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Late Boosting of the RV144 Regimen with AIDSVAX B/E and ALVAC-HIV in HIV-Uninfected Thai Volunteers, a Randomised Controlled Trial

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DECLARATION OF INTERESTS

J. T., and C. D. are employees of Sanofi Pasteur. C.D. is a shareholder at Sanofi Pasteur. S.P. was an employee of Sanofi Pasteur during the conduct of this study and analysis. F. S. is an employee of Global Solutions for Infectious Diseases. P.D. and Y.Z. are employees of Emmes, and receive other funding support for statistical analyses from the Henry M. Jackson Foundation. J.K. reports other from US Department of Defense, during the conduct of the study, non-financial support from GSK, and personal fees from Takeda, outside the submitted work. N.L.M. reports grants from US Army and NIAID/NIH, during the conduct of the study. M.R. reports grants from the Henry M. Jackson Foundation, during the conduct of the study. All other authors report no potential conflicts.

Data Sharing

Study protocol and informed consent documents are available online. Deidentified participant•level data and accompanying research resources are available upon request. Distribution of data will require compliance with all applicable regulatory and ethical processes.

SUMMARY

Background: The RV144 phase 3 vaccine trial in Thailand demonstrated that ALVAC-HIV (vCP1521) and AIDSVAX® B/E administration over six months resulted in a 31% efficacy in preventing HIV acquisition. In this trial, we assessed the immunologic impact of an additional vaccine boost to the RV144 regimen at varying intervals between the priming vaccine series and the boost.

Methods: RV306 is a double-blind, placebo-controlled, randomized clinical trial conducted in three clinical sites in Thailand. HIV-uninfected volunteers aged 20–40 randomly received the primary RV144 vaccine series at months 0, 1, 3, and 6, with no additional boost (Group I), additional AIDSVAX® B/E and ALVAC-HIV (vcp1521) at month 12 (Group II), AIDSVAX® B/E alone at month 12 (Group III), AIDSVAX® B/E and ALVAC-HIV at month 15 or 18 (Groups IVa or IVb), or placebo and were followed for 24 months. A randomization schedule was centrally generated with fixed sized strata for RIHES Chiang Mai (n=60) and combined Bangkok clinics (n=300). Primary outcomes were to assess the safety and tolerability of these vaccination regimens and characterize and compare cellular and humoral immune responses between the RV144 series alone and late boosts at different timepoints. Safety and tolerability outcomes were assessed by evaluating local and systemic reactogenicity and adverse events in all participants. Primary immunogenicity outcomes were evaluated by comparing peak humoral responses (HIV-specific IgG and IgA ELISA) and cellular responses (HIV-specific intracellular cytokine staining and polyfunctionality) two weeks post final vaccination among per-protocol participants who completed all vaccinations. This trial is registered at (ClinicalTrials.gov (NCT01931358); clinical follow up is now complete.

Findings: Between 28 October 2013 and 29 April 2014, 367 participants were enrolled, of whom 27 were assigned active vaccination in Group I, 102 in Group II, 101 in Group III, 52 in Group IVa, 51 in Group IVb, and 34 combined placebo across all groups. Late boosting did not induce vaccine-related serious adverse events. There were no significant differences in the occurrence or severity of local or systemic reactogenicity across active groups. Groups with late boosts (Groups II, III, IVa, and IVb) had increased peak plasma IgG binding antibody levels against gp70 V1V2 relative to Group I vaccine recipients with no late boost (gp70V1V2 92TH023 adjusted $p < 0{\cdot}02$ for each; gp70V1V2 Case A2 adjusted $p<0{\cdot}0001$ for each). Boosting at month 12 (Groups II and III) did not increase gp120 responses compared to the peak responses after the RV144 priming regimen at month 6; however, boosting at month 15 (Group IVa) improved responses to gp120 A244gD- D11 ($p=0{\cdot}0003$), and boosting at month 18 (Group IVb) improved responses to both gp120 A244gD- D11 ($p<0{\cdot}0001$) and gp120 MNgD- D11 ($p=0{\cdot}0016$). Plasma IgG responses were significantly lower among vaccine recipients boosted at month 12 (pooled Groups II+III) than at month 15 (Group IVa; adjusted $p < 0{\cdot}0001$ for each except for gp70 V1V2 CaseA2 $p = 0{\cdot}0142$) and at month 18 (Group IVb; all adjusted $p < 0{\cdot}001$). Boosting at month 18 versus month 15 resulted in a significantly higher plasma IgG response to gp120 antigens (all adjusted $p < 0{\cdot}01$) but not gp70 V1V2 antigens. CD4+ functionality and polyfunctionality scores after stimulation with HIV-1 Env peptides (92TH023) increased with delayed boosting: month 18 (Group IVb) > month 15 (Group IVa) > month 12 (Groups II+III) > no late boost (Group I). Groups with late boosts had increased both functionality and polyfunctionality scores relative to vaccine recipients with no late boost (all adjusted $p < 0{\cdot}05$, except for polyfunctionality score in Group I vs in Group IVb $p < 0{\cdot}01$).

Interpretation: Taken together, these results suggest that additional boosting of the RV144 regimen with longer intervals between the primary vaccination series and late boost improved immune responses and may improve the efficacy of preventing HIV acquisition.

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INTRODUCTION

The RV144 phase 3 trial (NCT00223080) conducted in Thailand has been the only vaccine trial to date demonstrating any efficacy in preventing HIV infection. Healthy volunteers who received the six-month vaccination regimen containing ALVAC-HIV (vcp1521) and AIDSVAX® B/E were 31% less likely to become HIV infected over 3·5 years of follow up than participants who received placebo [1]. In a post-hoc analysis, efficacy was 60% at 12 months, indicating that protective immunity may have waned rapidly [2]. The magnitude of plasma IgG antibodies to the scaffolded variable regions 1 and 2 (V1V2) of HIV-1 envelope was inversely correlated with risk, while plasma IgA antibody titers to HIV-1 gp120 envelope were directly correlated with risk [3]. Plasma IgA may increase risk by competitive binding to the gp120 C1 region of envelope on HIV, thus blocking IgG effector function to facilitate viral clearance [4]. HIV-specific polyfunctional CD4+ T cells capable of simultaneously producing multiple effector cytokines and other functional markers also correlated with protection in RV144 [5]. Increasing the magnitude of IgG anti-V1V2 responses while minimizing concomitant increases in plasma IgA and expanding CD4+ T cell function are key goals in improving the potential for protective efficacy of the RV144 regimen.

The RV305 clinical trial (NCT01435135) boosted 162 vaccine recipients from the RV144 trial six to eight years later, at months 0 and 6 with ALVAC-HIV and AIDSVAX® B/E, either alone or together. Long-term memory responses to HIV-1 antigens persisted, allowing boosting of HIV-specific plasma antibody responses to levels higher than observed in the RV144 trial [6]. Late boosting expanded a subdominant pool of envelope CD4 binding site-reactive memory B cells with increased somatic hypermutation, long third heavy chain complementarity determining regions (HCDR3), and Tier 2 neutralization capacity [7]. HIV-specific antibody responses were also present in cervicovaginal secretions, rectal secretions and seminal plasma [8].

However, a six to eight-year interval between priming and boosting would be logistically challenging and of limited impact in mobile populations most at risk for HIV infection. We conducted the RV306 trial (NCT01931358) to determine whether similar improvements in quality, magnitude or duration of humoral, cellular and mucosal responses could be afforded by boosting the RV144 regimen at either 12, 15, or 18 months post initial vaccination series, and to determine the optimal boosting interval for further clinical development (Figure 1).

