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HIV-1 envelope protein vaccine induces more robust antibody responses in infants than adults

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Title: HIV-1 envelope protein vaccine induces more robust antibody responses in infants than adults

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Abstract

Broadly neutralizing antibodies (bnAbs) develop more robustly in infants living with HIV-1 than adults, suggesting that vaccine-induced bnAbs may be more readily generated in infants. We evaluated a recombinant HIV-1 CH505TF gp120 monomer adjuvanted with GLA-SE in infants without HIV-1, born to mothers living with HIV-1, in a phase 1 randomized, placebo-controlled trial (HVTN135). Two weeks post-fifth dose, all vaccinated infants developed vaccine-specific CH505-positive B cells, IgG CH505TF gp120-specific binding antibodies (bAbs), and vaccine-matched Tier 1A neutralizing antibodies (nAbs); 86% had CD4 binding-site- (CD4bs) specific IgG+ B cells and 43% had CD4bs-directed bAbs based on reduced binding to a CD4bs mutant (Δ371I). Compared to healthy adults from a harmonized phase 1 trial (HVTN115), infant IgG bAb titers and Tier 1A nAb ID50 titers were higher. Maternal antibody interference was not detected. Infant bAbs to routine immunizations were unaffected. These data support that infants can respond to a candidate adjuvanted HIV-1 vaccine with a greater magnitude of HIV-specific humoral responses than adults.

Main Text:

Introduction

Induction of HIV-1 (HIV) broadly neutralizing antibodies (bnAbs) by vaccination is a major focus in HIV prevention research due to the ability of bnAbs to neutralize diverse HIV strains and prevent acquisition of susceptible HIV strains. However, inducing bnAbs by vaccination has proven challenging, likely due to complex B cell maturation pathways required for most bnAbs *(1)*. B cell immunogen lineage design is a vaccine concept that uses immunogens tailored to HIV strains isolated from individuals living with HIV who developed bnAbs. The goal is to stimulate rare precursor cells and guide their development into bnAbs via sequential immunizations based upon known viral evolution *(2)*. A key question is whether lineage-directed vaccines administered at birth may leverage features of the distinct early life immune system for increased immunogenicity compared to adults.

Studies of infants living with HIV have demonstrated that two years after acquiring HIV, the neutralization breadth in some infants is comparable to the breadth observed in the top 1% of adult neutralizers several years post HIV acquisition *(3)*. One bnAb lineage isolated from an infant who had acquired HIV developed with lower levels of somatic hypermutation and different variable gene usage than typically seen in bnAbs isolated from adults *(4)*. Another study of neutralizing Ab (nAb) responses in children living with HIV demonstrated that 70% of children who were slow progressors neutralized $\geq$50% of viruses tested, while only 15% of adults living with HIV clade C were able to do so within similar timeframes *(5)*. Children also had higher neutralization titers than adults. Development of bnAbs in children living with HIV is also associated with more responses targeting multiple bnAb epitopes than typically seen in adults *(6)*.

Infants may also demonstrate differences from adults with respect to the quality of a vaccine-induced humoral immune response. Antiviral activities, such as Ab-dependent cellular cytotoxicity (ADCC) and

Ab-dependent cellular phagocytosis (ADCP), can contribute to vaccine-mediated reduction in HIV acquisition *(7, 8)*. Infants born to mothers living with HIV had higher ADCC activity and FcγIIa and FcγRIIIa binding was associated with improved survival *(9, 10)*. In addition to increases in the breadth of response, infants vaccinated with similar doses of Envelope- (Env) specific gp120 vaccines developed higher binding Abs to the specific V1V2 loop – a response associated with protection in the RV144 vaccine trial – when compared to adults receiving the same vaccine *(11)*. Thus, the magnitude, breadth, and rate of affinity maturation of desirable HIV antibodies may be improved in infants compared to adults.

Studying an investigative HIV vaccine in an infant population is also warranted, given that an effective vaccine may be optimally delivered in infancy or early childhood to leverage the existing robust infrastructure for delivery of childhood vaccines and to allow time for maturation of B cell evolution before sexual debut. While observational evidence in the context of chronic HIV infection supports distinct anti-HIV immunity in newborns vs adults, limited data are available comparing adult and neonatal HIV vaccine-elicited responses. Therefore, we leveraged data from two clinical trials: HVTN115 (adults) (NCT03220724) and HVTN135 (infants) (NCT04607408). HVTN 115, a Phase 1 clinical trial conducted in adults, sought to initiate bnAb lineages utilizing the gp120 derivative of the CH505 transmitted/founder (TF) virus isolated from an African person recently infected with HIV*(12)*. After the experimental vaccine was established to be safe in adults, a harmonized trial was conducted in HIV-exposed but uninfected infants: HVTN 135 evaluated the same CH505TF gp120 vaccine similarly adjuvanted with GLA-SE *(13)*; the vaccine regimens and time points for specimen collection were matched as closely as possible between the two trials. To evaluate any differences in vaccine-induced immunity between neonatal and adult immune systems, we compared the immune responses between adults in HVTN 115 and infants in HVTN 135 using a common set of validated immunological assays and at the same gp120 protein dose. While bnAb precursors were not observed in either adults or infants,

we demonstrate that CH505TF gp120 HIV vaccination elicits different immune profiles in infants compared to adults, with infants eliciting higher levels of some Ab specificities than adults who received the same HIV vaccine. Future studies should pursue determining whether newer bnAb-inducing vaccine regimens can facilitate unique priming and/or shepherding of germline-boosted responses to generate vaccine-induced bnAb lineages in infants.

Results

Infant analysis cohort

In HVTN 135, a randomized, placebo-controlled phase 1 study conducted in South Africa, healthy infants born to mothers living with HIV were enrolled within 5 days of birth (**Figure 1** and **Figures S1, S2**). Infants were HIV-negative via DNA PCR at birth and received HIV prophylaxis; none acquired HIV through 2 years of follow-up *(13)*. Immunizations were given at enrollment and Weeks 8, 16, 32, and 54 using a schedule designed to minimize overlap with the Republic of South Africa (RSA) Essential Programme on Immunization (EPI) vaccination schedule (**Figure 1A**), while harmonizing with the adult administration schedule in HVTN 115 (**Figures S2, S3**). In Part A, infants were randomized 5:2 to 20 μg CH505TF gp120/ 2.5 μg GLA-SE (Low adjuvant dose) or placebo; in Part B, infants were randomized 1:1 to 20 μg CH505TF gp120/ 5 μg GLA-SE (Target dose) or placebo; and in Part C, infants were randomized 16:5:6 to the Target dose, 5 μg CH505TF gp120/ 5 μg GLA-SE (Low protein dose), or placebo (**Figure 1B**). Of the 38 infants enrolled, 32 (84%) (24/28 vaccine recipients; 8/10 placebo recipients) received all five immunizations and are included in the analysis of HIV vaccine responses. Seventeen (53.1%) were female sex and 52.6% were breastfed at birth (**Table S1**). Median age at first study vaccine dose was 4 days. All infants received BCG vaccine within the first five days of life as part of EPI vaccination schedule, with the majority (26/38) receiving BCG on Day 2 of life (**Table S2**). All infants received antiretroviral therapy (ART) prophylaxis and none acquired HIV through 2 years of follow-up. As expected by virtue of randomization, maternal and infant characteristics were balanced across HIV vaccine and placebo groups. A detailed description of the study cohort and safety outcomes is published previously *(13)*.

Infant responses to EPI immunizations appear unaffected by HIV vaccination

Using the Pediatric Vaccine Multiplex Assay (PVMA) *(14)* at Week 56 (W56), two weeks post-fifth dose, we assessed whether infants who received standard EPI vaccines (**Figure 1**) had evidence of interaction

with the study vaccine. Thirty five of 38 enrolled infants (92%) received the recommended 3 doses of DTaP-IPV-HepB-Hib vaccine prior to W56 and an additional 4 were missing specimens at W56; among the 31 infants remaining, all had protective levels of anti-HepB IgG Abs (100%; 95% CI: 89% to 100%) and 94% (95% CI: 79% to 98%) had protective levels of diphtheria-specific Abs (**Table 1**), using WHO-endorsed protective thresholds. Protective levels of tetanus-specific Abs were moderately frequent (68%; 95% CI: 50% to 81%) while protective levels of HiB-specific Ab levels were infrequent (39%; 95% CI: 24% to 56%). All infants had anti-pertussis Abs detected (100%; 95% CI: 89% to 100%). There were no significant differences in protective or detectable response rates to the EPI vaccines between CH505TF gp120 vaccine and placebo groups ($p > 0.55$ by Fisher's exact test). Low-to-moderate correlations were seen between EPI vaccine responses and CH505-specific binding and nAb responses at W56 among vaccine recipients (correlations ranging from -0.33 to 0.47; **Figures S4** and **S5**) based on Binding Antibody Multiplex Assay (BAMA) and TZM-bl neutralization assay. This population does not receive Rubella vaccination as part of the EPI vaccines and only one infant had a detectable response above the threshold for protection (3%; 95% CI: 0.5% to 15%; **Table S3**). RSV is not included in the South African vaccine schedule for either mothers or infants, but RSV infection is common *(15)* and 73% of infants [95% CI: 56% to 85%] had detectable RSV Abs.

No evidence of HIV Env-reactive maternal antibody interference for HIV vaccine in infants

Maternal antibody was measured by binding antibody to gp41, as the gp41 epitope was not present in the study vaccine. Maternal anti-gp41 Abs were detected using BAMA in all infants at high levels at baseline but declined by W56, with some residual W56 anti-gp41 Abs seen in all vaccine groups (Low Adjuvant Dose 20% gp41 positive [95% CI: 4% to 63%]; Target Dose: 43% [95% CI: 21% to 67%]; Low Protein Dose: 25% [95% CI: 5% to 70%]) and the placebo group (57% [95% CI: 25% to 84%]) (**Figure 2A**). We observed no increase in anti-gp41 responses among vaccinated infants, demonstrating that vaccine-specific antibodies were induced. Among CH505TF vaccine recipients, W56 bAb responses to the

vaccine-matched CH505TF gp120 antigen had low correlation with baseline binding responses to CH505TF gp120 which are maternally derived (corr = 0.24), suggesting that maternal antibody did not inhibit the vaccine response (**Figure 2B**). Similarly, low levels of correlation were observed between W56 binding antibody responses to CH505TF gp120 and W56 binding Abs to gp41 (corr = 0.03; **Figure S6A**) and between W56 nAb responses to CH505TF and baseline binding Abs to CH505TF gp120 (corr = -0.19; **Figure S6B**); these correlations were studied among vaccine recipients.

