Safety and immunogenicity of a polyvalent DNA–protein HIV vaccine with matched Env immunogens delivered as a prime–boost regimen or coadministered in HIV-uninfected adults in the USA (HVTN 124): a phase 1, placebo-controlled, double-blind randomised controlled trial

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Summary

Background An effective HIV vaccine will most likely need to have potent immunogenicity and broad cross-subtype coverage. The aim of the HIV Vaccine Trials Network (HVTN) 124 was to evaluate safety and immunogenicity of a unique polyvalent DNA–protein HIV vaccine with matching envelope (Env) immunogens.

Methods HVTN 124 was a randomised, phase 1, placebo-controlled, double-blind study, including participants who were HIV seronegative and aged 18–50 years at low risk for infection. The DNA vaccine comprised five plasmids: four copies expressing Env gp120 (clades A, B, C, and AE) and one *gag* p55 (clade C). The protein vaccine included four DNA vaccine-matched GLA-SE-adjuvanted recombinant gp120 proteins. Participants were enrolled across six clinical sites in the USA and were randomly assigned to placebo or one of two vaccine groups (ie, prime–boost or coadministration) in a 5:1 ratio in part A and a 7:1 ratio in part B. Vaccines were delivered via intramuscular needle injection. The primary outcomes were safety and tolerability, assessed via frequency, severity, and attributability of local and systemic reactogenicity and adverse events, laboratory safety measures, and early discontinuations. Part A evaluated safety. Part B evaluated safety and immunogenicity of two regimens: DNA prime (administered at months 0, 1, and 3) with protein boost (months 6 and 8), and DNA–protein coadministration (months 0, 1, 3, 6, and 8). All randomly assigned participants who received at least one dose were included in the safety analysis. The study is registered with ClinicalTrials.gov (NCT03409276) and is closed to new participants.

Findings Between April 19, 2018 and Feb 13, 2019, 60 participants (12 in part A [five men and seven women] and 48 in part B [21 men and 27 women]) were enrolled. All 60 participants received at least one dose, and 14 did not complete follow-up (six of 21 in the prime–boost group and eight of 21 in the coadminstration group). 11 clinical adverse events deemed by investigators as study-related occurred in seven of 48 participants in part B (eight of 21 in the prime–boost group and three of 21 in the coadministration group). Local reactogenicity in the vaccine groups was common, but the frequency and severity of reactogenicity signs or symptoms did not differ between the prime–boost and coadministration groups (eg, 20 [95%] of 21 in the prime–boost group *vs* 21 [100%] of 21 in the coadministration group had either local pain or tenderness of any severity [p=1·00], and seven [33%] *vs* nine [43%] had either erythema or induration [p=0·97]), nor did laboratory safety measures. There were no delayed-type hypersensitivity reactions or vasculitis or any severe clinical adverse events related to vaccination. The most frequently reported systemic reactogenicity symptoms in the active vaccine groups were malaise or fatigue (five [50%] of ten in part A and 17 [81%] of 21 in the prime–boost group *vs* 15 [71%] of 21 in the coadministration group in part B), headache (five [50%] and 18 [86%] *vs* 12 [57%]), and myalgia (four [40%] and 13 [62%] *vs* ten [48%]), mostly of mild or moderate severity.

Interpretation Both vaccine regimens were safe, warranting evaluation in larger trials.

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Introduction

Despite progress in HIV diagnosis rates, the linkage to care and treatment, and the increasing availability of chemoprophylaxis, an estimated 1·3 million new HIV infections occurred in 2022 worldwide.[1] The development of a safe and effective HIV vaccine remains an important objective towards ending the HIV epidemic.

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Research in context

Evidence before this study

We searched PubMed for studies published anytime until June 1, 2023, with no language restrictions. The search terms were "HIV", "DNA", "prime-boost", "vaccine", and "clinical trial". Additional searching and background research were conducted through discussions with coauthors and perusal of cited papers. An effective vaccine remains an elusive but crucial aim on the path towards ending the HIV epidemic. The unique challenges posed by the virus have stymied the effectiveness of numerous clinical trials, although correlates-of-protection analyses have provided useful insights that have guided the next generation of vaccine candidates. Emerging vaccines vary not only in immunogens, but also in vaccine platform, administration regimen, and adjuvant. DNA vaccines, like mRNA vaccines, offer several advantages over other platforms, including optimisation, precision of design, and relative ease of production. More importantly, immunogenicity of DNA vaccines has been substantially improved when used as a prime immunisation followed by the boost of adjuvanted protein vaccines. DNA vaccines in our platform are delivered in saline by the conventional needle injection into human muscle tissue, offering an excellent safety and tolerability profile. A previous study evaluated an earlier generation of the polyvalent DNA-protein HIV vaccine described in this study. Immunogenicity results were encouraging and long lasting, but it was recognised further screening and optimisation could be performed and modifications could be made to improve the safety profile.

Added value of this study

This phase 1 human study evaluates the safety and immunogenicity of the second generation of a polyvalent DNA–protein HIV vaccine in either coadministration or DNA prime–protein boost regimens. The DNA vaccine contained inserts expressing four gp120 antigens (A, B, C, and AE) and an optimised *gag* gene from subtype C. The protein vaccine consisted of four recombinant gp120 proteins matching the prime. Previous HIV vaccine studies have tended to use fewer vaccine subtypes or mismatched DNA–protein immunogens. In addition to being safe, the vaccine described in this study showed immunogenicity that was strong in both breadth and magnitude. Specific importance was placed on established correlates of protection, such as IgG3 response rates and magnitudes to gp120 and gp140 antigens and CD4 cell responses.

Implications of all the available evidence

The immunogenicity results of this trial (HIV Vaccine Trial Network [HVTN] 124) represent an important new benchmark in HIV vaccine research by showing a wide range of potent and broad immune responses in healthy adult volunteers. Consistency in study design and analyses have permitted direct comparison with other vaccine trials (HVTN 096, HVTN 105, HVTN 111, etc) and results from this trial, particularly in participants who received prime–boost, compared favourably. More rigorous comparisons are currently being performed, but the immunogenicity of the polyvalent DNA–protein HIV vaccine shown in HVTN 124 represents a promising first step in HIV vaccine development, warranting continued evaluation in larger and more advanced clinical trials.

There are several biological challenges to developing an effective HIV vaccine, including the diversity of virus sequences of both conserved and more variable envelope glycoproteins (Env), the conformational structure of Env shielding binding sites from neutralising antibodies, and the need for sterilising immunity to prevent latency following acute infection. Data from the partially efficacious vaccine in the RV144 trial and non-human primate studies suggest that a vaccine eliciting an advantageous balance of binding antibodies, antibody effector functionality, and cellular immunity offers an opportunity for protective efficacy against HIV infection. Moreover, the antibody response targeting the Env V1V2 region has been specifically highlighted as a correlate of protection, even in efficacy trials of unsuccessful vaccines in which this key response was low.[2,3]

DNA vaccines offer several unique advantages over traditional adjuvanted protein vaccines. Notably, they permit design optimisation based on pathogen sequences, entail relative ease in manufacturing and stability, and lead to more representative and conformational antigens expressed in vivo.[4] However, the immunogenicity of HIV DNA vaccines is low in humans when administered alone.[5] Contrastingly, when HIV DNA vaccines are administered concurrently or sequentially with adjuvanted protein vaccines, immunogenicity, including both polyfunctional antibody and cellular responses, is substantially improved.[6–8]

The HIV Vaccine Trials Network (HVTN) 124, presented in this Article, builds on a previously described study that evaluated an early generation of polyvalent DNA–protein HIV vaccine, DP6-001.[7] DP6-001 induced high-titre antibody responses to the five homologous gp120 antigens in all 27 participants. There were cross-subtype binding antibodies, heterologous neutralising activities,[7] broad recognition of gp70 V1V2 from multiple virus subtypes, and antibody-dependent cellular cytotoxicity (ADCC).[9] In addition, participants developed Env-specific cell-mediated immunity, with polyfunctional, multisubtype, Env-specific CD4 cell responses.[10] Although two cases of vasculitis led to cessation of DP6-001,[11] the polyfunctionality of immune responses inspired continued

effort in this approach. To mitigate against this risk, glucopyranosyl lipid adjuvant-stable oil-in-water emulsion (GLA-SE) was selected as an alternative adjuvant in place of QS21 to improve safety.

To improve upon the immunogenicity and safety outcomes of the DP6-001 trial, a panel of gp120 immunogens from 62 primary HIV-1 isolates was screened in animal studies to identify those best able to elicit broad antibody responses.[12] A new polyvalent Env formulation was constructed, which elicited higher antibody responses in preclinical studies against a panel of gp70 V1V2 antigens from diverse subtypes and broader neutralising antibody responses against a panel of pseudotyped viruses compared with the early polyvalent formulation used in DP6-001.[12] Thus, the objective of HVTN 124 was to evaluate the safety and immunogenicity of an optimised, second-generation polyvalent DNA–protein HIV vaccine consisting of DNA inserts expressing four gp120 antigens (ie, subtypes A, B, C, and AE) and an optimised *gag* gene from subtype C, and the four matching GLA-SE-adjuvanted recombinant gp120 proteins in both prime–boost and coadministration regimens.

Methods

Study design and participants

HVTN 124, a phase 1, placebo-controlled, double-blind randomised controlled trial, was designed to evaluate the safety and immunogenicity of a vaccine consisting of polyvalent, multisubtype, DNA plasmids with matching adjuvanted gp120 proteins (polyvalent DNA–protein HIV vaccine). The trial was conducted across six US sites (Case Western Reserve University, Cleveland, OH; Emory University, Atlanta, GA; Fenway Community Health Center, Boston, MA; University of Alabama, Birmingham, AL; University of Pennsylvania, Philadelphia, PA; and University of Rochester, Rochester, NY). Part A of the study was designed to evaluate protein–GLA-SE vaccine safety; part B was designed to compare immunogenicity between prime–boost and coadministration regimens, with continued safety monitoring. Institutional biosafety committees and institutional review boards and ethics committees at each site approved the protocol, which is available online.[13]

Participants were recruited through advertisements on print and social media, and in-person recruitment according to procedures established at each site. Participants were included if they were HIV-seronegative, aged 18–50 years, at low risk for HIV acquisition, without a serious underlying medical condition, on no systemic immunosuppressive medication, and met laboratory screening criteria, which are detailed in the protocol.[13] Data on gender were self-reported, with the following options: transgender male (female to male), transgender female (male to female), gender queer, gender variant or gender non-conforming, female, male, self-identified (specified), and prefer not to answer. Written informed consent was obtained from each participant before engagement in any trial activity. The study was registered with ClinicalTrials.gov (NCT03409276).

Randomisation and masking

Participants were randomly assigned to active vaccine or placebo in a 5:1 ratio in part A. In part B, participants were randomly assigned in a 1:1 ratio to one of two study groups (prime–boost or coadministration); within those groups they were further randomly assigned in a 7:1 ratio to either active vaccine or placebo. A statistical research assistant generated the randomisation sequence, which was computer-generated through a web-based system with balanced blocks and further stratified by self-reported sex assigned at birth in part B. Assignment details were provided via this web-based system to the site pharmacist, who was responsible for maintaining security of the treatment assignments. Participants and staff (except site pharmacists) were masked to treatment assignments. Syringes with the study product obscured by coloured tape were delivered to sites for administration.

Procedures

In part A, we planned for ten participants to receive the protein (400 µg) and GLA-SE (5 µg) vaccine and two to receive placebo at baseline and month 2 by intramuscular injection. The study was then paused to ensure safety of the protein–GLA-SE vaccine. In part B, we planned for 48 participants to receive one of two blinded study-treatment regimens or relevant placebos. The prime–boost group (n=21) received DNA vaccine (2 mg, left arm) plus placebo (right arm) intramuscularly at baseline, month 1, and month 3, followed by protein–GLA-SE vaccine (400 µg, right arm) plus placebo (left arm) at months 6 and 8. The coadministration group (n=21) received both the DNA vaccine (left arm) and protein–GLA-SE vaccine (right arm) intramuscularly at the same doses at baseline and months 1, 3, 6, and 8. The part B placebo group (n=6) received placebo injections (both arms) at all timepoints. Safety was monitored over 8 months in participants in part A and over 14 months in participants in part B. Reactogenicity, including systemic and local symptoms, vaccine-related lesions, and lymph nodes, was assessed in all participants for 7 days after each injection. Participants recorded symptoms using a post-vaccination symptom log. Sites contacted participants daily between day 1 and day 3 after vaccination. Symptoms were followed up to resolution if persistent beyond day 7. Participants returned to the clinic 2 weeks after each vaccination and 48 weeks after the first vaccination for clinical evaluation and laboratory testing of haematology and serum chemistry parameters. Adverse events were graded using the US Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events and assessed for their relationship to the study product by the local clinical investigators. Immunogenicity endpoints were assessed on serum samples from participants in

part B collected 2 weeks after the last immunisation (ie, month 8·5). Laboratory staff performing immunogenicity assays were masked to treatment assignments.

The DNA vaccine contained equal amounts (0·4 mg) of five DNA plasmids in the vector pSW3891.[7] Four plasmids contained codon-optimised gp120 gene sequences: three from primary isolates of HIV-1 subtypes (A, B, and C), and one from the CRF01_AE consensus sequence. The fifth plasmid contained a codon-optimised *gag* gene from subtype C. The recombinant protein vaccine contained equal amounts (100 µg each) of the four gp120 proteins (A, B, C, and AE) matching the *env* genes used in the DNA vaccine, produced in stable Chinese hamster ovary cell lines by Waisman Biomanufacturing (Madison, WI, USA) under conditions compliant with good manufacturing practices. The gp120 proteins were highly glycosylated, as previously reported.[14] GLA-SE adjuvant was provided by the Access of Advanced Health Institute (Seattle, WA, USA).

See Online for appendix

HIV-1 specific IgG, IgG3, and IgA binding antibody responses against a panel of 35 antigens (appendix pp 5–14) were measured on a Bio-Plex 200 instrument (Bio-Rad, Hercules, CA, USA) with a standardised, customised HIV-1 Luminex assay, as previously described.[3,15–17]

Neutralising antibodies against HIV-1 were measured as a function of reductions in the expression of Tat-regulated luciferase (Luc) reporter gene in TZM-bl cells.[18] The assay measured neutralisation titres against a panel of 14 Env-pseudotyped viruses that had tier 1A, 1B, and 2 phenotypes (appendix pp 15, 16).

ADCC was measured by two assays (GranToxiLux [ADCC–GTL] using the LSR II high-throughput sampler [BD Bioscience, San Jose, CA, USA] and luciferase [ADCC–Luc]). For the ADCC–GTL assay, target cells were a clonal isolate of the CEM.NKRCCR5 CD4 cell line coated with recombinant gp120s of virus subtypes as described.[19] Each plate had one standardised positive and negative control in duplicate. Flow cytometry was used to quantify the frequency of granzyme B-positive cells. ADCC was quantified as net percent granzyme B activity. A modified version of a previously published luciferase assay[20] measured percent reduction in relative luminescence units of infectious molecular clone-infected target cells following exposure to peripheral blood mononuclear cells (PBMCs) in the presence of serial dilutions of participants' serum. The area under the net percent granzyme B activity versus $\log_{10}$ (dilution) curve was calculated as ADCC response.

Intracellular cytokine staining, with a 17-colour panel, was assayed to evaluate HIV-specific CD4 and CD8 cell responses of cryopreserved PBMCs to two potential T-cell epitope (PTE) Env and two PTE Gag peptide pools, both pooled by sequence.[21] The total response was the sum of the two Env or Gag peptide pools. Criteria for cell viability, numbers of T cells analysed, and background response in the absence of specific peptide pool stimulation were used to determine the acceptability of an assay. The background-adjusted percent of CD4 and CD8 cells expressing IFNγ or IL-2 (or both)—and each functional profile for IFNγ, IL-2, TNF-α, CD40L, IL-4, and granzyme B—were analysed. To assess positivity for a peptide pool within a T-cell subset, a two-by-two contingency table was constructed with the number of cells positive for IFNγ or IL-2 and the number of cells negative for IFNγ and IL-2, for both the stimulated and the negative control data.[22] Combinatorial Polyfunctionality Analysis of Single Cells, a computational framework for unbiased polyfunctionality analysis of antigen-specific T-cell subsets, was performed.[23]

Outcomes

The primary outcomes of this analysis characterised the safety and tolerability of the vaccine regimens: frequency, severity, and attributability of local and systemic reactogenicity and adverse events; laboratory safety measures, including alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine, white blood cells, haemoglobin, platelets, lymphocyte count, and neutrophil count; and attributability of any early discontinuations.

The secondary outcomes characterised vaccine immunogenicity: magnitude and breadth of serum Env-specific IgG, IgG3, and IgA binding antibody responses (binding antibody multiplex assay); serum neutralising antibody responses against tier 1A, tier 1B, and selected tier 2 viruses (TZM-bl assay); ADCC activities against HIV-1 subtypes A, B, C, and AE (ADCC–GTL and ADCC–Luc assays); and HIV-specific CD4 and CD8 cell responses (intracellular cytokine staining assay), all measured 2 weeks after the final injection. All prespecified endpoints are listed in detail in the protocol, available online.[13]

Statistical analysis

All enrolled participants who received at least one vaccination at the enrolment visit were included in the safety analysis. With ten active vaccine recipients in part A and 21 per regimen in part B, there was a 90% chance of observing at least a single adverse event if the true rate of an event was more than or equal to 20·6% for the vaccine regimen in part A and more than or equal to 10·4% for each vaccine regimen in part B. For immunogenicity outcomes, we performed analyses among the participants receiving the full vaccination series (per protocol). With 15 prime–boost recipients and 13 coadministration recipients receiving the full series and providing specimens 2 weeks after the last vaccination, if the observed response rate for each vaccine regimen was 80%, then the 95% CI for the true response rate would be 55–93% for the prime–boost regimen and 50–92% for the coadministration regimen.

For safety analyses, the number and percentage of participants who had each type of reactogenicity sign or symptom, or adverse event, were tracked. When a sign

or symptom was recorded, each participant's reactogenicity events were counted once under the maximum severity. Wilcoxon rank sum tests were used for testing differences in severity between vaccine regimens.

For immunogenicity analyses, positive responses were established for each assay described. Response rates and 95% CIs were estimated with the Wilson score method.[24] Response rates were compared between vaccine regimens by use of the Barnard's test.[25] Response magnitudes between the two vaccine regimens among participants with a positive response were compared with an independent *t* test for binding antibody titres and neutralising antibody titres, and with a Wilcoxon rank sum test for IgA, ADCC, and T-cell responses. Magnitude-breadth curves characterising the magnitude of ADCC and number of antigens with a positive response were generated. The area under the magnitude-breadth curve was compared between vaccine regimens with the Wilcoxon rank sum test. Various comparisons across trials were performed to contextualise results, which was made possible through a central laboratory and standardised HVTN laboratory protocols. All analyses were performed using R version 4.03.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between April 19, 2018 and Feb 13, 2019, a total of 60 participants were assessed for eligibility, enrolled, and randomly assigned (figure 1). The study population included 34 (57%) participants assigned female sex at birth, 17 (28%) non-White individuals, and two (3%) of Latinx ethnicity. The median age was 26·5 years (IQR 23–34; table 1). All 12 participants in part A completed the study and had no clinical adverse events (table 2). 32 (76%) of 42 participants in part B assigned to the active vaccination groups completed follow-up, although three missed one or two vaccination visits (figure 1).

Both DNA and protein vaccines were well tolerated, and the frequency and severity of reactogenicity signs or

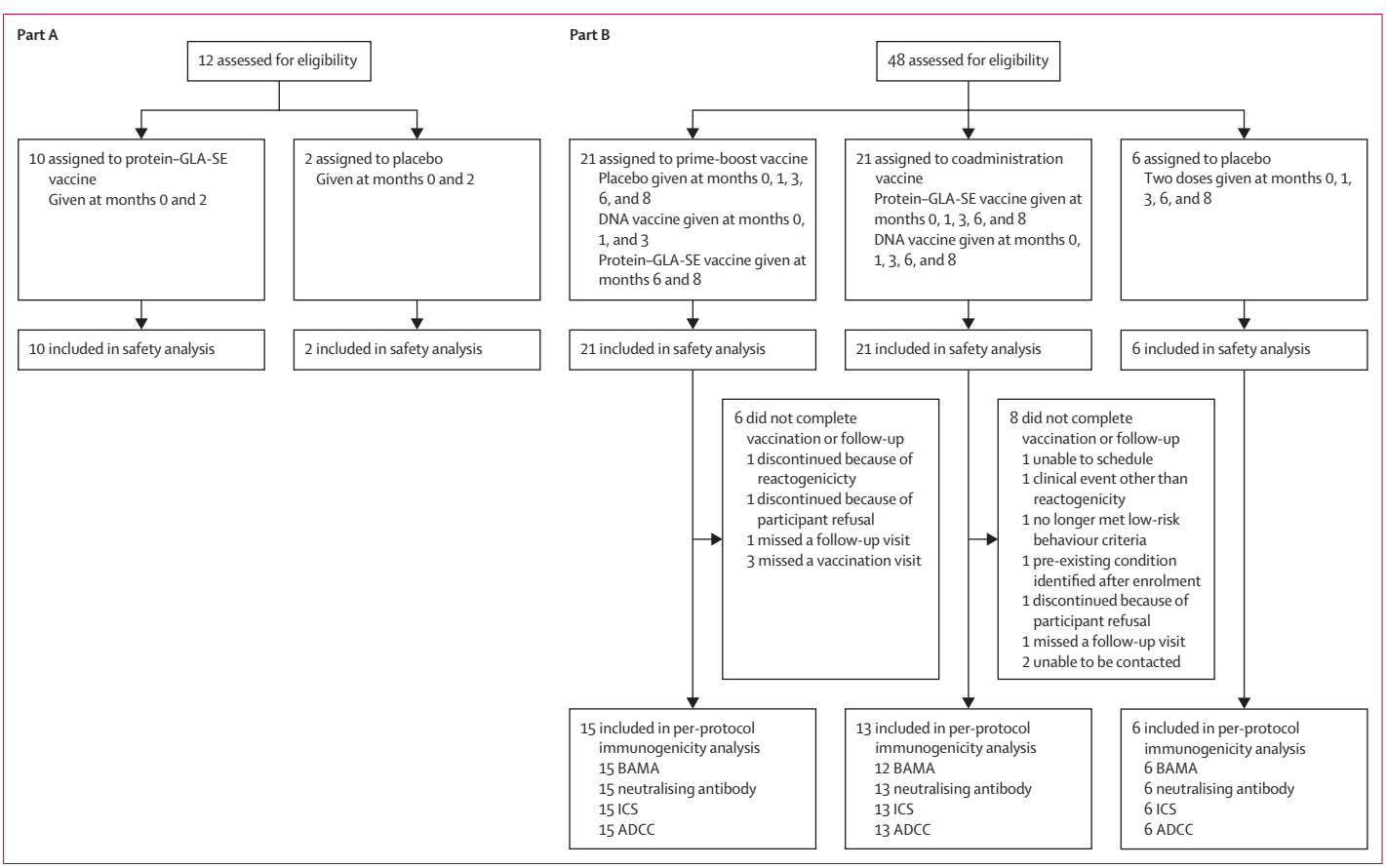

Figure 1: **Study profile**
HIV Vaccine Trials Network 124 was conducted in two parts: part A focused on safety of the protein–GLA-SE vaccine and part B compared DNA prime–protein boost with coadministration vaccine regimens, incorporating measures of immunogenicity and safety. The safety analysis was performed in all participants who received at least one assigned dose and the immunogenicity analyses were restricted to participants who received the full vaccination series (per protocol). ADCC=antibody-dependent cellular cytotoxicity. BAMA=binding antibody multiplex assay. ICS=intracellular cytokine staining.