METHODS

Study Design and Participants

Volunteers were healthy, HIV-uninfected Thai volunteers at low risk for HIV-1. Female participants agreed to contraception 45 days prior to first vaccination and for 3 months following final vaccination. All volunteers provided written informed consent. The study was approved by ethical review boards at the Walter Reed Army Institute of Research, Thai Ministry of Public Health, Royal Thai Army Medical Department, Faculty of Tropical Medicine, Mahidol University, Chiang Mai University, and Chulalongkorn University Faculty of Medicine, and conducted in accordance with Good Participatory Practice principles [(9)]. Volunteers were enrolled at either the Vaccine Trial Centre, Faculty of Tropical Medicine, Mahidol University in Bangkok, Thailand, the Royal Thai Army Clinical Research Center, AFRIMS in Bangkok, Thailand, or the Research Institute for Health Sciences (RIHES), Chiang Mai University in Chiang Mai, Thailand. Eligible volunteers were healthy, HIV-uninfected male and female volunteers between age 20 and 40, who were at low risk for HIV infection per investigator assessment, had normal clinical screening laboratory tests, were not pregnant or lactating, used adequate birth control for at least 3 months post final vaccination, and successfully completed a Test of understanding.

ALVAC-HIV (vCP1521) (manufactured by IDT Biologika, Germany, for Sanofi Pasteur) and AIDSVAX® B/E vaccine (manufactured by Genentech Inc. for Global Solutions for Infectious Diseases, formerly VaxGen) were administered as per the schedule in Figure 1 and formulated, reconstituted, and administered as in the RV144 trial [(1)]. Additional details are in the Appendix page 3).

All groups received the original RV144 series, ALVAC-HIV at months 0 and 1 (study weeks 0 and 4) followed by ALVAC-HIV and AIDSVAX® B/E at months 3 and 6 (study weeks 12 and 24), or placebo. Group I control volunteers did not receive any additional boosting; Group II received ALVAC-HIV and AIDSVAX® B/E or placebo at month 12 (study week 48), Group III received AIDSVAX® B/E alone or placebo at month 12, Group IVa received ALVAC-HIV and AIDSVAX® B/E or placebo at month 15 (study week 60), and Group IVb received ALVAC- HIV and AIDSVAX® B/E or placebo at month 18 (study week 72) (Figure 1).

Randomization and Masking

Volunteers were randomized into groups and to receive vaccine or placebo at a ratio of 10 to 1 per group in a blinded manner. The statistical center (Rockville, MD, USA) produced the block-randomized sequence by computer-generated random numbers, which were provided independently to each study site. Placebo recipients from all groups were combined for safety and immunogenicity analyses.

Procedures

HIV infection status was determined at screening and at months 0, 6, 12, and 24. Volunteers recorded local and systemic reactions on a diary card for 3 days following vaccination. Adverse events (AEs) occurring up to 3 months after last vaccination and all serious adverse events (SAEs) throughout the trial were recorded. Safety laboratory assessments including urine dipstick, complete blood cell count with differential, plasma creatinine and liver enzymes were obtained at baseline and months 6, 12, and 24. Female participants underwent urine pregnancy testing at baseline, immediately prior to each vaccination and/or optional invasive procedures, and at study completion.

HIV-1-specific plasma IgG and IgA ELISA antibody responses were assessed using rgp120 and scaffold proteins [(6)]. Capture antigens included V1V2 sequences from both subtype AE and Subtype B HIV-1 Env (gp70 V1V2 92TH023 and gp70 V1V2 Case A2) [(3, 10)], and HIV-1 Env gp120 proteins matched to sequences in AIDSVAX® B/E without the gD tag and with an 11 amino acid N-terminal deletion [(11)], represented as gp120 A244gD- D11 and gp120 MNgD- D11. Durability of IgG response was assessed for each participant by estimating the decline in $\log_{10}$ IgG from peak to 6 months post final vaccination. As no visit was scheduled at month 21 (6 months post peak for Group IVa), the midpoint of month 18 and month 24 was used to impute the $\log_{10}$ endpoint titer of Group IVa at that time [(12)]. Neutralizing antibodies were measured in TZM-bl cells [(13)].Tier 2 neutralization was assessed using a panel of 11 CRF01_AE pseudoviruses, and a global panel [(14, 15)].

Intracellular cytokine staining was performed as previously described [(6)], and functionality scores and polyfunctionality scores were calculated via COMPASS analyses [(5)]. Antigen specific cellular proliferation was assessed by quantification of CFSE (5–6-carboxyfluoresceindiacetate succimidyl ester) low CD4+ and CD8+ T cells, central memory (CM; CD27+/CD45RO+) and effector memory (EM: CD27−/CD45RO+) T cells. Detailed assay methods are in the Appendix pages 4–5.

Outcomes

Primary outcomes were to assess the safety and tolerability of these vaccination regimens and characterize and compare cellular and humoral immune responses between the RV144 series alone and late boosts at different timepoints. Safety and tolerability outcomes were assessed by evaluating local and systemic reactogenicity and adverse events in all participants. Primary humoral immunogenicity outcomes were evaluated by comparing peak humoral responses two weeks post final vaccination among per-protocol participants who completed all vaccinations by quantifying the relative change in magnitude of the HIV-specific plasma IgG and IgA response to subtype B and AE gp120 and V1V2 antigens. Primary cellular immunogenicity outcomes were evaluated by comparing peak CD4+ and CD8+ T cell responses two weeks post final vaccination among per-protocol participants who completed all vaccinations by quantifying the relative change in magnitude in HIV-specific intracellular cytokine staining and polyfunctionality after stimulation with HIV peptide pools. Secondary outcomes were to evaluate and compare lymphoproliferation responses and innate responses between vaccination regimens; the latter evaluation remains ongoing.

Statistical Analysis

The study was powered (>80%) to detect >20% differences in response rates between individual boost arms (n = 100) and the non-boost arm (n = 27) with two-tailed 5% level test while also permitting detection of mean differences of 0·4 standard deviations between boost arms. Immune responses were assessed two weeks after final vaccination in each Group. ELISA endpoint titers of immunoglobulin (Ig) and 50% inhibitory dose (ID50) of TZM-bl neutralizing antibody within a group were summarized by GMT with associated 95% confidence intervals based on the normal distribution. CD4+ TH023-specific functionality and polyfunctionality scores were calculated by COMPASS using a Bayesian approach to jointly model all cell subsets and are shown as group medians and interquartile ranges [5]. The responses over time within each group were assessed by positive incremental area under the curve (AUC) from month 0 to month 24. Comparisons of two groups were conducted using Mann-Whitney U tests for continuous variables and Barnard's unconditional exact tests for binary responses. All reported p-values for pairwise comparisons were adjusted by step-down Bonferroni methods to control the familywise error rate across all pairs for a given assay. All tests were 2-sided at the adjusted α=0·05 level. Analyses were conducted in SAS v9·4 (SAS Institute, Inc., Cary, NC). Additional details are in the Appendix pages 6–7.