Vaccine-induced Abs in infants were at least as high as infection-induced Abs in mothers living with HIV

At baseline, HIV-specific Ab responses in the infant measured using BAMA, which represent maternally transferred Ab), were similar to responses in the mother. The estimated geometric mean (GM) infant-to-mother AUC ratio among all mother-infant pairs was 0.98 [95% CI: 0.95 to 1.01] for gp41-specific bAbs and 0.87 [95% CI: 0.80 to 0.94] for CH505TF gp120-specific binding Abs. After 5 doses of CH505TF gp120 vaccination, descriptively, infant immune responses directed at the immunogen were higher on average and less variable than the HIV-specific responses in their mothers living with HIV: at W56, the geometric mean (GM) AUC was 14825 (geometric standard deviation: 1.84) in vaccinated infants versus 8452 (geometric standard deviation: 4.73) in mothers (**Figure 2C**). As expected, most of the infants in the placebo group (86% [95% CI: 49% to 97%]; 6/7) had undetectable CH505TF gp120 binding responses at W56, highlighting the waning of HIV-specific maternally transferred Abs (**Figure 2D**).

Functional vaccine-induced immune responses to HIV vaccination in infants

At W56, two weeks after the 5th and final immunization, positive and high magnitude neutralization titers (ID50) to a vaccine-matched strain that exhibits a Tier 1A phenotype (CH505.w4.3) *(16)*, measured via TZM-bl assay, were observed in all infant vaccine recipients; ID50s were significantly higher than in infants who received placebo ($p < 0.001$; **Figure 3A**). Geometric mean ID50 titers were 1,585 [95% CI:

796 to 3,155] among all vaccinated infants and 271 [95% CI: 37 to 1,964], 3,502 [95% CI: 1,812 to 6,770] and 900 [95% CI: 187 to 4,338] in the Low Adjuvant Dose, Target Dose, and Low Protein Dose groups, respectively. Much lower frequency and magnitude of responses were observed to the Tier 2 vaccine strain (CH505TF), with one low response (ID50 < 100) identified in each vaccine group and in the placebo group (**Figure S7A**). Low-magnitude responses to a Tier 1B virus modified to detect CH103 bnAb lineage precursors (CH505TF.gly4/GnT1^{-}) were also rare; these were detected in the Low Adjuvant Dose group (n = 1) and the Target Dose group (n = 2), all at ID50 titers < 50 (**Figure S7B**). One placebo recipient had positive, low-magnitude nAb responses to the CH505TF and CH505TF.gly4/GnT1 viruses at W56, suggesting residual maternal antibody. The CH505TF immunogen did not elicit heterologous tier 2 neutralization responses, likely necessary for a vaccine to prevent transmission of circulating strains.

Moderately strong and universally positive IgG binding Ab responses to the vaccine-matched CH505TF gp120 antigen were observed in all vaccine groups at W56, with magnitudes of response significantly higher in the vaccine vs. placebo group ($p < 0.001$) (**Figure 3B**). Geometric mean AUCs were 14,825 [95% CI: 11,382 to 19,310] among all vaccinated infants, and 7,742 [95% CI: 3,839 to 15,616], 17,731 [95% CI: 12,985 to 24,212], and 17,848 [95% CI: 9,567 to 33,295] in the Low Adjuvant Dose, Target Dose, and Low Protein Dose groups, respectively.

All vaccine recipients had positive and moderate levels of CH505+ B cells, measured using flow-cytometry-based B cell phenotyping, with levels significantly higher in vaccine vs. placebo group ($p < 0.001$; **Figure 3C**). The proportion of CH505-specific IgA B cells, and plasmablast responses were not meaningfully higher in vaccine versus placebo groups (**Figure S8A, C**). CH505-specific IgG B cells were seen in all vaccine groups and higher in magnitude than among placebo recipients (**Figure S8B**). Descriptively, the percent of CH505+ B cells was similar between dose groups (GM percent CH505+ B cells were 0.08 [95% CI: 0.06 to 0.10] among all vaccinated infants, and 0.11 [95% CI: 0.04 to 0.35] , 0.07

[95% CI: 0.04 to 0.10] , and 0.07 [95% CI: 0.04 to 0.15] in the Low Adjuvant Dose, Target Dose, and Low Protein Dose groups, respectively; **Figure 3C**).

At W56, binding antibody responses to the gp70 CH505TF V1V2 antigen were frequent (87.0%) and magnitudes of response were higher in the vaccine vs. placebo group ($p < 0.001$; **Figure 3D**) indicating that the CH505TF gp120 vaccine induced V1V2-specific binding Ab responses in nearly all recipients. Magnitudes of response were comparable across the vaccine groups (GM AUCs were 3569 [95% CI: 1,243 to 10,244] among all vaccinated infants, and 1787 [95% CI: 131 to 24,451], 3097 [95% CI: 618 to 15,510], and 13922 [95% CI: 4,147 to 46,738] in the Low Adjuvant Dose, Target Dose, and Low Protein Dose groups, respectively) and modest in magnitude relative to the CH505TF gp120 IgG responses.

In addition to binding to the monomeric CH505TF gp120 Env antigen, serum binding Ab responses were assessed against a stabilized SOSIP HIV Env trimer expressing CH505TF gp120 epitopes. IgG binding response magnitudes to the SOSIP antigen, and Ab binding profile suggestive of CD4bs CH103- and CH106-like Abs, were low among vaccinated infants and not different from the placebo group (**Figure S9**, **S10**).

To provide an assessment of heterologous binding, IgG binding Ab responses at baseline and W56 were measured against consensus HIV Env gp120 and gp140 antigens and the Clade C 1086 gp120 antigen. All vaccine recipients exhibited positive binding responses to the consensus Env antigens and the 1086C gp120 antigen at W56 (**Figure S11**). No placebo-group responses to these antigens were observed at the same time point. Descriptively, infant responses were similar or higher in magnitude compared to mothers living with HIV (**Figure S12** as compared to **Figure 3** and **Figure S11**).

ADCP and ADCC function induced by vaccination in infants

Since antibody Fc effector functions have been found to be associated with survival in infants born to mothers living with HIV *(9, 10)*, we examined whether these responses could be elicited by vaccination

using an ADCP assay employing flow cytometric-based technology. At W56, the median phagocytosis score of the pooled vaccine groups increased ~2-fold relative to baseline, whereas among placebo recipients ADCP activity generally decreased between baseline and W56, likely due to the decline in maternal Abs (GM ratio of W56 vs. baseline mean phagocytosis score, 2.0 vaccine [95% CI: 1.6 to 5.0] vs. 0.3 placebo [95% CI: -0.3 to 1.5]; p = 0.01; **Figure 4A**). In contrast, ADCC function declined from baseline to W56, in a similar fashion across vaccine and placebo groups (GM ratio of W56 vs. baseline ADCC peak % loss luciferase, 0.2 vaccine [95% CI: 0.4 to 0.6] vs. 0.2 placebo [95% CI: 0.1 to 0.5; p = 0.2; **Figure 4B and Figure S13**).

In addition to the ADCP and ADCC cell-based functional assays, binding antibody engagement with FcγRIIa H131 and FcγRIIIa F158 was measured by multiplexed human Fc receptor-binding assays to further evaluate the functional activity of the induced Abs (**Figures S14** and **S15**). At W56, among vaccine recipients, FcγRIIa CH505TF gp120-specific binding Ab magnitudes were similar to those at baseline, whereas a distinct decline was observed in the placebo group as maternal Abs waned (**Figure S22A**). Response rates were 100% across all vaccine groups and 0% in the placebo arm. Notably, binding IgG titers and FcγRIIa engagement against the gp70 CH505TF V1V2 antigen were low to undetectable at baseline (**Figure S22B**). However, by W56, following the final vaccination, engagement magnitudes in the vaccine groups had increased considerably and were comparable to those observed against the CH505TF gp120 antigen. FcγRIIIa F158 CH505TF gp120-specific binding antibody magnitudes among vaccinees tended to decrease between baseline and W56, albeit more slowly than in the placebo group (**Figure S15A**). FcγRIIIa F158 engagement against the gp70 CH505TF V1V2 antigen was low or undetectable at baseline, increased dramatically in vaccine groups and decreased in the placebo group (**Figure S15B**). Conversely, FcγRIIa H131 and FcγRIIIa F158 engagement by SOSIP trimer-specific Abs and Abs specific for HIV antigens not in the vaccine, attributable to maternal Abs, decreased between baseline and W56 across all groups (**Figures S16** and **S17**).

Role of GLA-SE adjuvant and Env protein doses in HIV vaccine regimen studied in infants

Descriptively, humoral and B cell immune responses to the HIV vaccine were lower for the Low Adjuvant Dose group (2.5 μg dose of the GLA-SE adjuvant) versus the Target Dose group (5 μg GLA-SE; **Figures 3, S7-17**). Week 56 GM nAb ID50 titers to the Tier 1A vaccine strain lineage (CH505.w4.3) was 271 [95% CI: 37 to 1,964] with the Low Adjuvant Dose versus 3502 [95% CI: 1,812 to 6,770] with the Target Dose (**Figure 3A**). The Low Protein Dose (5 μg CH505TF) tended to yield comparable or slightly lower immune responses compared to the Target Dose (20 μg CH505TF; **Figures 3, S7-17**). The ratios of GM immune response comparing the low versus target protein dose groups with respect to CH505.w4.3 nAb ID50 titer, CH505TF gp120 bAb AUC, CH505+ B cells, gp70 CH505TF V1V2 bAb AUC were 0.26, 1.25, 1.13, and 4.50, respectively (**Figure 3**).

Higher levels of CH505-specific antibodies in infants compared to adults

To evaluate whether vaccine induced immunity was improved in infants compared to adults, we leveraged data from HVTN 115, a randomized, placebo-controlled double-blinded phase 1 study conducted in the USA among healthy adults living without HIV (**Figure 1B and Figure S3**) *(17)*. One arm of HVTN 115 Part A evaluated a nearly identical regimen to the Target Dose studied among infants in HVTN 135: in the Corresponding Dose group, adults received CH505TF gp120 at 20 μg adjuvanted with 10 μg GLA-SE at enrollment and Weeks 8, 16, 32, and 52. Differences between the infant Target Dose and adult Corresponding Dose regimens were the higher dose of GLA-SE adjuvant in adults versus infants (10 μg versus 5 μg) and the slight difference in timing of dose 5; adults received the 5^{th} dose at Week 52 while infants received it at Week 54. Baseline characteristics of the adults included in the analysis are shown in **Table S4.**

Across the set of vaccine-matched antigens, binding and nAb CH505-specific vaccine effects, measured by fold-differences in vaccine versus placebo groups, tended to be higher in infants versus adults (**Figure**

5A). Among the largest differences was an over 10-fold higher vaccine effect on CH505.w4.3 nAb ID50 (GM ID50 = 3,502 infants [95% CI: 1,812 to 6,770] versus 257 adults [95% CI: 139 to 477]; **Figure 5B**) and an approximately 10-fold higher vaccine effect on FcγRIIIa F158 CH505TF gp120 (GM net MFI = 2,415 infants [95% CI: 943 to 6,188] versus 256 adults [95% CI: 85 to 775]; **Figure 5C**). Given that 400 μg of CH505TF gp120 dosage was optimal for immunogenicity in adults *(17)*, these data also suggest differences in immunogen dosing required for optimal immunogenicity in infants and adults.

