T cell responses induced by ChAdOx1 nCoV-19 (AZD1222) vaccine to wild-type SARS-CoV-2 among people living with and without HIV in South Africa

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ABSTRACT

Objective(s):

This study aimed to investigate SARS-CoV-2-specific T cell responses 14 days after single-dose ChAdOx1 nCoV-19 (AZD1222) vaccination in black Africans living with and without HIV in South Africa, as well as determine the effect of AZD1222 vaccination on cell-mediated immune responses in people living with HIV (PLWH) with prior SARS-CoV-2 infection.

Methods:

A total of 70 HIV-uninfected people and 104 PLWH were prospectively enrolled in the multicentre, randomised, double-blinded, placebo-controlled, phase Ib/IIa trial (COV005). Peripheral blood mononuclear cells were collected from trial participants 14 days after receipt of first dose of study treatment (placebo or AZD1222 vaccine). T cell responses against the full-length spike (FLS) glycoprotein of wild-type (WT) SARS-CoV-2 and mutated S-protein regions found in the Alpha, Beta, and Delta variants were assessed using an ex vivo ELISpot assay.

Results:

Among AZD1222 recipients without preceding SARS-CoV-2 infection, T cell responses to FLS of WT SARS-CoV-2 were similarly common in PLWH and HIV-uninfected people (30/33, 90.9% vs. 16/21, 76.2%; p=0.138); and magnitude of response was similar among responders (78 vs. 56 SFCs/10^6 PBMCs; p=0.255). Among PLWH, AZD1222 vaccinees with prior SARS-CoV-2 infection, displayed a heightened T cell response magnitude compared to those without prior infection (186 vs. 78 SFCs/10^6 PBMCs; p=0.001); and similar response rate (14/14, 100% vs. 30/33, 90.9%; p=0.244).

Conclusions:

Our results indicate comparable levels of protection against Covid-19 following AZD1222 vaccination in HIV-uninfected people and PLWH. Our results additionally show that hybrid immunity acquired through SARS-CoV-2 infection and AZD1222 vaccination, may confer heightened protection.

KEY WORDS:

AZD1222; ChAdOx1 nCoV-19; People living with HIV; SARS-CoV-2; T cell responses

MAIN TEXT

Introduction

The non-replicating simian adenovirus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine (ChAdOx1 nCoV-19 or AZD1222), is efficacious in protecting against symptomatic coronavirus disease 2019 ('Covid-19') due to wild-type (WT) SARS-CoV-2 [1], as well as protecting against severe Covid-19 due to variants of concern. AZD1222 includes the full-length spike glycoprotein (FLS) gene of WT SARS-CoV-2 and induces humoral and cell-mediated immune responses after vaccination [2-5]. Cell-mediated immune responses, particularly epitope-specific T cells producing interferon-gamma (IFN-γ), can be detected using enzyme-linked immunospot (ELISpot) from day-7 and peak at day-14 following the first dose of AZD1222 [1, 2, 4]. No significant changes in cellular immunity have been observed between the first and second homologous AZD1222 dose, hence day-14 likely represents the peak of cellular responses [1].

The magnitude and duration of spike-specific IFN-γ producing T cells to WT SARS-CoV-2 elicited by AZD1222, have been shown to be similar between people living with HIV (PLWH) and without HIV in a study from the United Kingdom [4]. Nevertheless, there is a paucity of information on AZD1222 and other Covid-19 cell-mediated immune responses in PLWH, including the effect of SARS-CoV-2 infection prior to vaccination on T cell responses. Here we report on SARS-CoV-2-specific T cell responses, 14 days after the first dose of study injection (placebo or AZD1222 vaccine) against WT SARS-CoV-2 in HIV-uninfected people and PLWH in South Africa. We also report on the effect of AZD1222 vaccination on cell-mediated immune responses in those previously infected with SARS-CoV-2.

Methods

Participants

Participants were enrolled in the multicentre, randomised, double-blinded, placebo-controlled, phase Ib/IIa trial (COV005) in South Africa, which assessed the safety and immunogenicity of ChAdOx1 nCoV-19 (AZD1222) vaccine in HIV-uninfected people and PLWH as described previously [5, 6]. In the phase Ib component of the study, we enrolled participants between 18-65 years to evaluate T cell responses in HIV-uninfected people (n=70) and PLWH (n=104). Participants were fully informed about trial procedures and possible risks before providing written consent. Details of the parent study, including interim results on the safety, vaccine efficacy and humoral immune responses have been published previously [5]. Summarised information about study design and participant enrolment are described in the supplementary text.

Laboratory procedures

SARS-CoV-2 infections at baseline and throughout the trial were identified with nucleic acid amplification tests (NAATs) [5]. Baseline SARS-CoV-2 serostatus (anti-nucleocapsid IgG) was also determined. Peripheral blood mononuclear cells (PBMCs) were collected from trial participants on day-14 following receipt of study treatment and cryopreserved [4, 5]. T cell responses against FLS of WT SARS-CoV-2, as well as against selectively mutated S-protein regions found in the Alpha, Beta, and Delta variants of concern were assessed using cryopreserved PBMCs and an *ex vivo* ELISpot assay with commercially available peptide antigens as detailed in the supplementary text. T cell responses were quantified by IFN-γ producing T cells expressed as spot-forming cells per million (SFCs/10^6) PBMCs by subtracting unstimulated background responses from antigen-stimulated responses. Positive responders were defined as having >10 SFCs/10^6 PBMCs. Additional details about laboratory procedures are provided in the supplementary text.

Statistical analysis

Summarised demographic and clinical characteristics are reported as medians with interquartile ranges for quantitative variables (age and $CD4^+$ counts), and as counts with percentages (proportions) for categorical variables. T cell responses for positive responders are summarised

as geometric mean SFCs/10^6 PBMCs with 95% confidence intervals ($CI_{95\%}$). The Chi-square test and student's t-test (unpaired) were used for comparing proportions and geometric means, respectively. Statistical analysis and graphical representation were performed using R (v.4.02) and GraphPad Prism (v.9.3.1; GraphPad Prism Software, LLC), respectively. *P* values <0.05 were considered statistically significant.

Study approvals

The study forms part of the COV005 trial which is registered with ClinicalTrials.gov (NTC04444674), and the Pan African Trials Registry (PACTR202006922165132). The trial's protocol (v.6.0) [7] was approved by the South African Health Products Regulatory Authority, and the human ethics committees of the University of Oxford, University of the Witwatersrand, Stellenbosch University, and University of Cape Town.

Results

We enrolled 70 HIV-uninfected people and 104 PLWH in the study. T cell responses were evaluated at day-14 following the first dose of study injection (Supplementary Digital Fig. S1). Sixteen participants (HIV-uninfected: n=9; PLWH: n=7) were excluded from the analysis: study withdrawal (n=1), PBMCs not collected (n=8), non-viable PBMCs at the time of analysis (n=5), and no baseline (day-0) SARS-CoV-2 serology (n=2). Ten participants (eight HIV-uninfected and two PLWH) with a positive SARS-CoV-2 NAAT before or at baseline, as well as nine participants (eight HIV-uninfected and one PLWH) with a positive SARS-CoV-2 NAAT between days 0-14, were also excluded.