Role of the funding source

Funders contributed to, reviewed, and approved the RV306 study design and outcomes and reviewed data. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Screening of 608 individuals was conducted to enroll 360 volunteers (Figure 2), enrolled from October 28, 2013 to April 29, 2014. There was no significant difference in demographic factors among volunteers in each group, and distribution of Group allocation was relatively similar across each of the three study sites: Mahidol University, Bangkok, Thailand, Royal Thai Army, Armed Forces Medical Research Institute of Science, Bangkok, Thailand, and Research Institute for Health Sciences and Faculty of Public Health, Chiang Mai University, Chiang Mai, Thailand (Table 1). A total of 367 volunteers received their initial vaccination. Seven volunteers who withdrew after initial vaccination during the study enrollment period were replaced with additional volunteers per protocol specifications. Of these 360 volunteers who received initial vaccination, 348 (96·7%) received the initial RV144 vaccination series over 6 months. An additional 14 volunteers withdrew prior to the late boost, with a disproportionate number of withdrawals (n=7) occurring in Group IVb due to unavailability of investigational product toward the end of the vaccination period. A total of 334/360 (92·7%) planned volunteers received all vaccinations and completed all study visits (Figure 2). Study deviations did not affect the primary and secondary outcomes of the study.

Serious adverse events (SAEs) were reported in 18 (5%) volunteers. Seventeen of them were from the active treatment groups with one in Group I, eight in Group II, three in Group III, three in Group IVa, and two in Group IVb. None of the SAEs were considered related to vaccine administration, and all but one SAE, a limb injury not related to vaccination, resolved without sequelae. There was no difference in distribution of adverse events across active treatment groups, and all related AEs had resolved by study end. A summary of AEs is on Table 2, with additional details in the Appendix pages 8–15.

Most participants experienced a local reaction after any vaccination, compared to the placebo group (Barnard's exact test p=0·0011). All severe local reactions resolved without sequalae. There were no significant differences in the occurrence or severity of local reactogenicity across active groups. No statistically significant differences in the proportion of participants with systemic reaction were observed among groups. All severe local and systemic reactions resolved without sequelae. Overall, the proportion of participants with local reactogenicity was not statistically significantly different between females and males; however, females were more likely to report a systemic reaction (142/191 vs. 104/176 or 74·4% vs. 59·1%, p=0·0021). Additional details on local and systemic reactogenicity are depicted in Appendix page 15.

No volunteers were enzyme immunoassay (EIA) reactive at study entry. Of the 317 vaccine recipients at month 6·5 (2 weeks after the RV144 vaccination regimen), 28 (9·0%) became EIA reactive. After boosting, EIA reactivity in Group III (12/97; 12·38%) was similar to Group IVb (7/50; 14·00%), while Group I had a similar EIA reactivity rate (2/27; 7·41%) to Group II and Group IVa (5/96; 5·21% and 2/47; 4·26%, respectively). Of 28 samples with EIA reactivity, 36% (n=10) were negative by Western blot testing while 61% (n=17) (60·72%) had indeterminate Western blots. All but one of these volunteers had HIV-1 RNA below the limit of detection (<50 copies/mL). One vaccine recipient in Group IVa with reactive EIA and a positive Western blot had a new diagnosis of HIV infection confirmed by nucleic acid testing. For the remaining participants, EIA reactivity waned rapidly, as no vaccine recipient from any group was EIA reactive at month 12, 6 months after the RV144 vaccination regimen. At the end-of-study, 2 (0·65%) of 310 vaccine recipients, both in Group III, remained EIA reactive with an indeterminate Western blot, with HIV-1 RNA below limit of detection (<50 copies/mL).

Immune responses among vaccine recipients who completed all vaccinations were analyzed. More than 99% (302/303 for HIV-1 Env gp120 and gp70V1V2 Case A2, 303/303 for HIV-1 Env gp70V1V292TH023) of participants who received active vaccinations developed measurable IgG antibodies. Plasma IgG binding antibody levels to HIV-1 Env gp120 (A244gD- D11 and MNgD- D11) and V1V2 (gp70V1V2 92TH023 and gp70V1V2 Case A2) antigens are depicted in Figure 3, expressed as geometric mean endpoint titer (GMT). Groups with late boosts (Groups II, III, IVa, and IVb) had increased peak plasma IgG binding antibody levels against gp70 V1V2 relative to Group I vaccine recipients with no late boost (gp70V1V2 92TH023 adjusted p values were 0.0084, 0.0184, <0.0001, and <0.0001 when compared Group I to Groups II, III, IVa, and IVb, respectively; gp70V1V2 Case A2 adjusted p<0·0001 for each). Boosting at month 12 (Groups II and III) did not increase gp120 responses compared to the peak responses after the RV144 priming regimen at month 6; however, boosting at month 15 (Group IVa) improved responses to gp120 A244gD- D11 (p=0·0003), and boosting at month 18 (Group IVb) improved responses to both gp120 A244gD- D11 (p<0·0001) and gp120 MNgD- D11 (p=0·0016). Similarly, area under the log GMT curve (AUC) of plasma IgG responses to all antigens was significantly lower in Group I than in any other group (adjusted p < 0·0001 for each pairwise comparison).

Similar to results in the RV305 trial [6], inclusion of ALVAC-HIV elicited similar responses to boosting with AIDSVAX® B/E alone, and no significant differences in plasma IgG responses to any antigen were found between Groups II and III. Therefore, results from month 12 late boost Groups II and III were pooled for analyses of the effect of timing of late boost on immune responses. Plasma IgG responses were significantly lower among vaccine recipients boosted at month 12 (pooled Groups II+III) than at month 15 (Group IVa; adjusted p < 0·0001 for each except for gp70 V1V2 CaseA2 p = 0·0142) and at month 18 (Group IVb; adjusted p<0·0001 for each except for gp70 V1V2 CaseA2 p=0·0007). Boosting at month 18 versus month 15 resulted in a significantly higher plasma IgG response to gp120 antigens (gp120A244gD-D11 adjusted p=0.0040, gp120MNgD-D11 adjusted p=0.0085) but not gp70 V1V2 antigens. The IgG AUC did not significantly differ among any pair of late boost groups (II+III, IVa, and IVb) for any antigen.

Durability of plasma IgG responses was quantified for each participant by estimating the decline in $\log_{10}$ IgG from peak to 6 months post final vaccination. The median decrease of plasma IgG responses to both gp120 antigens was 0·068 fold per week in Group I, compared with 0·041 per week each in Groups II+III, IVa, and IVb, corresponding to a median decrease of 32-fold in Group I and 8-fold in other groups (adjusted p < 0·0001 for each). The rate of GMT decline to gp70 V1V2 92TH023 was significantly higher in Group IVb than in Group II+III (0·068 vs 0·055 per week, p < 0·0001); however, no significant differences in decay rate to gp70 V1V2 Case A2 was found between any pair of groups.

As gender distribution was largely similar across groups, differences in IgG responses by sex were assessed among all vaccine recipients and within groups. Overall peak GMT was similar between males and females in responses to gp120 A244gD- D11 and gp70 V1V2 92TH023, but higher in females than males overall in responses to gp120 MNgD- D11 (female (n=158): GMT (95%CI)=22,542 [20,417, 24,887]; male (n=145): GMT (95%CI)=19,034 [17,116, 21,167]; p=0·041) and gp70 V1V2 Case A2 (female: GMT (95%CI)=1,809 [1,548, 2,114]; male: GMT (95%CI)=1,347 [1,168, 1,553]; p=0·0038). However, there was no effect of gender on peak immune responses within each group, except for plasma IgG responses to gp70 V1V2 Case A2 in Group II: female (n=49): GMT (95%CI)=2,215 (1,807, 2,715); male (n=46): GMT (95%CI)=1,114 (863, 1,439); adjusted p = 0·0007.

Plasma IgA responses are depicted in Figure 4. Unlike plasma IgG, groups with late boosts had no significant increase in plasma IgA HIV-1 Env and V1V2 binding antibody GMT over month 6 responses, either when comparing two weeks post vaccination or total AUC among groups.