These differences between infants and adults should be interpreted in the context of similar levels of anti-gp41 IgG bAbs post-5th dose, recognizing the absence of gp41 in the study vaccine: GM AUCs were 2,297 in infants [95% CI: 1,697 to 3,110] and 2,153 in adults [95% CI: 794 to 5,836]. This similarity in anti-gp41 bAbs lends support to the notion that the differences documented between infant and adult CH505-specific immune responses are vaccine-induced.

V1V2-directed binding antibodies were higher in infants versus adults

Vaccine-induced FcγRIIa H131 gp70 CH505TF V1V2 and FcγRIIIa F158 gp70 CH505TF V1V2 were also >10-fold higher in infants versus adults (FcγRIIa gp70 CH505TF V1V2: GM net MFI = 7,315 infants [95% CI: 2,167 to 24,692] versus 21 adults [95% CI: 1 to 432]; FcγRIIIa: gp70 CH505TF V1V2: GM net MFI = 2,688 infants [95% CI: 867 to 8,332] versus 14 adults [95% CI: 1 to 205]) (**Figure 5D and 5E**).

CD4bs B cell responses were similar in infants versus adults

CH505TF gp120 vaccination of infants elicited CD4bs specific memory B cells – binding wild-type CH505TF gp120 protein but not the CD4-binding site knock-out (KO) mutant, CH505TF gp120 Δ37I-- at high frequencies, comparable to those observed in adults (85.7% infants versus 77.8% adults) (**Figure 6**). Moderately high rates of CD4bs-directed bAbs were detected in infants based on binding to wildtype CH505TF gp120 that was reduced to a mutant with a specific point disruption in the CD4bs (Δ371I). IgG differential binding to CH505TF gp120 WT and Δ371I-mutant, FcγRIIa H131 binding, and FcγRIIIa F158

differential binding was detected in 43% [95% CI: 21% to 67%], 43% [95% CI: 21% to 67%], and 57% [95% CI: 33% to 79%] of infants, respectively, and these responses were never detected in adults. However, there was no evidence of differential binding to a different CD4bs epitope (D368) using the same assay, suggesting a different type of CD4bs Ab elicited by the CH505TF gp120.

We found no evidence for CD4bs neutralizing antibodies in polyclonal serum. Neutralizing Abs to the CH505TF.gly4/GnT1- virus, an indicator of potential CH103 bnAb precursors, were rare in infants (14% [95% CI: 4% to 40%]; 2 of 14) (**Figure 6**) and their magnitude was not reduced against a mutant virus with a CD4bs KO mutation introduced (CH505TF.gly4.S365P/GnT1-; **Figure S18A**). Low titers of ID50 nAbs to CH0505s.G458Y.N279K.2/GnT1, a screening tool used to identify potential CH235 bnAb precursors, were detected in 93% of infants (95% CI: 69% to 99%; 13 of 14) and only 14% of adults (95% CI: 3% to 51%; 1 of 7) (**Figure 6**). However, neutralization was not reduced when a CD4bs KO mutation was introduced (CH280D; **Figure S18B**), suggesting that polyclonal neutralization of these precursor viruses did not occur via a CH235-like mechanism and that the neutralization of the screening virus was unlikely to be analogous to CH235.

Immunogenetic features of B cell receptor (BCR) repertoire elicited by vaccine in infants

CH505TF gp120+ B cells were isolated from infant PBMCs at W34, 2 weeks post 4th immunization, for seven infants across different vaccine groups selected based on having the highest CH505.w4.3 nAb ID50 titers at W56 (**Figure S19A**). As expected, we found no evidence for antigen-reactive memory B cells in five placebo recipients with PBMCs available at W34 (**Figure S19B**). To assess vaccine-induced B cells elicited in infants, we flow sorted vaccine-matched CH505TF gp120+ total B cells, including memory B cells, (**Figure S19C**) from PBMCs at W34 in the seven vaccinated infants. These sorted total B cells were then analyzed in the 10x Genomics single cell immune profiling assay that generated paired heavy and light chain genes per B cell (**Figure S19D**). CH505TF gp120-negative total B cells from PBMCs from 3

placebo recipients were used for comparison; minimal CH505TF gp120+ B cells in these individuals were likely due to low level B cell cross-reactivity and were not studied. This approach provided the opportunity to collectively compare candidate vaccine-induced CH505TF gp120+ B cells from infant vaccine recipients (n = 174) to CH505TF gp120- B cells from infant placebo recipients (n = 34,833).

We found that CH505TF gp120+ compared to CH505TF gp120- B cells used a variety of immunoglobulin (Ig) heavy (IgH) chain genes (**Figure S19E**). The BCRs used by CH505TF gp120+ and CH505TF gp120- B cells had similar HCDR3 length distribution (**Figure S19F**). However, both total and IgG-only CH505TF gp120+ B cells had higher somatic hypermutation (SHM) frequencies compared to CH505TF gp120- B cells in placebo recipients (**Figure S19G**). As expected, class-switched IgG B cells were enriched among CH505TF gp120+ compared to CH505TF gp120- B cells, while the reverse was true for IgD and IgM B cells (**Figure S19H**).

BCR repertoire of infants and adults revealed differences used by both populations in response to HIV CH505TF gp120 vaccine

We analyzed previously-generated BCR data from adults who received optimal immunogen dosing at 400 μg CH505TF gp120 at 2 weeks post-5th vaccination (W54) for comparison with BCR data available from the representative infants studied who received ≤20 μg CH505TF gp120 but had maximal tier nAb magnitude as described above. Vaccine-induced B cells in infants and adults used a variety of IgH genes (**Figure S20A**). VH1-24 CH505TF gp120+ B cells were detected in adults, but not infants. The BCRs used by CH505TF gp120+ B cells in infants and adults had similar HCDR3 length distribution (**Figure S20B**), but CH505TF gp120+ B cells in adults sequenced after the fifth dose (week 52 after first vaccination) had higher SHM frequencies compared to infants sequenced after the fourth dose (week 34 after first vaccination) (median 0.0451 versus 0.0363; **Figure S20C**). IgG B cells were similarly used by the majority of CH505TF gp120+ B cells in infants and adults (**Figure S20D**). IgA CH505TF gp120+ B cells were higher

in adults and IgM CH505TF gp120+ B cells in infants (3.2% vs. 0.6%, respectively). However, IgM+ CH505TF gp120+ B cells were higher in infants compared to adults (12.1% versus 5.8%), at the post-fourth dose (infants) and post-fifth dose (adults), respectively.

Since VH1-69-using Abs were enriched among CH505TF gp120+ B cells in infants compared to adult vaccinees (16.0% versus 7.8%, respectively**; Figure S19I**) and given their roles in shaping effective Ab responses to different pathogens, including influenza *(18)* and HIV *(19)*, we next investigated the allelic variants in each population. We found that CH505TF gp120+ B cells in infants predominantly used the 54F allelic variant of VH1-69 (*12) (**Figure S21A**), while adults used a greater variety of alleles with a majority of 54L allelic variants (**Figure S21B**). This pattern was also seen in placebo recipient infants and placebo recipient adults who showed a predominance for 54F and 54L allelic variants, respectively, indicating that the different baseline B cell repertoire in adult and infant populations may influence the vaccine elicited responses.

Discussion

Here we present a systematic comparison of TLR4-adjuvanted, germline-targeting HIV immunogen vaccine responses in infants and adults. The HVTN 135 trial was conducted to determine whether the CH505TF gp120 + GLA-SE HIV vaccine could be given to infants safely and to evaluate the infant immune response to the vaccine; a previous publication supports safety of the vaccine regimen in infants *(13)*). The HVTN 135 trial was designed to be harmonized with the HVTN 115 study in adults with the intent to compare infant and adult immune responses to this novel adjuvanted HIV Env immunogen. Comparison of immune responses between these populations revealed that infants elicited Ab responses with a magnitude and quality that were comparable to, and often higher than adults. We also established that maternal Abs did not interfere with vaccine humoral immunogenicity in infants and the vaccine did not alter infant humoral responses to EPI vaccinations.

Few studies have systematically compared vaccine-elicited immune responses in infants and adults, particularly in the context of early phase vaccine trials. While receiving only a quarter of the adult dose of mRNA COVID-19 vaccine (25 µg versus 100 µg mRNA-1273), infants and toddlers (0.5-5 years of age) generated comparable levels of anti-SARS-CoV-2 IgG Abs to adults despite diminished IgA and IgM responses *(20)*. In the context of HIV vaccine development, prior studies have demonstrated that HIV vaccination in infants was immunogenic *(21, 22)* and studies that compared infant and adult vaccine regimens based on the partially efficacious RV144 vaccine *(7)* revealed that potentially protective V1V2 IgG responses were higher in infants and potentially non-protective serum Env IgA responses were lower *(11, 23)*. Our study is distinct in evaluating a vaccine intended to induce bnAb lineages in adults and infants with harmonized immunization schedules, specimen collection time points, and immunological assays.

The context of neonatal immunity is critical to consider when investigating new HIV vaccines in infants. For example, maternal HIV Abs are passively transferred from mother to infant and maternally derived antibodies have been previously shown to dampen the effect of B cell activation *(24, 25)*; however, this effect is, by definition, short-lived as maternal Abs wane over the first year of life. To minimize the contribution of maternally transferred antibodies, we evaluated primary immunogenicity two weeks after the last vaccination, 56 weeks after birth. We measured maternally-transferred Abs at baseline and found no association between maternal and vaccine-elicited Abs, suggesting that these antibodies do not interfere with the immune responses at the end of the five-dose regimen.