The remaining 139 participants comprised of 99.3% black Africans (138/139), 69 of whom received AZD1222 (HIV-uninfected: n=22; PLWH: n=47) and 70 placebo (HIV-uninfected: n=23; PLWH: n=47; Table 1). Underlying comorbidities included 21.6% (30/139) with obesity, 8.6% (12/139) with hypertension and 10.1% (14/139) with chronic respiratory conditions, which were similar between HIV-uninfected people and PLWH as previously described [5]. Seventy-two percent (68/94) of PLWH were female and the overall median age was 40 years (IQR: 33-46); whilst in the HIV-uninfected group, 37.8% (17/45) were female with a median age of 34 years (IQR: 26-42). The median $CD4^+$ T cell count for PLWH was 680 cells/µL (IQR: 503-911) with median HIV-1 viral loads of 10 copies/mL (IQR: 10-50).

Among HIV-uninfected people who were anti-N IgG seronegative at baseline, 76.2% (16/21) and 54.5% (12/22; p=0.137) of AZD1222 and placebo recipients respectively, exhibited T cell responses above the cut-off threshold against WT FLS (Fig. 1a). Geometric mean IFN-γ producing T cells of positive responders were 56 ($CI_{95\%}$: 35-90) and 36 ($CI_{95\%}$: 20-64) SFCs/10^6 PBMCs in AZD1222 and placebo recipients, respectively; p=0.195. Among PLWH who were SARS-CoV-2 anti-N IgG seronegative at enrolment, the proportion of those with T cell responses was higher in AZD1222 (90.9%, 30/33) compared with placebo recipients (66.7%, 20/30; p=0.018; Fig. 1b). Also, the magnitude of IFN-γ producing T cells of positive responders was higher in AZD1222 (78 SFCs/10^6 PBMCs; $CI_{95\%}$: 55-109) than placebo recipients (33 SFCs/10^6 PBMCs;

$CI_{95\%}$: 25-42; p<0.001). Placebo group responders were similar in HIV-uninfected people and PLWH (54.5% vs. 66.7%; p=0.375) who were anti-N seronegative. Among AZD1222 recipients without preceding SARS-CoV-2 infection, T cell responses were more common in PLWH compared with HIV-uninfected people (90.9% vs. 76.2%; p=0.138); and magnitude of response was similar among the responders (78 vs. 56 SFCs/10^6 PBMCs; p=0.255).

In PLWH who had SARS-CoV-2 infection prior to study injection, all AZD1222 recipients (100%, 14/14) and 82.4% (14/17; p=0.098) of the placebo group demonstrated T cell responses to WT FLS (Fig. 1c). The T cell response magnitude in AZD1222 recipients (186 SFCs/10^6 PBMCs; $CI_{95\%}$: 130-267), however, was higher than the placebo group (60 SFCs/10^6 PBMCs; $CI_{95\%}$: 41-88; p<0.001) among responders. Also, PLWH vaccinated with AZD1222 following prior SARS-CoV-2 infection, displayed an increased T cell response magnitude compared to vaccinated PLWH without prior SARS-CoV-2 infection (186 vs. 78 SFCs/10^6 PBMCs; p=0.001); and similar response rate (100% vs. 90.9%; p=0.244).

We also investigated cellular immune responses to selectively mutated S-protein regions found in the Alpha, Beta, and Delta variants. No significant differences (p>0.05) were found between WT and variant pools, regardless of HIV-status, SARS-CoV-2 anti-N IgG serostatus, or receipt of placebo and AZD1222 (Supplementary Digital Fig. S2-S5).

Discussion

We show that T cell responses to FLS of WT SARS-CoV-2 14 days after single-dose AZD1222 vaccination was similar in black Africans living with and without HIV who were SARS-CoV-2 anti-N IgG seronegative when vaccinated. Our findings of similar T cell responses in PLWH and HIV-uninfected AZD1222 recipients among those who were anti-N seronegative at enrolment, corroborate the observations of a study in predominantly white Europeans which also investigated AZD1222 induced IFN-γ producing T cells using the ELISpot method [4].

We also show that T cell responses following AZD1222 vaccination was heightened in PLWH who had been infected by SARS-CoV-2 prior to vaccination compared with those who were anti-N seronegative at enrolment. In PLWH, prior SARS-CoV-2 infection in the placebo group was associated with similar T cell responses and magnitude of response compared with the AZD1222 recipients who were SARS-CoV-2 anti-N IgG seronegative. Also, single-dose AZD1222 vaccination following SARS-CoV-2 infection in PLWH elicited a greater T cell response magnitude compared to vaccinated PLWH without prior SARS-CoV-2 exposure, suggesting an additive benefit of vaccination following primary infection. The observation of preceding SARS-CoV-2 infection inducing higher T cell responses in AZD1222 vaccinees among PLWH, is similar to that reported in HIV-uninfected people following administration of a single-dose messenger RNA vaccine (BNT162b2) in convalescent individuals [8-10].

We observed a modest T cell response in placebo recipients who were anti-N seronegative at enrolment in PLWH (66.7%) and HIV-uninfected people (54.5%); which is likely attributable to cross-reactive T cells due to exposure to endemic human coronaviruses [11]. Cross-reactive T cells have also been found in SARS-CoV-2 anti-N IgG seronegative Europeans comprising the control group who had received the meningococcal conjugate vaccine (MenACWY) in a phase 1/2 single-blind, randomised controlled AZD1222 trial [1].

We could not differentiate cellular immune responses between placebo and AZD1222 recipients using the ELISpot method; which can be considered a limitation. Our inability to differentiate T

cell responses between the placebo and AZD1222 groups, may be ascribed to the Alpha, Beta, and Delta variant pools. These pools only contained S-protein immunodominant T cell epitopes that are affected by variant-specific mutations in contrast to the entire FLS pool that can contribute more epitopes to elicit greater T cell responses. Poly-epitopic T cell responses induced by Covid-19 vaccines are relatively unaffected by mutations contributing to antibody-evasiveness, and even less so following T cell responses induced by past infections from heterologous variants [12-14]. The persistent high vaccine effectiveness against severe Covid-19 has been attributed to the relative conservation of T cell immunity from vaccination and natural infection, even when effectiveness against SARS-CoV-2 infection and mild Covid-19 have diminished due to antibody waning or relative antibody-evasiveness of some variants.

Our study only investigated quantitative T cell responses following receipt of study treatment between HIV-uninfected participants and PLWH and did not investigate qualitative differences in T cell polyfunctionality and other cellular phenotypes. Another limitation of this study includes the small sample size of HIV-uninfected participants who were anti-N IgG seropositive at baseline, probably due to the different time of enrolment of the two HIV groups, which precluded comparisons between seropositive participants living with and without HIV. Also, the restrictive enrolment criteria of PLWH, prevents generalisability to the overall black African population living with HIV in South Africa.

In conclusion, due to the similarity of cellular immune responses between HIV-uninfected people and PLWH on stable antiretroviral therapy, a similar level of protection is expected against severe Covid-19 following AZD1222 vaccination. Additionally, hybrid immunity acquired through SARS-CoV-2 infection and vaccination, may confer heightened protection; which is particularly important in countries where vaccines are belatedly rolled out only after a significant percentage of the population have developed immunity.

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Author contributions

W.C.M. and G.K. prepared this manuscript. S.A.M., A.L.K., J.G., L.F., C.L.C., G.K. and F.P. enrolled trial participants, as well as collected data and samples. W.C.M., C.K.M., N.J.M., and R.L. processed samples. W.C.M. and G.K. generated data. W.C.M., G.K. and A.I. analysed data by performing statistical analyses. W.C.M., G.K., C.K.M., A.L.K., J.G., L.F., F.P., W.A.B., M.C.N., C.L.C, S.C.G., T.L., A.J.P., and S.A.M. interpreted the results. All authors had full access to this study's data and had final responsibility for the decision to submit to publication.