symptoms were similar between the prime–boost and coadministration groups. No participant developed a delayed-type hypersensitivity reaction or vasculitis, or any severe clinical adverse events related to vaccination. One participant in the prime–boost group developed a severe grade elevation in aspartate aminotransferase concentration 21 days after the second DNA vaccination, deemed by the investigator as not related to the vaccine. The elevated aspartate aminotransferase concentration was asymptomatic, resolved after 14 days, and the participant continued in the study without further issue. There were 11 clinical adverse events deemed by investigators as study-related in seven participants in part B (eight in the prime–boost group and three in the coadministration group):

	Part A		Part B			Total (N=60)
	Placebo (N=2)	Protein (N=10)	Placebo (N=6)	Prime–boost (N=21)	Coadministration (N=21)	
Sex						
Male	1 (50%)	4 (40%)	3 (50%)	8 (38%)	10 (48%)	26 (43%)
Female	1 (50%)	6 (60%)	3 (50%)	13 (62%)	11 (52%)	34 (57%)
Gender						
Transgender male	1 (50%)*	0	0	0	0	1 (2%)
Transgender female	0	1 (10%)	0	0	0	1 (2%)
Gender queer	0	0	0	0	0	0
Gender variant or gender non-conforming	0	0	0	0	1 (5%)	1 (2%)
Male	1 (50%)	2 (20%)	3 (50%)	7 (33%)	9 (43%)	22 (37%)
Female	0	7 (70%)	2 (33%)	13 (62%)	11 (52%)	33 (55%)
Self-identified (specified)	1 (50%; non-binary transgender)*	0	1 (17%; non-binary)	1 (5%; non-binary)	0	3 (5%)
Prefer not to answer	0	0	0	0	0	0
Ethnicity						
Hispanic or Latinx	0	0	0	1 (5%)	1 (5%)	2 (3%)
Other	2 (100%)	10 (100%)	6 (100%)	20 (95%)	20 (95%)	58 (97%)
Race						
White	1 (50%)	7 (70%)	4 (67%)	14 (67%)	17 (81%)	43 (72%)
Black or African American	1 (50%)	0	1 (17%)	3 (14%)	0	5 (8%)
Asian	0	2 (20%)	0	2 (10%)	2 (10%)	6 (10%)
American Indian or Alaska Native	0	0	0	0	0	0
Native Hawaiian or other Pacific Islander	0	0	0	0	0	0
Other	0	1 (10%)	1 (17%)	2 (10%)	2 (10%)	6 (10%)
Age, years						
<18	0	0	0	0	0	0
18–20	0	4 (40%)	0	3 (14%)	0	7 (12%)
21–30	1 (50%)	3 (30%)	4 (67%)	12 (57%)	10 (48%)	30 (50%)
31–40	0	1 (10%)	2 (33%)	6 (29%)	9 (43%)	18 (30%)
41–50	1 (50%)	2 (20%)	0	0	2 (10%)	5 (8%)
>50	0	0	0	0	0	0
Median (IQR)	36·5 (31–42)	22·5 (19–36)	29 (28–34)	25 (23–31)	31 (24–34)	26·5 (23–34)
Range	26–47	18–47	27–36	19–40	21–48	18–48
Vaccination frequencies						
Part A: day 0	2 (100%)	10 (100%)	..	..	..	12 (100%)
Part A: day 56	2 (100%)	10 (100%)	..	..	..	12 (100%)
Part B: day 0	..	..	6 (100%)	21 (100%)	21 (100%)	48 (100%)
Part B: day 28	..	..	6 (100%)	21 (100%)	19 (90%)	46 (96%)
Part B: day 84	..	..	5 (83%)	19 (90%)	18 (86%)	42 (88%)
Part B: day 168	..	..	6 (100%)	17 (81%)	17 (81%)	40 (83%)
Part B: day 224	..	..	6 (100%)	19 (90%)	14 (67%)	39 (81%)

Data are n (%) unless otherwise specified. *One participant self-reported as both transgender male and self-identified non-binary transgender, therefore the total number of participants remains 60.

Table 1: **Participant demographics and rates of vaccine administration**

	Part A		Part B			Total (N=60)	p value across all groups (part A and B)	p-value for prime–boost *vs* coadministration
	Placebo (N=2)	Protein–GLA-SE (N=10)	Placebo (N=6)	Prime–boost (N=21)	Coadministration (N=21)			
Maximum local reactogenicity								
Pain							0·0007	0·91
None	2 (100%)	5 (50%)	5 (83%)	3 (14%)	1 (5%)	16 (27%)	..	..
Mild	0	4 (40%)	1 (17%)	12 (57%)	15 (71%)	32 (53%)	..	..
Moderate	0	1 (10%)	0	5 (24%)	4 (19%)	10 (17%)	..	..
Severe	0	0	0	1 (5%)	1 (5%)	2 (3%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Tenderness							0·0006	1·00
None	2 (100%)	3 (30%)	4 (67%)	1 (5%)	0	10 (17%)	..	..
Mild	0	6 (60%)	2 (33%)	15 (71%)	17 (81%)	40 (67%)	..	..
Moderate	0	1 (10%)	0	4 (19%)	3 (14%)	8 (13%)	..	..
Severe	0	0	0	1 (5%)	1 (5%)	2 (3%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Pain or tenderness*							0·0006	1·00
None	2 (100%)	3 (30%)	4 (67%)	1 (5%)	0	10 (17%)	..	..
Mild	0	6 (60%)	2 (33%)	13 (62%)	15 (71%)	36 (60%)	..	..
Moderate	0	1 (10%)	0	6 (29%)	5 (24%)	12 (20%)	..	..
Severe	0	0	0	1 (5%)	1 (5%)	2 (3%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Erythema							0·29	0·80
None or not gradable	2 (100%)	10 (100%)	6 (100%)	14 (67%)	13 (62%)	45 (75%)	..	..
Grade 1	0	0	0	0	2 (10%)	2 (3%)	..	..
Grade 2	0	0	0	2 (10%)	4 (19%)	6 (10%)	..	..
Grade 3	0	0	0	5 (24%)	2 (10%)	7 (12%)	..	..
Grade 4	0	0	0	0	0	0	..	..
Induration							0·091	0·79
None or not gradable	2 (100%)	10 (100%)	6 (100%)	16 (76%)	14 (67%)	48 (80%)	..	..
Grade 1	0	0	0	1 (5%)	3 (14%)	4 (7%)	..	..
Grade 2	0	0	0	0	2 (10%)	2 (3%)	..	..
Grade 3	0	0	0	4 (19%)	2 (10%)	6 (10%)	..	..
Grade 4	0	0	0	0	0	0	..	..
Erythema or induration							0·12	0·97
None or not gradable	2 (100%)	10 (100%)	6 (100%)	14 (67%)	12 (57%)	44 (73%)	..	..
Grade 1	0	0	0	0	3 (14%)	3 (5%)	..	..
Grade 2	0	0	0	2 (10%)	4 (19%)	6 (10%)	..	..
Grade 3	0	0	0	5 (24%)	2 (10%)	7 (12%)	..	..
Grade 4	0	0	0	0	0	0	..	..
Maximum systemic reactogenicity								
Malaise or fatigue							0·13	0·44
None	1 (50%)	5 (50%)	2 (33%)	4 (19%)	6 (29%)	18 (30%)	..	..
Mild	1 (50%)	5 (50%)	1 (17%)	8 (38%)	8 (38%)	23 (38%)	..	..
Moderate	0	0	2 (33%)	7 (33%)	6 (29%)	15 (25%)	..	..
Severe	0	0	1 (17%)	2 (10%)	1 (5%)	4 (7%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Myalgia							0·56	0·43
None	1 (50%)	6 (60%)	4 (67%)	8 (38%)	11 (52%)	30 (50%)	..	..
Mild	1 (50%)	4 (40%)	2 (33%)	9 (43%)	7 (33%)	23 (38%)	..	..
Moderate	0	0	0	2 (10%)	1 (5%)	3 (5%)	..	..
Severe	0	0	0	2 (10%)	2 (10%)	4 (7%)	..	..
Life threatening	0	0	0	0	0	0	..	..

(Table 2 continues on next page)

	Part A		Part B			Total (N=60)	p value across all groups (part A and B)	p-value for prime–boost *vs* coadministration
	Placebo (N=2)	Protein–GLA-SE (N=10)	Placebo (N=6)	Prime–boost (N=21)	Coadministration (N=21)			
(Continued from previous page)								
Headache							0·084	0·066
None	1 (50%)	5 (50%)	2 (33%)	3 (14%)	9 (43%)	20 (33%)	..	..
Mild	1 (50%)	5 (50%)	3 (50%)	10 (48%)	8 (38%)	27 (45%)	..	..
Moderate	0	0	1 (17%)	8 (38%)	2 (10%)	11 (18%)	..	..
Severe	0	0	0	0	2 (10%)	2 (3%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Nausea							0·71	0·43
None	1 (50%)	8 (80%)	4 (67%)	12 (57%)	15 (71%)	40 (67%)	..	..
Mild	1 (50%)	2 (20%)	1 (17%)	6 (29%)	4 (19%)	14 (23%)	..	..
Moderate	0	0	1 (17%)	3 (14%)	2 (10%)	6 (10%)	..	..
Severe	0	0	0	0	0	0	..	..
Life threatening	0	0	0	0	0	0	..	..
Vomiting							0·53	0·98
None	2 (100%)	10 (100%)	6 (100%)	17 (81%)	18 (85·7%)	53 (88%)	..	..
Mild	0	0	0	3 (14%)	1 (5%)	4 (7%)	..	..
Moderate	0	0	0	1 (5%)	2 (10%)	3 (5%)	..	..
Severe	0	0	0	0	0	0	..	..
Life threatening	0	0	0	0	0	0	..	..
Chills							0·022	0·19
None	2 (100%)	10 (100%)	6 (100%)	11 (52%)	15 (71%)	44 (73%)	..	..
Mild	0	0	0	4 (19%)	4 (19%)	8 (13%)	..	..
Moderate	0	0	0	4 (19%)	0	4 (7%)	..	..
Severe	0	0	0	2 (10%)	2 (10%)	4 (7%)	..	..
Arthralgia							0·051	0·044
None	2 (100%)	8 (80%)	6 (100%)	10 (48%)	17 (81%)	43 (72%)	..	..
Mild	0	1 (10%)	0	8 (38%)	2 (10%)	11 (18%)	..	..
Moderate	0	1 (10%)	0	1 (5%)	1 (5%)	3 (5%)	..	..
Severe	0	0	0	2 (10%)	1 (5%)	3 (5%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Maximum systemic symptoms							0·26	0·51
None	1 (50%)	3 (30%)	2 (33%)	3 (14%)	5 (24%)	14 (23%)	..	..
Mild	1 (50%)	6 (60%)	0	8 (38%)	8 (38%)	23 (38%)	..	..
Moderate	0	1 (10%)	3 (50%)	8 (38%)	6 (29%)	18 (30%)	..	..
Severe	0	0	1 (17%)	2 (10%)	2 (10%)	5 (8%)	..	..
Life threatening	0	0	0	0	0	0	..	..
Temperature							0·17	0·63
None (<37·9°C)	2 (100%)	10 (100%)	6 (100%)	15 (71%)	16 (76%)	49 (82%)	..	..
Grade 1 (38–38·5°C)	0	0	0	2 (10%)	3 (14%)	5 (8%)	..	..
Grade 2 (38·6–39°C)	0	0	0	3 (14%)	2 (10%)	5 (8%)	..	..
Grade 3 (39–39·9°C)	0	0	0	0	0	0	..	..
Grade 4 (≥40°C)	0	0	0	1 (5%)	0	1 (2%)	..	..

Data are n (%). Some percentages do not add to 100 due to rounding. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits. For erythema and induration, grade 1 was defined as affecting 2·5 cm to <5 cm in any diameter or 6·25 cm^2 to <25 cm^2, with no or ungradable complications; grade 2 was defined as affecting 5 cm to <10 cm in any diameter or 25 cm^2 to <100 cm^2; grade 3 was defined as severe complications or >10 cm in any diameter or >100 cm^2; and grade 4 was defined as potentially life-threatening complications. p values correspond to Kruskal–Wallis test. *Pain or tenderness was included as its own category in addition to the individual events, as prespecified in the protocol.

Table 2: **Summary of local and systemic reactogenicity events for participants in part A and part B**

four participants with injection site pruritus (three in the prime–boost group; one in the coadministration group), three with rashes (two in the prime–boost group; one in the coadministration group), one with abdominal pain (prime–boost group), one with hyperhidrosis (prime–boost group), one with nightmares (prime–boost group), and one with

pruritus (coadministration group). Local reactogenicity was common, although less frequent and of lesser severity among participants in part A than in part B (table 2). In part B, 41 (98%) of 42 vaccine participants had injection site pain or tenderness (20 [95%] of 21 in the prime–boost group and 21 [100%] of 21 in the coadministration group); six (29%) of 21 in the prime–boost group and five (24%) of 21 in the coadministration group were of moderate severity and two (5%) were of severe severity (one in each treatment group). Erythema or induration was reported by 16 (38%) of 42 vaccine participants in part B; these signs extended at least 10 cm in greatest diameter or at least 100 cm^2 in area (ie, grade 3) in seven (17%) participants (five [24%] of 21 in the prime–boost group and two [10%] of 21 in the coadministration group). The most frequently reported systemic reactogenicity symptoms were malaise or fatigue, headache, and myalgia, mostly of mild or moderate severity (table 2). Laboratory measures of safety did not differ between vaccination groups (appendix pp 21–38).

Among part B participants, five (24%) of 21 prime–boost recipients and one (5%) of 19 coadministration recipients developed vaccine-induced seropositivity (based on serum samples collected at the last study visit or at a participant's last visit if they left the study early) on a panel of commercially available assays.

Serum HIV-1 Env-specific binding IgG response rates and magnitudes were measured against the four autologous vaccine gp120 antigens (figure 2A), a multi-clade breadth panel of 13 heterologous gp120 and gp140 antigens (figure 2B), and a panel of 16 heterologous gp70-V1V2 antigens (figure 3A; appendix pp 5–8) 2 weeks after the last immunisation. High IgG response rates and magnitudes to autologous gp120 antigens were observed in both treatment regimens (figure 2A; appendix pp 5–8); all 15 participants receiving the prime–boost regimen and 11 (92%) of 12 receiving the coadministration regimen had positive responses with corresponding geometric means of individual-specific median titres to the four antigens of 10 938 (SD 5469) in the prime–boost group and 7332 (3666) in the coadministration group (p=0·048). Geometric mean titres (GMTs) were higher against clade A, C, and AE gp120 antigens in the prime–boost group compared with the coadministration group and were similar against clade B gp120 between the two groups (appendix p 5).

High IgG response rates were also observed against the heterologous gp120 and gp140 antigen panel (clades A, B, C, AE, BC, and consensus) in participants of both the prime–boost group (14 [93%] of 15 participants against BORI_D11gp120 and 15 [100%] of 15 against all the other measured antigens) and the coadministration group (11 [92%] of 12 for each antigen tested; figure 2B; appendix pp 5–8). GMTs were consistently higher in the prime–boost group (ranging from 7332 [SD 3666] against BORI_D11gp120 to 40 135 [12 040] against 1086C_D7gp120) than in the coadministration group (ranging from 3641 [2185] against BORI_D11gp120 to 36 316 [3632] against 1086C_D7gp120) across all 13 antigens, with nine (69%) being significant (figure 2B; appendix pp 5–8).

In addition, participants in the prime–boost group had similar (13 [81%] of 16 tested antigens) or significantly

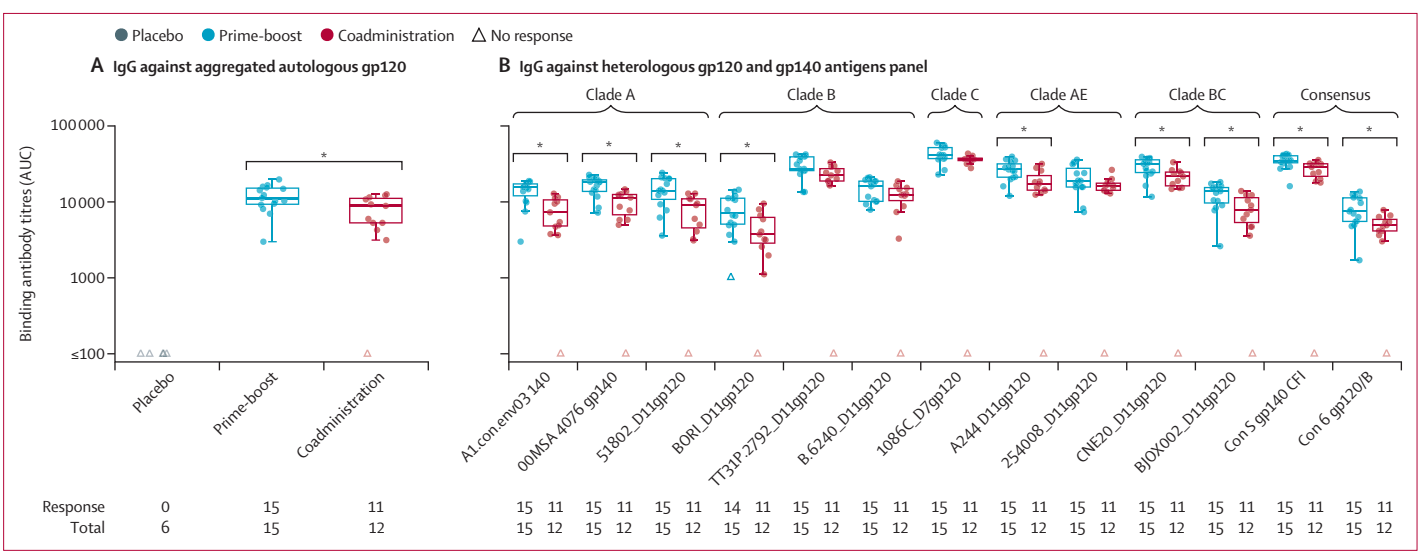

Figure 2: **gp120 and gp140 antibody response**

IgG titres for participants receiving prime–boost (blue), coadministration (red), and placebo (grey) against aggregated autologous gp120 antigens (A; non-aggregated results are presented in appendix [pp 5–8]) and a heterologous gp120 and gp140 panel (B). Response rates and relevant clades are summarised above each plot. Dots represent individual participants; participants with a non-response are shown as triangles. The line splitting the boxplots in two is the median, the bottom edge of the box is the 25th percentile, the top edge is the 75th percentile, the values at which the horizontal lines stop correspond to the most extreme datapoints that are no more than 1·5 times the IQR away from the median, or if no such datapoints exist, the data extremes. AUC=area under the curve. *p<0·05, precise p values are reported in appendix (pp 5–8).

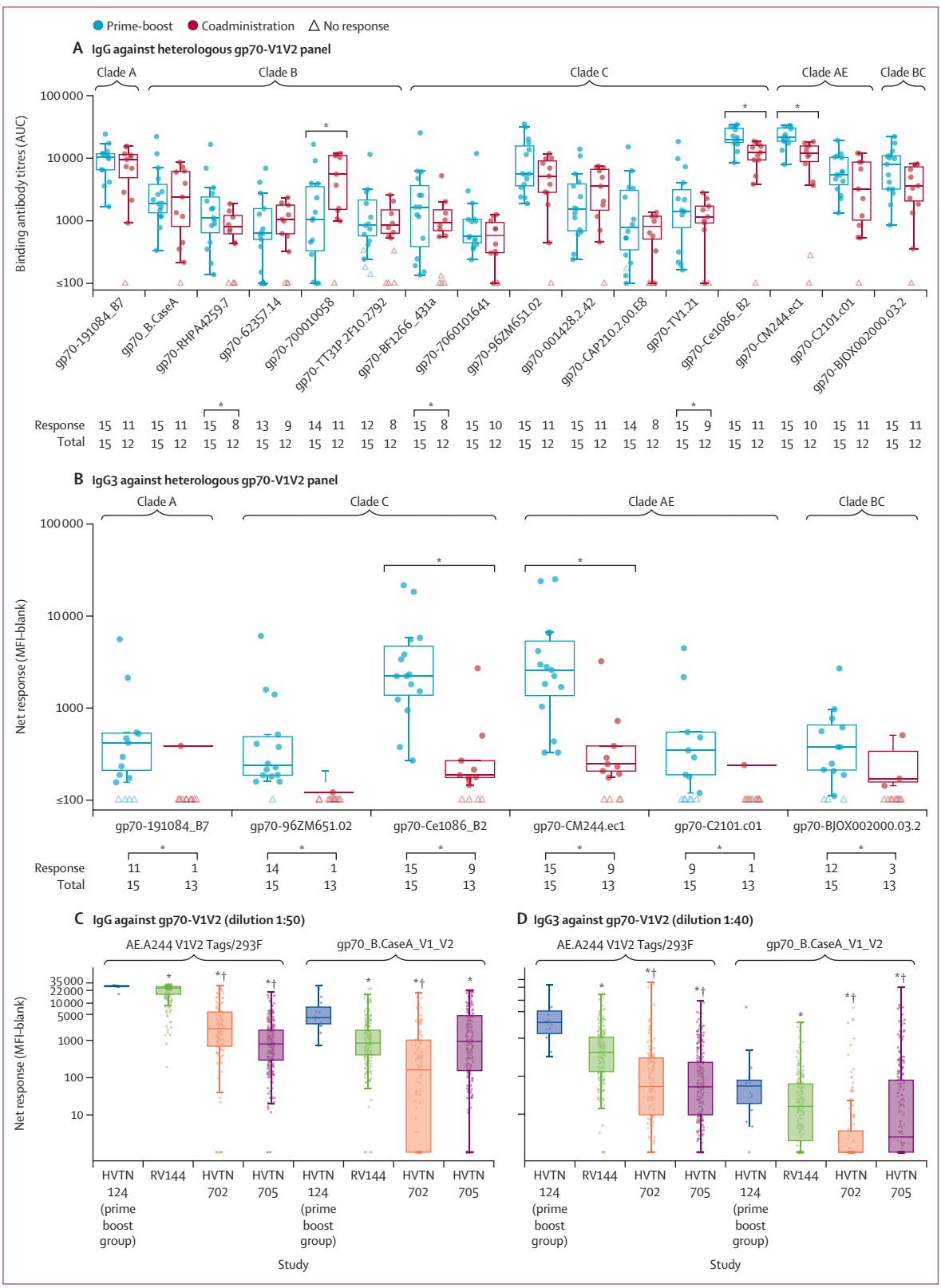

Figure 3: **gp70-V1V2 antibody response**

IgG (A) and IgG3 (B) responses in the prime–boost (blue) and coadministration (red) groups against gp70-V1V2 panels. Response rates and relevant clades are summarised above each plot. Dots represent individual participants; participants with a non-response are shown as triangles. IgG (C) and IgG3 (D) responses against antigens common across the HVTN 124, RV144, HVTN 702, and HVTN 705 trials are provided to contextualise our results with those of other contemporary HIV vaccine trials; results for the prime–boost group only are presented for the HVTN 124 trial. Net response (MFI–blank) is the IgG or IgG3 response measured by MFI minus a sample-specific background measure (known as blank). Dots represent individual participants. The line splitting the boxplots in two is the median, the bottom edge of the box is the 25th percentile, the top edge is the 75th percentile, the values at which the horizontal lines stop correspond to the most extreme datapoints that are no more than 1·5 times the IQR away from the median, or if no such datapoints exist, the data extremes. HVTN=HIV Vaccine Trials Network. MFI=mean fluorescence intensity. *p<0·05 compared with HVTN 124 prime–boost group (precise p values in the appendix pp 5–8). †p<0·05 compared with RV144 (precise p values in the appendix p 17).

higher (three [19%]) response rates than those in the coadministration group for each of the gp70-V1V2 antigens tested (80–100% of participants had a response in the prime–boost group *vs* 67–92% in the co-administration group; figure 3A; appendix pp 5–8). There was greater variability among titres to gp70-V1V2 antigens compared with the autologous gp120 antigens and the heterologous gp120 and gp140 antigen panel. GMTs to gp70-V1V2 were similar (against 13 [81%] of 16 antigens) or significantly higher (against two [13%]) for participants in the prime–boost group versus the coadministration group (appendix pp 5–8).