After sequential immunization of infants with CH505TF gp120, HIV Env-specific binding Abs – including against the CD4bs and V1V2 regions – were detectable with levels comparable to or higher than those observed in adults. Notably, IgG responses directed at the V1V2 epitope in infants were higher when compared to adults who received the same vaccine regimen. Although V1V2-specific Abs are typically not associated with potent neutralization, binding anti-V1V2 IgG responses have been correlated with protective immunity in non-human primate studies *(26)* and HIV vaccine efficacy trials *(7, 8, 27, 28)*.

HVTN 135 was also designed to consider whether there was a dose effect of the immunogen or the adjuvant. Although the Target Dose (20 µg CH505TF gp120) and Low Protein Dose (5 µg CH505TF gp120) yielded similar CH505TF gp120 IgG responses, the Target Dose induced greater neutralization ID50 titers against CH505.w4.3, suggesting that protein dose may influence the neutralizing capacity of the Ab response. Adjuvant dose appeared to have a more beneficial impact on eliciting CH505TF gp120-binding IgG Ab titers than HIV protein dose. In contrast to previous studies where some immune responses were improved with a lower protein dose, we found no evidence that a low dose of protein was superior across any readout we tested *(23)*.

Some Fc effector-mediated responses (FcγRIIa and FcγRIIIa) to CH505TF V1V2 were markedly higher in infants compared to adults. These findings suggest that the vaccine-induced V1V2-specific binding Ab responses capable of engaging FcγRIIa may have contributed to the observed approximate >2-fold

improvement in ADCP activity at Week 56. However, the vaccine also elicited greater FcγRIIIa engagement with V1V2-specific binding Abs, and this enhanced V1V2-binding Ab specificity did not appear to improve ADCC activity from baseline levels, as measured by the ADCC luciferase assay, suggesting that the differences in adults versus infants may depend on the type of effector readout.

We did not find evidence for induction of bnAb precursors in either infants or adults *(17)*, likely related to the inability of a soluble gp120 immunogen to induce such responses. In infants, there was a moderate frequency of vaccine-induced CD4bs-directed Abs that demonstrated differential binding to the CH505TF gp120 WT and Δ371I-mutant, but no differential binding using the D368R-mutant, suggesting candidate CD4bs Abs with a Tier 1 nAb phenotype. Moreover, we found no evidence for precursors of Tier 2 neutralizing CD4bs bnAb lineages, CH103 or CH235, in mediating neutralization in polyclonal serum. However, there was a 10-fold higher nAb response against a Tier 1A virus in infants compared to adults and Tier 1b nAbs were only detected in infants. Although the Abs in infants were strain-specific Tier 1 antibodies, the difference from adults is encouraging and strongly suggests that when presented with the same immunogen, the infants more effectively mature the Ab response to affinity mature to neutralization *(29)*. That infants can mount targeted Ab responses capable of both neutralization and Fc-mediated effector functions, with increased magnitude of these responses compared to adults, further supports continued investigation of HIV vaccines in infants. This broader immune response may represent the type of coordinated activity necessary to establish effective immunity against HIV infection. Overall, our observations suggest that the neonatal immune environment may be more permissive to initiating bnAb lineages, possibly due to reduced regulatory barriers and a more adaptable B cell repertoire *(3, 30)*. Therefore, our data suggest that bnAb-inducing vaccines may be more effective in infants supporting their continued development in this age group.

Analysis of the BCR repertoire in infants and adults revealed an intrinsic difference that may shape the Ab response to different pathogens and vaccine immunogens, including HIV vaccines. Investigation of B

cell repertoires in vaccinated infants revealed an enrichment of immunoglobulin genes (including VH1-2, VH1-46, and VH1-69) associated with known HIV nAbs in CH505TF gp120+ versus gp120- B cells *(31–33)*. This vaccine-induced repertoire also exhibited somatically mutated immunoglobulin genes used by class-switched IgG+ cells, features linked to robust antiviral and neutralizing antibody responses *(32, 33)*. However, it remains to be determined if Ig genes used by Abs in infants have more or less rare or improbable mutations linked to maturation of bnAb lineages as previously described *(34)*. The BCR repertoire of vaccine-induced CH505TF gp120+ B cells differed between infants and adults, with infants showing greater enrichment of VH1-69 and VH4-39 gene usage compared to adults, who uniquely display VH1-24+ cells. These differences may reflect age-related distinctions in B cell maturation and isotype selection that may impact vaccine-elicited immunity, or population differences, noting that HVTN115 was conducted in the US whereas HVTN135 was conducted in South Africa.

This study has several limitations. First, we evaluated only repeated administration of a single protein HIV vaccine immunogen to determine whether it could prime or induce precursors of CD4bs bnAb lineages, and to enable a direct comparison to a completed adult trial with known immunogenicity. From the standpoint of bnAb lineage development, most current vaccination approaches intended to prime and boost bnAb lineages towards mature bnAb status use serial vaccinations with different immunogens to drive directed B cell evolution. Second, the infant and adult vaccine trials differed according to dose of adjuvant (5 μg GLA-SE in infants vs 10 μg GLSA-SE in adults) due to the need to use a lower dose in infants as is standard for many licensed vaccines. Since we observed enhanced immune responses in infants in general, we think it is unlikely that the lower dose of GLA-SE is responsible for the differences in immune responses. Importantly, the same 20 μg CH505TF protein dose was compared between adult and infant studies. While this was the Target Dose for infants it was the lowest dose studied in adults, and among adults the 400 μg dose was found to have induced improved immune responses. Also, factors other than age may differ between infants and adults, including race and

ethnicity which differ notably between the South African infants and US adults. Infants had been potentially exposed to HIV and passively acquired maternal antibodies that bound to the vaccine immunogens — this could plausibly have led to distinct immunity independent of age *(35)*. Additionally, since infants were receiving a series of EPI vaccinations, the effect of other adjuvants cannot be excluded. The sample size for this phase 1 trial is necessarily small, leading to low precision in estimating rates and levels of immune response. Nonetheless, large differences were seen in infant vs. adult immune responses. Finally, a caveat of our BCR repertoire analyses is that it may be affected by the differences in timing and dosing among the infants versus adults: infants were studied post-fourth immunization compared to post-fifth immunization in adults, and with a 5 or 20 μg immunogen dose in infants compared to 400 μg dosage in adults.

Given the difficulty of eliciting bnAbs following vaccination, an HIV vaccine that can fully engage innate, cellular, and humoral immunity will likely be necessary to prevent HIV acquisition given repeated exposures over a lifespan. Our results suggest that infant vaccination with an HIV neoantigen induces immune responses of a quantity and quality that supports continued vaccine evaluation in pediatric populations. Moreover, following an initial period of vulnerability during the peripartum period in the context of maternal HIV infection, infants have a long window before sexual debut. Therefore bnAb-predicated vaccine strategies that may require months-to-years for the B cell evolution required to produce antibodies capable of providing protective immunity could work well for this population.

With the emergence of even more promising HIV antigens *(32, 36, 37)* and pediatric adjuvants *(38–46)* , a GLA-SE-adjuvanted CH505TF gp120 vaccine is unlikely to be advanced further in clinical development. Indeed, human *in vitro* modeling suggests that other adjuvants including TLR7/8 agonist may have greater effect in infants *(47)*. Overall, our results support continued discovery and development of adjuvanted HIV vaccines intended to induce bnAbs in infant populations. Our results comparing infants to adults suggest that there are promising vaccine candidates that might be successful in infants but not

in adults. Indeed, traditional clinical pathways that only test candidate vaccines in adults with an eventual age de-escalation could hamper identification of an efficacious pediatric HIV vaccine. The combination of very reassuring safety data *(13)*, a lack of interference with routine immunizations, lack of evidence for maternal antibody interference, evidence for distinct and enhanced B cell responses, and potential for leveraging the existing and highly effective neonatal vaccine delivery infrastructure provide a strong rationale for the continued development and evaluation of an infant HIV vaccine.

Materials and Methods

Experimental design

HVTN 135 was a randomized, placebo-controlled double-blinded phase 1 trial conducted at a single site in Soweto, South Africa (**Figure 1** and **Figure S1**), clinicaltrials.gov ID#NCT04607408 registered October 29, 2020 (https://clinicaltrials.gov/study/NCT04607408?term=HVTN%20135&viewType=Card&rank=1). Participants were healthy infants born to mothers living with HIV, enrolled within 5 days of birth; infants had a negative HIV DNA PCR test at birth and received prophylaxis with none acquiring HIV through 2 years of follow-up *(13)*. Full eligibility criteria are included in the study protocol (see **Supplementary Information**). The trial was approved by the South African Health Products Regulatory Authority (SAHPRA) and the University of the Witwatersrand Human Research Ethics Committee (HREC), reference number 190914B. The study was approved by HREC on January 21, 2020 and by SAHPRA on February 20, 2020. The funding source was the Division of AIDS (DAIDS) of the National Institute of Allergy and Infectious Diseases (NIAID) of the US National Institutes of Health (NIH). Members of the DAIDS helped to draft the initial manuscript, but analysis was conducted independently. The funder had no role in data collection or statistical analyses.

HVTN 115 was a three-part randomized, placebo-controlled double-blinded phase 1 study conducted at four sites in the USA (**Figure 1 and Figure S2**). Healthy adults living without HIV between the ages of 18 and 50 at low risk of HIV acquisition were eligible. Primary results on HVTN 115 safety and immunogenicity are in submission *(17)*. This manuscript considers HVTN 115 Part A only as this regimen was directly comparable to HVTN 135. The protocol was approved by a central IRB (Advarra, reference number 00042693) for the multisite HVTN 115 study and registered at ClinicalTrials.gov (NCT03220724).

Both studies were sized to detect moderately frequent adverse events following vaccination with high probability (e.g., 90% chance of observing an event occurring with 12% frequency or more given 18

participants in the infant Target Dose group) and to enable reasonably precise characterization of immunogenicity in each vaccine group, allowing for historical rates of loss-to-follow-up and missed participant visits (e.g. an expected 95% confidence interval for an immune response observed in 3 of 13 infants expected to have immune responses measured post-fifth dose of 8.2% to 50.3%).

Randomization and masking

HVTN 135 vaccinations were given at enrollment and Weeks 8, 16, 32, and 54 *(13)*. This schedule was designed to minimize overlap with the Republic of South Africa (RSA) Essential Programme on Immunization (EPI) vaccination schedule (**Figure 1A**), while harmonizing as closely as possible with the adult administration schedule in HVTN 115; as a result the only difference was that adults in HVTN 115 received injection 5 at Week 52 while infants in HVTN 135 received injection 5 at Week 54. In HVTN 135, study vaccine was not given within 7 days of EPI vaccines for doses 2-5.