Data availability

Anonymised participant data will be made available upon request directed to the corresponding author.

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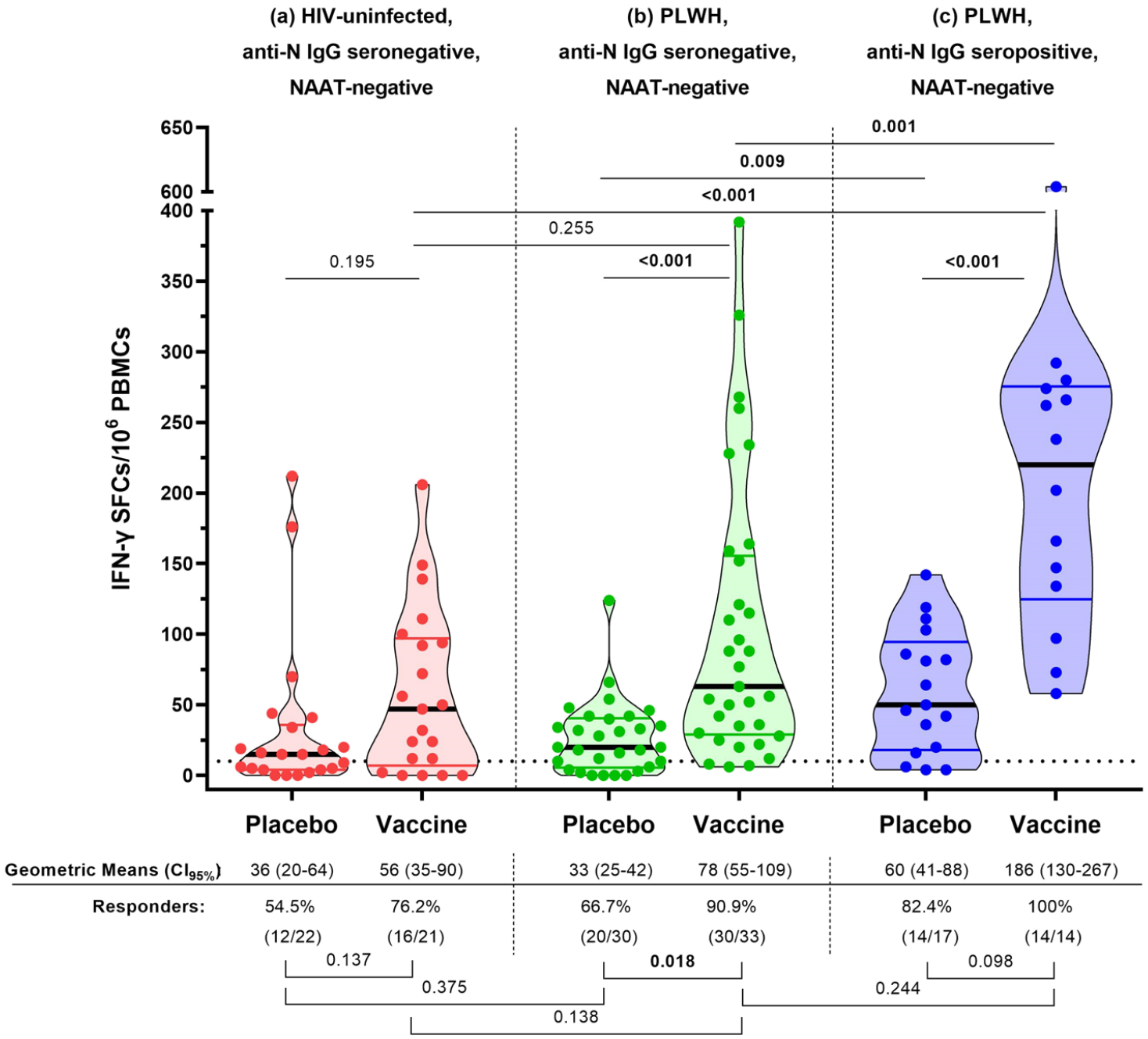
FIGURES

Fig. 1. IFN-γ T cell responses against FLS of WT SARS-CoV-2 in placebo and AZD1222 recipients living with and without HIV.

(a) T cell responses in HIV-uninfected people without evidence of prior SARS-CoV-2 infection (placebo: n=22; AZD1222: n=21) at baseline. **(b)** T cell responses in PLWH without evidence of prior SARS-CoV-2 infection (placebo: n=30; AZD1222: n=33) at baseline. **(c)** T cell response in PLWH with evidence of prior SARS-CoV-2 infection (placebo: n=17; AZD1222: n=14). Individual points represent the total IFN-γ spot-forming-cells per million (SFCs/10^6) PBMCs of each participant. Bold black lines and coloured lines represent the median and interquartile ranges respectively. The dotted line represents the lower limit of positive responders at 10 SFCs/10^6 PBMCs. Geometric mean SFCs/10^6 PBMCs (95% confidence intervals) were exclusively determined for positive responders using normalized data. The Chi-square test and student's t-test (unpaired) were used for comparisons of proportions and geometric means, respectively (significant *P* values are indicated in bold). Denotations: FLS=full-length spike glycoprotein; IFN-γ=interferon-gamma; IgG=immunoglobulin G; anti-N=anti-nucleocapsid protein of SARS-CoV-2; NAAT=nucleic acid amplification test; PBMC=peripheral blood mononuclear cells; PLWH=people living with HIV.

Fig. 1


(a) HIV-uninfected, anti-N IgG seronegative, NAAT-negative
(b) PLWH, anti-N IgG seronegative, NAAT-negative
(c) PLWH, anti-N IgG seropositive, NAAT-negative
IFN-γ SFCs/10^6 PBMCs
650
600
400
350
300
250
200
150
100
50
0
0.001
0.009
<0.001
0.255
0.195
<0.001
<0.001
Placebo
Vaccine
Placebo
Vaccine
Placebo
Vaccine
Geometric Means (CI95%)
36 (20-64)
56 (35-90)
33 (25-42)
78 (55-109)
60 (41-88)
186 (130-267)
Responders:
54.5% (12/22)
76.2% (16/21)
66.7% (20/30)
90.9% (30/33)
82.4% (14/17)
100% (14/14)
0.137
0.018
0.098
0.375
0.244
0.138

TABLES

Table 1: Baseline demographics for SARS-CoV-2 NAAT-negative participants, stratified by baseline SARS-CoV-2 serostatus and HIV status*

Variable	Overall	Anti-N IgG Seronegative			Anti-N IgG Seropositive		
	HIV-uninfected people						
Number tested	**Total (N=45)**	**Total (n=43)**	**Placebo (n=22)**	**Vaccine (n=21)**	**Total (n=2)**	**Placebo (n=1)**	**Vaccine (n=1)**
Median age, years (IQR)	34 (26-42)	34 (26-42)	33 (26-44)	34 (24-40)	34 (33-36)	31 (31-31)	37 (37-37)
Sex							
Female	17 (37.8)	16 (37.2)	8 (36.4)	8 (38.1)	1 (50)	0 (0)	1 (100)
Male	28 (62.2)	27 (62.8)	14 (63.6)	13 (61.9)	1 (50)	1 (100)	0 (0)
Race							
Black	45 (100)	43 (100)	22 (100)	21 (100)	2 (100)	1 (100)	1 (100)
Body-mass index (BMI)							
Underweight (0 to <18.5)	5 (11.1)	5 (11.6)	4 (18.2)	1 (4.8)	0 (0)	0 (0)	0 (0)
Normal (18.5 to <25)	19 (42.2)	19 (44.2)	10 (45.5)	9 (42.9)	0 (0)	0 (0)	0 (0)
Overweight (25 to <30)	11 (24.4)	9 (20.9)	2 (9.1)	7 (33.3)	2 (100)	1 (100)	1 (100)
Obese (≥30)	10 (22.2)	10 (23.3)	6 (27.3)	4 (19.0)	0 (0)	0 (0)	0 (0)
Hypertension	1 (2.2)	1 (2.3)	0 (0)	1 (4.8)	0 (0)	0 (0)	0 (0)
Current alcohol user	20 (44.4)	19 (44.2)	11 (50)	8 (38.1)	1 (50)	0 (0)	1 (100)
Current smoker	23 (51.1)	22 (51.2)	11 (50)	11 (52.4)	1 (50)	0 (0)	1 (100)