IgG3 response rates, previously correlated with decreased risk for HIV infection,[16] were higher in the prime–boost group versus the coadministration group against many of the antigens tested (appendix pp 9–12). Response rates in the prime–boost group ranged from 13% to 100% against the 16 gp70-V1V2 antigens, compared with 0% to 69% in the coadministration group ($p<0·05$ for nine comparisons). Among participants with a response, median magnitudes in prime–boost recipients ranged from 178 (IQR 155–214) to 2596 (1366–5345). IgG3 responses rates were positive in at least nine (60%) of 15 participants against six gp70-V1V2 antigens, with corresponding geometric means of magnitudes ranging from 393 (SD 427; gp70–96ZM651.02) to 2437 (3188; gp70-CM244.ec1; figure 3B; appendix pp 9–12). In a post-hoc analysis, we found that the magnitudes of both IgG and IgG3 responses against two gp70-V1V2 antigens (AE.A244 V1V2 Tags/293F, gp70_B.CaseA_V1_V2) that had been shown to be correlated with lower risk of HIV infection in the RV144 trial,[16] were higher in the prime–boost group of HVTN 124 than in other preventive vaccine studies (ie, RV144, HVTN 702, and HVTN 705; figure 3C, D; appendix p 17).

Serum HIV-1 Env-specific IgA response rates and magnitudes, previously correlated with increased HIV acquisition risk,[3] were low against autologous gp120 antigens, ranging from 13% to 53% in the prime–boost group and 8% to 50% in the coadministration group (appendix pp 13–14).

As measured by the ADCC–GTL assay, ADCC response rates varied by both treatment group and the four gp120 clades of coated targets. The mean (magnitude-breadth) curve was higher in the prime–boost group versus the coadministration group (median AUC-magnitude breadth 20·7 [IQR 18·5–25·8] *vs* 15·1 [8·5–16·5], $p=0·0031$; appendix p 2). Similarly, ADCC–GTL response rates were higher in participants given prime–boost versus coadministration against clade C TV1 (14 [93%] of 15 *vs* seven [54%] of 13, $p=0·017$) and clade AE gp120s (14 [93%] *vs* 4 [31%], $p=0·0004$) targets, although magnitudes among participants with a positive response were not significantly different against target cells between the two regimens (appendix p 18). By the ADCC–Luc assay, ADCC responses were evaluated with three different target cells infected with subtype B, C, or AE infectious molecular clones (appendix p 18). Response rates ranged from 80% to 100% in participants in the prime–boost group and 39% to 100% in participants in the coadministration group, and magnitudes again were not significantly different. However, the mean magnitude-breadth curve showed higher magnitude and breadth of ADCC–Luc responses in the prime–boost group versus the coadministration group (median AUC-magnitude breadth 33·7 [IQR 24·2–52·2] *vs* 24·1 [15·6–30·5], $p=0·025$; appendix p 2).

Serum antibody titres capable of neutralising 50% (ID_{50}) and 80% (ID_{80}) of input virus were measured against a panel of Env-pseudotyped viruses with tier 1A, 1B, and 2 neutralisation phenotypes by the TZM-bl assay (appendix pp 15–16). Neutralising antibody responses were observed in each participant in both treatment groups against the tier 1A isolate MW965.26 with similar ID_{50} GMTs (appendix pp 3, 15–16). Tier 1B neutralising antibody activities were observed against clade AE, B, C, and AG viruses, but with more variation in magnitude than for tier 1A, and more variation in magnitude between the prime–boost group then the coadministration group (appendix p 3). All participants in both treatment regimens had high neutralising antibody titres against clade AE TH023.6 (GMT 971 [SD 835] in the prime–boost group *vs* 801 [730] in the coadministration group). Similar neutralising antibody response rates were observed in both regimens against clade AG virus Dj263.8 (seven [47%] of 15 participants in the prime–boost group *vs* six [46%] of 13 in the coadministration group) but at lower titres (GMT of 21 [SD 14] in the prime–boost group and 21 [10] in the coadministration group). More participants in the prime–boost group than in the coadministration group had detectable neutralising antibody responses against clade C isolates 6644.v2.c33 (14 [93%] *vs* nine [69%]) and 1107356.07 (two [13%] *vs* one [8%]), and clade AG isolate T27.11 (ten [67%] *vs* one [8%]), although titres were low and only for T27.11 did response rates significantly differ between the vaccine regimens (appendix pp 15–16). Low-level tier 2 neutralisation was only detected in one coadministration recipient against the autologous 92UG037.1 isolate (appendix p 3). No placebo recipient had a positive neutralising antibody response.

Neutralising antibody titres in the prime–boost group also compared favourably with past candidate HIV vaccine trials. Responses to tier 1B TH023.6 isolate were significantly higher in this study than those observed with prime–boost or coadministration of DNA and New York vaccinia virus plus protein (HVTN 096), prime–boost of the canary pox virus ALVAC and ALVAC plus protein (HVTN 097), and the prime–boost or coadministration of a different DNA–protein regimen (HVTN 105; appendix pp 3, 19), with participants with a positive response in HVTN 124 having a GMT that was

more than eight times higher than those in HVTN 096, HVTN 097, and HVTN 105.

The response rate of CD4 cells expressing IFNγ or IL-2 to either Env peptide pool was significantly higher in participants in the prime–boost group (15 [100%] of 15) compared with those in the coadministration group (five [38%] of 13, p=0·0002), although the magnitude of responses among participants who had a response did not differ (figure 4A; appendix p 20). A single recipient of the prime–boost regimen had CD4 cell responses to either of the Gag peptide pools, and no participants in either group had measurable CD8 cell responses. There were no cellular immune responses among participants who received placebo. Polyfunctionality analysis of CD4 cells revealed Env-specific T cells expressing combinations of one to four functional markers (figure 4B). The subsets including IFNγ were higher in participants of the prime–boost group compared with the coadministration group, whereas those subsets not including IFNγ were similar between groups (appendix p 20).

A visual representation of the rate and magnitude for each of the measured humoral and cellular immune responses by group is depicted in the appendix (p 4). The differences in response rates, defined as the maximum proportion of participants who responded to any of the antigens tested in each panel, shows that the prime–boost regimen was superior to the coadministration regimen with respect to ADCC (both ADCC-Luc and ADCC-GTL assays) and CD4 cell responses to Env (appendix p 4). Furthermore, the prime–boost regimen had a higher mean percentile of response magnitudes among participants with a positive response in each immunological assay, except for tier 1A virus neutralisation and CD4 cell responses (appendix p 4).

Discussion

HVTN 124 tested the safety and immunogenicity of a second-generation polyvalent DNA–protein HIV vaccine formulation including four matched gp120 antigens in both DNA and protein components, in either DNA prime–protein boost or coadministration regimens. Immunogenicity was robust and the vaccine was safe; there were no serious cutaneous adverse events.

HVTN 124 was driven by the hypothesis that matching DNA and protein vaccines by subtype and sequence is essential to maximise both antibody and cellular immune responses, and that using a dual-platform prime–boost regimen is superior to co-administration. Previous HIV vaccine studies have combined DNA or viral-vector-priming immunogens derived from only one or two virus subtypes, administered concurrently or sequentially with a protein vaccine derived from one or two virus subtypes with typically mismatched *env* sequences to those in the priming series.[8,26–28] Results from these trials differ considerably from ours. In HVTN 105, two priming immunisations with DNA-HIV-PT123 (subtype C ZM/96 *gag*, Zm96 *gp140*, and CN54 *pol-nef*) followed by two administrations of AIDSVAX B/E (subtypes B MN gp120 and AE A244 gp120) was compared with their

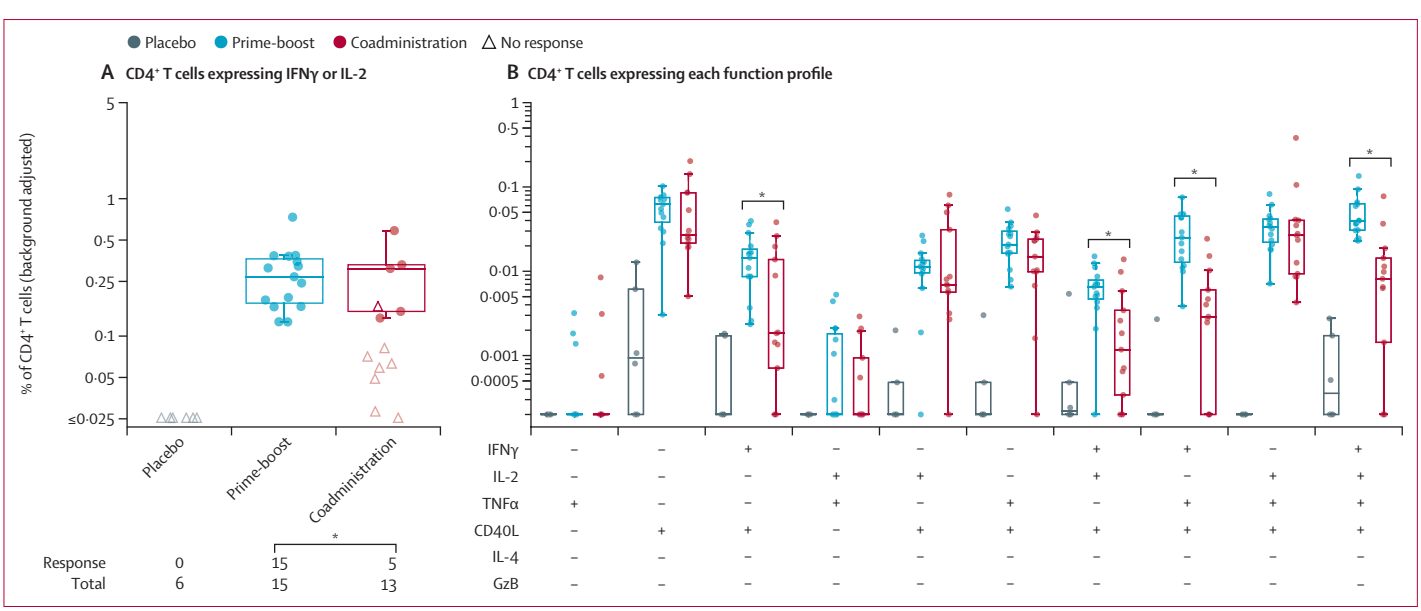

Figure 4: **CD4 cell response**

Response rates and magnitudes of CD4 cells expressing IFNγ or IL-2 (A) and of CD4 cells expressing each function profile (B) from participants receiving prime–boost (blue), coadministration (red), and placebo (grey) in response to Env peptides. There were no measurable CD8 T cell responses (appendix p 4). The line splitting the boxplots in two is the median, the bottom edge of the box is the 25th percentile, the top edge is the 75th percentile, the values at which the horizontal lines stop correspond to the most extreme datapoints that are no more than 1·5 times the IQR away from the median, or if no such datapoints exist, the data extremes. GzB=granzyme B.*p<0·05 (precise p values in the appendix p 20).

coadministration.[8] Lower concentrations of IgG binding antibodies to autologous gp120 were observed in recipients of the prime–boost regimen at the maximum immunogenicity timepoint compared with those receiving coadministration, whereas CD4 cell responses and ADCC were similar between dosing regimens. In HVTN 111, DNA-HIV-PT123 was administered as a prime followed by a bivalent subtype C Env protein boost comprising TV1.C and 1086.C; coadministration was likewise compared.[28] The prime–boost regimen, although matched for subtype, again yielded antibody responses that were considered inferior to coadministration, whereas CD4 cell immune responses were higher in participants given prime–boost. Therefore, the elicitation of immune responses by immunogens can be influenced by whether they are matched by subtype and sequence, and whether they are administered sequentially or concurrently. Of note, coadministration of DNA and protein vaccines appears to have an advantage of earlier elicitation of antibody responses compared with the prime–boost strategy,[8,26] and a limitation of our study is the absence of immunogenicity data following the end of the priming (and early coadministration) timepoint. Similarly, long-term follow-up immunogenicity analyses were not performed, leaving open the possibility that the durability of response between regimens could differ.

The immunogenicity results observed in HVTN 124 lend further credence to the value of both matching prime–boost antigens and the polyvalent Env antigen approach, because elicited immune responses were broad as well as robust. Binding antibodies, ADCC activities, and tier 1B neutralising antibodies were reactive against viruses or viral antigens across multiple major HIV-1 subtypes. High binding antibody response rates and magnitudes were observed to the four autologous vaccine gp120 antigens, a heterologous panel of gp120 and gp140 antigens, and a panel of gp70-V1V2 antigens in both prime–boost and coadministration groups. Polyfunctional antibody activities, including ADCC and neutralising antibodies against tier 1B viruses, were also observed.

Antibodies against the V1V2 region of HIV-1 Env are of particular interest as a correlate of protection based on efficacy analyses of the RV144 trial.[3] In HVTN 124, high antibody response rates were detected to a panel of heterologous gp70-V1V2 antigens, including one clade A, five clade B, seven clade C, two clade AE, and one BC antigen, with geometric mean titres exceeding 10^3 against a majority. Of potentially greater implication, IgG3 antibodies were also detected against gp70 V1V2 antigens across different subtypes. IgG3 antibodies against V1V2 have also been identified as an important correlate of protection, evidence for which was supported by comparisons between the immune responses between samples collected from RV144 and HVTN 702 participants.[2] The higher IgG and IgG3 titres against multiple V1V2 antigens observed in HVTN 124 participants receiving prime–boost compared with those in RV144, HVTN702, and HVTN705 suggest that the tested polyvalent DNA–protein HIV vaccine formulation might offer a better chance at eliciting protective immune responses compared with the vaccines evaluated in these other trials.

Neutralising antibody responses rates against tier 2 viruses were low in HVTN 124, although all participants developed neutralising responses against clade AE TH023.6 at modest titres and more than 50% of participants receiving the prime–boost regimen had neutralising antibodies against clade AG T271.11 and clade C 6644.v2.c33, other tier 1B viruses, albeit at low titres. Nevertheless, this result was noteworthy as most candidate vaccines fail to generate substantial tier 1B neutralising antibody responses. The rate, magnitude, and breadth of neutralising antibody responses against tier 1B viruses observed in HVTN 124 were similar to or exceeded those elicited by other prime–boost vaccine combinations that advanced to efficacy studies.[27,29]

With regard to administration strategy, the prime–boost regimen consistently outperformed the co-administration regimen, and elicited similar or greater response rates and magnitudes for each immunological outcome assayed. The prime–boost group had a higher response rate and magnitude of ADCC responses than the coadministration group, particularly against infectious molecular clone-transfected targets. Of particular note, IgG3 response rates and magnitudes to gp120 and gp140 and gp70-V1V2 antigens were also higher in the prime–boost group. This disparity between dosing regimens was also observed in CD4 cell responses. 100% of participants in the prime–boost group had an Env-specific CD4 cell response (*vs* 38% in the coadministration group), representing one of the few times that an HIV vaccine was able to elicit HIV antigen-specific CD4 cell responses in all immunised volunteers and lending credence to the hypothesis that DNA prime is beneficial to the CD4 cell response;[4] the significant proportion of polyfunctional CD4 cells among participants who had a CD4 cell response was also particularly noteworthy.

Overall, these results compare favourably with immunogenicity outcomes in contemporary HIV vaccine trials evaluating DNA or viral-vectored prime–protein boost combinations. Comparisons are often restricted by differences in antigens used to evaluate immune responses across studies. When similar assays with identical antigens were used, selected comparisons could be made: IgG binding antibodies titres to subtype C 1086.C gp120 were of higher magnitude in HVTN 124 participants receiving prime–boost compared with those observed in HVTN 100 (evaluating the vaccine combination of ALVAC-HIV and bivalent subtype C gp120 consisting of TV1.C and 1086.C),[27] and were of similar or higher magnitude to subtype AE A244 and to 1086.C in HVTN 105.[11] The percentage of participants with a CD4 cell response to any HIV Env potential T-cell epitope

was greater among HVTN 124 participants receiving prime–boost than those in the HVTN 096, HVTN 105, and HVTN 111 trials.[8,26,28] Additionally, ADCC response rates to TV1 gp120-coated or infected targets were higher in the HVTN 124 participants receiving prime–boost compared with those receiving the trivalent and tetravalent Ad26 mosaic gp140 vaccine administered together with clade C gp140 in HVTN 117.[30]

Previous candidate HIV vaccines have had insufficient immunogenicity or breadth to offer protection against the diversity of circulating virus quasispecies. Modest prevention of incident HIV infection was observed in only one trial, RV144,[31] which evaluated a vaccine regimen consisting of a priming canary pox virus-vectored (ALVAC-HIV vcp1521) clade AE vaccine, followed by a bivalent, recombinant gp120 protein subunit (AIDSVAX B/E) boost. Correlates of protection from HIV acquisition observed in the RV144 trial include IgG binding antibodies to V1V2 antigens, IgG3 binding antibodies to C.1086 V1V2, high concentrations of ADCC in the presence of low concentrations of IgA to Env, and CD4 cell polyfunctionality.[3,16,17,23,32] Although no protection from HIV acquisition was observed in the HVTN 702 trial, which sought to optimise the RV144 vaccine combination for the southern African epidemic,[33] the combination of high concentrations of IgG A244 V1V2 binding antibodies and vaccine-matched CD4 cell responses were found to correlate with lower HIV-1 risk.[2] The recipients of prime–boost in HVTN 124 had each of these immunological correlates of protection.

In conclusion, the polyvalent DNA prime with matched polyvalent protein boost vaccine regimen evaluated in this trial was safe and immunogenic. Furthermore, immunogenicity outcomes showed a combination of both magnitude and breadth. Humoral and cellular immune responses were enhanced by a DNA prime–protein boost dosing strategy compared with immunogen coadministration. These responses compared very favourably to those observed in contemporaneous, early-phase vaccine candidate studies and were of magnitudes that exceeded those associated with vaccine protection in efficacy trials. We recognise these results are based on small sample sizes and are subject to inherent biases (eg, selection and measurement biases), but we nevertheless believe further evaluation of this combination in larger trials with more robust statistical methods is warranted.

Contributors

IF, SSL, STR, SW, and SL wrote and edited the original draft of the manuscript. IF, ETO, KHM, DAC, MCK, and SE served as primary investigators at clinical trial sites. MNP and VLM contributed to study product design. SSL, NG, HZ, KES, JRH, SCDR, DEM, MSS, DCM, GF, and GDT conducted the formal analysis. IF, JGK, and LC provided supervision. MAA contributed to the oversight of study conduct, data analysis and interpretation, and editing of this manuscript. IF, GDT, LC, and SL acquired funding. All authors provided feedback on the final manuscript. SSL and HZ accessed and verified the data. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

IF, NG, KES, JRH, SE, SCDR, DEM, DCM, GDT, LC, and SL report support for the present manuscript from National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID), Division of AIDS, and the HIV Vaccine Trials Network (HVTN) paid to their institutions. SCDR and DEM report support for the present manuscript from the Bill & Melinda Gates Foundation, and grants or contracts outside of the submitted work from Janssen, Sanofi, and Moderna. IF reports grants or contracts outside of the submitted work from Janssen, Gilead, Moderna, Pfizer, and Sanofi paid to his institution, consulting fees from Gilead and ViiV, and participation or payment from Janssen for participation on a data safety monitoring board or advisory board. JRH reports support for attending meetings or travel from the HVTN. MCK reports grants or contracts from NIAID outside of the submitted work and participation on a data safety monitoring board or advisory board for the Worcester HIV Vaccine. SL reports royalties or licences from Cosmic Bliss Sciences related to polyvalent DNA–protein HIV vaccine IP from the University of Massachusetts Medical School, Worcester, MA, USA, and reports stock ownership of Cosmic Bliss Sciences since 2023. All other authors declare no competing interests.

Data sharing

All data presented in this Article will be available upon publication from https://atlas.scharp.org/cpas/project/HVTN%20Public%20Data/HVTN%20124/begin.view.

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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Frank I, Li SS, Grunenberg N, et al. Safety and immunogenicity of a polyvalent DNA–protein HIV vaccine with matched Env immunogens delivered as a prime–boost regimen or coadministered in HIV-uninfected adults in the USA (HVTN 124): a phase 1, placebo-controlled, double-blind randomised controlled trial. *Lancet HIV* 2024; **11:** e285–99.