Plasma TZM-bl neutralizing antibodies against a panel of tier 1 pseudoviruses (PSVs) is shown in Figure 5. 100% of all vaccine recipients had detectable neutralization activity at least at one time point, whereas placebo recipients had no detectable neutralization. Late boosting at any time point improved infectious dose, 50% (ID50) neutralization titers to

Subtype AE and C PSVs over no late boosting in Group I (TH023.6 adjusted p<0.0001 for each except for comparison of Group I vs Group II p=0.0003, MW965.26 adjusted p <0·0001 for each), whereas only late boosting at month 15 or month 18 improved neutralization activity against Subtype B MN.3 (Group IVa p=0·0207; Group IVb p=0·0030). Response rates to subtype AE and C viruses increased with late boosting when compared to no late boosting in Group I (TH023.6 adjusted p values were 0.0001, 0.0076, 0.0052, and 0.0076 when compared Group I to Groups II, III, IVa, and IVb, respectively, MW965.26 adjusted p < 0·0001 for each). No significant differences in response rates were found against subtype B MN.3 and SF162.LS. Similar to plasma binding IgG, no significant difference was seen between month 12 boosting with AIDSVAX® B/E with or without ALVAC-HIV (Groups II versus III) for any PSV. Little or no detectable increase in neutralizing activity was seen in placebo recipients at any time point or against the negative control PSV, MuLV, for any tested sample. Little tier 2 virus neutralization was observed after initial vaccination or boost. When neutralization of tier 2 pseudoviruses was detected, titers were low. For a subset of plasma samples, IgG was depleted from the plasma to confirm that the low titer neutralizing activity observed in the whole plasma was IgG mediated (Appendix page 16)

T-cell responses were measured after stimulation with HIV-1 peptide pools for 92TH023 Env, LAI Gag and the V2 loop by intracellular cytokine staining. ALVAC-HIV boosting did not appear to affect cellular responses, as there were no significant differences in ICS, functionality, polyfunctionality, or antigen-specific cellular proliferation between month 12 boosting with AIDSVAX® B/E with or without ALVAC-HIV (Groups II versus III). Data from these groups were then combined for subsequent comparison on the effect of the interval between the priming series and late boost on cellular immune responses.

Envelope-specific CD4+ T cells were readily detected in vaccine recipients expressing IFNγ (34%), IL-2 (39%) or TNFα (18%) after the primary vaccination scheme. Two weeks after boosting at 12, 15 or 18 months, envelope-specific CD4+ T cells expressing IFNγ (32%), IL-2 (30%) or TNFα (21%) were detected, whereas responses in participants not receiving a boost waned considerably. Envelope-specific IFNγ response magnitude ranged up to 0·25% of CD4+ T cells after primary vaccination and was maintained at levels up to 0·33% after the late boost. Magnitude of the envelope-specific IL-2 response was similar and maintained at levels up to 0·46% after the late boost. No differences were observed in the frequency of responders or magnitude of the envelope-specific CD4+ T-cell response after the 12-, 15- or 18-month boosts (Appendix page 17). Minimal responses were detected for CD4+ T-cell responses against V2 loop peptides or HIV-1 Gag peptides, and CD8+ T-cell responses to all peptides.

As depicted in Appendix page 18, CD4+ functionality and polyfunctionality scores after stimulation with HIV-1 Env peptides (92TH023) increased with delayed boosting: month 18 (Group IVb) > month 15 (Group IVa) > month 12 (Groups II+III) > no late boost (Group I). Groups with late boosts had increased both functionality (adjusted p values were 0.0316, 0.0316, and 0.0174 when compared Group I to Groups II+III, IVa, and IVb, respectively) and polyfunctionality (adjusted p values were 0.0183, 0.0156, and 0.0082 when compared Group I to Groups II, III, IVa, and IVb, respectively) scores relative to vaccine recipients with no late boost. A heatmap of posterior probabilities for functional CD4 T cell subsets two weeks post final vaccination is depicted in Appendix page 19. Boosting at month 18 improved both functionality and polyfunctionality scores over boosting at month 12 (functionality score adjusted p=0.0321, polyfunctionality score adjusted p=0.0363). A post hoc regression analysis showed a significant association between timing of the boost and peak functionality (p<0·0001) and polyfunctionality (p<0·0001). None of the comparisons were significant for CD8+ T cell functionality scores. To further characterize envelope-specific T cell responses, CD4+ T cell proliferation was assessed upon stimulation with 92TH023 Env peptide pools using a carboxyfluorescein succinimidyl ester (CFSE)-based assay. CD4+ T cell proliferation was detected two weeks after the primary RV144 vaccination series (month 6) in 70/79 (89%) vaccine recipients with a median magnitude of 4·94%. CFSElow CD4+ T cells. After six months (month 12), proliferative responses decreased significantly in Group I participants in the absence of a late boost to 5/9 volunteers (56% response rate; median CD4+CFSElow: 1·45%, p=0·0078, Appendix page 20). However, late boosting re-stimulated the antigen-specific CD4+ T cell proliferation at two weeks following late boosts at month 12, 15 and 18, with 22/31 volunteers (71% of response rate; median CD4+CFSElow: 3·38%), 15/18 volunteers (83% response rate; median CD4+CFSElow: 8·72%) and 14/18 volunteers (78% response rate; median CD4+CFSElow: 5·89%), respectively. There was no significant difference in response rate or median response frequency between late boost groups. In addition, the proliferation of both effector memory (TEM; CD45RO+CD27−) and central memory (TCM; CD45RO +CD27+) CD4+ T cells was maintained by the late boost.

DISCUSSION

One potential strategy to improve upon the partial efficacy of the RV144 vaccination regimen is to boost waning immune responses using additional vaccinations. We conducted a detailed evaluation of the effect of an additional boost with AIDSVAX® B/E with or without ALVAC-HIV on safety and immune responses and assessed the impact of varying the interval between priming and boosting.

Vaccination at month 12 with AIDSVAX® B/E with or without ALVAC-HIV, or at month 15 or 18 with both vaccines, significantly improved humoral and cellular immunogenicity relative to participants who did not receive a late boost to the RV144 vaccine regimen. Because plasma IgG binding to scaffolded gp70 V1V2 was an inverse correlate of risk in RV144 while plasma IgA binding to gp120 was a direct correlate of risk [3], it is encouraging that late boosting resulted in an increased plasma IgG to IgA ratio over time. The fact that late boosting increased the durability of plasma IgG responses is important given that responses in the RV144 trial were not durable and may have contributed to waning efficacy [2]. These data support the incorporation of a late boost, consistent with the HVTN 702 Phase 2b/3 efficacy trial currently ongoing in the Republic of South Africa (NCT02968849), where participants receive the primary RV144 vaccination series over six months with late boosts at months 12 and 18 [16].