Healthcare worker	1 (2.2)	1 (2.3)	1 (4.5)	0 (0)	0 (0)	0 (0)	0 (0)
Median CD4+ count, cells/μL (IQR)	NA	NA	NA	NA	NA	NA	NA
People living with HIV							
Number tested	**Total (N=94)**	**Total (n=63)**	**Placebo (n=30)**	**Vaccine (n=33)**	**Total (n=31)**	**Placebo (n=17)**	**Vaccine (n=14)**
Median age, years (IQR)	40 (33-46)	37 (33-45)	40 (36-44)	36 (31-47)	43 (40-47)	44 (40-47)	44 (33-48)
Sex							
Female	68 (72.3)	45 (71.4)	23 (76.7)	22 (66.7)	23 (74.2)	14 (82.4)	9 (64.3)
Male	26 (27.7)	18 (28.6)	7 (23.3)	11 (33.3)	8 (25.8)	3 (17.6)	5 (35.7)
Race							
Black	93 (98.9)	62 (98.4)	30 (100)	32 (97)	31 (100)	17 (100)	14 (100)
White	1 (1.1)	1 (1.6)	0 (0)	1 (3)	0 (0)	0 (0)	0 (0)
Body-mass index (BMI)							
Underweight (0 to <18.5)	5 (5.3)	4 (6.3)	0 (0)	4 (12.1)	1 (3.2)	1 (5.9)	0 (0)
Normal (18.5 to <25)	40 (42.6)	24 (38.1)	11 (36.7)	13 (39.4)	16 (51.6)	5 (29.4)	11 (78.6)
Overweight (25 to <30)	29 (30.8)	24 (38.1)	11 (36.7)	13 (39.4)	5 (16.1)	4 (23.5)	1 (7.1)
Obese (≥30)	20 (21.3)	11 (17.5)	8 (26.7)	3 (9.1)	9 (29.0)	7(41.2)	2 (14.3)
Hypertension	11 (11.7)	6 (9.5)	2 (6.7)	4 (12.1)	5 (16.1)	5 (29.4)	0 (0)
Respiratory illness	14 (14.9)	8 (12.7)	6 (20)	2 (6.2)	6 (19.4)	3 (17.6)	3 (21.4)
Current alcohol drinker	43 (45.7)	25 (40)	9 (30)	16 (48.5)	18 (58.1)	9 (52.9)	9 (64.3)

Current smoker	31 (33)	21 (33.3)	9 (30)	12 (36.4)	10 (32.3)	5 (29.4)	5 (35.7)
Healthcare worker	2 (2.1)	2 (3.2)	1 (3.3)	1 (3)	0 (0)	0 (0)	0 (0)
Median CD4+ count, cells/µL (IQR)	680 (503-911)	667 (504-907)	585 (489-852)	746 (570-940)	695 (505-916)	739 (532-920)	642 (499-874)
Median CD4+ percentage (IQR)	35.6 (29.7-39.4)	36.3 (29.9-41.2)	34.8 (28.1-39.3)	36.9 (31.7-41.3)	34.8 (30.1-37.8)	34.8 (28.3-38.6)	34.8 (32.3-37.2)
HIV-1 viral load <50 copies/mL	26 (27.7)	19 (30.2)	12 (40)	7 (21.2)	7 (22.6)	6 (35.3)	1 (7.1)
ARTs							
Boosted PI + 1 NRTI	3 (3.2)	2 (3.2)	2 (76.7)	0 (0)	1 (3.2)	1 (5.9)	0 (0)
Boosted PI + 2 NRTIs	3 (3.2)	1 (1.6)	1 (3.3)	0 (0)	2 (6.5)	1 (5.9)	1 (7.1)
INSTI + 2 NRTIs	10 (10.6)	7 (11.1)	4 (13.3)	3 (9.1)	3 (9.7)	1 (5.9)	2 (14.3)
NNRTI +2 NRTIs	51 (54.3)	39 (61.9)	17 (56.7)	22 (66.7)	12 (38.7)	8 (47.1)	4 (28.6)
ART duration							
<1 year	8 (8.5)	5 (7.9)	3 (10)	2 (6.1)	3 (9.7)	1 (5.9)	2 (14.3)
1 to <5 years	24 (25.5)	20 (31.7)	9 (30)	11 (33.3)	4 (12.9)	1 (5.9)	3 (21.4)
≥5 years	35 (37.2)	24 (38.1)	12 (40)	12 (36.4)	11 (35.5)	9 (52.9)	2 (14.3)

*Data are n (%) unless otherwise stated. Data exclude participants with a positive SARS-CoV-2 NAAT before or on the day of receiving study injection (placebo or AZD1222 vaccine), as well as participants that acquired SARS-CoV-2 infection within 14 days following study injection. Denotations: ART=antiretroviral treatment; IgG=immunoglobulin G; HIV=human immunodeficiency virus; INSTI=integrase strand transfer inhibitor; IQR=interquartile rage; anti-N=anti-nucleocapsid protein of SARS-CoV-2; NA=not applicable; NAAT=nucleic acid

amplification test; NNRTI=non-nucleoside reverse transcriptase inhibitor; NRTI=nucleoside or nucleotide reverse transcriptase inhibitors; PI=protease inhibitor; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

SUPPLEMENTARY DIGITAL CONTENT

Methods

Study design and enrolment

Between June 24, 2020, and July 29, 2020, and between August 17, 2020, and November 12, 2020, 70 HIV-uninfected people and 104 people living with HIV (PLWH) were enrolled in a randomised, double-blinded, placebo-controlled, phase Ib/IIa trial (COV005) at two South African sites (Vaccines and Infectious Diseases Analytics (VIDA) Research Unit; Wits Reproductive Health and HIV Institute (RHI) Research Centre), for intensive vaccine safety and immunogenicity monitoring [5, 6]. Enrolled participants were healthy adults between 18-65 years with confirmed HIV statuses. Detailed inclusion and exclusion criteria of participants are described in the trial protocol [7]. PLWH had additional requirements for eligibility, which included stable antiretroviral therapy (ART) for at least three months, and human immunodeficiency virus type 1 (HIV-1) viral loads of less than 1000 copies per mL within two weeks of trial randomisation. Enrolled participants were randomly assigned (1:1) to the placebo group (injected with 0.9% sodium chloride per dose), and the AZD1222 vaccine group (injected with 5×5^{10} virus particles per dose).

Processing of peripheral blood mononuclear cells

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples by density gradient centrifugation using Lymphoprep™ (STEMCELL Technologies) and cryopreserved in liquid nitrogen as previously described [4]. Cryopreserved PBMCs were thawed as previously described [15] and counted using a hemacytometer (Bright-line Improved Neubauer 0.1 mm, Sigma-Aldrich) and light microscope (DM500, Leica Microsystems).