Supplementary Figure 1. Antibody-dependent cell-mediated cytotoxicity. Antibody-dependent cell-mediated cytotoxicity (ADCC) in Prime-Boost (blue), Coadministration (red), and Placebo (grey) recipients was assessed via Granzyme B activity (A) and percent loss of luciferase activity (B) and quantified by the area under the magnitude-breadth curve (AUC-MB) 2

Supplementary Figure 2. Neutralizing antibody activity. Neutralizing antibody responses of Prime-Boost (blue), Coadministration (red), and Placebo (grey) participants to tier 1A (A), tier 1B (B), and tier 2 (C) viruses (*p<0.05, precise p-values are reported in Supplementary Table 3, Appendix pages 15-16). Response rates are summarized above each plot. Non-responders are shown as triangles Neutralizing antibody responses to tier 1B virus TH023.6 are also shown and compared against comparable groups from other HVTN 096, HVTN 097, and HVTN 105 HIV vaccine trials (D) (*p<0.05 compared to HVTN 124 Prime-Boost, #p<0.05 compared to HVTN 124 Coadministration, precise p-values are reported in Supplementary Table 6, Appendix page 19). 3

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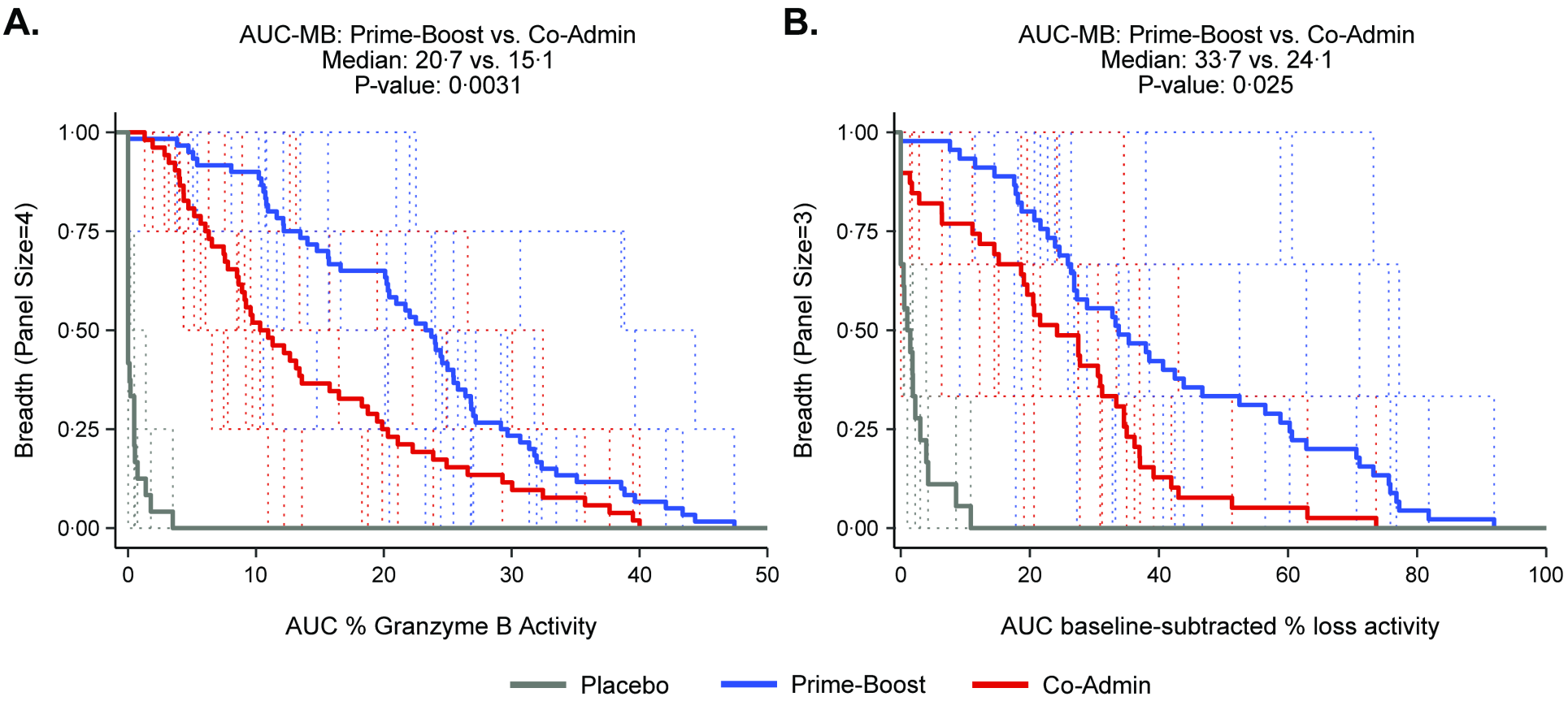

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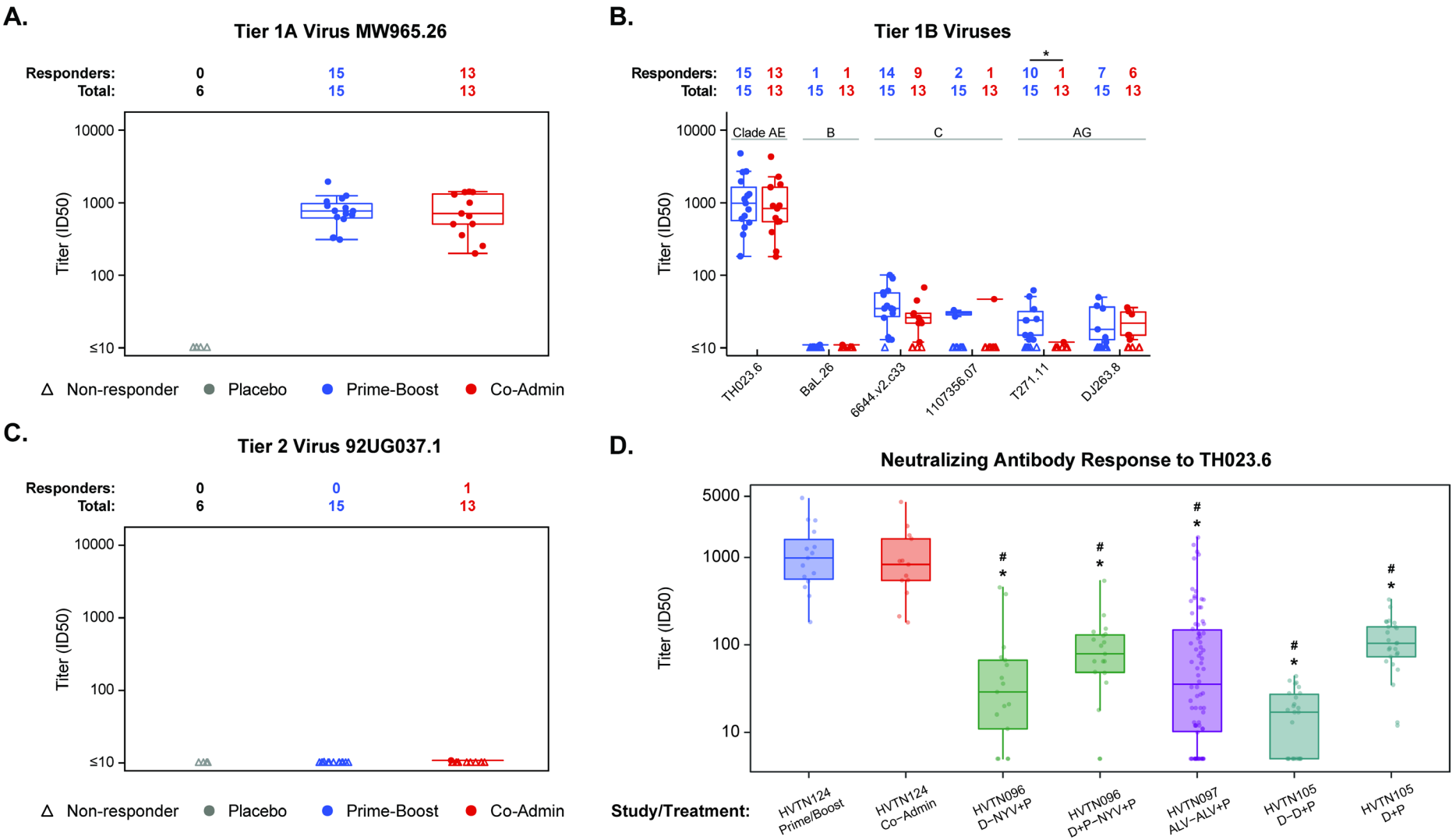

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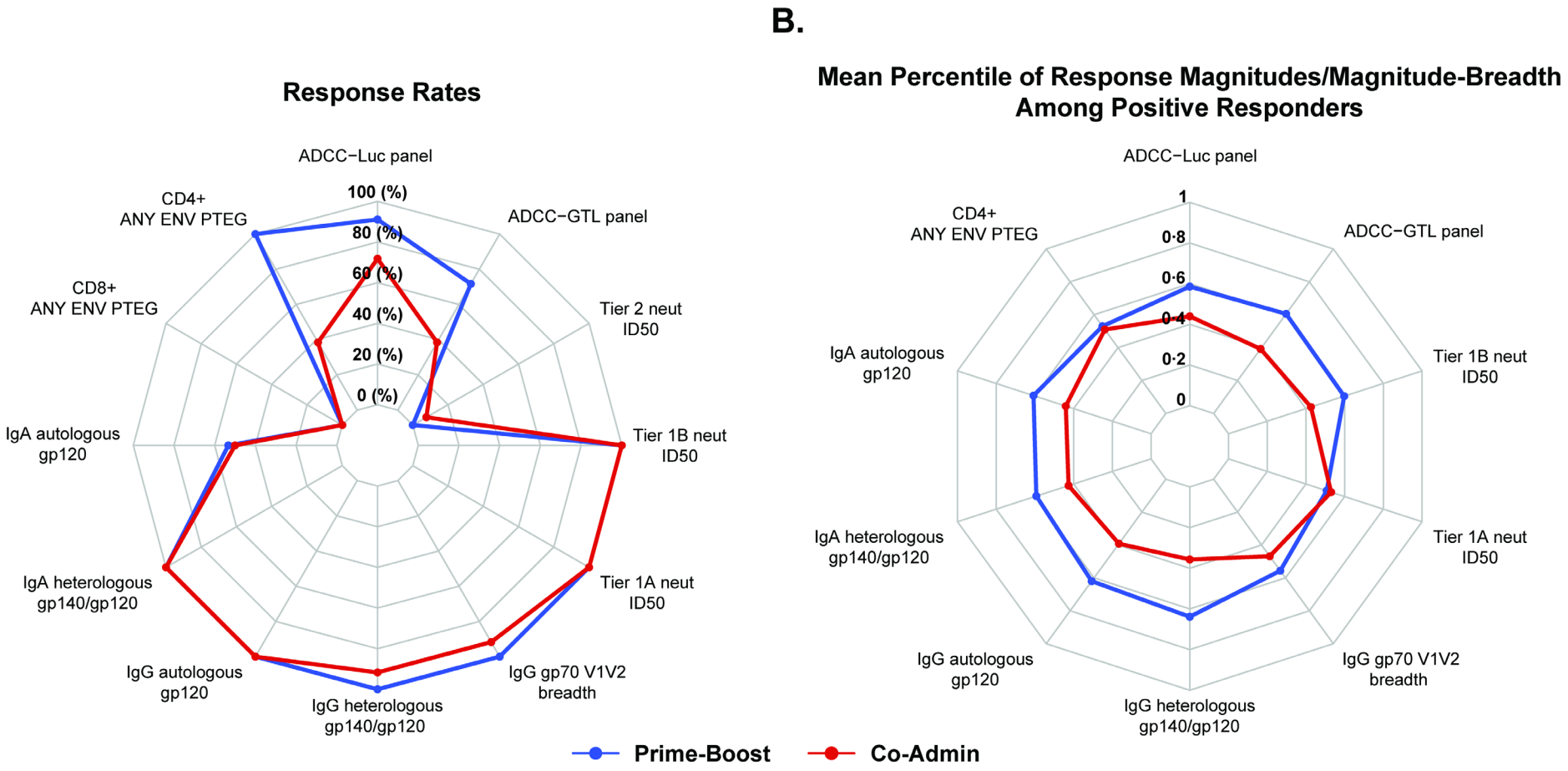

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Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
IgG	Autologous gp120	gp120-A	N (%, 95% CI)	15 (100·0%, 79.6-100.0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	13360 (6680)	8103 (3241)	**0·0089**
			Median (IQR)	15527 (11736, 17254)	9702 (6270, 11187)	
			Range	(3942, 27583)	(4632, 11900)	
		gp120-B	N (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	6634 (3317)	6634 (3981)	0·95
			Median (IQR)	6793 (4872, 9282)	7345 (4476, 10226)	
			Range	(1697, 12984)	(1898, 12481)	
		gp120-C	N (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	12088 (4835)	8103 (4052)	**0·032**
			Median (IQR)	13317 (10019, 15672)	8980 (5998, 11667)	
			Range	(3456, 24361)	(2872, 13011)	
		gp120-AE	N (%, 95% CI)	15 (100·0%, 79·6-100·0%)	12 (100·0%, 75·8-100·0%)	1·0
			Geometric Mean (SD)	9897 (4949)	6634 (3981)	0·057
			Median (IQR)	8950 (8483, 14693)	5871 (5387, 9842)	
			Range	(2525, 16062)	(2548, 13201)	
	Aggregated autologous gp120	gp120-A, -B, -C, -AE	N (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	10938 (5469)	7332 (3666)	**0·048**
			Median (IQR)	11230 (9313, 15178)	8860 (5295, 11134)	
			Range	(2990, 19765)	(3141, 12587)	
	Heterologous gp120/gp140 Panel	A1.con.env03 140 CF	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	13360 (6680)	7332 (3666)	**0·0035**
			Median (IQR)	15507 (12034, 17252)	7294 (4820, 10632)	
			Range	(2999, 18829)	(3651, 12838)	
		00MSA 4076 gp140	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	14765 (4429)	8955 (3582)	**0·0026**
			Median (IQR)	18073 (13741, 19500)	11411 (6770, 12520)	
			Range	(7219, 22703)	(4988, 14635)	
		51802_D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	13360 (6680)	7332 (3666)	**0·0087**
			Median (IQR)	14086 (10802, 20231)	9078 (4549, 10978)	
			Range	(3603, 24160)	(3110, 12894)	
		BORI_D11gp120	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	11 (91·7%, 64·6-98·5%)	1·0
			Geometric Mean (SD)	7332 (3666)	3641 (2185)	**0·011**
			Median (IQR)	7238 (5095, 11182)	3736 (2863, 6256)	
			Range	(2996, 14451)	(1114, 9504)	
		TT31P.2792_D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	29733 (11893)	22026 (4405)	0·065

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
			Median (IQR)	27188 (25827, 39202)	22343 (18839, 27404)	
			Range	(13494, 42223)	(16442, 33036)	
		B.6240_D11gp120/293F	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	13360 (4008)	10938 (5469)	0·22
			Median (IQR)	16059 (10166, 18775)	12502 (10404, 15031)	
			Range	(7886, 21078)	(3282, 18667)	
		1086C_D7gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	40135 (12040)	36316 (3632)	0·10
			Median (IQR)	41320 (37180, 51917)	36321 (35008, 37662)	
			Range	(22951, 60116)	(27857, 43259)	
		A244 D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	24343 (7303)	18034 (5410)	**0·014**
			Median (IQR)	26730 (21148, 31676)	17350 (14277, 22208)	
			Range	(12016, 39408)	(12434, 31698)	
		254008_D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	19930 (9965)	16318 (3264)	0·28
			Median (IQR)	18996 (15697, 27693)	16398 (14220, 17636)	
			Range	(7348, 35957)	(12908, 26409)	
		CNE20_D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	26903 (8071)	19930 (5979)	**0·023**
			Median (IQR)	31733 (24363, 35830)	21676 (16265, 24709)	
			Range	(11685, 39253)	(14791, 33311)	
		BJOX002_D11gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	12088 (6044)	7332 (3666)	**0·037**
			Median (IQR)	13841 (9676, 15454)	7857 (5354, 11366)	
			Range	(2612, 18230)	(3589, 13910)	
		Con S gp140 CFI	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	32860 (6572)	26903 (5381)	**0·015**
			Median (IQR)	35031 (32748, 40510)	28894 (21733, 31783)	
			Range	(16109, 42846)	(17962, 35646)	
		Con 6 gp120/B	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	18034 (9017)	12088 (3627)	**0·026**
			Median (IQR)	18961 (13992, 28602)	12486 (10482, 14886)	
			Range	(4315, 34509)	(7645, 19754)	
	gp70 V1V2 Breadth Panel	gp70-191084_B7	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	8955 (6269)	6634 (5971)	0·41
			Median (IQR)	10508 (6527, 11795)	9562 (4876, 11441)	
			Range	(1685, 24382)	(922, 15521)	

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
		gp70_B.CaseA	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	2441 (2685)	1998 (2598)	0·65
			Median (IQR)	1915 (1342, 3816)	2397 (812, 6121)	
			Range	(336, 22158)	(217, 8603)	
		gp70-RHPA4259.7	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	8 (66·7%, 39·1-86·2%)	**0·021**
			Geometric Mean (SD)	1212 (1576)	812 (406)	0·33
			Median (IQR)	1106 (643, 2398)	798 (619, 1216)	
			Range	(138, 16648)	(432, 1869)	
		gp70-62357.14	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	9 (75·0%, 46·8-91·1%)	0·60
			Geometric Mean (SD)	812 (1056)	992 (695)	0·58
			Median (IQR)	644 (505, 1564)	1050 (621, 1780)	
			Range	(74, 6918)	(320, 2349)	
		gp70-700010058	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	11 (91·7%, 64·6-98·5%)	1·0
			Geometric Mean (SD)	898 (1526)	4447 (4447)	**0·012**
			Median (IQR)	1052 (331, 3521)	5550 (1524, 11081)	
			Range	(76, 16693)	(993, 11981)	
		gp70-TT31P.2F10.2792	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	8 (66·7%, 39·1-86·2%)	0·59
			Geometric Mean (SD)	1097 (1206)	992 (595)	0·77
			Median (IQR)	864 (574, 2152)	845 (633, 1496)	
			Range	(244, 11530)	(531, 2576)	
		gp70-BF1266_431a	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	8 (66·7%, 39·1-86·2%)	**0·021**
			Geometric Mean (SD)	1339 (2009)	1097 (877)	0·77
			Median (IQR)	1654 (386, 3650)	931 (695, 1498)	
			Range	(136, 25444)	(558, 5242)	
		gp70-7060101641	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	10 (83·3%, 55·2-95·3%)	0·14
			Geometric Mean (SD)	812 (812)	493 (443)	0·16
			Median (IQR)	562 (445, 1053)	582 (309, 939)	
			Range	(239, 11893)	(62, 1260)	
		gp70-96ZM651.02	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	7332 (7332)	4447 (4447)	0·19
			Median (IQR)	5639 (3622, 15701)	5177 (2849, 9062)	
			Range	(1880, 35248)	(445, 11826)	
		gp70-001428.2.42	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	1636 (1963)	2697 (2697)	0·28
			Median (IQR)	1549 (691, 3882)	3567 (1476, 6642)	
			Range	(241, 14015)	(456, 7457)	
		gp70-CAP210.2.00.E8	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	8 (66·7%, 39·1-86·2%)	0·087
			Geometric Mean (SD)	898 (1347)	602 (602)	0·44
			Median (IQR)	777 (344, 3069)	813 (510, 1182)	

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
			Range	(70, 15174)	(65, 1357)	
		gp70-TV1.21	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	9 (75·0%, 46·8-91·1%)	**0·048**
			Geometric Mean (SD)	1480 (2072)	992 (1191)	0·44
			Median (IQR)	1410 (779, 3138)	1158 (922, 1716)	
			Range	(164, 18467)	(51, 2811)	
		gp70-Ce1086_B2	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	19930 (7972)	10938 (5469)	**0·0030**
			Median (IQR)	20052 (17841, 30047)	12372 (9410, 16078)	
			Range	(8510, 34608)	(3853, 18665)	
		gp70-CM244.ec1	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	10 (83·3%, 55·2-95·3%)	0·14
			Geometric Mean (SD)	22026 (8811)	9897 (5938)	**0·0032**
			Median (IQR)	21683 (18728, 30496)	12040 (8843, 15689)	
			Range	(7940, 33493)	(3666, 18352)	
		gp70-C2101.c01	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	5432 (3802)	2981 (3577)	0·15
			Median (IQR)	5479 (3761, 10297)	3212 (1023, 8687)	
			Range	(1330, 19345)	(532, 12090)	
		gp70-BJOX002000.03.2	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	11 (91·7%, 64·6-98·5%)	0·34
			Geometric Mean (SD)	6003 (5403)	3294 (3294)	0·11
			Median (IQR)	8050 (3212, 10926)	3619 (2071, 7280)	
			Range	(856, 22240)	(358, 8106)	
	Other	gp41	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-24·2%)	1·0
		p24	n (%, 95% CI)	1 (6·7%, 1·2-29·8%)	0 (0·0%, 0·0-24·2%)	0·51
			Geometric Mean (SD)	1552 (NA)		NA
			Median (IQR)	1552 (1552, 1552)		
			Range	(1552, 1552)		

*: Barnard's test was used for response rate comparison between two vaccine regimens. Two-sided independent t-test was used for magnitudes titres among positive responders between two vaccine regimens only if there are at least 4 positive responders per vaccine regimen; otherwise, p-value is missing (NA). Bolded p-values indicate for significance with $p<0·05$.

Supplementary Table 1B. Summary of IgG3 response rates and titres among positive responders by isotype, antigen, and vaccine regimen, with comparisons between the two vaccine regimens (Prime-Boost and Coadministration) in Part B based on the Per Protocol (PP) data.

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
IgG3	Autologous gp120	gp120-A	N (%, 95% CI)	14 (93·3%, 70·2-98·8%)	2 (15·4%, 4·3-42·2%)	**<0·0001**
			Geometric Mean (SD)	1094 (1539)	749 (1241)	NA
			Median (IQR)	751 (476, 1634)	1325 (778, 1871)	
			Range	(122, 15676)	(232, 2418)	
		gp120-B	N (%, 95% CI)	12 (80·0%, 54·8-93·0%)	2 (15·4%, 4·3-42·2%)	**0·0007**
			Geometric Mean (SD)	530 (768)	749 (775)	NA
			Median (IQR)	265 (178, 2315)	959 (659, 1258)	
			Range	(135, 6778)	(360, 1557)	
		gp120-C	N (%, 95% CI)	13 (86·7%, 62·1-96·3%)	2 (15·4%, 4·3-42·2%)	**0·0002**
			Geometric Mean (SD)	799 (830)	116 (19)	NA
			Median (IQR)	1039 (312, 1626)	117 (110, 124)	
			Range	(187, 5080)	(103, 130)	
	Aggregated autologous gp120	gp120-A, -B, -C	N (%, 95% CI)	13 (86·7%, 62·1-96·3%)	2 (15·4%, 4·3-42·2%)	**<0·0001**
			Geometric Mean (SD)	943 (1085)	601 (809)	NA
			Median (IQR)	804 (392, 2008)	894 (563, 1226)	
			Range	(191, 6778)	(232, 1557)	
	Heterologous gp120/gp140 Panel	A1.con.env03 140 CF	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	1 (7·7%, 1·4-33·3%)	**<0·0001**
			Geometric Mean (SD)	554 (519)	133 (NA)	NA
			Median (IQR)	594 (284, 1185)	133 (133, 133)	
			Range	(106, 2352)	(133, 133)	
		00MSA 4076 gp140	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	1 (7·7%, 1·4-33·3%)	**<0·0001**
			Geometric Mean (SD)	637 (541)	148 (NA)	NA
			Median (IQR)	477 (336, 1340)	148 (148, 148)	
			Range	(174, 2206)	(148, 148)	
		51802_D11gp120	n (%, 95% CI)	9 (60·0%, 35·8-80·2%)	0 (0·0%, 0·0-22·8%)	**0·0009**
			Geometric Mean (SD)	565 (773)		NA
			Median (IQR)	371 (240, 773)		
			Range	(145, 14175)		
		BORI_D11gp120	n (%, 95% CI)	7 (46·7%, 24·8-69·9%)	0 (0·0%, 0·0-22·8%)	**0·0044**
			Geometric Mean (SD)	256 (301)		NA
			Median (IQR)	164 (149, 211)		
			Range	(132, 3518)		
		TT31P.2792_D11gp120	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	2 (15·4%, 4·3-42·2%)	**0·0002**
			Geometric Mean (SD)	701 (902)	127 (22)	NA
			Median (IQR)	654 (240, 1049)	128 (120, 136)	
			Range	(164, 22000)	(112, 144)	
		B.6240_D11gp120/293F	n (%, 95% CI)	10 (66·7%, 41·7-84·8%)	0 (0·0%, 0·0-22·8%)	**0·0002**
			Geometric Mean (SD)	712 (1214)		NA

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
			Median (IQR)	338 (250, 901)		
			Range	(156, 22000)		
		1086C_D7gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	7 (53·8%, 29·1-76·8%)	**0·0038**
			Geometric Mean (SD)	2023 (2600)	246 (133)	**0·018**
			Median (IQR)	2476 (913, 4983)	193 (160, 368)	
			Range	(242, 22000)	(146, 569)	
		A244 D11gp120	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	3 (23·1%, 8·2-50·3%)	**0·0001**
			Geometric Mean (SD)	1131 (1447)	314 (140)	NA
			Median (IQR)	1119 (526, 2596)	252 (243, 388)	
			Range	(142, 15912)	(233, 524)	
		254008_D11gp120	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	1 (7·7%, 1·4-33·3%)	**0·0001**
			Geometric Mean (SD)	470 (626)	160 (NA)	NA
			Median (IQR)	398 (213, 572)	160 (160, 160)	
			Range	(122, 22000)	(160, 160)	
		CNE20_D11gp120	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	2 (15·4%, 4·3-42·2%)	**0·0002**
			Geometric Mean (SD)	1030 (1226)	123 (37)	NA
			Median (IQR)	967 (627, 1301)	126 (113, 139)	
			Range	(153, 22000)	(100, 152)	
		BJOX002_D11gp120	n (%, 95% CI)	11 (73·3%, 48·0-89·1%)	0 (0·0%, 0·0-22·8%)	**<0·0001**
			Geometric Mean (SD)	449 (499)		NA
			Median (IQR)	374 (247, 568)		
			Range	(112, 8237)		
		Con S gp140 CFI	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	4 (30·8%, 12·7-57·6%)	**0·0004**
			Geometric Mean (SD)	2525 (3150)	204 (122)	**0·0032**
			Median (IQR)	3034 (1070, 6202)	240 (139, 332)	
			Range	(254, 15400)	(102, 344)	
		Con 6 gp120/B	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	1 (7·7%, 1·4-33·3%)	**<0·0001**
			Geometric Mean (SD)	568 (788)	102 (NA)	NA
			Median (IQR)	486 (278, 616)	102 (102, 102)	
			Range	(102, 22000)	(102, 102)	
	gp70 V1V2 Breadth Panel	gp70-191084_B7	n (%, 95% CI)	11 (73·3%, 48·0-89·1%)	1 (7·7%, 1·4-33·3%)	**0·0003**
			Geometric Mean (SD)	468 (517)	388 (NA)	NA
			Median (IQR)	418 (211, 534)	388 (388, 388)	
			Range	(156, 5608)	(388, 388)	
		gp70_B.CaseA	n (%, 95% CI)	3 (20·0%, 7·0-45·2%)	1 (7·7%, 1·4-33·3%)	0·53
			Geometric Mean (SD)	815 (1535)	111 (NA)	NA
			Median (IQR)	496 (331, 3518)	111 (111, 111)	
			Range	(167, 6540)	(111, 111)	