In the RV305 trial, boosting RV144 vaccine recipients after 6 to 8 years demonstrated that immune responses to AIDSVAX® B/E were higher after the initial boost than after the second boost six months later [6], suggesting that a delayed interval between primary vaccination series and late boosting may generate stronger immunity to booster vaccinations. In this study, boosting at month 15 or 18 resulted in stronger humoral and cellular responses than boosting at month 12, and longer boosting intervals also improved neutralizing antibody responses. While vaccine boosts at any time point improved neutralization of tier 1 and CRF01_AE transmitted founder PSVs, only boosting at month 15 or month 18 improved neutralization of subtype B MN.3. This may be due to the preferential expansion of subdominant HIV-neutralizing B cell clonal lineages as demonstrated in the RV305 trial [7], which coincided with increased somatic hypermutation and third heavy chain complementarity determining regions (HCDR3) length of HIV-1 envelope CD4 binding site-reactive antibodies, both properties of broadly neutralizing antibodies [17–20]. Analyses of HCDR3 length and somatic hypermutation in RV306 vaccinees are ongoing, along with quantification of polyclonal avidity, Fc-effector profiles and IgG subclass distribution, given that differences in these factors may impact vaccine efficacy [21–23]. Because the RV144 regimen induced weak neutralization and may have prevented HIV infection through largely non-neutralizing mechanisms [24–26], investigations to assess the impact of late boosting on non-neutralizing antibody effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) are also ongoing.

T cell polyfunctionality is known to be involved in viral control in HIV-infected individuals [27], and is an inverse correlate of acquisition in healthy volunteers [5]. Late boosting improved envelope-specific CD4+ T-cell polyfunctionality, and increasing the interval between prime and boost further improved polyfunctionality. Antigen-specific CD4+ T-cell cytokine responses are also associated with proliferative capacity. In HIV-infected individuals, HIV-specific CD4+ T-cell proliferation has been associated with control of viral replication and prevention of disease progression [28]. In healthy individuals, HIV-specific T-cell proliferation has been induced by other vaccine regimens [29], and has been associated with protection from HIV acquisition in a cohort of Kenyan sex workers [30]. Longitudinal analyses in RV306 clearly demonstrate that late boosting is required to maintain this response, as envelope-specific CD4+ T-cell proliferation decreased significantly six months post vaccination without boosting. Waning of these responses without late boosting may have contributed in part to the waning efficacy over time in RV144 [2]. As CD4+ T cells are known to provide help to B-cell activation and differentiation, we are currently investigating the relationship of CD4+ T-cell polyfunctionality and proliferative capacity to plasmablast and memory B-cell functions. Overall, some analyses were limited by smaller or unequal group sizes or lack of extended follow up allowing for prolonged analyses of durability of responses.

Taken together, these data support the possibility that addition of a late boost to the RV144 vaccine regimen may improve protective efficacy by improving humoral immunogenicity, neutralization capacity and cellular polyfunctionality while maintaining antigen-specific CD4+ proliferation. Furthermore, lengthening the interval between primary vaccination series and late boosting may be beneficial in improving antigen-specific immune responses. However, when vaccinating persons at greater risk for HIV infection, it is possible that delaying a late boost may extend the period of suboptimal immune protection from HIV acquisition between priming and boosting, thus increasing risk of infection prior to the late boost. Conversely, a delayed interval between primary vaccination series and boosting may be readily achievable in a pediatric population, where a primary vaccination series could be administered in childhood with a delayed boost in adolescence, an age of very high HIV risk across the globe. Therefore, the optimal interval between priming and boosting is subject to multifactorial determinants and may be population-specific.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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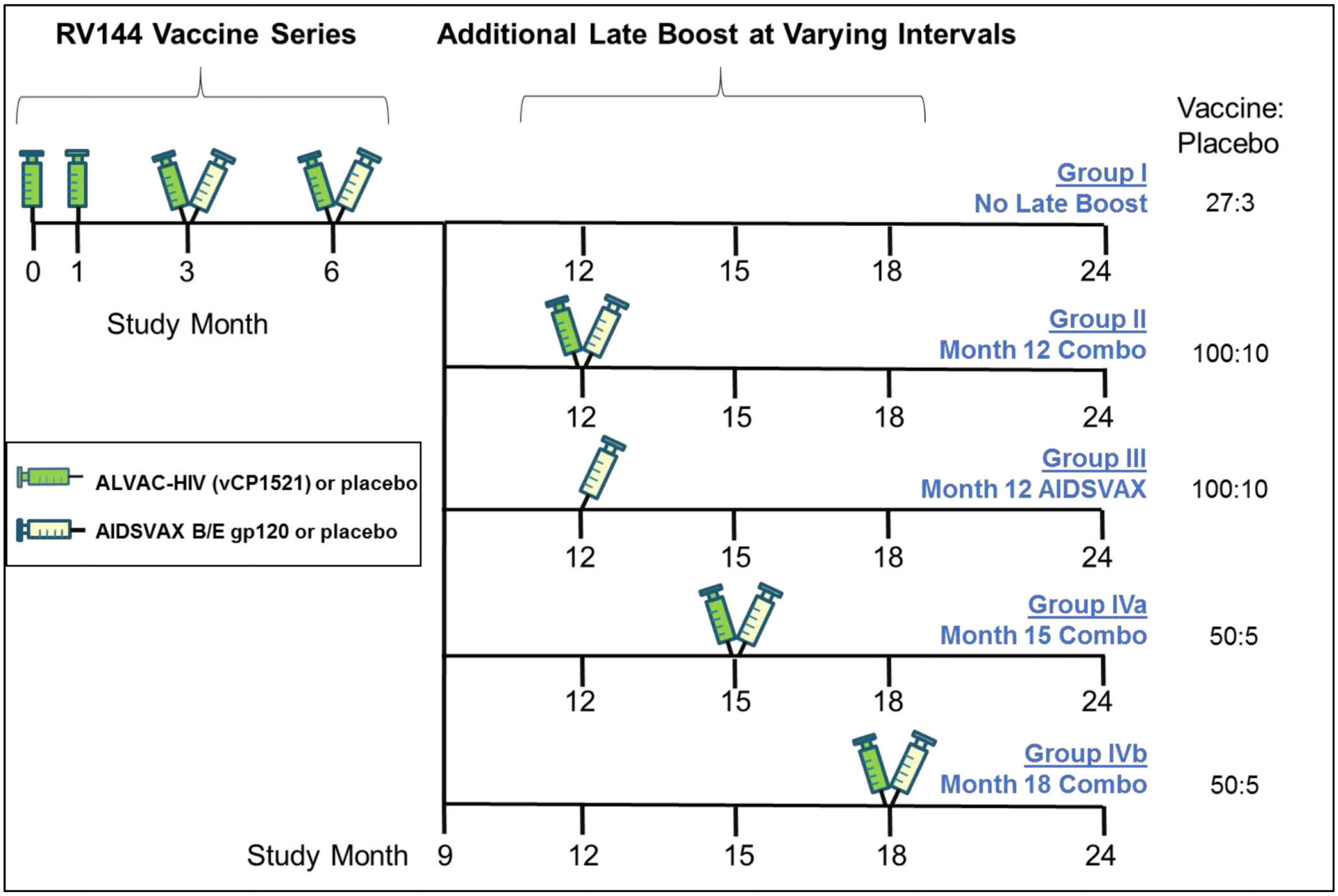

Figure 1: RV306 study design

Each RV306 participant received ALVAC-HIV and AIDSVAX® B/E either alone or in combination (abbreviated Combo), at the indicated time points. Participants were randomized to 1 of 5 groups and further randomized within each group to receive either vaccine product or placebo injections at the ratio indicated for each group displayed on the right. Participants were followed for 24 months in total.