Peptide antigen pools

Commercially available PepTivator® SARS-CoV-2 peptide pools (Miltenyi Biotec) were used as antigens for T cell stimulation. Peptide pools Prot_S1 (Cat# 130-127-048) and Prot_S (Cat# 130-126-701) were combined to represent the FLS of WT SARS-CoV-2 [13, 14]. Prot_S B.1.1.7 (Cat# 130-127-844), Prot_S B.1.351 (Cat# 130-127-958), and Prot_S B.1.617.2 (Cat# 130-128-763), were included to represent selectively mutated regions found in the S-protein of the Alpha, Beta, and Delta variants of

concern. Three corresponding reference pools (Cat# 130-127-841, Cat# 130-127-952, and Cat# 130-128-761), containing 30-34 homologous peptides of WT SARS-CoV-2 (WT pools: partial S-protein regions of WT SARS-CoV-2) were included as controls for the variant pools.

***Ex vivo* IFN-γ enzyme-linked immunospot assay**

Cryopreserved PBMCs were tested in a blinded fashion using an *ex vivo* IFN-γ enzyme-linked immunospot (ELISpot) assay with Human IFN-γ ELISpot^{BASIC} kits (Mabtech AB). A total of 2-2.5 x 10^5 PBMCs were stimulated in duplicate with 1 µg/mL peptide pools for 18-20 hours. Peptide pools were titrated to identify the lowest concentration that can elicit an IFN-γ response. Unstimulated negative controls and 10 µg/mL Phytohemagglutinin-L (Sigma-Aldrich) positive controls were included. ELISpot plates were counted using an ELISpot plate reader (Motorized Zeiss Axio Imager M1, Carl Zeiss) and KS ELISpot software (v.4.11). Results are expressed as spot-forming-cells per million (SFCs/10^6) PBMCs, calculated by subtracting mean background responses in unstimulated control wells from mean responses in antigen-stimulated wells. Positive responders were defined as having >10 SFCs/10^6 PBMCs.

Supplementary figures

Fig. S1. Study profile stratified by SARS-CoV-2 sero- and molecular status.

Denotations: ELISpot=enzyme-linked immunosorbent spot; IFN-γ=interferon-gamma; IgG=immunoglobulin G; anti-N=anti-nucleocapsid protein of SARS-CoV-2; NAAT=nucleic acid amplification test; PBMC=peripheral blood mononuclear cells.

Fig. S2. IFN-γ T cell responses against the partial S-protein region of the Alpha variant in AZD1222 and placebo recipients living with and without HIV in South Africa.

(a) T cell responses in HIV-uninfected people without evidence of prior SARS-CoV-2 infection at baseline. **(b)** T cell responses in PLWH without evidence of prior SARS-CoV-2 infection at baseline. **(c)** T cell response in PLWH with evidence of prior SARS-CoV-2 infection. Denotations: FLS=full-length spike glycoprotein; IFN-γ=interferon-gamma; IgG=immunoglobulin G; anti-N=anti-nucleocapsid protein of SARS-CoV-2; NAAT=nucleic acid amplification test; PBMC=peripheral blood mononuclear cells; PLWH=people living with HIV; WT Pool=wild-type reference pool.

Fig. S3. IFN-γ T cell responses against the partial S-protein region of the Beta variant in AZD1222 and placebo recipients living with and without HIV in South Africa.

Identical figure legend as Fig. S2.

Fig. S4. IFN-γ T cell responses against the partial S-protein region of the Delta variant in AZD1222 and placebo recipients living with and without HIV in South Africa.

Identical figure legend as Fig. S2.

Fig. S5. IFN-γ T cell responses against FLS of WT SARS-CoV-2, as well as against the Alpha, Beta, and Delta variants in SARS-CoV-2 anti-N IgG seronegative AZD1222 recipients living with and without HIV.

(**a**) T cell responses against FLS of WT SARS-CoV-2. (**b**) T cell responses against the Alpha variant of concern. (**c**) T cell responses against the Beta variant. (**d**) T cell responses against the Delta variant of

concern. Denotations: FLS=full-length spike glycoprotein; IFN-γ=interferon-gamma; PBMC=peripheral blood mononuclear cells; PLWH=people living with HIV; WT Pool=wild-type reference pool.