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
		gp70-RHPA4259.7	n (%, 95% CI)	4 (26·7%, 10·9-51·9%)	0 (0·0%, 0·0-22·8%)	**0·048**
			Geometric Mean (SD)	391 (441)		NA
			Median (IQR)	327 (173, 801)		
			Range	(154, 1782)		
		gp70-62357.14	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	1 (7·7%, 1·4-33·3%)	0·71
			Geometric Mean (SD)	185 (48)	112 (NA)	NA
			Median (IQR)	188 (171, 205)	112 (112, 112)	
			Range	(154, 222)	(112, 112)	
		gp70-700010058	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	0 (0·0%, 0·0-22·8%)	0·21
			Geometric Mean (SD)	737 (1472)		NA
			Median (IQR)	1603 (891, 2315)		
			Range	(180, 3026)		
		gp70-TT31P.2F10.2792	n (%, 95% CI)	3 (20·0%, 7·0-45·2%)	0 (0·0%, 0·0-22·8%)	0·093
			Geometric Mean (SD)	345 (361)		NA
			Median (IQR)	334 (228, 666)		
			Range	(123, 998)		
		gp70-BF1266_431a	n (%, 95% CI)	4 (26·7%, 10·9-51·9%)	0 (0·0%, 0·0-22·8%)	**0·048**
			Geometric Mean (SD)	656 (1000)		NA
			Median (IQR)	528 (326, 1793)		
			Range	(138, 5172)		
		gp70-7060101641	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	0 (0·0%, 0·0-22·8%)	0·21
			Geometric Mean (SD)	293 (288)		NA
			Median (IQR)	367 (257, 477)		
			Range	(146, 587)		
		gp70-96ZM651.02	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	1 (7·7%, 1·4-33·3%)	**<0·0001**
			Geometric Mean (SD)	393 (427)	121 (NA)	NA
			Median (IQR)	238 (186, 489)	122 (122, 122)	
			Range	(159, 6075)	(122, 122)	
		gp70-001428.2.42	n (%, 95% CI)	4 (26·7%, 10·9-51·9%)	1 (7·7%, 1·4-33·3%)	0·23
			Geometric Mean (SD)	181 (69)	121 (NA)	NA
			Median (IQR)	178 (155, 214)	121 (121, 121)	
			Range	(115, 291)	(121, 121)	
		gp70-CAP210.2.00.E8	n (%, 95% CI)	4 (26·7%, 10·9-51·9%)	0 (0·0%, 0·0-22·8%)	**0·048**
			Geometric Mean (SD)	188 (103)		NA
			Median (IQR)	171 (141, 242)		
			Range	(110, 396)		
		gp70-TV1.21	n (%, 95% CI)	3 (20·0%, 7·0-45·2%)	1 (7·7%, 1·4-33·3%)	0·53
			Geometric Mean (SD)	515 (295)	130 (NA)	NA
			Median (IQR)	616 (444, 717)	130 (130, 130)	

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=12)	p-value*
			Range	(271, 817)	(130, 130)	
		gp70-Ce1086_B2	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	9 (69·2%, 42·4-87·3%)	**0·018**
			Geometric Mean (SD)	2433 (2950)	281 (260)	**0·023**
			Median (IQR)	2228 (1380, 4690)	187 (176, 268)	
			Range	(269, 21577)	(146, 2708)	
		gp70-CM244.ec1	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	9 (69·2%, 42·4-87·3%)	**0·018**
			Geometric Mean (SD)	2437 (3188)	361 (335)	**0·028**
			Median (IQR)	2596 (1366, 5345)	246 (207, 388)	
			Range	(329, 22000)	(176, 3224)	
		gp70-C2101.c01	n (%, 95% CI)	9 (60·0%, 35·8-80·2%)	1 (7·7%, 1·4-33·3%)	**0·0043**
			Geometric Mean (SD)	466 (556)	241 (NA)	NA
			Median (IQR)	348 (188, 548)	240 (240, 240)	
			Range	(119, 4469)	(240, 240)	
		gp70-BJOX002000.03.2	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	3 (23·1%, 8·2-50·3%)	**0·0038**
			Geometric Mean (SD)	410 (359)	232 (158)	NA
			Median (IQR)	376 (212, 656)	171 (157, 339)	
			Range	(111, 2692)	(144, 507)	
	Other	gp41	n (%, 95% CI)	1 (6·7%, 1·2-29·8%)	0 (0·0%, 0·0-22·8%)	0·51
			Geometric Mean (SD)	2343 (NA)		NA
			Median (IQR)	2343 (2343, 2343)		
			Range	(2343, 2343)		
		p24	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	0 (0·0%, 0·0-22·8%)	0·21
			Geometric Mean (SD)	1039 (355)		NA
			Median (IQR)	1069 (943, 1196)		
			Range	(816, 1322)		

*: Barnard's test was used for response rate comparison between two vaccine regimens. Two-sided independent t-test was used for magnitudes titres among positive responders between two vaccine regimens only if there are at least 4 positive responders per vaccine regimen; otherwise, p-value is missing (NA). Bolded p-values indicate for significance with $p<0·05$.

Supplementary Table 2. Summary of IgA response rates and magnitudes among positive responders by isotype, antigen, and vaccine regimen, with comparisons between two vaccine regimens (Prime-Boost and Coadministration) in Part B based on the Per Protocol (PP) data.

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
IgA	Autologous gp120	gp120-A	n (%, 95% CI)	5 (33·3%, 15·2-58·3%)	2 (16·7%, 4·7-44·8%)	0·35
			Geometric Mean (SD)	10938 (6563)	4024 (1610)	NA
			Median (IQR)	11835 (8753, 14463)	4112 (3492, 4731)	
			Range	(4182, 22000)	(2873, 5350)	
		gp120-B	n (%, 95% CI)	8 (53·3%, 30·1-75·2%)	6 (50·0%, 25·4-74·6%)	1·0
			Geometric Mean (SD)	2697 (3237)	1808 (1446)	0·49
			Median (IQR)	2636 (1348, 5083)	2031 (1116, 3196)	
			Range	(522, 20931)	(493, 4338)	
		gp120-C	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	2 (16·7%, 4·7-44·8%)	1·0
			Geometric Mean (SD)	22026 (0)	7332 (733)	NA
			Median (IQR)	21229 (21050, 21408)	7346 (7113, 7579)	
			Range	(20871, 21587)	(6880, 7813)	
		gp120-AE	n (%, 95% CI)	4 (26·7%, 10·9-51·9%)	1 (8·3%, 1·5-35·4%)	0·30
			Geometric Mean (SD)	5432 (1629)	11250 (NA)	NA
			Median (IQR)	4631 (4403, 5689)	11250 (11250, 11250)	
			Range	(4392, 8192)	(11250, 11250)	
	Heterologous gp140/gp120 panel	Con S gp140 CFI	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	10 (83·3%, 55·2-95·3%)	0·59
			Geometric Mean (SD)	1998 (2198)	1097 (1316)	0·15
			Median (IQR)	1752 (1323, 2752)	1034 (407, 2053)	
			Range	(262, 22000)	(264, 11759)	
		Con 6 gp120/B	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	2 (16·7%, 4·7-44·8%)	1·0
			Geometric Mean (SD)	6634 (6634)	4024 (0)	NA
			Median (IQR)	7941 (5596, 10285)	4107 (4050, 4165)	
			Range	(3252, 12630)	(3992, 4222)	
		1086C_D7gp120	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	12 (100·0%, 75·8-100·0%)	1·0
			Geometric Mean (SD)	5432 (7061)	9897 (4949)	0·23
			Median (IQR)	3427 (2967, 20564)	9151 (7173, 12648)	
			Range	(392, 22000)	(4607, 22000)	
		A1.con.env03 140 CF	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	4 (33·3%, 13·8-60·9%)	**0·020**
			Geometric Mean (SD)	812 (975)	812 (1381)	0·95
			Median (IQR)	569 (470, 1187)	1043 (402, 2586)	
			Range	(170, 22000)	(111, 5586)	
		00MSA 4076 gp140	n (%, 95% CI)	13 (86·7%, 62·1-96·3%)	4 (33·3%, 13·8-60·9%)	**0·0060**
			Geometric Mean (SD)	992 (1191)	602 (782)	0·48
			Median (IQR)	870 (666, 1039)	577 (286, 1325)	

Isotype	Panel	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
			Range	(170, 22000)	(155, 2827)	
	Other	gp41	n (%, 95% CI)	0(0·0%, 0·0-20·4%)	0(0·0%, 0·0-24·2%)	1·0
		p24	n (%, 95% CI)	2 (20·0%, 5·7-51·0%)	0 (0·0%, 0·0-39·0%)	0·33
			Geometric Mean (SD)	13360 (9352)		NA
			Median (IQR)	15080 (11619, 18540)		
			Range	(8159, 22000)		

*: Barnard's test was used for response rate comparison between two vaccine regimens. Wilcoxon rank sum test was used for magnitudes titres among positive responders between two vaccine regimens only if there are at least 4 positive responders per vaccine regimen; otherwise, p-value is missing (NA). Bolded p-values indicate for significance with $p<0·05$.

Supplementary Table 3. Summary of neutralizing antibody ID50/ID80 response rates and titres among positive responders by isotype, antigen, and vaccine regimen, with comparisons between the two vaccine regimens (Prime-Boost and Coadministration) in Part B based on the Per Protocol (PP) data.

Titre	Tier	Isolate	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
ID50	1a	MW965.26	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	13 (100·0%, 77·2-100·0%)	1·0
			Geometric Mean (SD)	726 (374)	704 (483)	0·86
			Median (IQR)	767 (617, 972)	711 (507, 1318)	
			Range	(311, 1948)	(200, 1423)	
	1b	TH023.6	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	13 (100·0%, 77·2-100·0%)	1·0
			Geometric Mean (SD)	971 (835)	801 (730)	0·59
			Median (IQR)	987 (566, 1640)	831 (547, 1637)	
			Range	(183, 4787)	(181, 4315)	
		BaL.26	n (%, 95% CI)	1 (6·7%, 1·2-29·8%)	1 (7·7%, 1·4-33·3%)	1·0
			Geometric Mean (SD)	11 (NA)	11 (NA)	NA
			Median (IQR)	11 (11, 11)	11 (11, 11)	
			Range	(11, 11)	(11, 11)	
		6644.v2.c33	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	9 (69·2%, 42·4-87·3%)	0·11
			Geometric Mean (SD)	36 (24)	28 (13)	0·20
			Median (IQR)	35 (27, 57)	26 (22, 30)	
			Range	(13, 101)	(12, 68)	
		1107356.07	n (%, 95% CI)	2 (13·3%, 3·7-37·9%)	1 (7·7%, 1·4-33·3%)	0·71
			Geometric Mean (SD)	30 (4)	47 (NA)	NA
			Median (IQR)	30 (28, 32)	47 (47, 47)	
			Range	(27, 33)	(47, 47)	
		T271.11	n (%, 95% CI)	10 (66·7%, 41·7-84·8%)	1 (7·7%, 1·4-33·3%)	**0·0013**
			Geometric Mean (SD)	24 (13)	12 (NA)	NA
			Median (IQR)	24 (15, 32)	12 (12, 12)	
			Range	(13, 62)	(12, 12)	
		DJ263.8	n (%, 95% CI)	7 (46·7%, 24·8-69·9%)	6 (46·2%, 23·2-70·9%)	1·0
			Geometric Mean (SD)	21 (14)	21 (10)	1·0
			Median (IQR)	18 (13, 36)	22 (15, 31)	
			Range	(10, 50)	(13, 36)	
		Bx08.16	n (%, 95% CI)	3 (20·0%, 7·0-45·2%)	0 (0·0%, 0·0-22·8%)	0·093
			Geometric Mean (SD)	16 (4)		NA
			Median (IQR)	16 (14, 18)		
			(Min, Max))	(12, 20)		
		1056-10.TA11.1826	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		6535.3	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		Q23.17	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0

Titre	Tier	Isolate	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
		ZM197M.PB7	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
	2	92UG037.1	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	1 (7·7%, 1·4-33·3%)	0·35
			Geometric Mean (SD)		11 (NA)	NA
			Median (IQR)		11 (11, 11)	
			(Min, Max))		(11, 11)	
		JR-FL	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
ID80	1a	MW965.26	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	13 (100·0%, 77·2-100·0%)	1·0
			Geometric Mean (SD)	252 (132)	229 (160)	0·75
			Median (IQR)	270 (190, 337)	190 (166, 452)	
			(Min, Max))	(101, 549)	(74, 504)	
	1b	1056-10.TA11.1826	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		6535.3	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		Bx08.16	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		Q23.17	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		ZM197M.PB7	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
	2	92UG037.1	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0
		JR-FL	n (%, 95% CI)	0 (0·0%, 0·0-20·4%)	0 (0·0%, 0·0-22·8%)	1·0

*: Barnard's test was used for response rate comparison between two vaccine regimens. Two-sided independent t-test was used for magnitude titres among positive responders between two vaccine regimens only if there are at least 4 positive responders per vaccine regimen; otherwise, p-value is missing (NA). Bolded p-values indicate for significance with $p<0·05$.

Supplementary Table 4. Comparison of IgG (dilution 1:50) and IgG3 (dilution 1:40) to gp70 V1V2 antigens between HVTN 124 Prime-Boost recipients and participants from other study regimens.

Isotype	Antigen	Study	GM (SD)	Median (IQR)	p-value (vs. HVTN 124)	p-value (vs. RV144)
IgG	AE.A244 V1V2 Tags/293F	HVTN 124 Prime-Boost	27876 (3673)	28876 (28158, 29401)	-	-
		RV144	19096 (13991)	25426 (17332, 27834)	0·0004	-
		HVTN 702	1369 (3223)	2067 (699, 5796)	<0·0001	<0·0001
		HVTN 705	705 (1087)	804 (297, 1882)	<0·0001	<0·0001
	gp70_B.CaseA_V1_V2	HVTN 124 Prime-Boost	4888 (4879)	4071 (2801, 8134)	-	-
		RV144	810 (1327)	850 (408, 1880)	<0·0001	-
		HVTN 702	61 (207)	162 (1, 1020)	<0·0001	<0·0001
		HVTN 705	544 (1600)	937 (155, 4614)	0·0012	0·89
IgG3	AE.A244 V1V2 Tags/293F	HVTN 124 Prime-Boost	2472 (3298)	2596 (1366, 5345)	-	-
		RV144	358 (624)	425 (132, 1061)	<0·0001	-
		HVTN 702	56 (136)	54 (10, 302)	<0·0001	<0·0001
		HVTN 705	50 (111)	52 (10, 231)	<0·0001	<0·0001
	gp70_B.CaseA_V1_V2	HVTN 124 Prime-Boost	46 (94)	55 (21, 79)	-	-
		RV144	14 (28)	16 (2, 63)	0·039	-
		HVTN 702	3 (6)	1 (1, 4)	<0·0001	<0·0001
		HVTN 705	10 (27)	2 (1, 79)	0·013	0·013

Supplementary Table 5. Summary of ADCC response rates and magnitudes among positive responders by isotype, antigen, and vaccine regimen, with comparisons between the two vaccine regimens (Prime-Boost and Coadministration) in Part B based on the Per Protocol (PP) data.

	Antigen	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
ADCC-GTL	gp120 A	n (%, 95% CI)	3 (20·0%, 7·0-45·2%)	2 (15·4%, 4·3-42·2%)	0·81
		Mean (SD)	23·0 (2·3)	20·7 (6·0)	NA
		Median (IQR)	22·5 (21·7, 24·0)	20·7 (18·6, 22·8)	
		Range	(21·0,25·5)	(16·5,24·9)	
	gp120 AE	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	4 (30·8%, 12·7-57·6%)	**0·0004**
		Mean (SD)	26·0 (8·9)	25·3 (7·0)	0·96
		Median (IQR)	25·7 (20·2, 30·6)	25·2 (19·8, 30·6)	
		Range	(14·0, 44·4)	(18·3, 32·4)	
	gp120 B	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	7 (53·8%, 29·1-76·8%)	0·17
		Mean (SD)	28·7 (8·3)	21·9 (4·8)	0·068
		Median (IQR)	27·5 (23·6, 31·0)	19·9 (19·1, 25·2)	
		Range	(16·6, 47·4)	(15·8, 29·3)	
	Clade C TV1	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	7 (53·8%, 29·1-76·8%)	**0·017**
		Mean (SD)	29·5 (7·8)	29·9 (10·8)	0·91
		Median (IQR)	26·6 (24·3, 34·4)	35·7 (21·7, 38·6)	
		Range	(20·1, 43·4)	(13·1, 40·0)	
ADCC-IMC	Clade AE CM235	n (%, 95% CI)	15 (100·0%, 79·6-100·0%)	13 (100·0%, 77·2-100·0%)	1·0
		Mean (SD)	52·9 (22·2)	38·3 (16·0)	0·058
		Median (IQR)	46·7 (36·9,73·1)	35·0 (27·7,41·9)	
		Range	(17·8, 91·9)	(19·1, 73·6)	
	Clade C TV1	n (%, 95% CI)	14 (93·3%, 70·2-98·8%)	10 (76·9%, 49·7-91·8%)	0·27
		Mean (SD)	38·5 (19·5)	25·2 (7·9)	0·19
		Median (IQR)	28·1 (26·0, 58·2)	24·5 (19·8, 32·7)	
		Range	(14·5, 73·2)	(14·5, 34·6)	
	Clade B WITO	n (%, 95% CI)	12 (80·0%, 54·8-93·0%)	5 (38·5%, 17·7-64·5%)	**0·036**
		Mean (SD)	38·3 (22·9)	32·0 (10·1)	0·88
		Median (IQR)	29·2 (22·5, 46·2)	37·0 (24·2, 37·1)	
		Range	(17·6, 77·2)	(18·6, 43·0)	

*: Barnard's test was used for response rate comparison between two vaccine regimens. Wilcoxon rank sum test was used for magnitudes among positive responders between two vaccine regimens only if there are at least 4 positive responders per vaccine regimen; otherwise, p-value is missing (NA). Bolded p-values indicate for significance with p<0·05.

Supplementary Table 6. Comparisons of neutralizing antibody ID50 titre to TH023.6 between participants receiving HVTN 124 study regimens and participants from other study regimens

Study Regimen	GM (SD)	Median (IQR)	p-value*	p-value&
HVTN124 Prime-Boost	971 (835)	987 (566, 1640)	-	-
HVTN124 Coadministration	801 (730)	831 (547, 1637)	-	-
HVTN096 D-NYV+P	51 (58)	42 (21, 72)	**<0·0001**	**<0·0001**
HVTN096 D+P-NYV+P	90 (69)	98 (65, 133)	**<0·0001**	**<0·0001**
HVTN097 ALV-ALV+P	82 (115)	87 (24, 208)	**<0·0001**	**<0·0001**
HVTN105 D-D+P	25 (9)	25 (18, 34)	**<0·0001**	**<0·0001**
HVTN105 D+P	95 (76)	104 (73, 161)	**<0·0001**	**<0·0001**

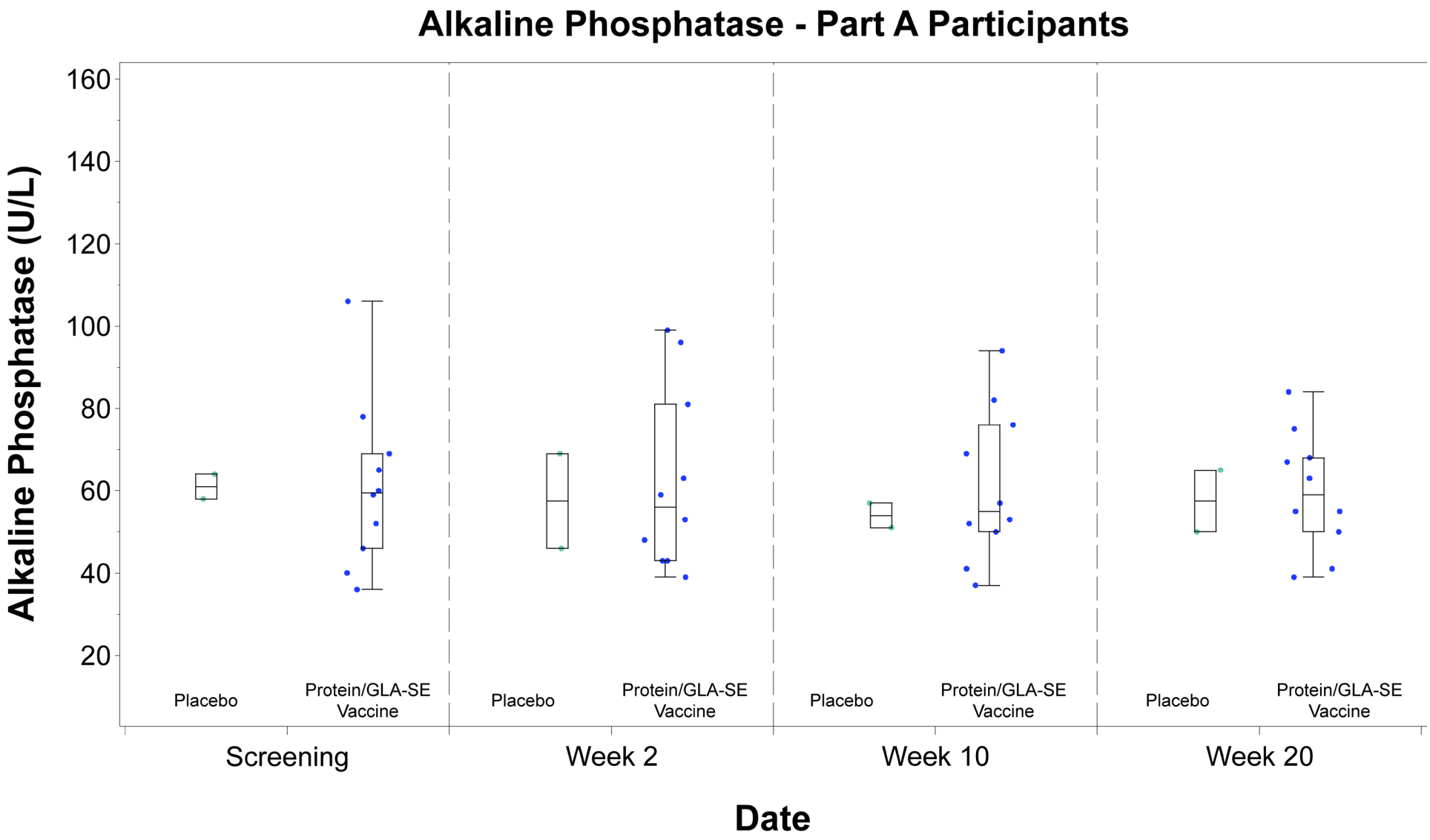
*Compared to HVTN 124 Prime-Boost
&Compared to HVTN 124 Coadministration

