., I.L., R.G., A.A., and M.G.G. contributed to the study design in further study amendments. L.B., J.M., A.C., J.C., J.C.L.B.Q., I.S., C. Busca, J.A., A.I., S.B., P.S., J.N., J.G.G., L.P.L., J.B., L.J.G.F., G.M.A., J.M.M., S.S., P.D., S.M., J.R.A., and B.M. contributed to clinical development of the study. M.F. and Y.T. conducted the VES pharmacodynamic analysis. I.L., A.A., M.G.G., J.R.A., and B.M. contributed to data management and overall study coordination. Y.A.S. and J.J.W. undertook the statistical analysis. D.L. and S.G. verified the data. L.B., C.Brander, J.M., and B.M. drafted the manuscript. E.V., D.S., J.R.A., J.J.W., and Y.C. revised the manuscript critically for important intellectual content. All authors reviewed and approved the final version of the manuscript.

Competing interests

C.Brander and B.M. are co-inventors of the HTI immunogen (patent application PCT/EP2013/051596). C.Brander, B.M., and I.M. are co-inventors of US patent Application No. 62/935,519 and US Appl. No. 62/851,546, which have relevance to the vaccine regimen used in this study. B.M. reports consultancy, advisory, and/or speaker fees from AELIX Therapeutics, Gilead Sciences, Janssen, ViiV, and MSD. J.M. reports advisory board and speaker fees and grant support from MSD, AbbVie, Boehringer Ingelheim, Gilead Sciences, ViiV Healthcare, Janssen-Cilag, and Bristol Myers Squibb. A.C. reports advisory and speaker fees and grant support from Gilead Sciences, Janssen, MSD, and ViiV Healthcare. P.S. reports advisory and/or speaker fees and/or support for attending meetings from Gilead Sciences, Janssen-Cilag, Merck Sharp & Dohme, Pfizer, and ViiV Healthcare, and has received a research grant from ViiV Healthcare, all outside of the submitted work. J.B. reports speaker fees and grant support from Gilead Sciences and MSD. J.C. reports speaker fees and grant support from Gilead Sciences and MSD. J.A. reports advisory board and speaker fees and grant support from Gilead Sciences, Janssen, MSD, ViiV; data and safety monitoring board membership with Grifols and HIPRA; and is an employee of the European AIDS Clinical Society (guidelines coordinator). A.I. reports advisory and speaker fees and grant support from Gilead Sciences, Janssen, MSD, Theratechnologies, and ViiV. S.M. reports speaking fees and research grants from Gilead Sciences, Janssen-Cilag, MSD, and ViiV. P.D. reports lecture and advisory board fees from ViiV Healthcare, MSD, GSK, Roche, Theratechnologies, Janssen & Cilag, and Gilead. Y.C., Y.T., R.G., D.S., D.L. J.J.W., S.G., and E.V. are employees and stockholders of Gilead Sciences, Inc. M.F. is an employee of ClinData Insight Inc, whose services were funded by Gilead Sciences, Inc. I.M. was an employee of AELIX Therapeutics, is currently an employee of Orion Biotechnology and is a consultant for Synklino. M.G.G. was an employee of AELIX Therapeutics at the time the research was conducted. J.M.M. has received consulting honoraria and/or research grants from Angelini, Contrafect, Cubist, Genentech, Gilead Sciences, Jansen, Lysovant, Medtronic, MSD, Novartis, Pfizer, and ViiV Healthcare, outside the submitted work. J.N. has received honoraria and/or speaking fees and/or financial support for attending conferences from AbbVie, Gilead Sciences, Janssen-Cilag, Merck Sharp & Dome, and ViiV Healthcare outside of the submitted work. C.Brander is the cofounder, shareholder, and CSO of AELIX. J.R.A. reports advisory and speaker fees and grant support from ViiV, Janssen, Gilead Sciences, MSD, and AELIX Therapeutics. The remaining authors declare that the research was conducted in the absence of any conflict of interest.

Additional information

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