Fig. S1

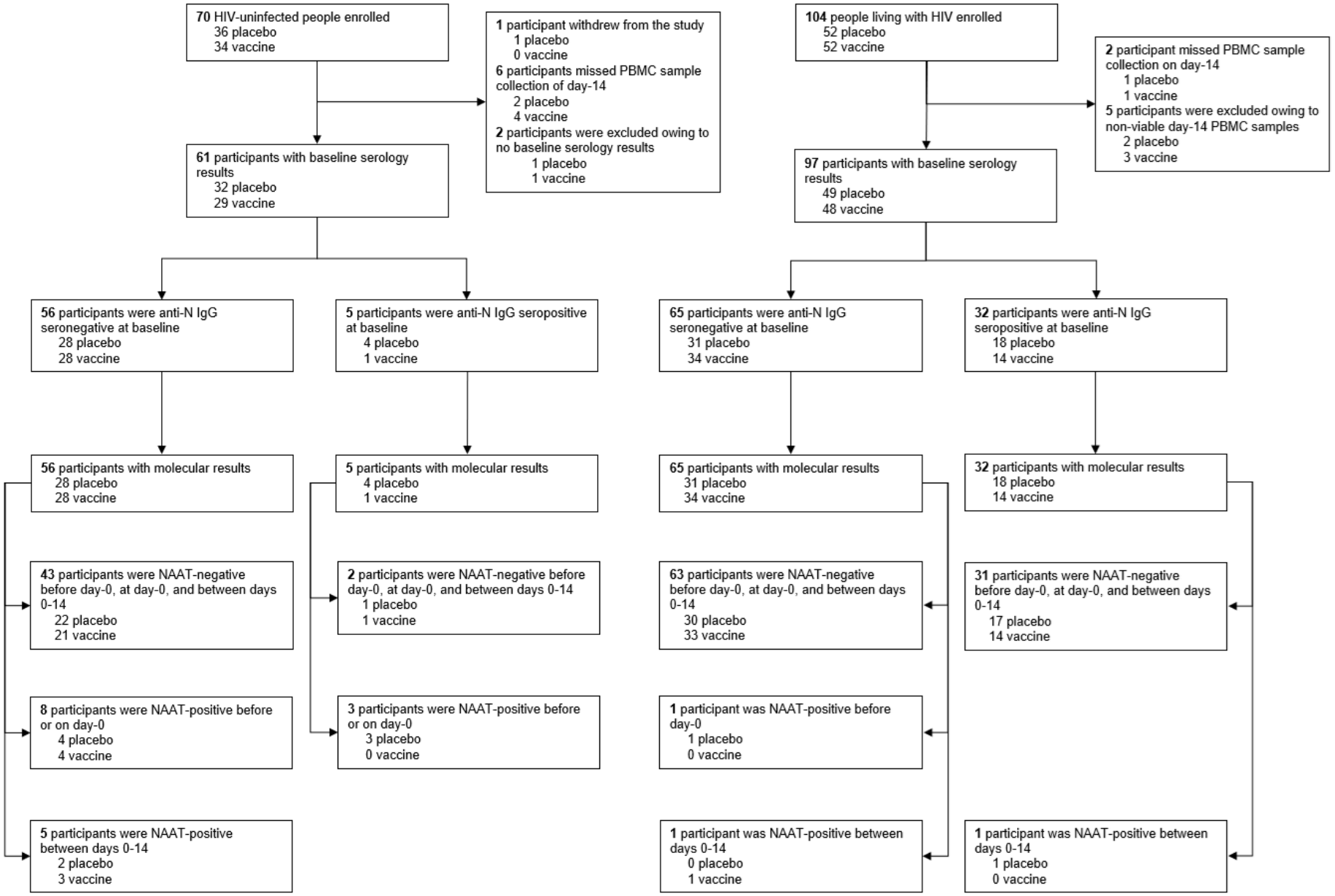

70 HIV-uninfected people enrolled
36 placebo
34 vaccine
1 participant withdrew from the study
1 placebo
0 vaccine
6 participants missed PBMC sample collection of day-14
2 placebo
4 vaccine
2 participants were excluded owing to no baseline serology results
1 placebo
1 vaccine
61 participants with baseline serology results
32 placebo
29 vaccine
104 people living with HIV enrolled
52 placebo
52 vaccine
2 participant missed PBMC sample collection on day-14
1 placebo
1 vaccine
5 participants were excluded owing to non-viable day-14 PBMC samples
2 placebo
3 vaccine
97 participants with baseline serology results
49 placebo
48 vaccine
56 participants were anti-N IgG seronegative at baseline
28 placebo
28 vaccine
5 participants were anti-N IgG seropositive at baseline
4 placebo
1 vaccine
65 participants were anti-N IgG seronegative at baseline
31 placebo
34 vaccine
32 participants were anti-N IgG seropositive at baseline
18 placebo
14 vaccine
56 participants with molecular results
28 placebo
28 vaccine
5 participants with molecular results
4 placebo
1 vaccine
65 participants with molecular results
31 placebo
34 vaccine
32 participants with molecular results
18 placebo
14 vaccine
43 participants were NAAT-negative before day-0, at day-0, and between days 0-14
22 placebo
21 vaccine
2 participants were NAAT-negative before day-0, at day-0, and between days 0-14
1 placebo
1 vaccine
63 participants were NAAT-negative before day-0, at day-0, and between days 0-14
30 placebo
33 vaccine
31 participants were NAAT-negative before day-0, at day-0, and between days 0-14
17 placebo
14 vaccine
8 participants were NAAT-positive before or on day-0
4 placebo
4 vaccine
3 participants were NAAT-positive before or on day-0
3 placebo
0 vaccine
1 participant was NAAT-positive before day-0
1 placebo
0 vaccine
5 participants were NAAT-positive between days 0-14
2 placebo
3 vaccine
1 participant was NAAT-positive between days 0-14
0 placebo
1 vaccine
1 participant was NAAT-positive between days 0-14
1 placebo
0 vaccine

Fig. S2

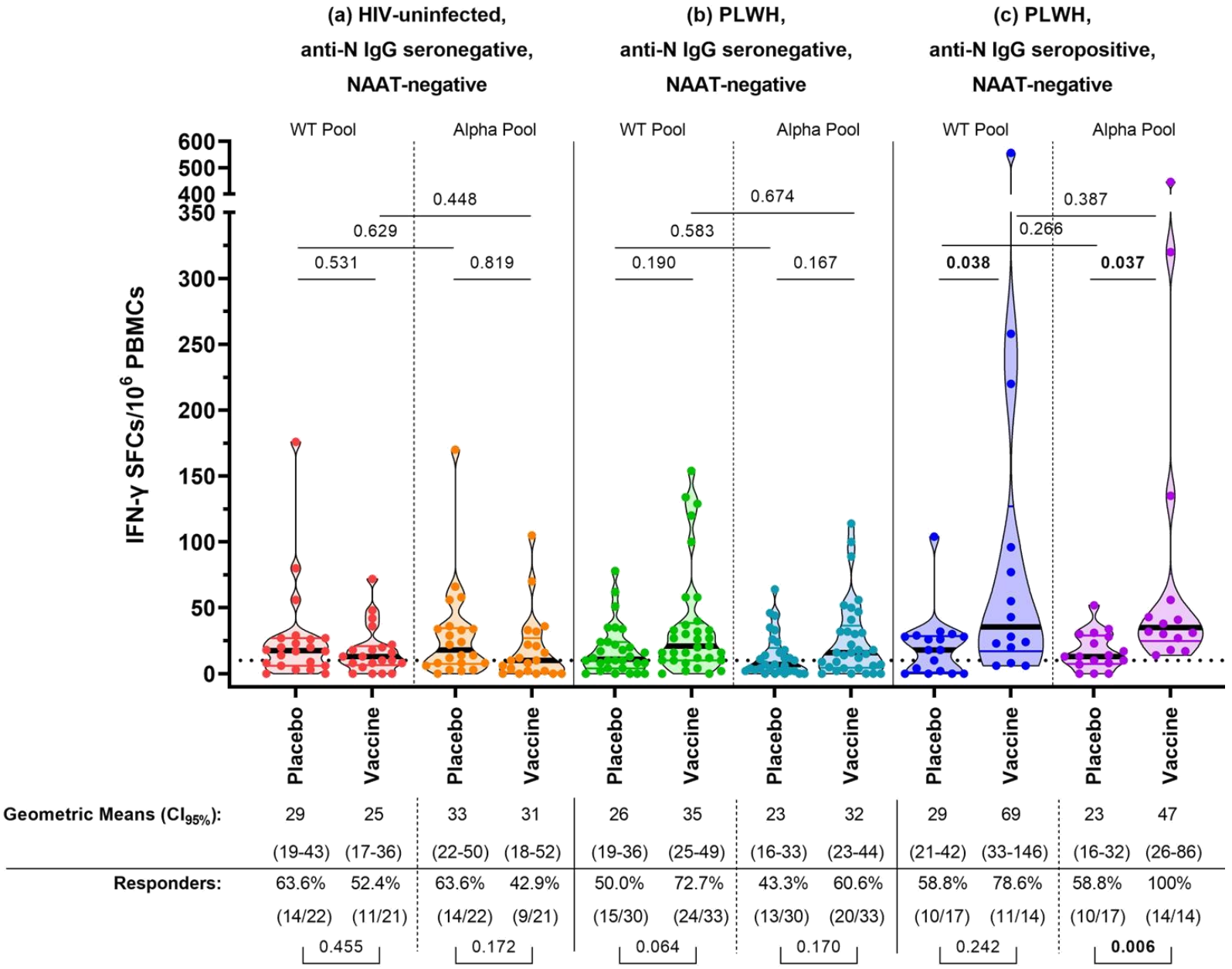

(a) HIV-uninfected, anti-N IgG seronegative, NAAT-negative
(b) PLWH, anti-N IgG seronegative, NAAT-negative
(c) PLWH, anti-N IgG seropositive, NAAT-negative
WT Pool
Alpha Pool
IFN-γ SFCs/10^6 PBMCs
600
500
400
350
300
250
200
150
100
50
0
0.531
0.629
0.448
0.819
0.190
0.583
0.674
0.167
0.038
0.266
0.387
0.037
Placebo
Vaccine
Geometric Means (CI95%):
29 (19-43)
25 (17-36)
33 (22-50)
31 (18-52)
26 (19-36)
35 (25-49)
23 (16-33)
32 (23-44)
29 (21-42)
69 (33-146)
23 (16-32)
47 (26-86)
Responders:
63.6% (14/22)
52.4% (11/21)
63.6% (14/22)
42.9% (9/21)
50.0% (15/30)
72.7% (24/33)
43.3% (13/30)
60.6% (20/33)
58.8% (10/17)
78.6% (11/14)
58.8% (10/17)
100% (14/14)
0.455
0.172
0.064
0.170
0.242
0.006