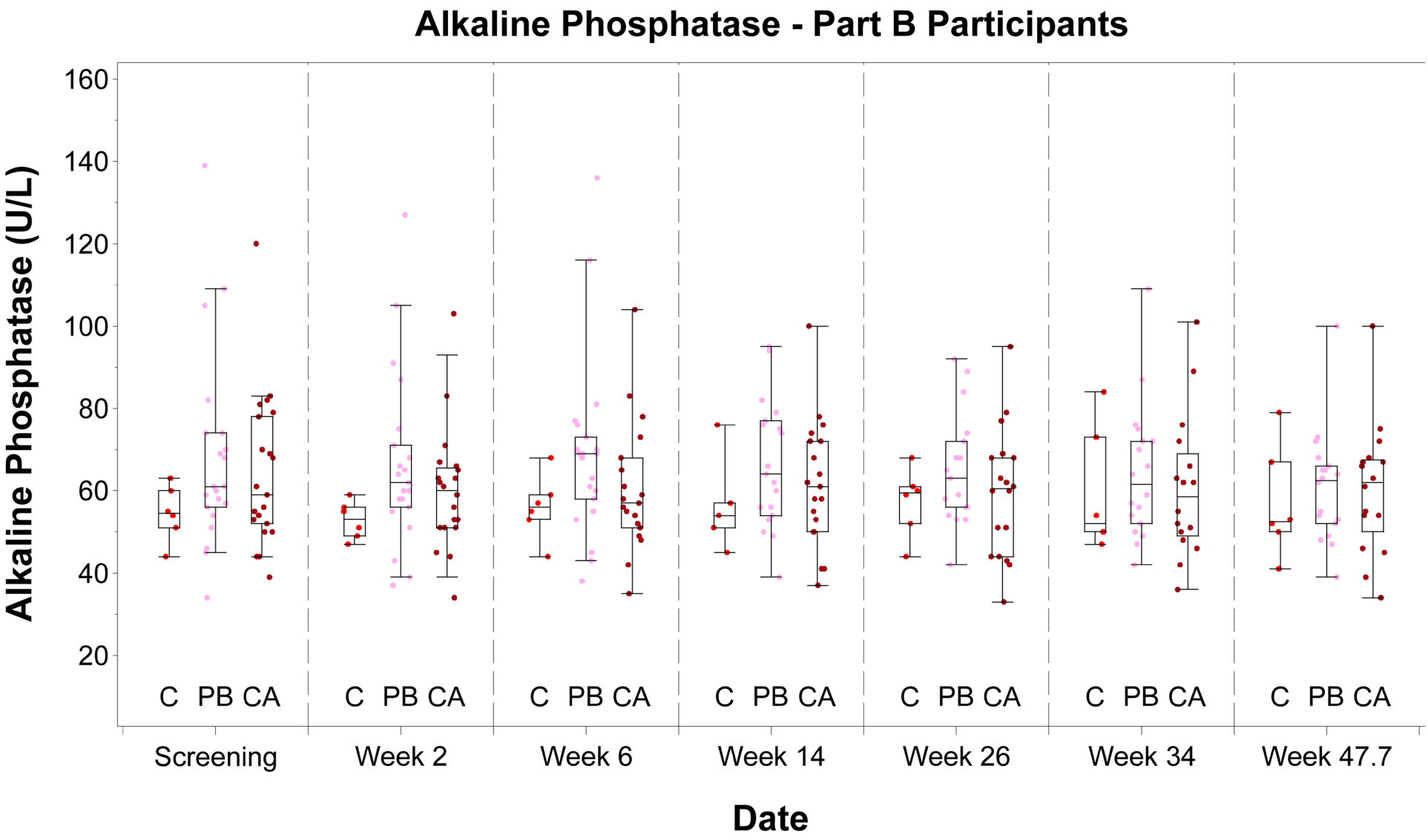
Supplementary Table 7. Summary of intracellular cytokine staining CD4+ T cell response rates and magnitudes among positive responders by T cell subset, vaccine regimen, antigen, and comparisons between the two vaccine regimens (Prime-Boost and Coadministration) in Part B based on the Per Protocol (PP) data

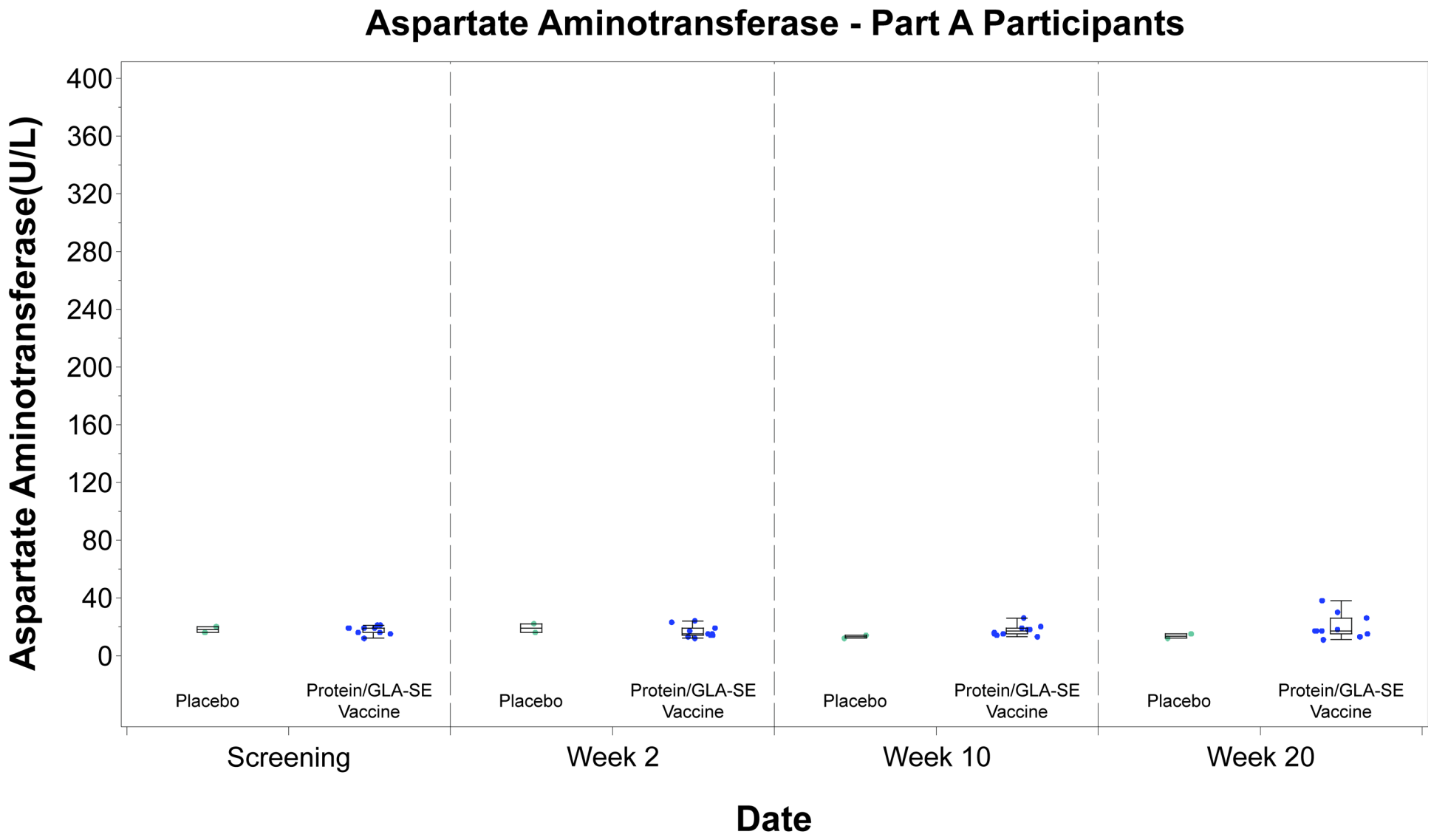
T Cell Subset	Antigen	Profile	Statistics	Prime-Boost (n=15)	Coadministration (n=13)	p-value*
CD4+	ANY ENV PTEG	IFNγ/IL2	N (%, 95% CI)	15 (100·0%, 79·6-100·0%)	5 (38·5%, 17·7-64·5%)	**0·0002**
			Mean (SD)	0·3 (0·2)	0·3 (0·2)	0·93
			Median (IQR)	0·3 (0·2, 0·4)	0·3 (0·2, 0·3)	
			Range	(0·1, 0·7)	(0·1, 0·6)	
CD4+	ANY ENV PTEG	TNFα+	Mean (SD)	0·000 (0·001)	0·001 (0·002)	0·85
			Median (IQR)	0·000 (0·000,0·000)	0·000 (0·000,0·000)	
			Range	(0·000,0·003)	(0·000,0·008)	
		CD40L+	Mean (SD)	0·053 (0·024)	0·052 (0·053)	0·39
			Median (IQR)	0·049 (0·038,0·073)	0·029 (0·024,0·061)	
			Range	(0·003,0·102)	(0·005,0·203)	
		IFNγ+CD40L+	Mean (SD)	0·014 (0·011)	0·007 (0·011)	**0·030**
			Median (IQR)	0·011 (0·007,0·017)	0·001 (0·000,0·010)	
			Range	(0·002,0·039)	(0·000,0·038)	
		IL2+TNFα+	Mean (SD)	0·001 (0·002)	0·001 (0·001)	0·57
			Median (IQR)	0·000 (0·000,0·002)	0·000 (0·000,0·001)	
			Range	(0·000,0·006)	(0·000,0·003)	
		IL2+CD40L+	Mean (SD)	0·013 (0·007)	0·022 (0·026)	0·45
			Median (IQR)	0·012 (0·010,0·015)	0·008 (0·006,0·036)	
			Range	(0·000,0·027)	(0·000,0·081)	
		TNFα+CD40L+	Mean (SD)	0·023 (0·012)	0·016 (0·012)	0·17
			Median (IQR)	0·021 (0·013,0·030)	0·013 (0·009,0·023)	
			Range	(0·007,0·055)	(0·000,0·046)	
		IFNγ+IL2+CD40L+	Mean (SD)	0·008 (0·005)	0·003 (0·004)	**0·014**
			Median (IQR)	0·007 (0·005,0·010)	0·001 (0·000,0·003)	
			Range	(0·000,0·019)	(0·000,0·014)	
		IFNγ+TNFα+CD40L+	Mean (SD)	0·028 (0·019)	0·005 (0·007)	**0·0002**
			Median (IQR)	0·022 (0·013,0·043)	0·003 (0·000,0·005)	
			Range	(0·004,0·075)	(0·000,0·024)	
		IL2+TNFα+CD40L+	Mean (SD)	0·037 (0·018)	0·059 (0·095)	0·44
			Median (IQR)	0·035 (0·025,0·044)	0·027 (0·012,0·041)	
			Range	(0·007,0·082)	(0·004,0·382)	
		IFNγ+IL2+TNFα+CD40L+	Mean (SD)	0·053 (0·033)	0·014 (0·020)	**0·0003**
			Median (IQR)	0·040 (0·031,0·065)	0·007 (0·001,0·015)	
			Range	(0·018,0·135)	(0·000,0·078)	

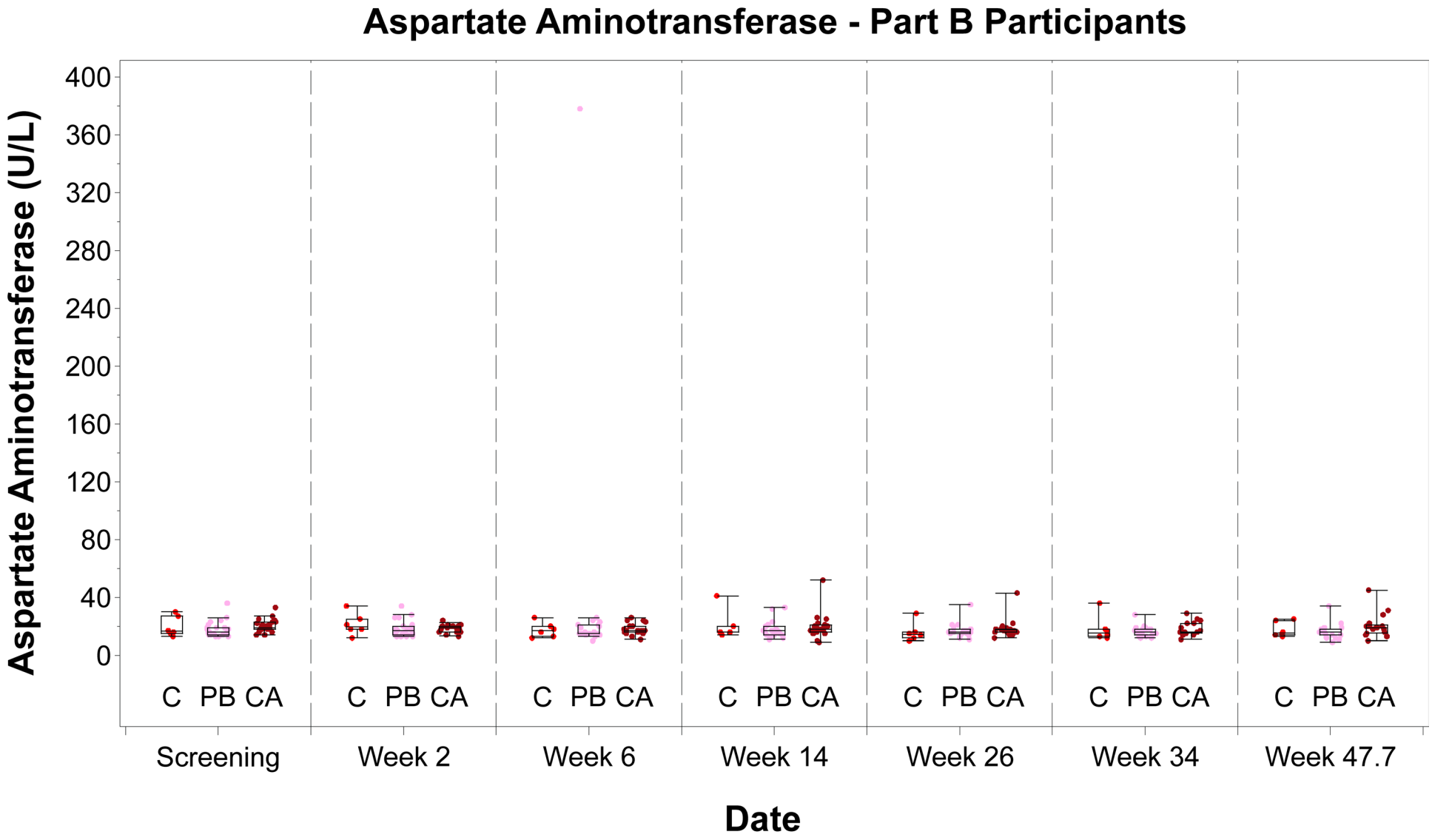
*: Barnard's test was used for response rate comparison between two vaccine regimens. Wilcoxon rank sum test was used for magnitudes between two vaccine regimens among positive responders for marginal response to IFNγ/IL2 and among all participants for the rest of T cells profiles. Bolded p-values indicate for significance with p<0·05.

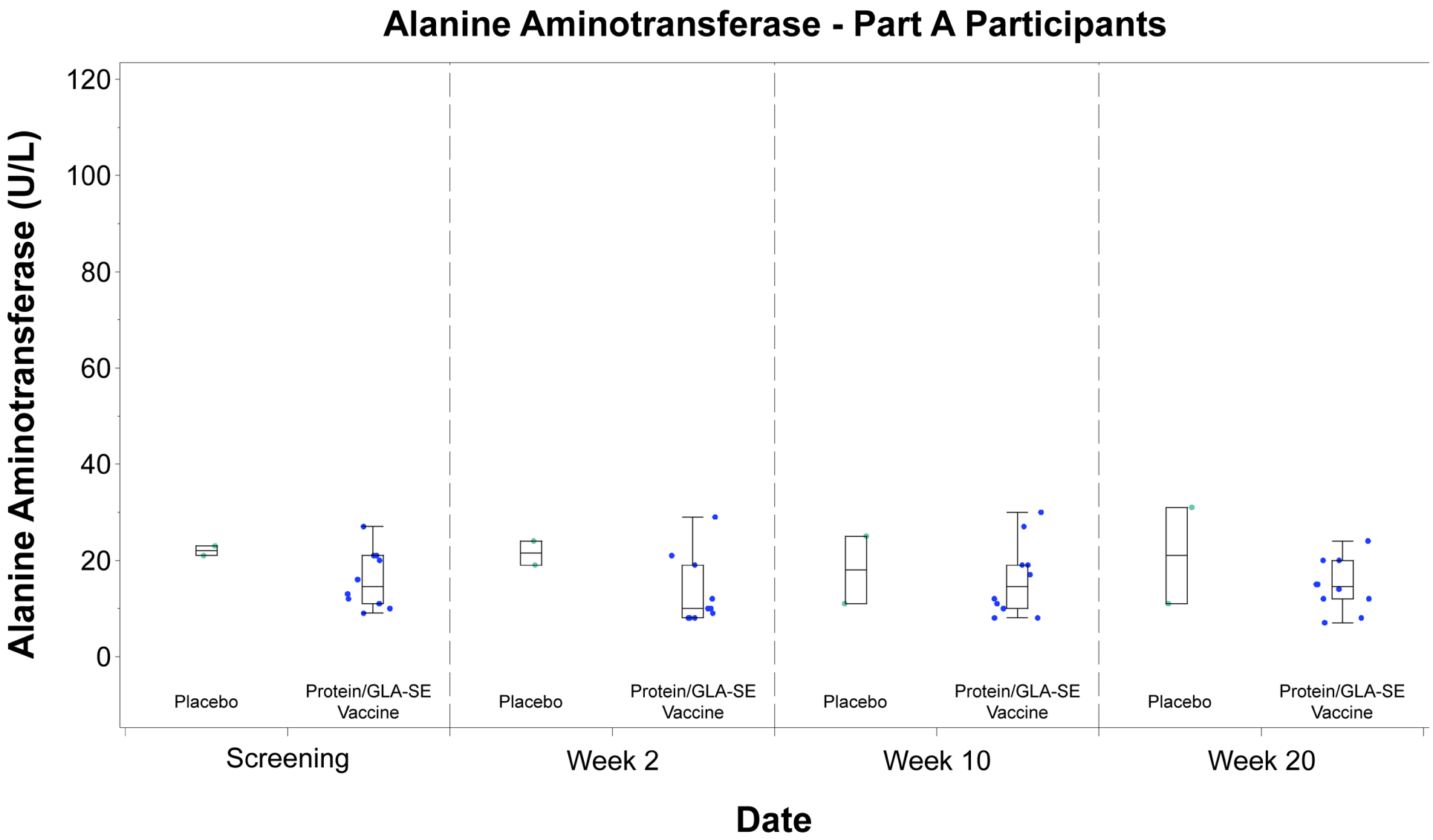
Laboratory Safety Data. Distributions of values of laboratory safety measures at baseline and follow-up visits post vaccination. Dots represent individual participants. The line splitting the boxplots in two is the median, the bottom edge of the box is the 25th percentile, the top edge is the 75th percentile, the values at which the horizontal lines stop correspond to the most extreme datapoints that are no more than 1·5 times the IQR away from the median, or if no such datapoints exist, the data extremes. C = Part B Placebo Control, PB = Prime-Boost, CA = Coadministration.