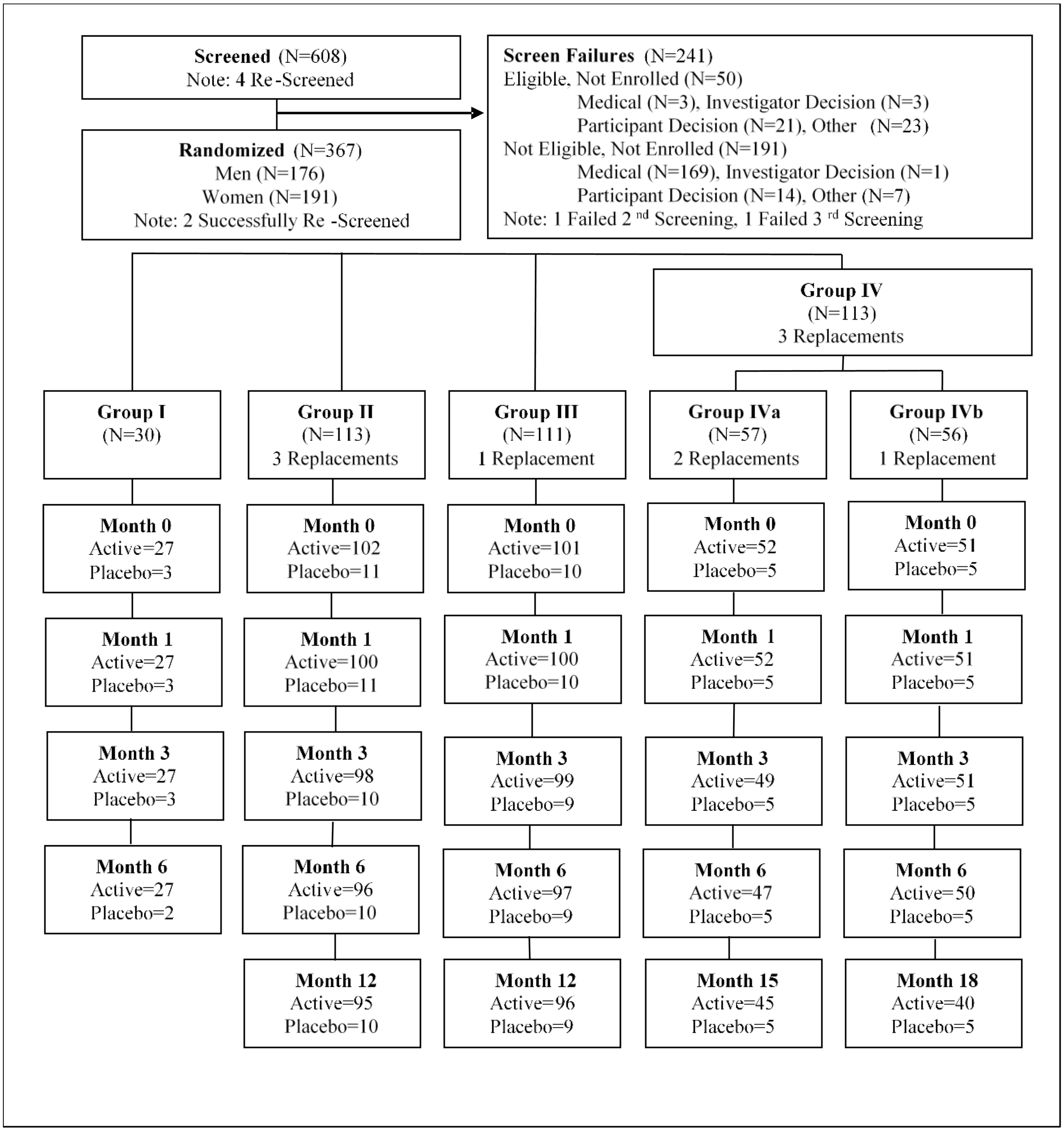

Figure 2: Trial profile

Participant screening and enrollment by study group.

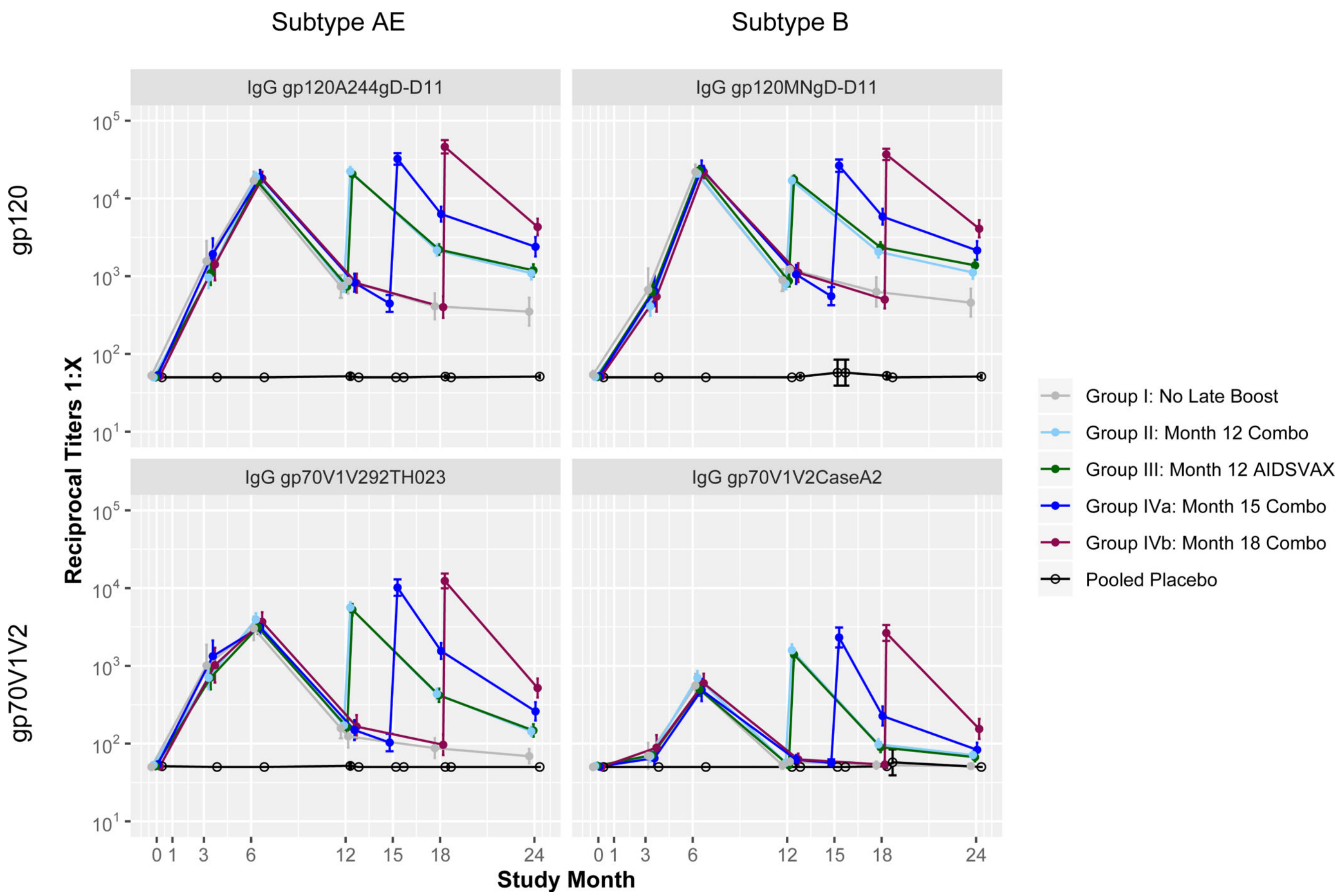

Figure 3: Plasma IgG HIV-1 Env and V1V2 binding antibody levels increase with late boosting

IgG binding antibody responses to HIV-1 gp120 and scaffolded gp70 V1V2 antigens. Reciprocal titers against gp120 A244gD- D11 (upper left), gp120 MNgD- D11 (upper right), gp70 V1V2 (92TH023) (lower left), and gp70 V1V2 (case A2) (lower right) are shown. Vaccination timepoints and the last visit are shown on the x axis. All peak immunogenicity measurements were performed two weeks post vaccination. Each panel graphically displays geometric mean titers, color-coded by group as per the legend. Error bars depict 95% confidence intervals. Volunteers completing all vaccinations are depicted. The responses against HIV-1 gp70 V1V2 in Group I were significantly lower than each of the late boosting groups (adjusted $p < 0{\cdot}02$ for each). The responses against HIV-1 gp120 in Group I were significantly lower than in Group IVb (adjusted $p < 0{\cdot}01$ for each). Late boosting significantly reduced decline of IgG antibodies to gp120 six months post boosting. Statistical significance was assessed using the Mann–Whitney U test with step-down Bonferroni adjustment.