Fig. S3

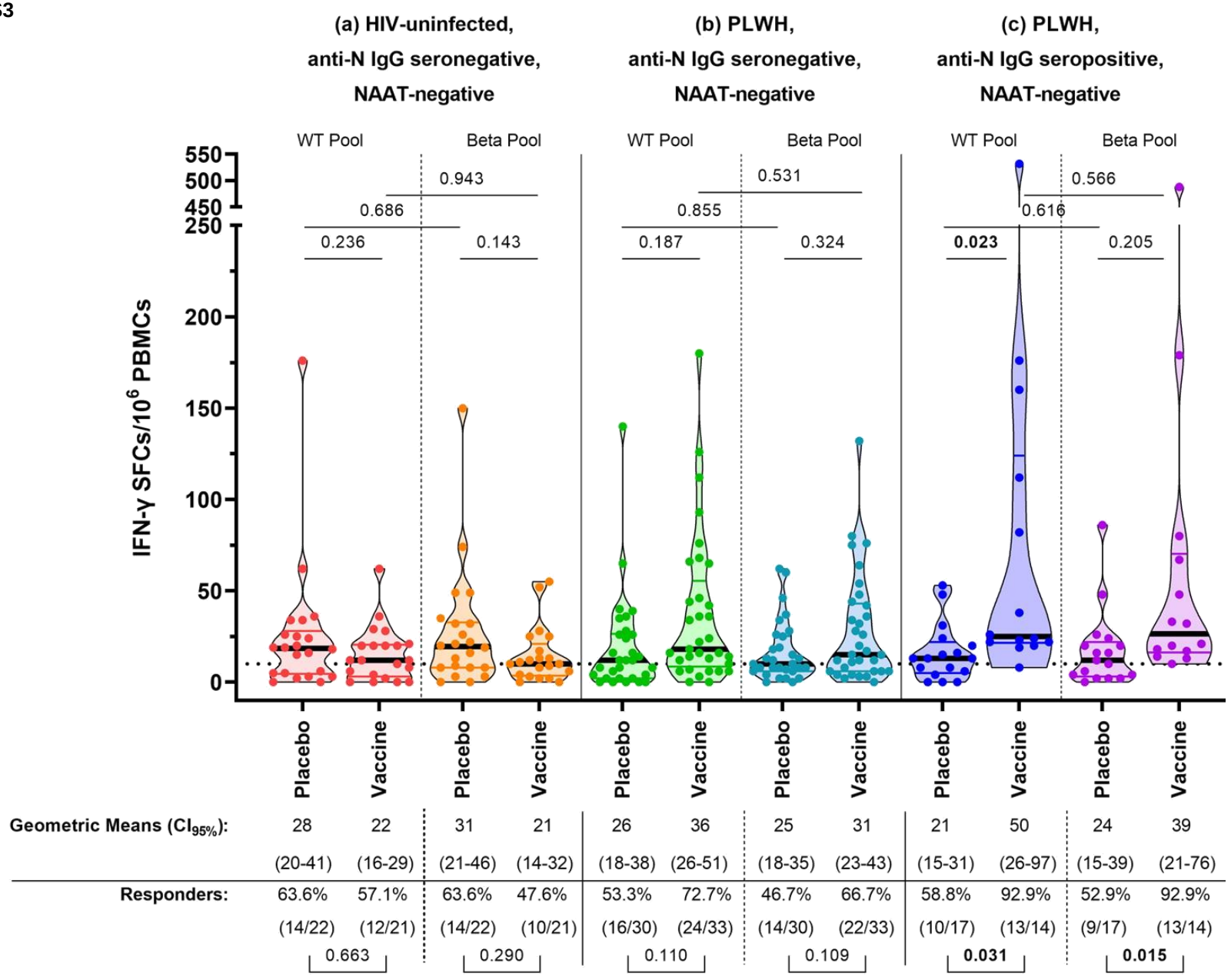

(a) HIV-uninfected, anti-N IgG seronegative, NAAT-negative
(b) PLWH, anti-N IgG seronegative, NAAT-negative
(c) PLWH, anti-N IgG seropositive, NAAT-negative
WT Pool
Beta Pool
IFN-γ SFCs/10^6 PBMCs
550
500
450
250
200
150
100
50
0
0.943
0.686
0.236
0.143
0.531
0.855
0.187
0.324
0.566
0.616
0.023
0.205
Placebo
Vaccine
Geometric Means (CI95%):
28 (20-41)
22 (16-29)
31 (21-46)
21 (14-32)
26 (18-38)
36 (26-51)
25 (18-35)
31 (23-43)
21 (15-31)
50 (26-97)
24 (15-39)
39 (21-76)
Responders:
63.6% (14/22)
57.1% (12/21)
63.6% (14/22)
47.6% (10/21)
53.3% (16/30)
72.7% (24/33)
46.7% (14/30)
66.7% (22/33)
58.8% (10/17)
92.9% (13/14)
52.9% (9/17)
92.9% (13/14)
0.663
0.290
0.110
0.109
0.031
0.015