HVTN 135 was conducted in three parts (**Figure 1B**) *(13)*. Part A provided initial safety data on the 20 μg CH505TF gp120 when combined with a 2.5 μg dose of the GLA-SE adjuvant; infants were randomized 5:2 to 20 μg CH505TF gp120/ 2.5 μg GLA-SE (Low adjuvant dose group) or placebo. Part B provided a preliminary safety assessment of the maximum targeted 5 μg dose of the GLA-SE adjuvant; infants were randomized 1:1 to 20 μg CH505TF gp120/ 5 μg GLA-SE (Target dose group) or placebo. Part C expanded the evaluation of the 20 μg CH505TF gp120/ 5 μg GLA-SE regimen and included for comparison a dose-sparing 5 μg CH505TF gp120/ 5 μg GLA-SE arm (Low protein dose group); infants were randomized 16:5:6 to 20 μg CH505TF gp120/ 5 μg GLA-SE, 5 μg CH505TF gp120/ 5 μg GLA-SE, or placebo.

HVTN 115 Part A participants were randomized 2:2:2:1 to receive CH505TF gp120 at 20 μg (Corresponding dose group), 100 μg, or 400 μg adjuvanted with 10 μg GLA-SE or placebo at enrollment and Weeks 8, 16, 32, and 52. Note that the dose of GLA-SE adjuvant was higher in adults versus infants (10 μg versus 5 μg).

For each study, randomization assignments were computer-generated and provided to the clinical site pharmacist through a web-based randomization system.

Study products

The CH505TF gp120 immunogen was derived from a clade C transmitted/founder HIV virus isolated from a single acutely HIV–infected person from Malawi (CH505) *(12, 48, 49)*. The adjuvant GLA-SE is an oil-in-water (OIW) stable emulsion containing the immunological adjuvant Glucopyranosyl Lipid A (GLA). CH505TF gp120 is manufactured by Berkshire Sterile Manufacturing and GLA-SE is manufactured by the Infectious Disease Research Institute (IDRI); both products were provided by the Division of AIDS (DAIDS) at the National Institute of Allergy and Infectious Diseases. The placebo was 0.9% Sodium Chloride.

Study procedures

In both HVTN 115 and 135, participants received study product at the indicated doses via injections administered intramuscularly in the thigh. Participants were followed for 24 months.

Specimen collection and HIV testing time points are summarized in **Figure S2**. Diagnostic tests for HIV acquisition utilized an HVTN-developed diagnostic algorithm that distinguishes between vaccine-induced antibodies and HIV acquisition *(50)*.

Study objectives

The primary objectives of HVTN 135 were to evaluate vaccine safety and tolerability and to evaluate the extent to which CH505TG gp120 + GLA-SE vaccination induces B-cell lineages potentially capable of generating a broadly neutralizing antibody response, as measured by HIV Env gp120-specific, CD4bs-specific and V1V2-specific serum IgG bAbs by BAMA, and quantification and phenotypic characterization of peripheral B cells capable of binding HIV Env gp120, the CD4bs, and the V1V2bs by flow cytometry.

Evaluating the impact of the HIV vaccine on EPI vaccine responses, evaluating vaccine-induced nAbs, and assessing Fc-mediated antibody effector functions were secondary objectives. Comparing infant responses in HVTN 135 versus adult responses in HVTN 115 and characterizing B cell lineages and evaluating the amount of B-cell receptor somatic hypermutation were exploratory objectives of the infant study.

Immunogenicity assays

Validated and standardized immunogenicity assays were run for the two studies. The primary immunogenicity time point was two weeks post-fifth vaccination, i.e. at W56 for HVTN 135 and Week 54 for HVTN 115. Infant immune responses at baseline were measured in infant blood except in 1 infant for which only cord blood was available for Binding antibody multiplex assay (BAMA), Fc receptor (FcR) binding assay, and antibody-dependent cellular phagocytosis (ADCP) assay.

Binding antibody multiplex assay (BAMA)

HIV binding Ab responses were measured using the validated HIV Binding Ab Multiplex Assay (BAMA), which employs Luminex xMAP technology to enable simultaneous detection of multiple HIV antigens *(51–53)*. The area under the curve (AUC) was calculated from the serial dilution curve, and a three-part positivity method using HVTN 115 Part A baseline MFI values was applied to post-enrollment serum samples to make a response call. Differential binding ratios between wildtype (CH505TF gp120) and mutants (CH505TF gp120_d371, CH505TF gp120_D368R) were used to assess CD4bs-binding Ab specificity (**Table S5**).

Fc receptor (FcR) binding assay

Vaccine-induced Abs that can engage cellular FcR were measured by multiplexed human Fc receptor-binding assays using Avi-tagged high-affinity FcγR proteins, FcγRIIa H131 and FcγRIIIa F158 *(8)*.

The antibody-dependent cellular phagocytosis (ADCP) assay

The capacity of vaccine-induced Abs to mediate phagocytosis was assessed by a qualified ADCP assay employing flow cytometric-based technology *(8, 54–56)*, to CH505TF gp120. A response was considered positive if the mean phagocytosis score was at or above an antigen-specific cutoff value (the 95th percentile of the baseline score for HVTN 115) and more than 3-times the median antigen-specific score for HVTN 115.

Neutralizing antibody assay

Neutralizing Abs against Tier 1 and Tier 2 strains of HIV were as previously described in TZM-bl cells *(57, 58)*. Neutralization titers were measured against the envelope (Env) -pseudotyped Tier 2 vaccine strain (CH505TF) and a vaccine strain lineage virus that exhibits a Tier 1A neutralization phenotype (CH505.w4.3), as well as against CH505TF.gly4/GnT1 and CH0505s.G458Y.N279K.2/GnT1, viruses modified to detect CH103 and CH235 bnAb lineage precursors, respectively; both lineages target the CD4 binding site (CD4bs). A response was considered positive if infection was reduced by at least 50% at the lowest serum dilution tested (1:10 dilution). Titers are the serum dilution at which infection was reduced by either 50% (ID50) or 80% (ID80) as measured by relative luminescence units (RLUs).

B cell phenotyping (BCP) assay

HIV Env-specific B cells induced by vaccination were identified and characterized using fluorescently labeled recombinant CH505 Env gp120 proteins in the context of a flow cytometry panel *(59)*. Total B cells are identified using doublet exclusion, lymphocyte scatter profile, a viability dye to exclude dead cells, and are negative for lineage markers: CD3, CD56 and CD14; and positive for CD19 and CD20 (**Figure S22**). B cells are further gated as IgD- and then IgG+. Candidate CD4-binding site-specific B cells bound wild-type CH505TF gp120 protein but not the CD4-binding site knock-out (KO) mutant, CH505TF gp120 Δ37I. The positivity threshold for a positive response was defined based on the 85th percentile of

the proportion of cells in the subset (IgD, IgG+, IgA+, antigen+ B cells) among placebo recipients at that visit. Data from Low IgD- B cells (<5000) and assay results deemed unreliable for analysis were excluded from the analysis.

Antibody Dependent Cellular Cytotoxicity (ADCC)

ADCC was assessed by a qualified GranToxiLux Ab-Dependent Cell Mediated Cytotoxicity (ADCC GTL) assay *(60)* with target cells, CEM.NKRCCR5 CD4+ T cell line *(61)* coated with recombinant CH505 gp120. The magnitude of response is the peak net percent granzyme B activity, defined as the percent of target cells positive for GTL (an indicator of granzyme B uptake) minus the percent of target cells positive for GTL when incubated with effector cells in the absence of a source of Abs. A positive response is defined as at least 8% granzyme B activity. ADCC was also assessed by a luciferase assay *(62)* with CEM.NKRCCR5 cells *(61)* infected with HIV CH505s.LucR.T2A.ecto/293T/17. The magnitude of response was calculated as the percentage of killing calculated as 100 * (RLU of target and effector well - RLU of test well)/ (RLU of target and effector well). A positive response was defined as percent killing of at least 15%.

Binding ELISA

384 well ELISA plates (Costar #3700) were coated directly with 10 µl Env or streptavidin (Thermo Fisher Scientific Inc. #S-888) diluted to 2 mcg/ml in 0.1M sodium bicarbonate overnight at 4C. The plates were then washed with PBS/0.1% Tween-20 and blocked for 60 minutes at room temperature with assay diluent (PBS/4%(w/v) whey protein/15% Normal Goat Serum/0.5% Tween-20/0.05% Sodium Azide). Plates coated with streptavidin were incubated for 60 minutes with 10 µl of biotinylated Env at 2mcg/ml followed by washing. Serum samples were titrated in a 12 by 3-fold serial dilution starting at 1:30 then 10 µl was added to assay plates for 90 minutes followed by washing. Goat anti-human IgG-HRP secondary antibody (Jackson ImmunoResearch C:109-035-008) diluted 1:15,000 in assay diluent without azide was added at 10 µl per well and incubated for 60 minutes. The plates were washed and detected

with 20 µl SureBlue Reserve (Seracare #5120-0081) for 15 minutes. The reaction was stopped with the addition of 20 µl HCL stop solution. Plates were read at 450 nm. Using Molecular Devices 'Softmax-Pro' software, absorbance values from each 12-point binding curve were used to calculate the Log Area Under the Curve (Log AUC). Baseline Log AUC was subtracted and plotted as the change in Log AUC (Δ Log AUC).

SOSIP Blocking

The ability of serum to block monoclonal antibody binding to HIV Env SOSIP trimer was measured by ELISA in 384 well plates (Costar #3700). Monoclonal antibodies with defined Env binding specificities were labeled with horseradish peroxidase (HRP) using Lightning-Link® kits (Novus Biologicals #701-0003). Plates were coated with 10 µl streptavidin (Thermo Fisher Scientific Inc. #S-888) diluted to 2 mcg/ml in 0.1M sodium bicarbonate overnight at 4C then washed with PBS/0.1% Tween-20 and blocked for 60 minutes at room temperature with assay diluent (PBS containing 4%(w/v) whey protein/15% Normal Goat Serum/0.5% Tween-20/0.05% Sodium Azide). Plates were incubated for 60 minutes with 10 µl of biotinylated Env followed by washing. Serum samples diluted to 1:50 were incubated in triplicate wells at 10 µl each for 90 minutes followed by washing. The HRP labeled test antibodies were added at 10 µl for 60 minutes. The working concentrations of the biotinylated Envs and HRP labeled antibodies used above were determined in prior optimization assays that allowed for optimal blocking of the unlabeled version of the same antibody. Plates were washed a final time and HRP-antibody binding was detected with the addition of 20 µl SureBlue Reserve (Seracare #5120-0081). The reaction was stopped with 20 µl HCL stop solution. Plates were read at 450nm. Percent blocking of each sample was calculated using absorbance values as follows: 100-((sample mean absorbance/0% blocking control mean absorbance) x 100).