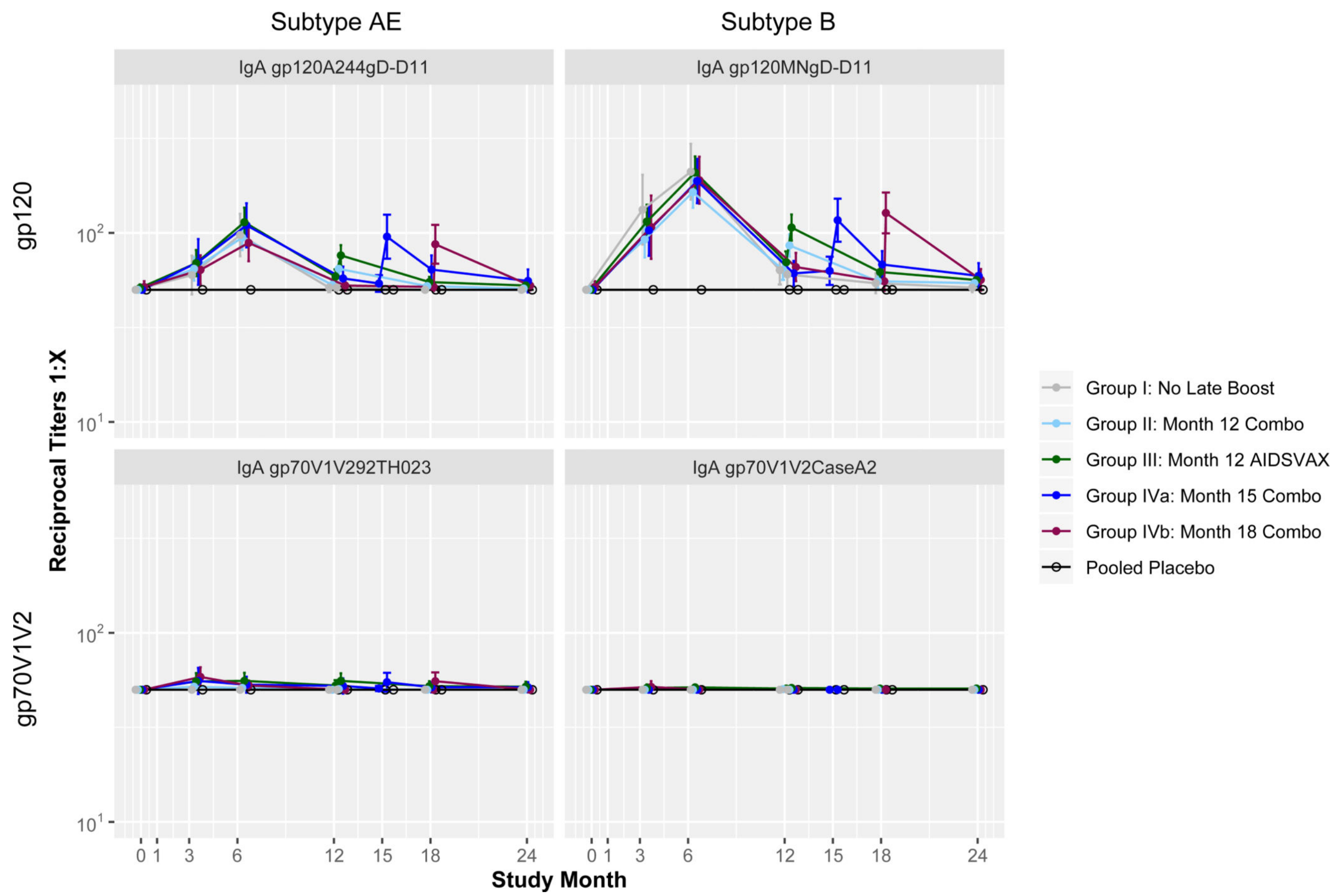

Figure 4: Plasma IgA HIV-1 Env and V1V2 binding antibody levels do not increase with late boosting

IgA binding antibody responses against HIV-1 gp120 and scaffolded variable regions 1 and 2 (V1V2) antigens. Reciprocal titers against gp120 A244gD- D11 (upper left), gp120 MNgD- D11 (upper right), gp70 V1V2 (92TH023) (lower left), and gp70 V1V2 (case A2) (lower right) are shown. Vaccination timepoints and the last visit are shown on the x axis. All peak immunogenicity measurements were performed two weeks post vaccination. Each panel graphically depicts geometric mean titers, color coded by group as per the legend. Error bars depict 95% confidence intervals. Volunteers completing all vaccinations are depicted, at vaccination time points as indicated above the x-axis.