Fig. S4

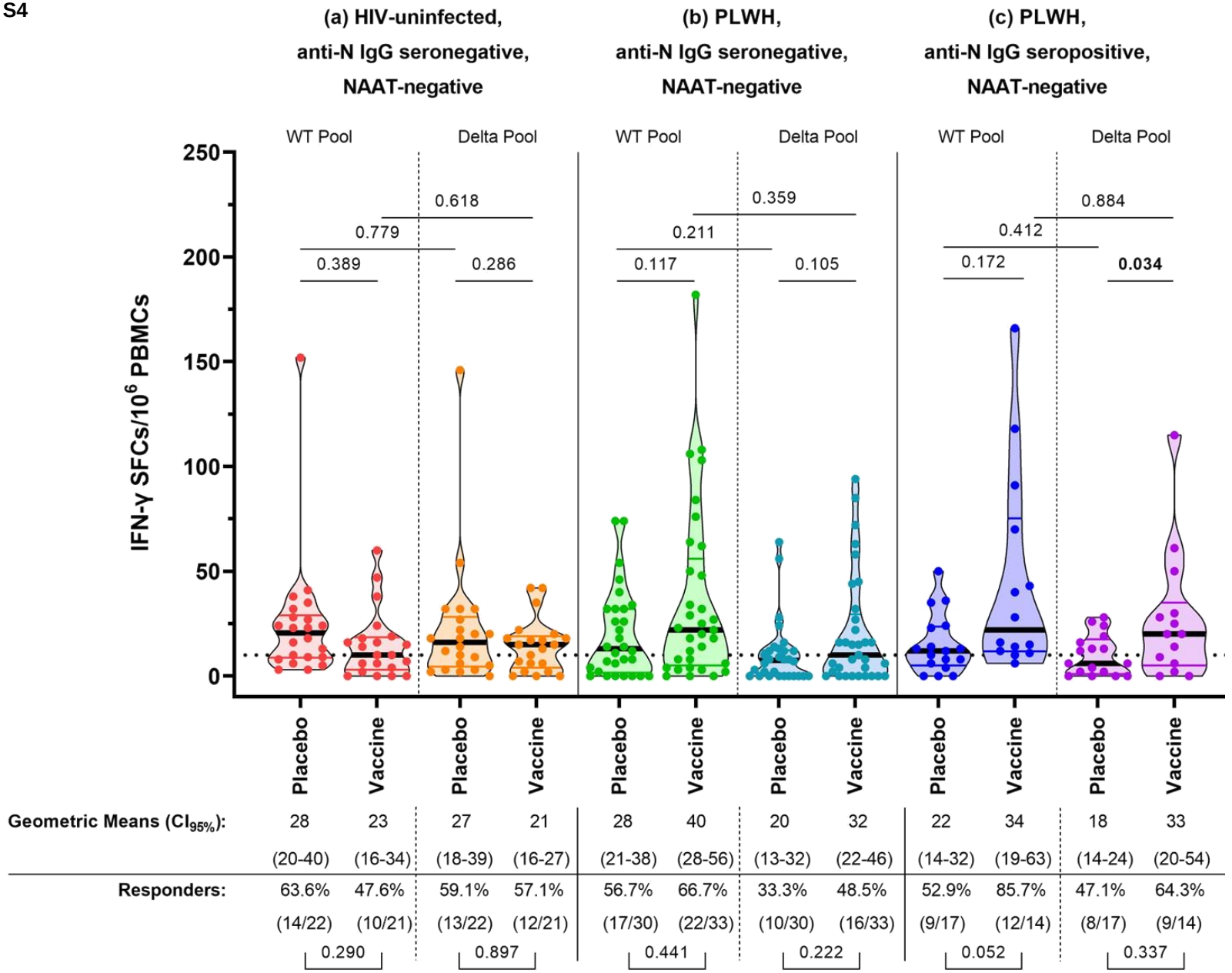
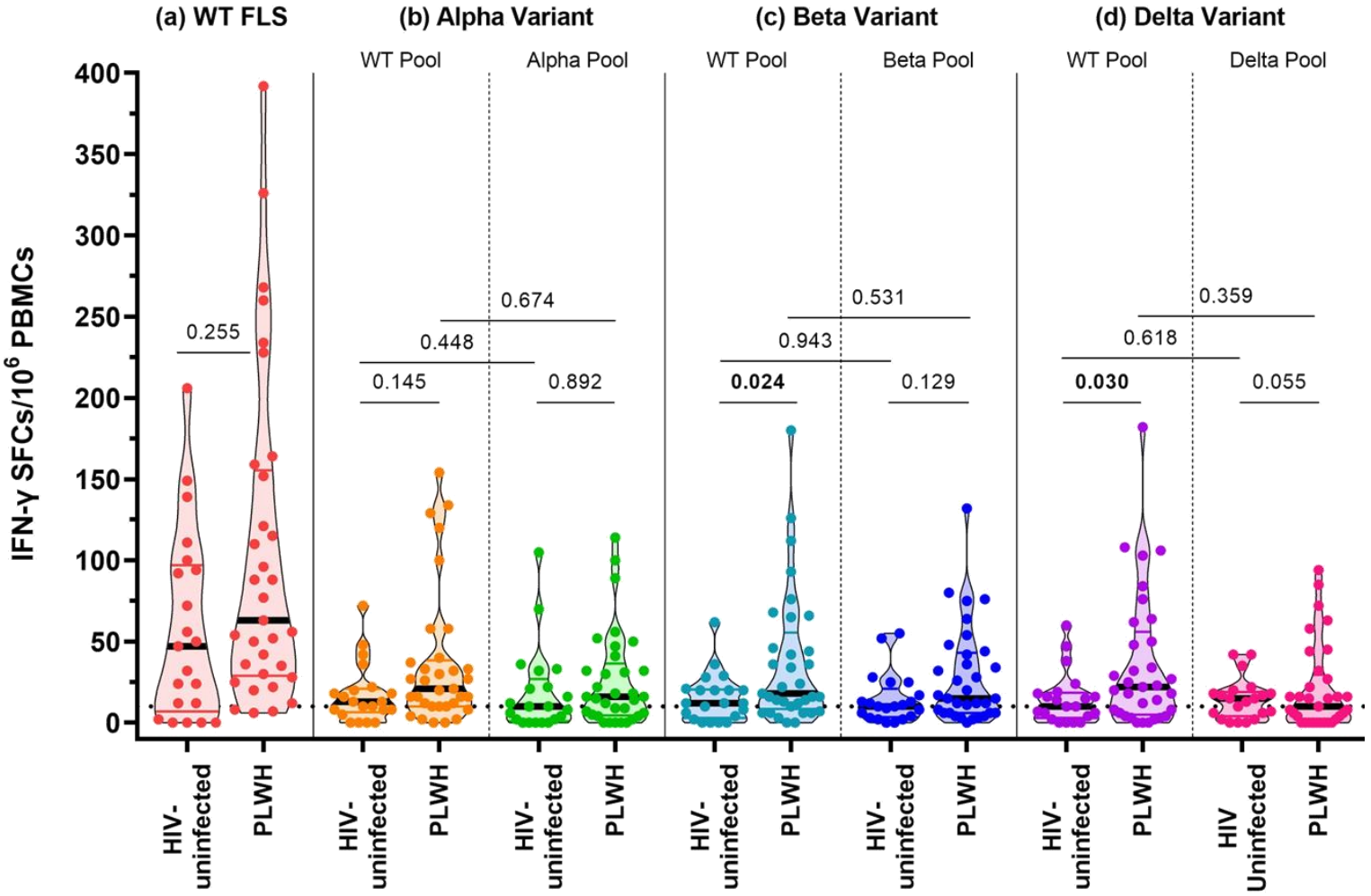

(a) HIV-uninfected, anti-N IgG seronegative, NAAT-negative
(b) PLWH, anti-N IgG seronegative, NAAT-negative
(c) PLWH, anti-N IgG seropositive, NAAT-negative
WT Pool
Delta Pool
IFN-γ SFCs/10^6 PBMCs
250
200
150
100
50
0
0.618
0.779
0.389
0.286
0.359
0.211
0.117
0.105
0.884
0.412
0.172
0.034
Placebo
Vaccine
Geometric Means (CI95%):
28 (20-40)
23 (16-34)
27 (18-39)
21 (16-27)
28 (21-38)
40 (28-56)
20 (13-32)
32 (22-46)
22 (14-32)
34 (19-63)
18 (14-24)
33 (20-54)
Responders:
63.6% (14/22)
47.6% (10/21)
59.1% (13/22)
57.1% (12/21)
56.7% (17/30)
66.7% (22/33)
33.3% (10/30)
48.5% (16/33)
52.9% (9/17)
85.7% (12/14)
47.1% (8/17)
64.3% (9/14)
0.290
0.897
0.441
0.222
0.052
0.337

Fig. S5


(a) WT FLS
(b) Alpha Variant
(c) Beta Variant
(d) Delta Variant
WT Pool
Alpha Pool
Beta Pool
Delta Pool
IFN-γ SFCs/10^6 PBMCs
400
350
300
250
200
150
100
50
0
0.255
0.674
0.448
0.145
0.892
0.531
0.943
0.024
0.129
0.359
0.618
0.030
0.055
HIV-uninfected
PLWH
HIV Uninfected
Geometric Means (CI95%):
56 (35-90) 78 (55-109)
25 (17-36) 35 (25-49)
31 (18-52) 32 (23-44)
22 (16-29) 36 (26-51)
21 (14-32) 31 (23-43)
23 (16-34) 40 (28-56)
21 (16-27) 32 (22-46)
Responders:
76.2% (16/21) 90.9% (30/33)
52.4% (11/21) 72.7% (24/33)
42.9% (9/21) 60.6% (20/33)
57.1% (12/21) 72.7% (24/33)
47.6% (10/21) 66.7% (22/33)
47.6% (10/21) 66.7% (22/33)
57.1% (12/21) 48.5% (16/33)
0.138
0.127
0.202
0.236
0.165
0.199
0.535