Infected Cells Antibody Binding Assay (ICABA)

The ability of vaccine-induced Abs to recognize infected cells was measured by ICABA utilizing HIV CH505 Infectious Molecular Clone (IMC) infected target cells *(63)*. CEM.CCR5.NKR cells infected with HIV CH505 and mock-infected cells (cells not exposed to the antigen) were tested in a 96-well plate without sera to assess background signal. The magnitude of response was defined as the %IgG+ infected cells at peak subtracting nonspecific (%IgG+ mock infected cells) and background (%IgG+ in the absence of sera) signal. A positive response was defined as a response above 5%.

Measurement of EPI vaccine responses

The Pediatric Vaccine Multiplex Assay (PVMA) utilized the same Luminex instrument and techniques as BAMA to assess serum samples for Abs against *Haemophilus influenzae* type B (HiB), diphtheria, tetanus, pertussis, rubella and respiratory syncytial virus (RSV) *(14)*. WHO endorsed protective thresholds were used: 0.01 IU/mL anti-hepB IgG, 0.15 µg/mL anti-HiB IgG, 0.1 IU/mL anti-tetanus IgG, 0.1 IU/mL anti-diphtheria IgG, and 10 IU/mL anti-rubella IgG. For pertussis and RSV, the lower limit of quantification (LLOQ; 0.686 IU/mL for Pertussis and is 0.0762 µg/mL for RSV) was used as the response threshold indicating Ab detection; protective thresholds were not available.

B cell receptor (BCR) sequencing methods

CH505TF gp120+ B cells were isolated from PBMCs from W34, 2 weeks post 4th immunization, in a subset of infant vaccinees in various dose groups selected on high W56 CH505.w4.3 nAb ID50 titers; 6 of 7 infants received 20 mcg CH505TF. These seven infants also had an increased frequency of CH505+ B cells at W34 and W56. Antigen+ (Ag+) B cells were flow sorted using vaccine-matched CH505TF gp120 from vaccine recipients, whereas Ag- B cells were collected from CH505TF gp120-negative B cells for 3 placebo recipients at W54, W56, or W74; time points were selected based on sample availability. The Ag+ and Ag- B cells were studied via 10x Genomics single cell immune profiling assay as previously

described *(64)* that generated paired variable heavy and light chain and transcriptomic sequences per B cell.

For comparison to adults, previously generated BCR repertoires elicited by CH505TF vaccination in HVTN 115 were used: PBMCs from 2 weeks post-5th vaccination (W54) were assayed for 12 participants who received 400 μg CH505TF gp120 adjuvanted with 10 μg GLA-SE.

Statistical methods

The analysis cohort included participants in the per-protocol cohort of each study, i.e., participants who received all scheduled HIV vaccine doses without error and within visit windows. One exception is for BCR repertoire analysis, comparing adults versus infants: among adults in HVTN 115 with BCR repertoire data, 10/12 received all five immunizations; 1 participant missed the 4th immunization and another participant missed the 5th immunization. The other exception is analyses of W56 immune responses to a given EPI vaccine which restricted to infants who received all doses of that vaccine through W56. Specifically, W56 Hepatitis B, HiB, tetanus, diphtheria, and pertussis responses were evaluated among infants who received 3 prior doses of DTaP-IPV-HepB-Hib vaccine.

HVTN 135 Parts B and C participants who received the same Target Dose (20 μg CH505TF gp120/ 5 μg GLA-SE) vaccine regimen were pooled for analysis. Within each study, participants randomized to placebo were pooled for analysis. Treatment groups were pooled for EPI analysis.

IgG antibody transfer ratios were calculated in HVTN 135 as the ratio of maternal to infant antibody level (AUC titer) at baseline.

For evaluating infant ADCC and ADCP functions in HVTN 135 accounting for maternal antibody influences, the elevation post-vaccination (calculated as the ratio of W56 versus baseline levels) was evaluated, restricting to participants with data at both time points.

Response rate confidence intervals were calculated using the Wilson score method *(65)* and for geometric mean responses using the normal approximation. Spearman rank correlation coefficients were used to describe associations between pairs of quantitative immune responses. For primary and secondary immune response endpoints measuring binding and neutralizing antibodies, B cells, and Fc functions to vaccine-matched antigens at W56, quantitative immune responses were compared between vaccine and placebo groups using Wilcoxon Rank Sum tests. EPI response rates were compared between vaccine and placebo groups using Fisher's exact tests. All tests were two-sided and 0.05-level.

Infants and adults were compared with respect to the vaccine effect size, measured by the ratio of immune response in vaccine versus placebo groups, restricting to the 20 μg CH505TF gp120/ 5 μg GLA-SE dose (Target Dose) group in HVTN 135 and the 20 μg CH505TF gp120/ 10 μg GLA-SE (corresponding dose) group in HVTN 115. The exception is the BCR repertoire analyses which compared infants in various dose groups in HVTN 135 with adults in the 400 μg CH505TF/ 10 μg GLA-SE group in HVTN 115.

Given the small sample sizes and exploratory nature of the comparisons among HVTN 135 vaccine groups and between infants and adults, these comparisons are descriptive; 95% confidence intervals are provided to characterize uncertainty but no statistical testing was done.

Data availability

Data underlying the findings of this manuscript, and metadata including the study protocol and Statistical Analysis Plan, will be made publicly available at Harvard Dataverse [https://dataverse.harvard.edu/dataverse/hvtn135]. Additional data from this study can be made available with explicit permissions from the HVTN, ethics committees, and by contacting the corresponding author.

Supplementary Materials

Supplemental Figures

Figure S1. HVTN 135 CONSORT diagram.

Figure S2. HVTN 135 and HVTN 115 HIV testing and specimen collection schedules.

Figure S3. Full schema for HVTN 115.

Figure S4. Infant IgG responses to EPI vaccines and infant neutralizing antibody responses to HIV-vaccine-matched antigens are not linearly related (Week 56).

Figure S5. Infant IgG responses to EPI vaccines and infant binding antibody responses to HIV-vaccine-matched antigens are not linearly related (Week 56).

Figure S6. Impact of maternal Ab on infant response to vaccine.

Figure S7. Infant neutralizing antibody responses at Week 56 to the Tier 2 vaccine virus (CH0505TF) and to a Tier 1A virus modified to detect CH103 bnAb lineage precursors (CH0505TF.gly4/GnT1).

Figure S8. Infant CH505-positive B cell responses at Week 56.

Figure S9. Infant binding IgG ELISA titers to vaccine matched CH505 stabilized trimer at baseline and Week 56.

Figure S10. Infant CH103 and CH106 conformational binding antibody response to CH505 at Week 56.

Figure S11. Infant IgG binding antibody responses at Week 56 to vaccine-mismatched antigens.

Figure S12. Mothers' IgG binding antibody responses at Week 56.

Figure S13. Infant CH505 vaccine-induced ADCP and ADCC responses.

Figure S14. Infant FcγRIIa responses at baseline and Week 56 to vaccine-matched antigens.

Figure S15. Infant FcγRIIIa responses at baseline and Week 56 to vaccine-matched antigens.

Figure S16. Infant FcγRIIa responses at baseline and Week 56 to vaccine-mismatched antigens.

Figure S17. Infant FcγRIIIa responses at baseline and Week 56 to vaccine-mismatched antigens.

Figure S18. CH103 and CH235 lineage neutralizing Antibody ID50.

Figure S19. B cell receptor repertoire analyses in HVTN 135 infants.

Figure S20. B cell receptor repertoire analyses in HVTN 135 infants vs. HVTN 115 adults.

Figure S21. Allelic variants of VH1-69 gene in the BCR repertoire of HVTN 135 infants and HVTN 115 adults.

Supplemental Tables

Table S1. HVTN 135 infant participant characteristics at enrollment.

Table S2. Timing of infant receipt of BCG vaccine by day of life.

Table S3. Infant binding antibody response to common pathogens.

Table S4. HVTN 115 adult participant characteristics.

Table S5. Antigen names.

Supplemental Methods

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Author contributions

NNM, JH, ASWD, HJ and GDT drafted the manuscript. SC, KW, MC, LZ, SS performed data analyses. AV served as clinical site PI and HVTN 135 co-chair, GG served as study chair, GDT as cochair, JK and MES were study chairs for HVTN 115. NNM served as the laboratory lead for HVTN135 and DM for HVTN115. WH was protocol team leader for both HVTN 135 and 115. LP and MBI were medical officers. TMM, OF, KO, KKS, GDT assisted in designing the HVTN 135 study. GF, WW, RP, KES, RP, PLM, DM, HK, GDT, JH, YC, GF generated immunological data. PK and MB analyzed immune data. BFH developed the CH505TF gp120 vaccine. LC, GDT secured funding. All authors reviewed and approved the manuscript.

Competing Interests

OL is a named inventor on patents relating to vaccine adjuvants (distinct from the GLA-SE in this study) and to human in vitro systems that model the safety and immunogenicity of adjuvants, vaccines and immunomodulators. He serves as a consultant to, and receives sponsored research from,

GlaxoSmithKline (GSK) and is a co-founder of A*RMR Sciences*. GDT is a scientific reviewer for the Gilead Scholars and received funding to her institution from BioNTech SE and UVax Bio LLC.

All other authors have no competing interests to declare.