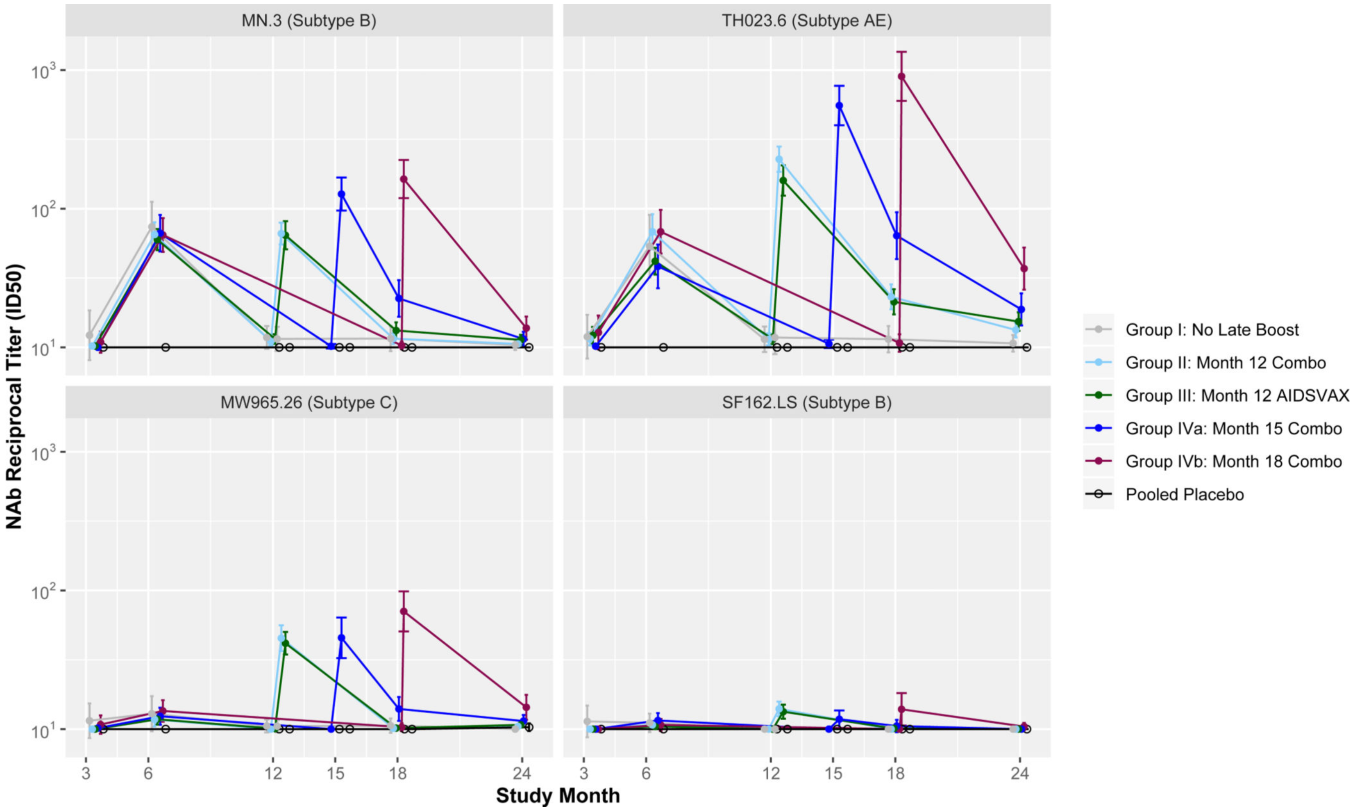

Figure 5: TZM-bl neutralizing antibody levels against subtype B MN.3, subtype AE TH023.6, and subtype C MW965.26 increase with late boosting

ID50 against MN.3 (upper left), TH023.6 (upper right), MW965.26 (lower left), and SF162.LS (lower right) are shown. Vaccination timepoints and the last visit are shown on the x axis. All peak immunogenicity measurements were performed two weeks post vaccination. Each panel graphically depicts ID50, color-coded by group as per the legend. Error bars depict 95% confidence intervals. Volunteers completing all vaccinations are depicted. The neutralizing antibody levels against TH023.6 and MW965.26 in Group I were significantly lower than each of the late boosting groups (adjusted $p < 0{\cdot}001$ for each). The responses against MN.3 in Group I were similar to responses observed in Groups II and III, but lower than in Groups IVa and IVb (adjusted $p \leq 0{\cdot}02$ for each). Statistical significance was assessed using the Mann–Whitney U test with step-down Bonferroni adjustment.

Table 1:

Demographics of Study Population by Group

		Group I (N=30)	Group II (N=113)	Group III (N=111)	Group IVa (N=57)	Group IVb (N=56)	Total (N=367)
Sex	Male	12 (40·0)	52 (46·0)	60 (54·1)	22 (38·6)	30 (53·6)	176 (48·0)
	Female	18 (60·0)	61 (54·0)	51 (45·9)	35 (61·4)	26 (46·4)	191 (52·0)
Age	Mean (SD)	28·6 (6·4)	28·6 (5·5)	28·8 (5·8)	27·0 (5·9)	27·2 (5·6)	28·2 (5·8)
	Range	(20, 39)	(20, 39)	(20, 39)	(20, 39)	(20, 39)	(20, 39)
Education Level	Primary school	7 (23·3)	24 (21·2)	14 (12·6)	14 (24·6)	8 (14·3)	67 (18·3)
	Secondary school	15 (50·0)	57 (50·4)	58 (52·3)	30 (52·6)	34 (60·7)	194 (52·9)
	Vocational	4 (13·3)	21 (18·6)	25 (22·5)	8 (14·0)	7 (12·5)	65 (17·7)
	Bachelor's degree	4 (13·3)	11 (9·7)	14 (12·6)	5 (8·8)	7 (12·5)	41 (11·2)
Occupation	No occupation	1 (3·3)	8 (7·1)	11 (9·9)	10 (17·5)	6 (10·7)	36 (9·8)
	Student	6 (20·0)	16 (14·2)	13 (11·7)	8 (14·0)	13 (23·2)	56 (15·3)
	Government Employee	2 (6·7)	7 (6·2)	10 (9·0)	4 (7·0)	6 (10·7)	29 (7·9)
	Employee	13 (43·3)	61 (54·0)	60 (54·1)	26 (45·6)	26 (46·4)	186 (50·7)
	Merchant/Self-employed	8 (26·7)	21 (18·6)	17(15·3)	9 (15·8)	5 (8·9)	60 (16·3)
Birth Place	Bangkok and suburb	20 (66·7)	74 (65·5)	60 (54·1)	27 (47·4)	33 (58·9)	214 (58·3)
	Chiang Mai province	4 (13·3)	10 (8·8)	11 (9·9)	7 (12·3)	4 (7·1)	36 (9·8)
	Other	6 (20·0)	29 (25·7)	40 (36·0)	23 (40·4)	19 (33·9)	117 (31·9)
Site	RIHES	4 (13·3)	19 (16·8)	19(17·1)	9 (15·8)	9 (16·1)	60 (16·3)
	RTA	13 (43·3)	48 (42·5)	46 (41·4)	24 (42·1)	24 (42·9)	155 (42·2)
	VTC	13 (43·3)	46 (40·7)	46 (41·4)	24 (42·1)	23 (41·1)	152 (41·4)
Vaccination Status	Dose 1	30	113	111	57	56	367
	Dose 2	30	111	110	57	56	364
	Dose 3	30	108	108	54	56	356
	Dose 4	29	106	106	52	55	348
	Dose 5	NA	105	105	50	45	305

There was no significant difference between groups for any variable listed.

Table 2:

Adverse events by severity, relatedness, seriousness and vaccination

		30-Day Post Dose [1]				All Treatment Emergent			
		Vaccine (N=333)		Placebo (N=34)		Placebo (N=34)		Vaccine (N=333)	
		# Event	# Participant	# Event	# Participant	# Event	# Participant	# Event	# Participant
Adverse Event	Any	377	188 (56·5)	41	20 (58·8)	816	259 (77·8)	87	24 (70·6)
	Mild	285	125 (37·5)	34	14 (41·2)	574	131 (39·3)	67	13 (38·2)
	Moderate	83	55 (16·5)	7	6 (17·6)	217	105 (31·5)	19	10 (29·4)
	Severe	9	8 (2·4)	0	0 (0)	25	23 (6·9)	1	1 (2·9)
Related Adverse Event	Any	33	23 (6·9)	2	2 (5·9)	34	24 (7·2)	2	2 (5·9)
	Mild	25	16 (4·8)	2	2 (5·9)	26	17 (5·1)	2	2 (5·9)
	Moderate	7	6 (1·8)	0	0 (0)	7	6 (1·8)	0	0 (0)
	Severe	1	1 (0·3)	0	0 (0)	1	1 (0·3)	0	0 (0)
Serious Adverse Event	Any	4	4 (1·2)	0	0 (0)	17	17 (5·1)	1	1 (2·9)
	Mild	0	0 (0)	0	0 (0)	0	0 (0)	0	0 (0)
	Moderate	2	2 (0·6)	0	0 (0)	8	8 (2·4)	0	0 (0)
	Severe	2	2 (0·6)	0	0 (0)	9	9 (2·7)	1	1 (2·9)

[1]30-day post any of the four doses for Group I, or any of the 5 doses for other groups