

1 **TITLE PAGE**

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3 **Limited humoral and specific T-cell responses after SARS-CoV-2 vaccination in**

4 **PLWH with poor immune reconstitution**

5 **Running title:**

6 COVID-19 vaccines in PLWH

7

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25

1 **ABSTRACT**

2

3 **Background.** We analyzed humoral and cellular immune responses induced by SARS-
4 CoV-2 mRNA vaccines in people living with HIV-1 (PLWH) with < 200 CD4⁺ T-cells.

5 **Methods.** Prospective cohort study including 58 PLWH with CD4⁺ T-cell counts < 200
6 cells/mm³, 36 with CD4⁺ T-cell counts > 500 , and 33 HIV-1-negative controls.
7 Antibodies against the SARS-CoV-2 Spike protein (anti-S IgG) and the receptor-
8 binding domain (anti-RBD IgG) were quantified before and four weeks after the first
9 and the second dose of BNT162b2 or mRNA-1273 (w8). Viral neutralization activity
10 and T-cell responses were also determined.

11 **Results.** At w8, anti-S/anti-RBD IgG responses increased in all groups ($P < 0.0001$).
12 Median (IQR) S-IgG and RBD-IgG at w8 were 153.6 (26.4; 654.9) and 171.9 (61.8;
13 425.8) in the HIV < 200 group compared to 245.6 (145; 824) and 555.8 (166.4; 1751) in
14 the HIV > 500 group, and 274.7 (193.7; 680.4) and 281.6 (181; 831.8) BAU/mL in
15 controls ($P < 0.05$). Neutralizing capacity and specific T-cell immune responses were
16 absent or reduced in 33% of the HIV < 200 group, compared with 3.7% in the HIV > 500
17 ($P = 0.0003$).

18 **Conclusion.** One third of PLWH with CD4⁺ T-cell counts < 200 cells/mm³ show low
19 anti-S/anti-RBD IgG levels, reduced in vitro neutralization activity against SARS-CoV-
20 2 and no vaccine-induced T-cells after receiving COVID-19 mRNA vaccines.

21

22 **Keywords:**

23 SARS-CoV-2 vaccine, people living with HIV (PLWH), anti-S IgG, anti-RBD IgG,
24 neutralizing antibodies

25

1 INTRODUCTION

2 The coronavirus disease-2019 (COVID-19) pandemic, caused by the severe acute
3 respiratory syndrome coronavirus 2 (SARS-CoV-2), constitutes an unprecedented threat
4 to the global health care system. Since the first case reported in Wuhan, China, close to
5 480 million cases have been documented worldwide, causing over 6 million deaths as of
6 March 2022 (<https://covid19.who.int/>).

7
8 Successful development of safe and effective vaccines has drastically reduced morbidity
9 and mortality associated with COVID-19[1,2]. Both, messenger RNA (mRNA) and
10 adenovirus-vectored vaccines show high effectiveness at preventing COVID-19 illness,
11 reducing severe disease and death[1,2]. Levels of binding and neutralizing antibodies
12 directed against the SARS-CoV-2 Spike protein (S) and the receptor binding domain
13 (RBD) correlate with vaccine efficacy[3–5].

14
15 People living with HIV (PLWH) are at higher risk to develop severe COVID-19
16 disease[6,7], especially those individuals aging 40 years or older, those with low CD4⁺
17 T-cell counts (<200 cells/mm³) and/or those with three or more comorbidities[8].
18 Consequently, PLWH with low CD4⁺ T-cell counts have received priority access to
19 SARS-CoV-2 vaccines in many vaccine roll-out programs. However, this population
20 was either excluded or underrepresented in pivotal phase 2/3 vaccine trials as these
21 studies only allowed inclusion of PLWH with high CD4⁺ T-cell counts on suppressive
22 ART[1]. While PLWH with well-controlled infection showed similar humoral responses
23 compared to general population[9–12], data on vaccine-induced responses from PLWH
24 at higher risk of severe COVID-19 disease are still scarce. This information is essential
25 for the clinical management of this group patients.

1 Our objective was to characterize humoral and cellular immune response after SARS-
2 CoV-2 vaccination in PLWH with low CD4⁺ T-cell counts and/or with suboptimal viral
3 suppression.

4

5 **MATERIAL AND METHODS**

6

7 **Study design**

8 Prospective observational single-center cohort study to investigate vaccine-specific
9 immune responses after 2 doses of mRNA-based SARS-CoV-2 vaccination in PLWH
10 with distinct levels of immune status compared to aged-matched HIV-negative controls.
11 The primary endpoint was to assess antibody levels against S and RBD (anti-S and anti-
12 RBD IgG) after primary (2-dose) vaccination. Antibody levels were measured in all
13 participants at baseline, 21 or 28 days after the first vaccination, and at 28 days after the
14 second vaccination (named w4 and w8 for consistency). Secondary endpoints included
15 determination of avidity, IgM/IgA measurements, neutralization activity and vaccine-
16 induced cellular immune responses, which were assessed at baseline and at w8 in a
17 subgroup of clinically representative individuals of the total cohort based on sample
18 availability.

19

20 All participants included in the study received two doses of mRNA SARS-CoV-2
21 vaccines (BNT162b2 -21 days apart- or mRNA-1273 -28 days apart-) following the
22 National Vaccination Plan for primary doses. Peripheral blood mononuclear cells
23 (PBMCs) and plasma samples were collected at baseline, at w4 and w8. Demographic
24 data were collected from electronic medical records.