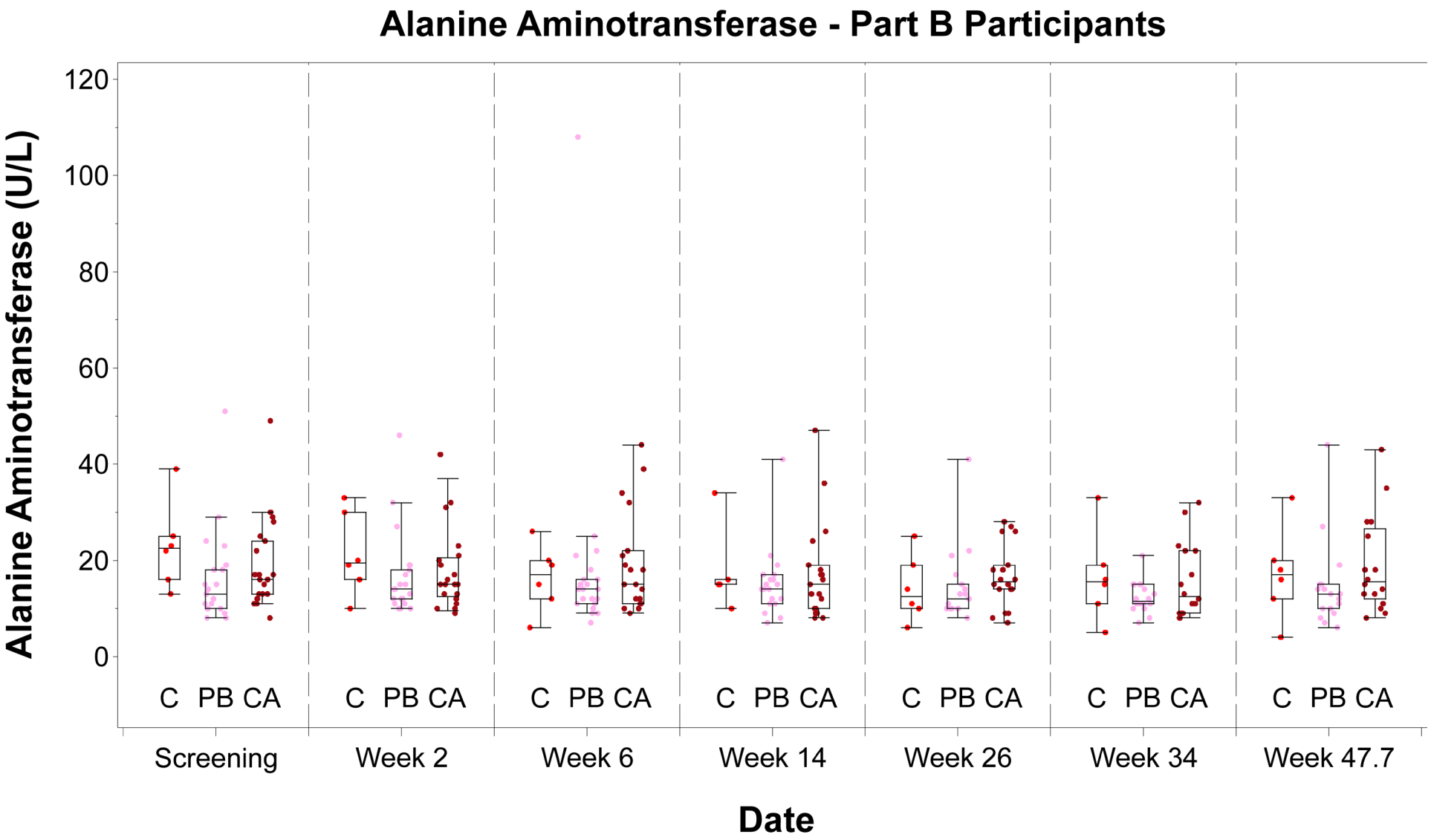


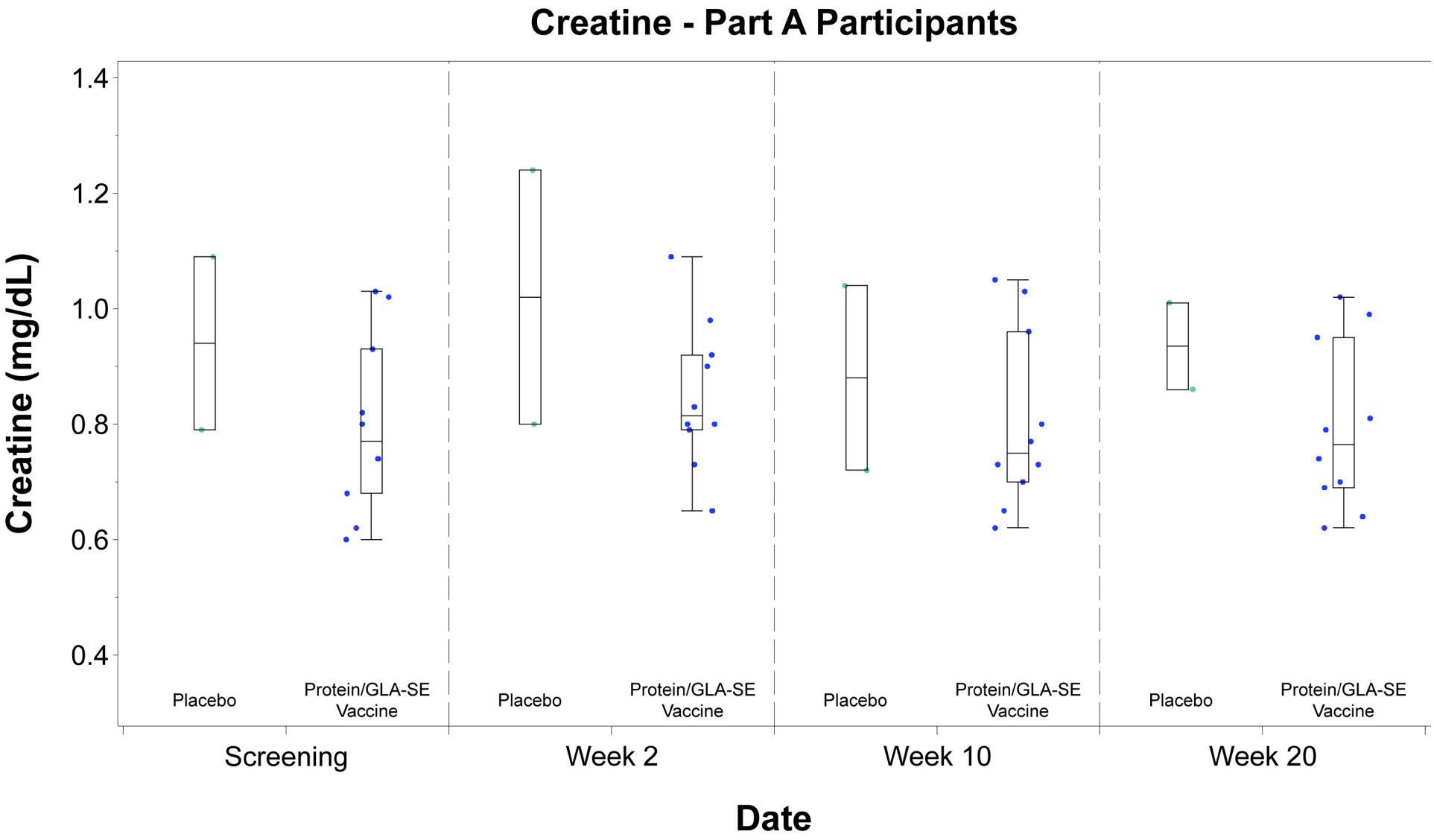

Alkaline Phosphatase - Part B Participants
Alkaline Phosphatase (U/L)
160
140
120
100
80
60
40
20
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

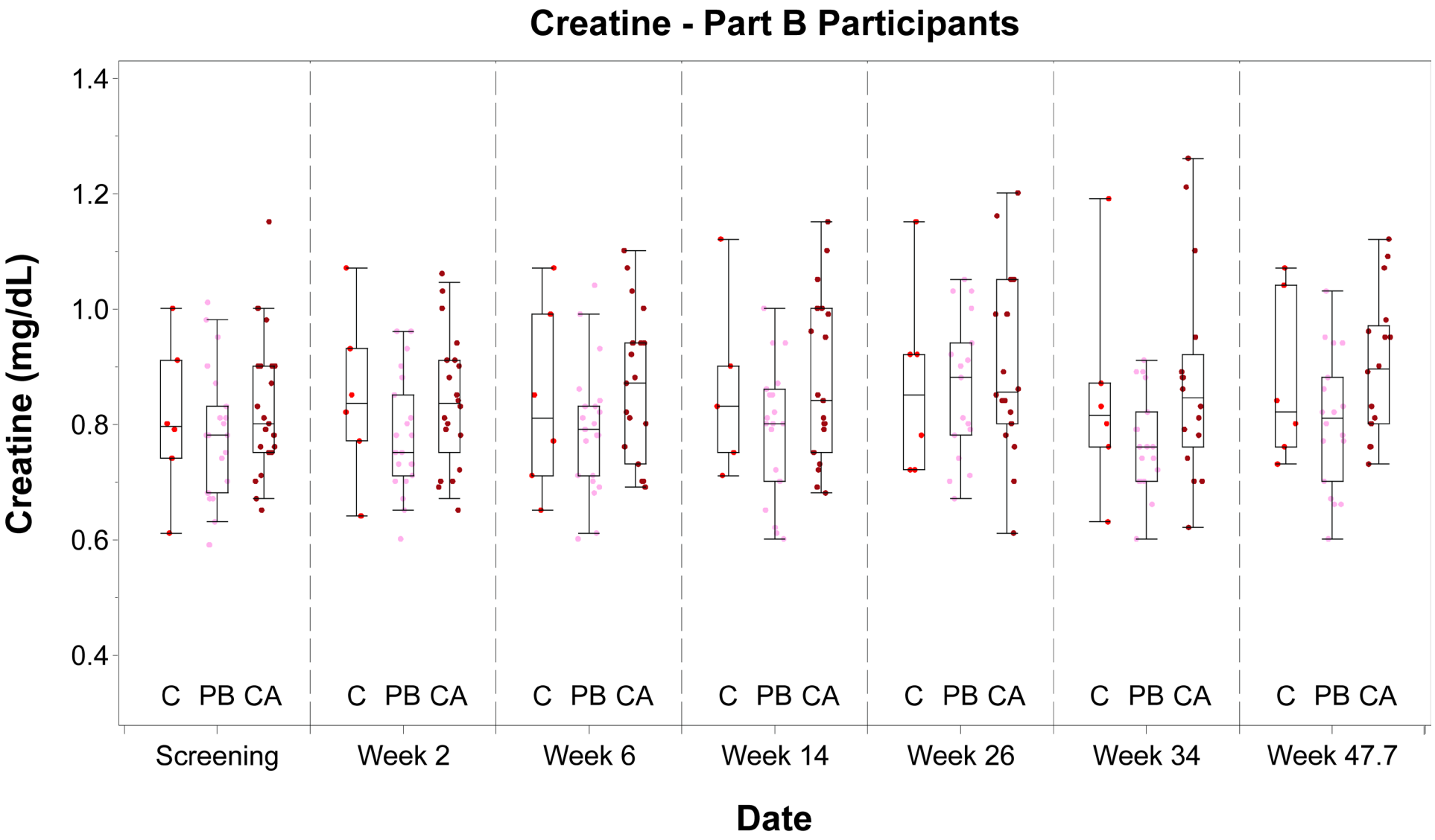

Aspartate Aminotransferase - Part A Participants
Aspartate Aminotransferase(U/L)
400
360
320
280
240
200
160
120
80
40
0
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date

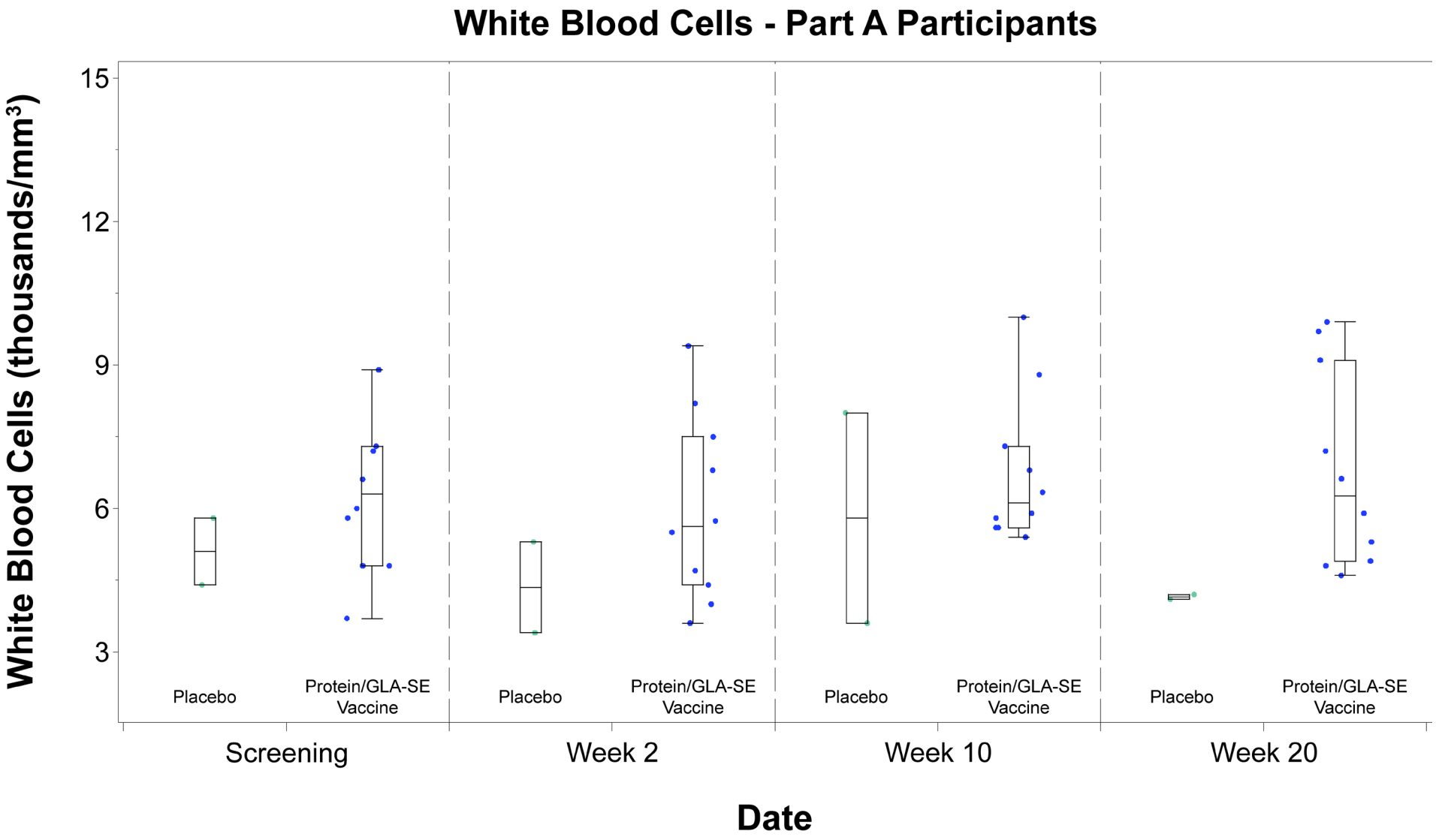

Aspartate Aminotransferase - Part B Participants
Aspartate Aminotransferase (U/L)
400
360
320
280
240
200
160
120
80
40
0
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

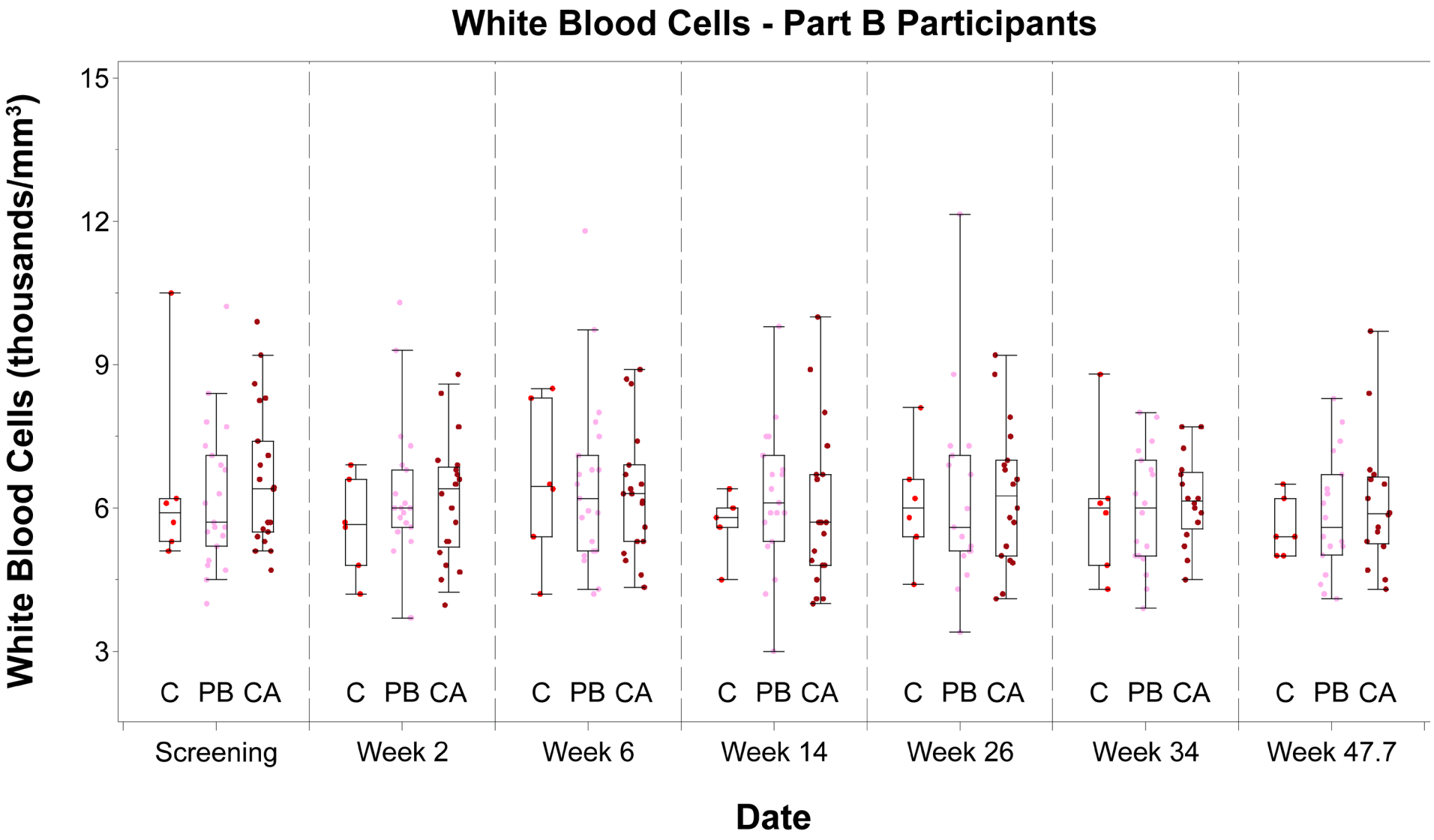

Alanine Aminotransferase - Part A Participants
Alanine Aminotransferase (U/L)
120
100
80
60
40
20
0
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date

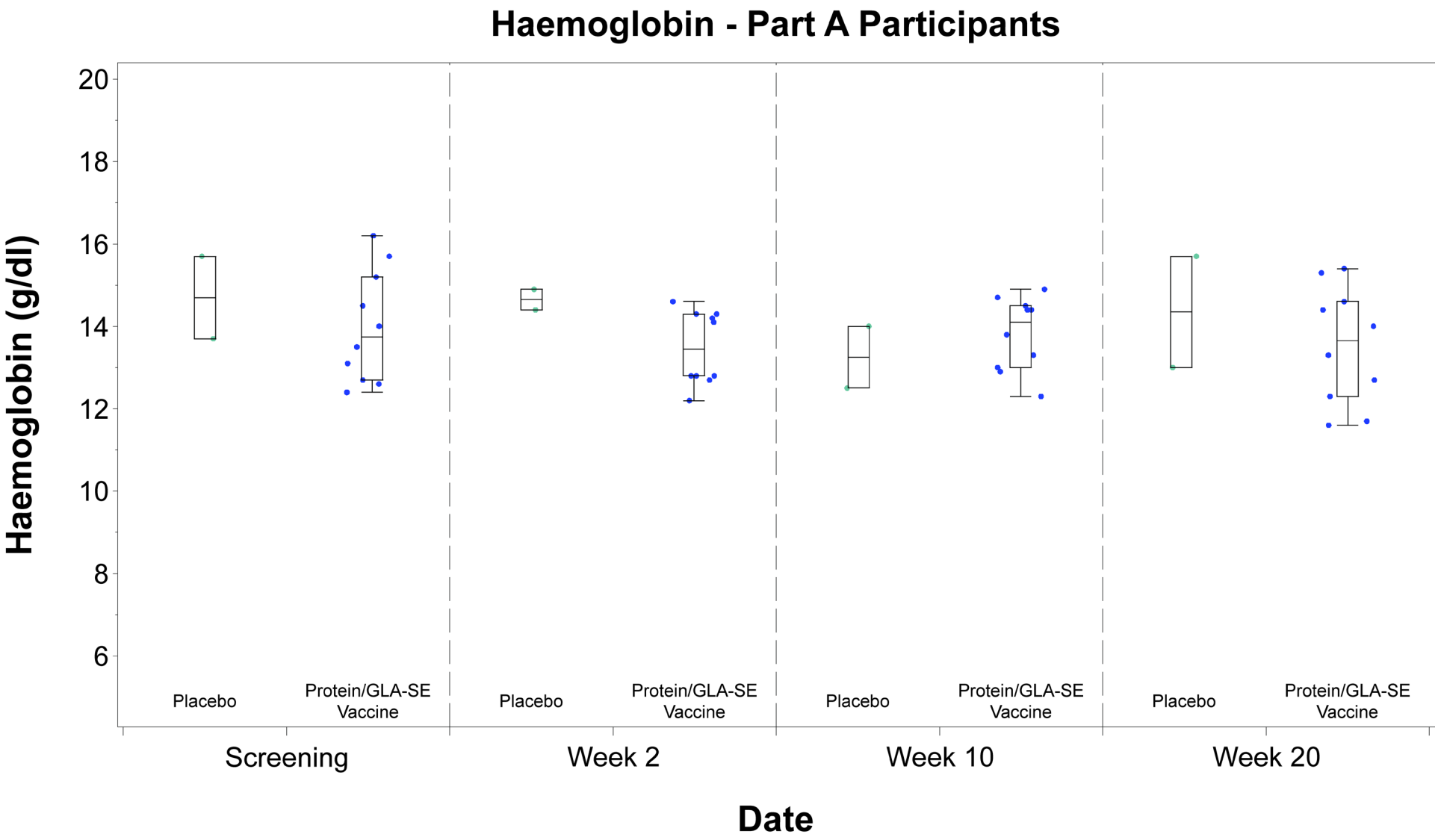

Alanine Aminotransferase - Part B Participants
Alanine Aminotransferase (U/L)
120
100
80
60
40
20
0
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

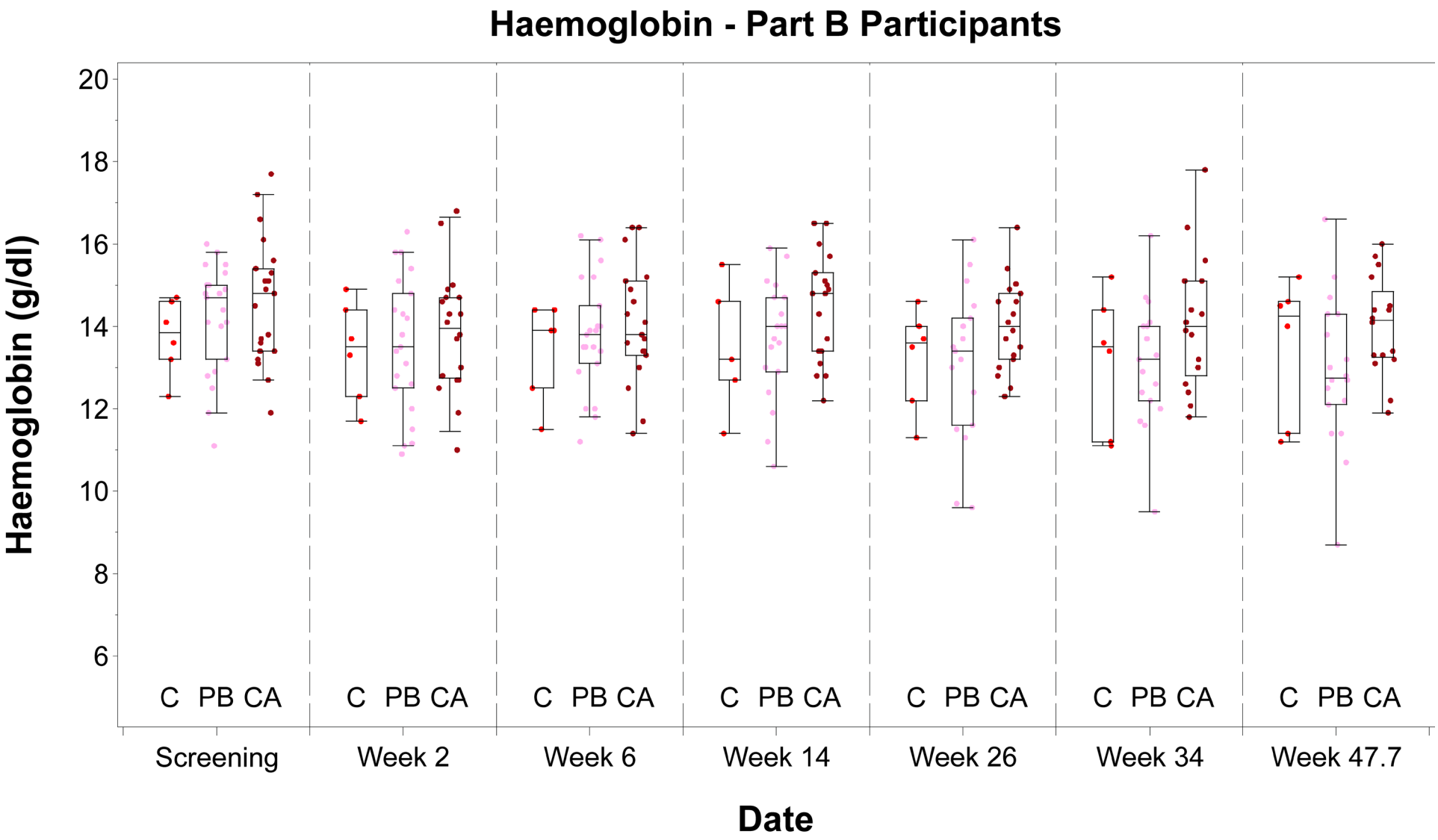

Creatine - Part A Participants
Creatine (mg/dL)
1.4
1.2
1.0
0.8
0.6
0.4
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date