Figures

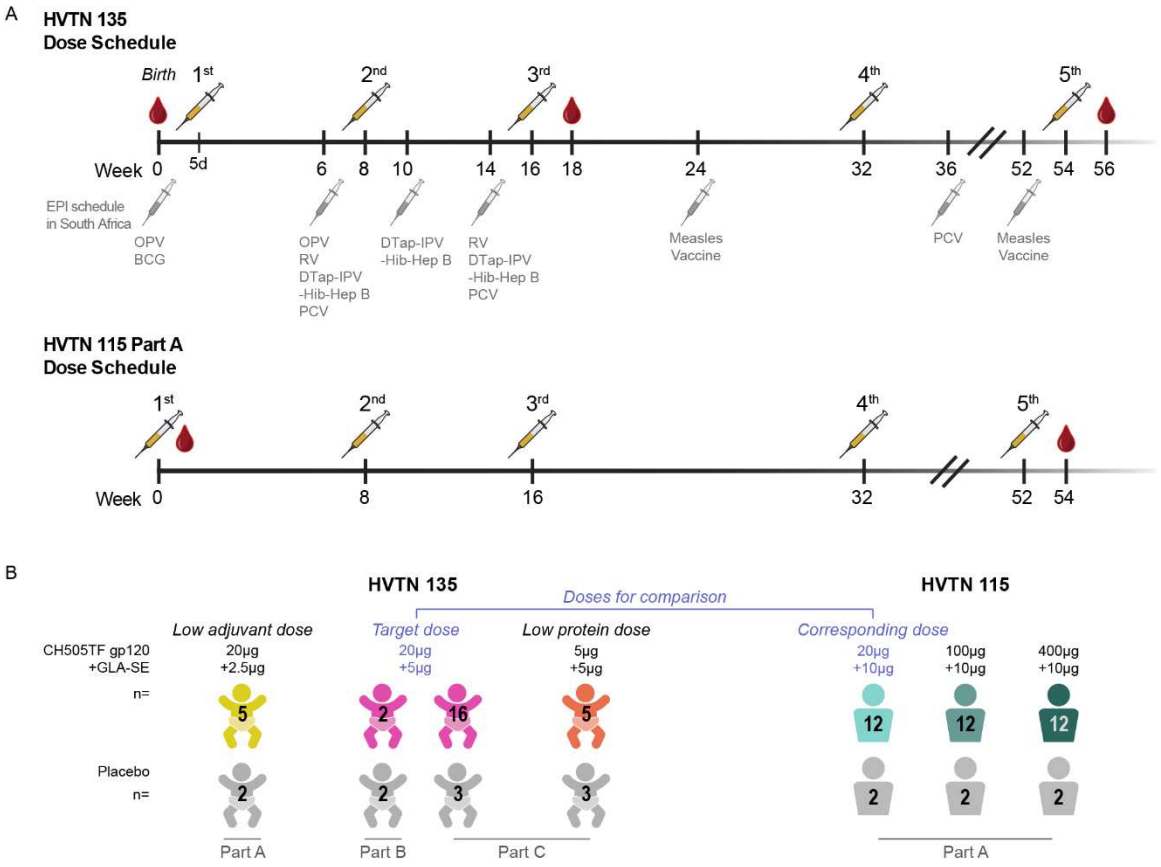


Figure 1. HVTN 115 and HVTN 135 vaccination schedules and study groups. A) HVTN 135 schedule of infant immunizations and blood draws in relation to standard infant immunizations, and HVTN 115 adult immunization schedule and blood draws; B) Schema for HVTN 135 and HVTN 115 Part A.

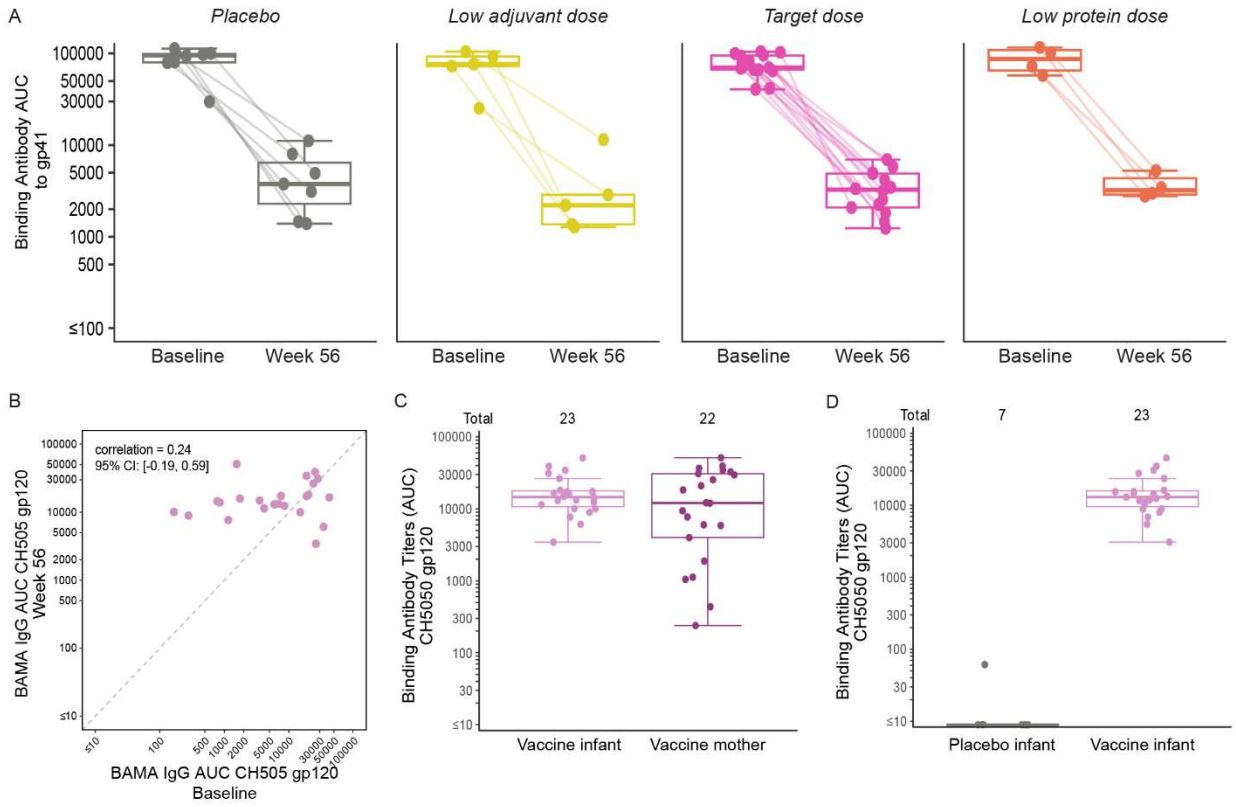


Figure 2. Maternal antibodies among infants in HVTN 135. A) IgG gp41-specific binding Abs at baseline and Week 56 in each treatment group. B) Association between baseline and Week 56 binding antibody responses to vaccine-matched CH505TF gp120 among infants receiving the vaccine. C) Week 56 IgG binding antibodies to CH505TF gp120 in infants receiving CH505 vaccine and their mothers at Week 56 who are living with HIV. D) Week 56 IgG binding antibodies to CH505TF gp120 in infants receiving CH505 vaccine or placebo

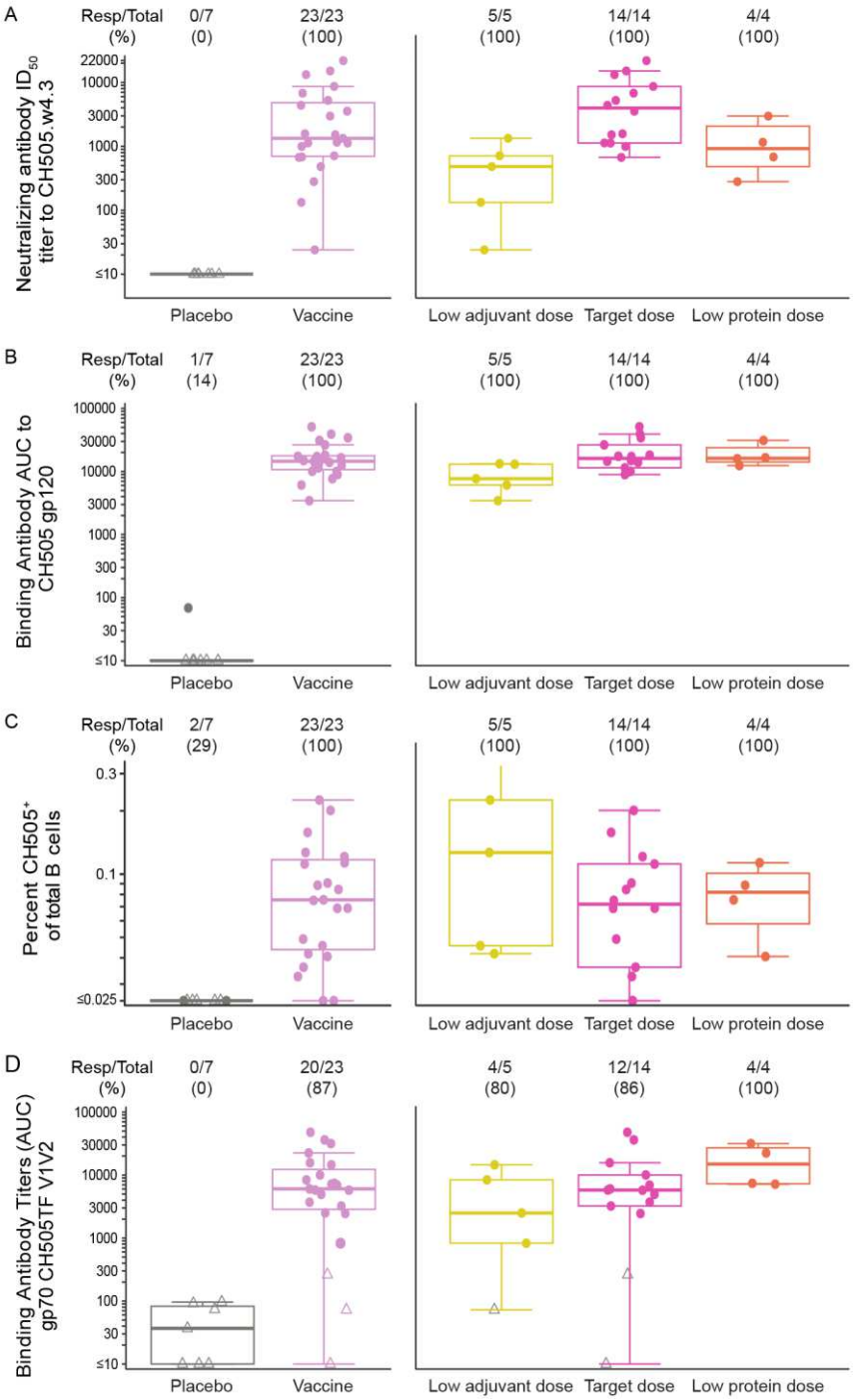


Figure 3. CH505 vaccine-induced humoral immune responses two weeks post-last vaccination among infants in HVTN 135. Immune responses are measured at Week 56 and shown for placebo and vaccine recipients on the left, and by vaccine dose group on the right. A) Neutralizing antibody ID50 titer to the Tier 1A vaccine strain, CH505.w4.3, by TZM-bl assay; B) IgG binding Ab responses to the vaccine-matched CH505TF gp120 by BAMA; C) B cell phenotyping measured by percent of B cells that are CH505+ by flow cytometry. D) IgG binding Ab responses to gp70 CH505TF V1V2 by BAMA. Filled circles are positive responders and open triangles are non-responders. The number of responders out of total and percentage are above the graphs.