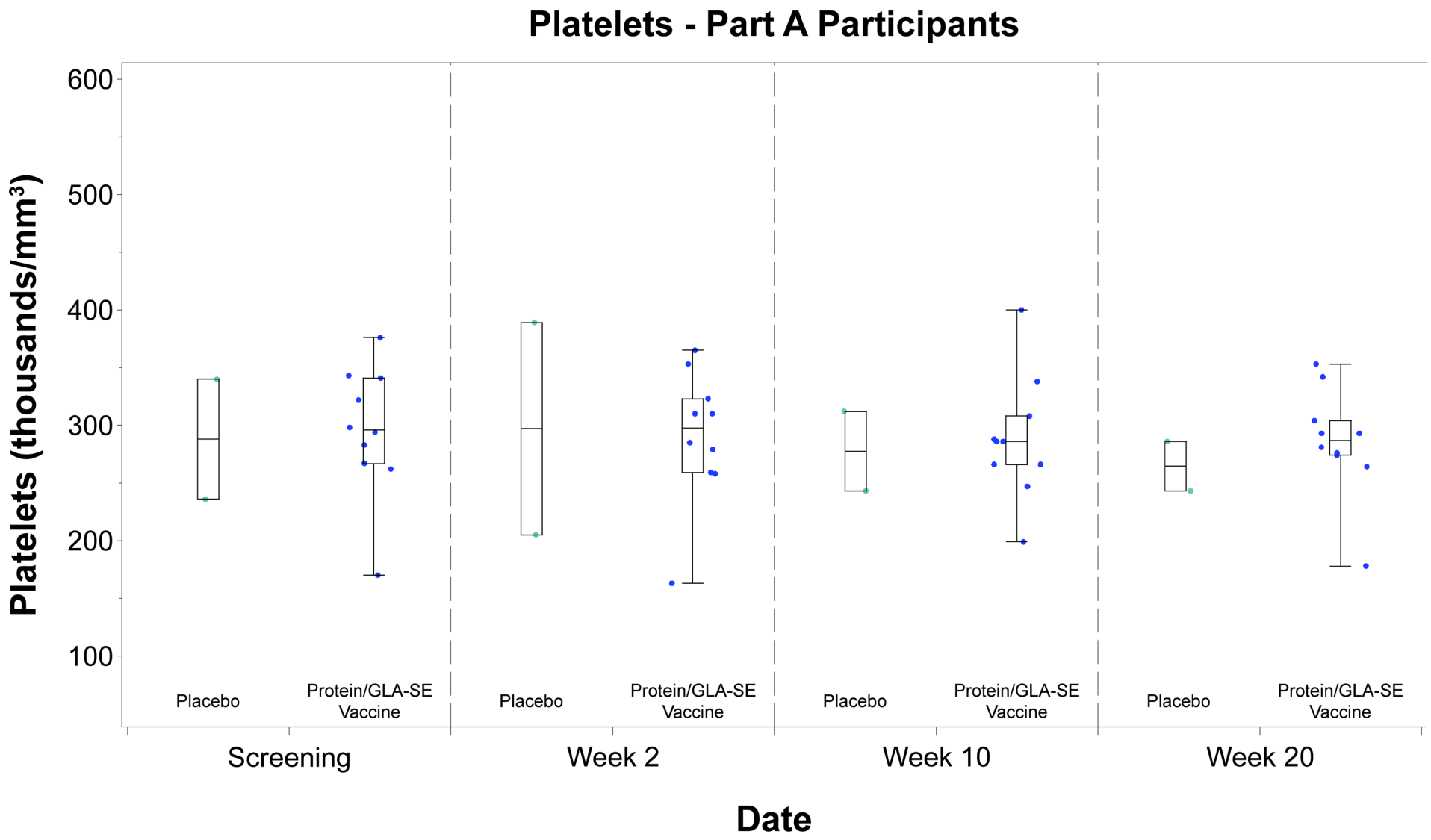

Creatine - Part B Participants
Creatine (mg/dL)
1.4
1.2
1.0
0.8
0.6
0.4
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

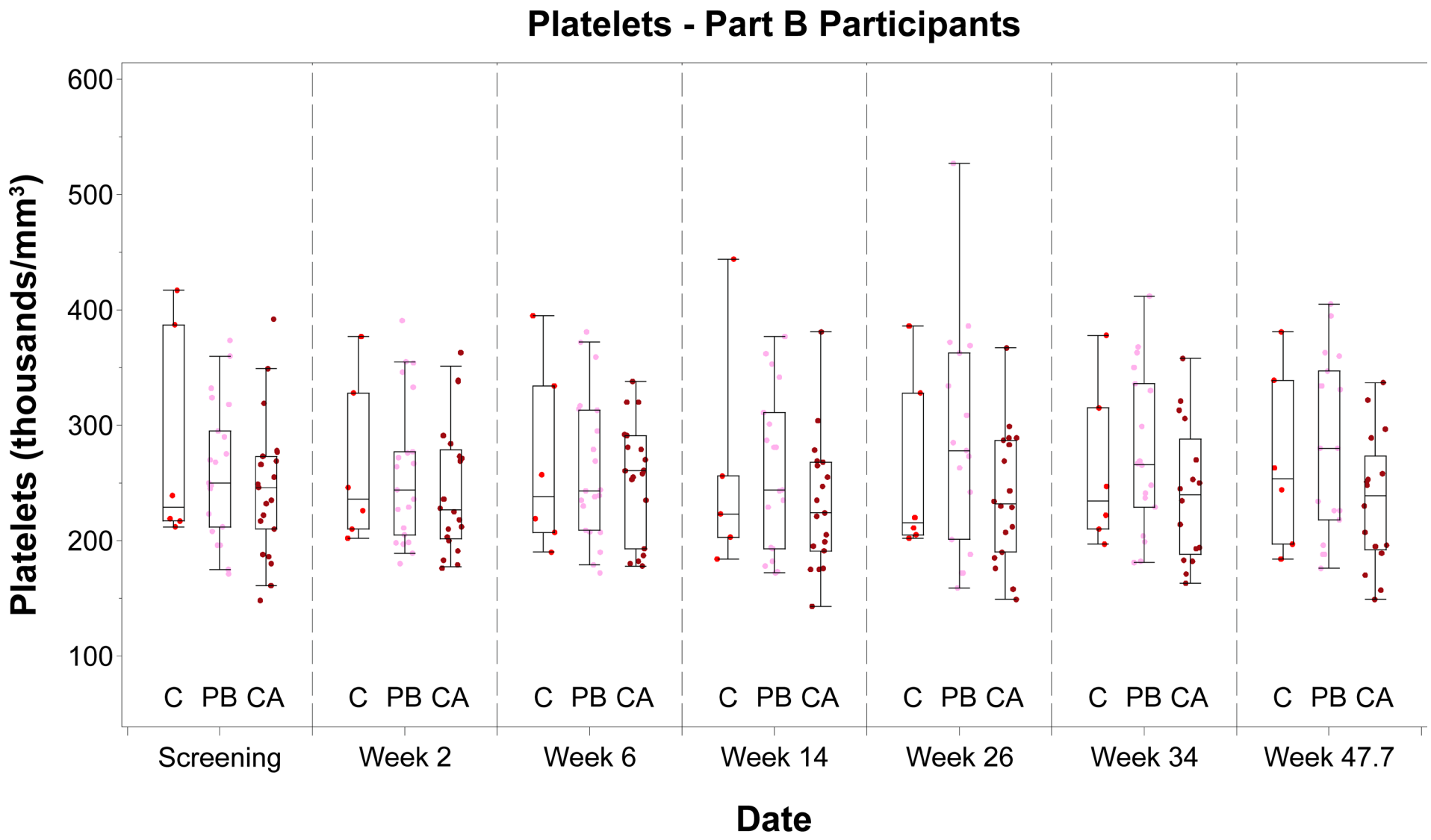

White Blood Cells - Part A Participants
White Blood Cells (thousands/mm³)
15
12
9
6
3
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date

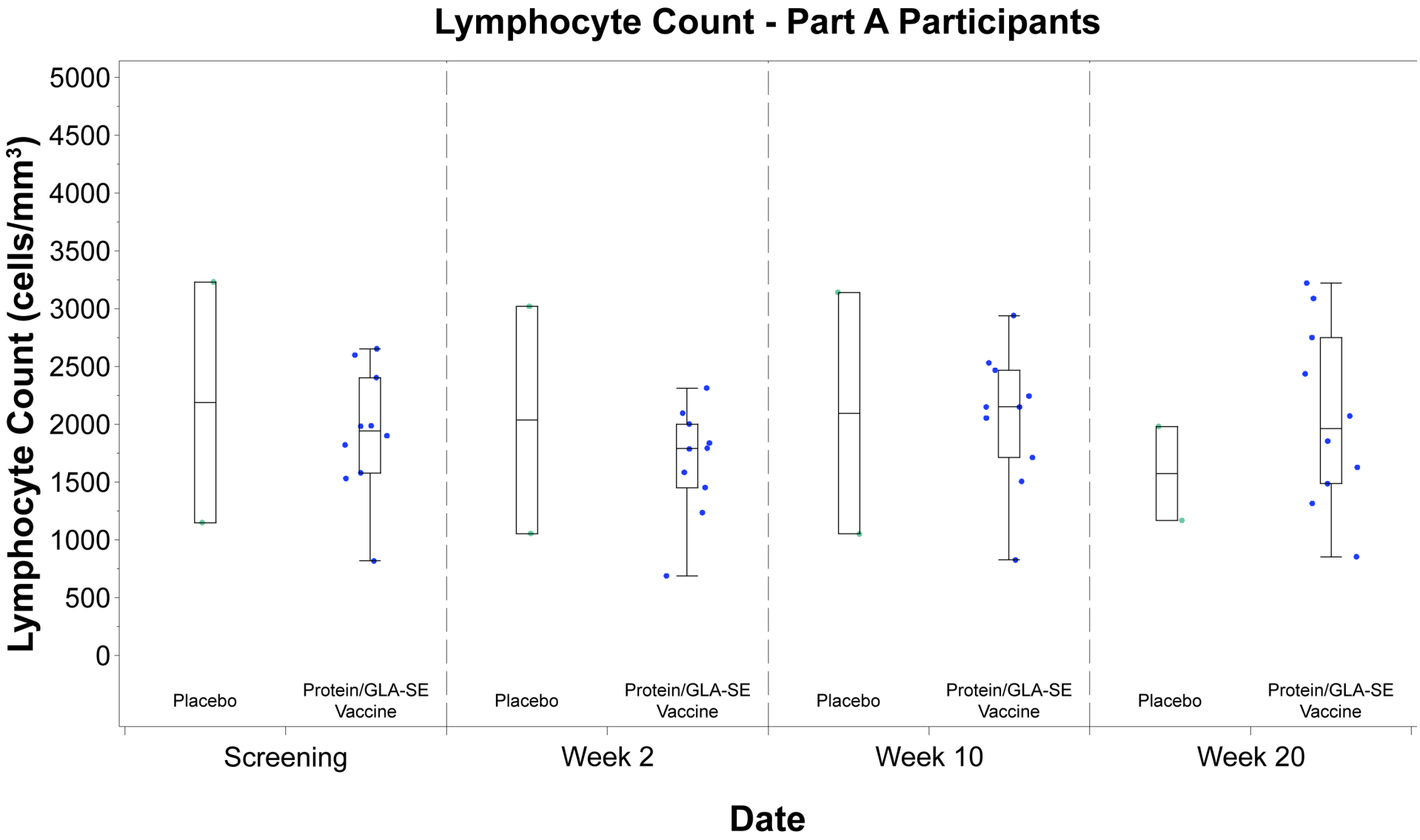

White Blood Cells - Part B Participants
White Blood Cells (thousands/mm³)
15
12
9
6
3
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

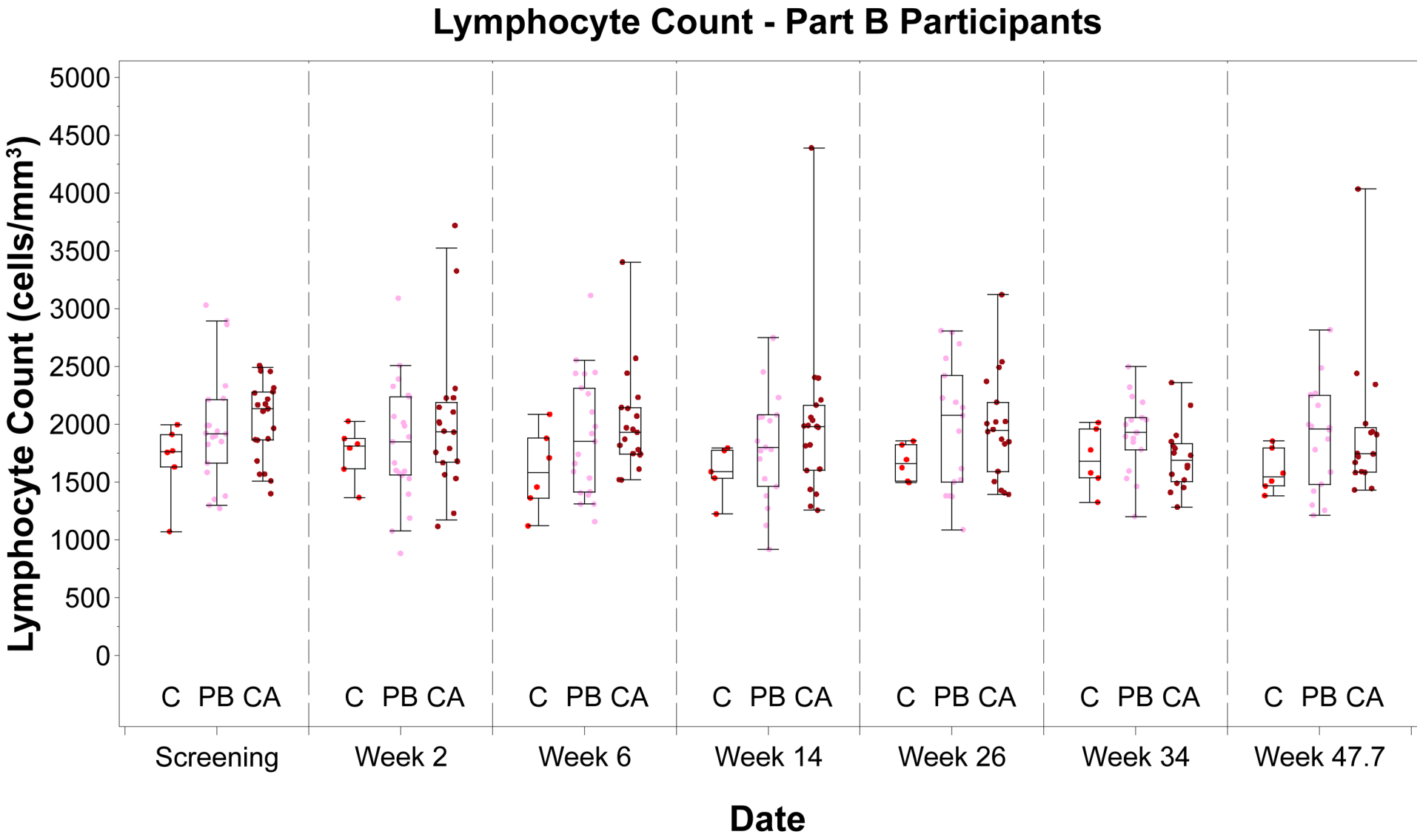

Haemoglobin - Part A Participants
Haemoglobin (g/dl)
20
18
16
14
12
10
8
6
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date

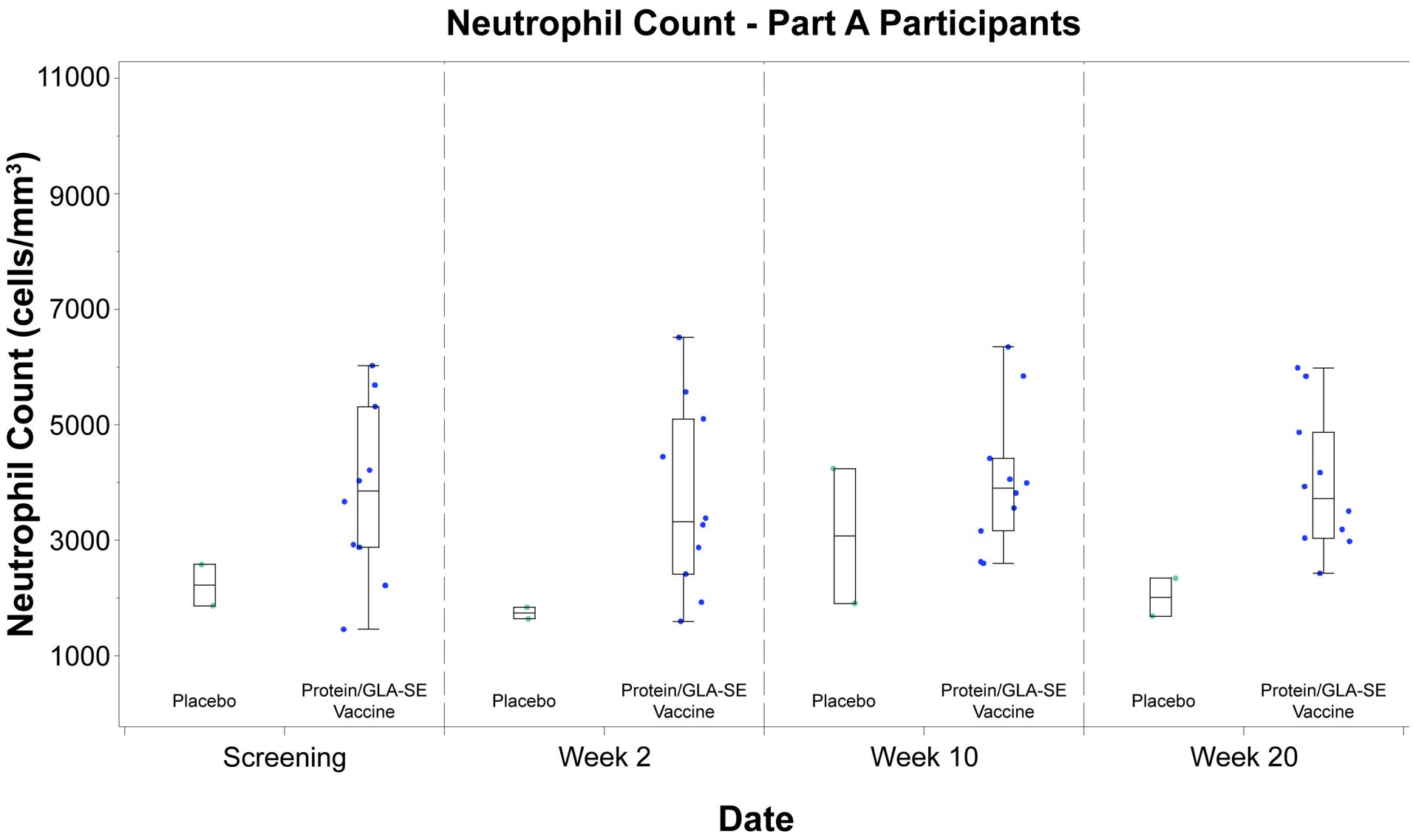

Haemoglobin - Part B Participants
Haemoglobin (g/dl)
20
18
16
14
12
10
8
6
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date

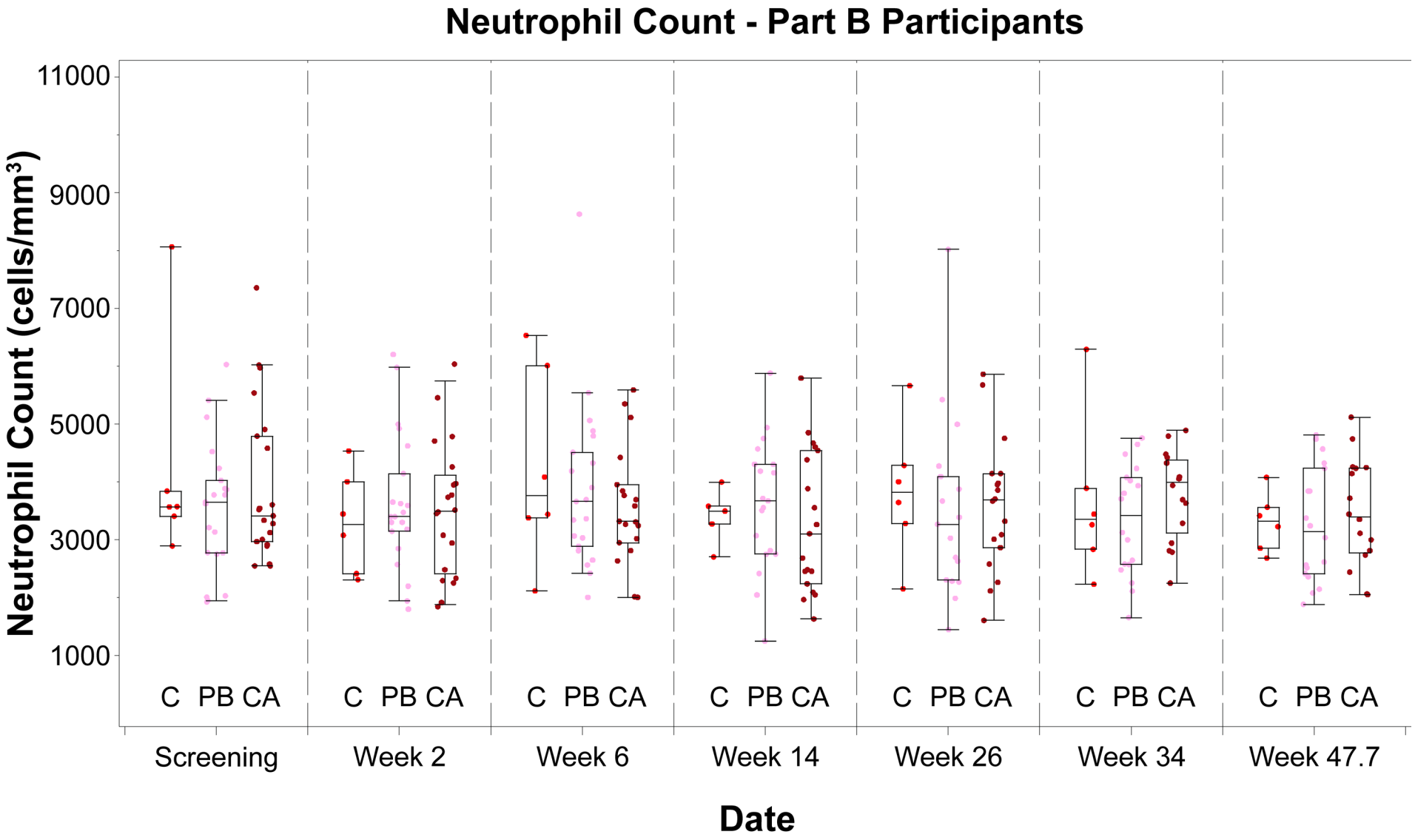

Platelets - Part A Participants
Platelets (thousands/mm³)
600
500
400
300
200
100
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date


Platelets - Part B Participants
Platelets (thousands/mm³)
600
500
400
300
200
100
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date


Lymphocyte Count - Part A Participants
Lymphocyte Count (cells/mm³)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date


Lymphocyte Count - Part B Participants
Lymphocyte Count (cells/mm³)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date


Neutrophil Count - Part A Participants
Neutrophil Count (cells/mm³)
11000
9000
7000
5000
3000
1000
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Placebo
Protein/GLA-SE Vaccine
Screening
Week 2
Week 10
Week 20
Date


Neutrophil Count - Part B Participants
Neutrophil Count (cells/mm³)
11000
9000
7000
5000
3000
1000
C PB CA
C PB CA
C PB CA
C PB CA
C PB CA
C PB CA
C PB CA
Screening
Week 2
Week 6
Week 14
Week 26
Week 34
Week 47.7
Date