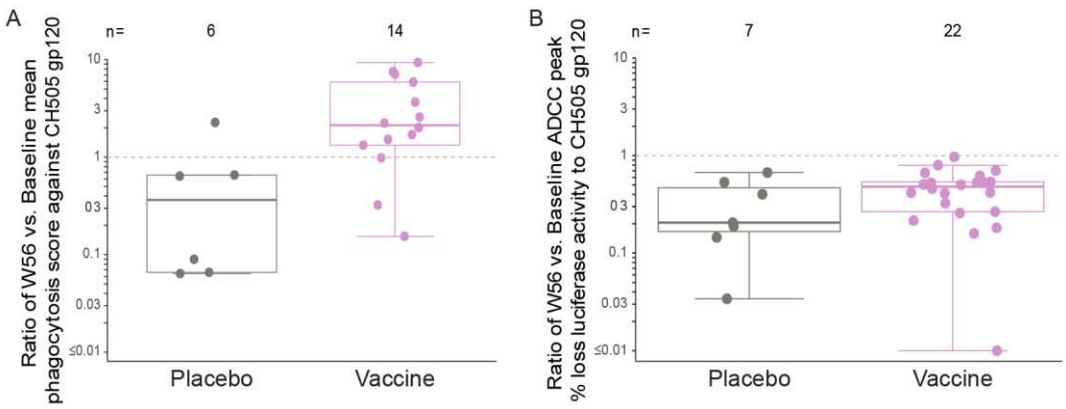


Figure 4. CH505 vaccine-induced ADCP and ADCC functions two weeks post-last vaccination among infants in HVTN 135. A) Ratio of Week 56 versus Baseline ADCP function against the vaccine-matched CH505TF gp120 as measured by mean phagocytosis score, by ADCP assay. B) Ratio of Week 56 versus Baseline ADCC function against the vaccine-matched CH505TF gp120 as measured by peak percent loss luciferase, by ADCC luciferase assay.

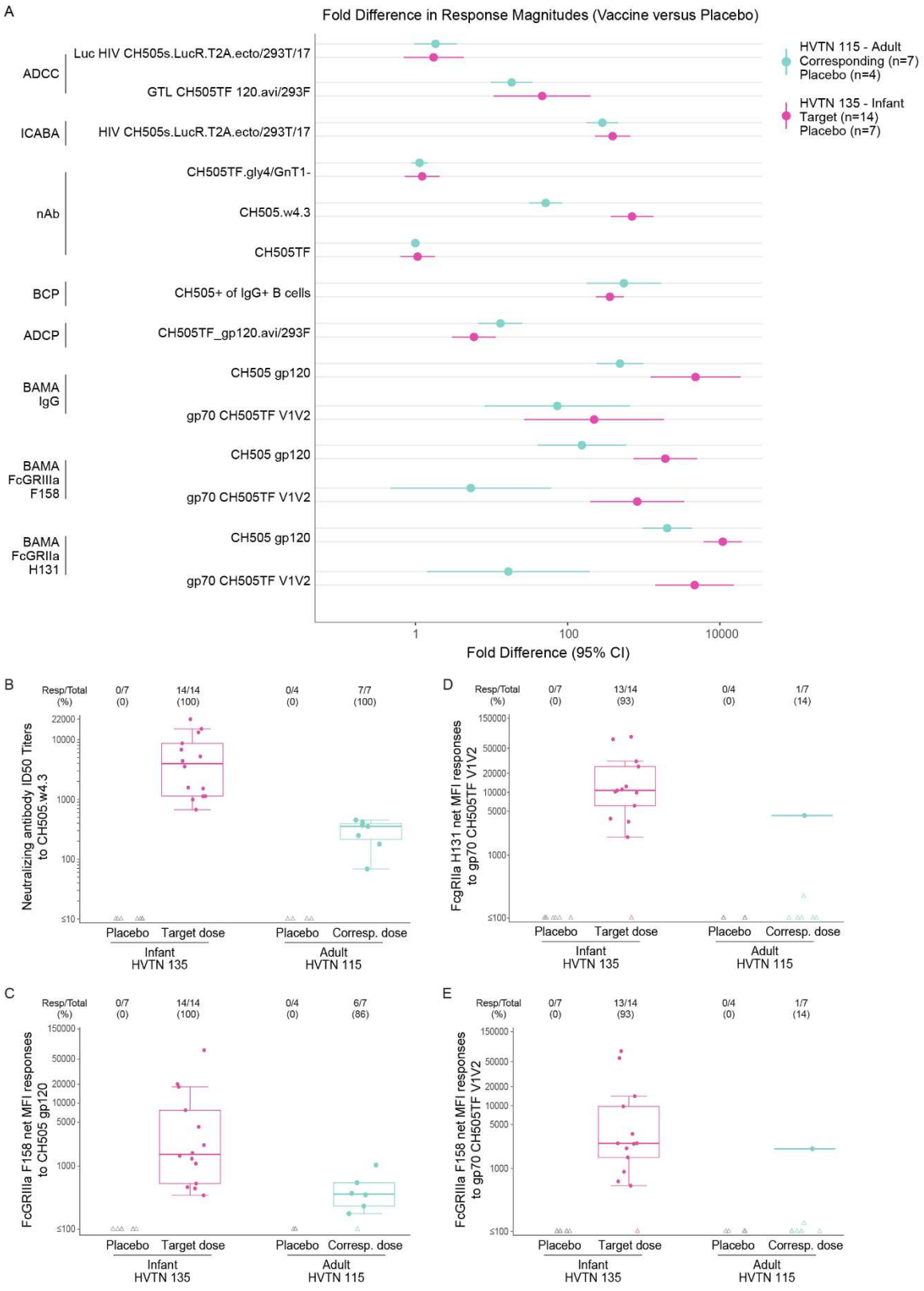


Figure 5. CH505 vaccine-induced immune responses for adults in HVTN 115 and infants in HVTN 135, two weeks post-last vaccination. A) Forest plot comparing vaccine immunogenicity, i.e. fold differences

vaccine versus placebo, for CH505 antigen-specific immune responses in adults versus infants. Boxplots of the four immune responses with the largest differences in vaccine effects, infants versus adults: B) Neutralizing antibody ID50 titer to the tier 1A vaccine strain, CH505.w4.3, by TZM-bl assay. C) FcγRIIIa F158 responses to the vaccine-matched CH505TF gp120 by BAMA. D) FcγRIIa H131 responses to the vaccine-matched CH505TF gp70 by BAMA. E) FcγRIIIa F158 responses to the vaccine-matched CH505TF gp70 by BAMA. Filled circles are positive responders and open triangles are non-responders. The number of responders out of total and percentage are above the graphs.

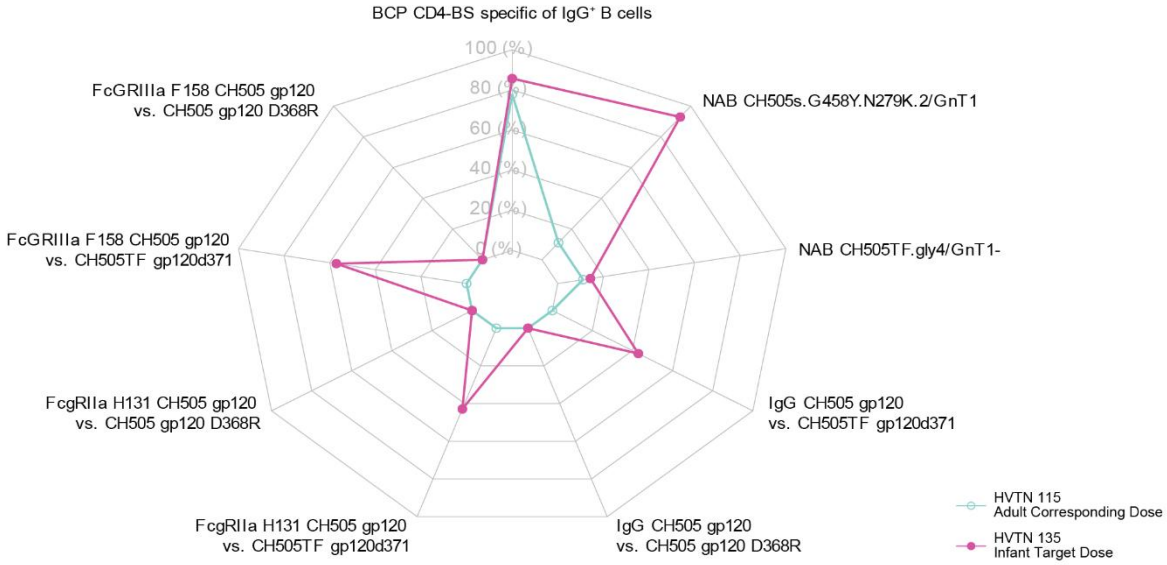


Figure 6. Rates of CD4 binding-site directed immune responses among adults in HVTN 115 and infants in HVTN 135, two weeks post-last vaccination. Radar plot comparing rates of CD4 binding-site-specific immune responses in CH505-vaccinated Infants vs adults.

Tables

Table 1. **Infant binding antibody responses to routine infant immunizations.** Number and percent of infants with protective binding antibody responses to each antigen, by vaccine versus placebo assignment, with 95% confidence interval and results of Fisher's exact test comparing rates between groups. For Pertussis, protective levels have not been established; therefore, detectable levels are reported. Antibody levels were assessed at Week 56 by PVMA and ELISA assays. Analyses are restricted to infants who received the recommended EPI immunizations up to Week 56.

	HVTN 135 Placebo				HVTN 135 Vaccine				
	Num. infants[1]	Num. protective[2]	%	95% CI	Num. infants[1]	Num. protective[2]	%	95% CI	p-value**
Haemophilus influenzae type B	8	4	50	(21.5, 78.5)	23	8	34.8	(18.8, 55.1)	0.58
Hepatitis-B	8	8	100	(67.6, 100)	23	23	100	(85.7, 100)	1.00
Diphtheria	8	7	87.5	(52.9, 97.8)	23	22	95.7	(79, 99.2)	0.55
Tetanus	8	6	75	(40.9, 92.8)	23	15	65.2	(44.9, 81.2)	0.73
Pertussis*	8	8	100	(67.6, 100)	23	23	100	(85.7, 100)	1.00

[1]Number of infants who received all relevant recommended EPI vaccine doses prior to Week 56 for a given antigen.

[2]Number of infants with protective antibody responses to each antigen.

*For Pertussis, protective levels have not been established and therefore number of infants with detectable levels are reported.

** Two-sided 0.05-level Fisher's exact test comparing vaccine and placebo groups.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HVTN135ProtocolVersion3.pdf
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- CONSORTchecklistHVTN135.docx