25

1 The study was approved by the Institutional Ethical Review Board at Hospital
2 Universitari Germans Trias I Pujol (HUGTiP) in Badalona, Spain (PI-21-108). All
3 participants gave written informed consent before enrollment. Biological biosafety
4 committee approved SARS-CoV-2 experiments at the BSL3 laboratory of the Center for
5 Bioimaging and Comparative Medicine (CSB-20-015-M3).

6

7 **Study population**

8 In April 2021, once vaccine-rollout prioritized PLWH with CD4⁺ T-cell counts <200
9 cells/mm³, we searched for individuals aging >18 years with <200 CD4⁺ T-cells/mm³
10 within the last year out of PLWH under regular care at HUGTiP. Exclusion criteria
11 included previous SARS-CoV-2 vaccination and known history of SARS-CoV-2
12 infection (Fig.1, Flow diagram)

13 PLWH with >500 CD4⁺ T-cell counts and HIV-1 negative controls, mostly represented
14 by healthcare workers, were included sequentially through opportunistic vaccination at
15 HUGTiP and were matched by age with HIV<200 group.

16

17 **Enzyme-linked immunosorbent assay**

18 We measured anti-S IgG and anti-RBD IgG antibody levels at enrollment, and at w4
19 and w8 in 127 participants who completed follow-up, except for one individual in the
20 HIV<200 group, whose results were not valid, and S-IgG avidity and anti-S IgM and
21 IgA antibodies in 52 randomly-selected participants (n=26 in HIV<200 group, n=13 in
22 HIV>500 group and n=13 in control group). Plates were coated overnight at 4°C with
23 the HIS.H8 antibody (ThermoFisher Scientific) at 2µg/mL, blocked with PBS+1%
24 bovine serum albumin (BSA, Miltenyi biotech) for two hours at room temperature (RT),
25 washed and incubated with: S, RBD or Nucleocapsid protein (all from Sino Biological)

1 at 1µg/ml or PBS/1%BSA to estimate sample background. A positive plasma sample
2 was used as standard, and a pool of 10 SARS-CoV-2 negative plasma samples served as
3 negative control. Samples were incubated for one hour (RT). Secondary antibodies:
4 HRP conjugated goat anti-human IgG, goat anti-human IgM, and goat anti-human IgA
5 (all from Jackson ImmunoResearch) were incubated for 30 minutes. O-
6 Phenylenediamine dihydrochloride (OPD) (Sigma Aldrich) was added and the
7 enzymatic reaction was stopped with 2M of H₂SO₄ (Sigma Aldrich). Signal was
8 evaluated as the optical density (OD) at 492 nm with noise correction at 620 nm.
9 Specific signal for each antigen was determined after subtracting background obtained
10 from antigen-free wells. Results are expressed as arbitrary units (AU/mL) according to
11 the standard[13]

12
13 S-IgG avidity was evaluated by ELISA. Plates were coated with S (Sino Biological) at
14 1µg/ml and were blocked with PBS/1%BSA (Miltenyi biotech) for two hours (RT).
15 Samples were diluted at 0.5 AU/mL and evaluated in quadruplicate for one hour (RT).
16 After washing, 2M Guanidine-HCl or PBS were added and incubated for 15 minutes
17 (RT). Bound antibodies were detected using HRP conjugated goat anti-human IgG
18 (Jackson ImmunoResearch). Plates were revealed using OPD (Sigma Aldrich).
19 Enzymatic reaction was stopped with 2M of H₂SO₄ (Sigma Aldrich). Signal was
20 evaluated as OD at 492 nm with noise correction at 620 nm. S-IgG avidity was
21 calculated as the ratio between mean signal obtained with and without guanidine
22 treatment.

23
24

1 **Viral isolation and titration**

2 SARS-CoV-2 was isolated from a nasopharyngeal swab collected in March 2020 in
3 Spain and propagated for two passages in Vero E6 cells. Virus stock was prepared by
4 collecting supernatant, sequenced as detailed previously[14] and titrated in 10-fold
5 serial dilutions to calculate the TCID₅₀/mL. Virus was used at an MOI of $7 \cdot 10^{-4}$ to
6 achieve 50% cytopathic effect 3 days post-infection.

7

8 **Neutralization Assay**

9 Neutralizing antibodies were measured in a randomly selected subgroup, representative
10 of the total cohort, of 42 individuals from HIV<200 group, 27 individuals from
11 HIV>500 group and 27 from control group, which were matched by age with HIV<200
12 group.

13 Neutralization assays were performed preincubating 60 TCID₅₀ of SARS-CoV-2 with
14 three-fold serial dilutions (1/60 to 1/14580) of heat-inactivated plasma samples for 1h at
15 37 °C. Pre-incubated viruses were added to 60.000 Vero E6 cells per well in 96-well
16 plates. 72 hours later, viral-induced cytopathic effect was measured using CellTiter-Glo
17 Luminescent cell viability reagent (Promega) and Luminoskan Plate Reader
18 (Thermofisher). Relative light units (RLU) were normalized, and ID₅₀ (the dilution
19 inhibiting 50% of the infection) was calculated by plotting and fitting plasma dilution
20 log vs. response to a 4-parameter equation as previously described[5,13,15].

21

22 **Characterization of SARS-CoV-2-specific T-cell responses by ELISpot**

23 Cellular responses were analyzed by IFN- γ ELISpot in those 42 individuals from the
24 HIV<200 group who were tested for neutralizing activity. After quality control, valid
25 results were available from 30 individuals.

1 A peptide library of 425 15-18mers (overlapping by 10 or 11 aminoacids, Synpeptide,
2 China) was selected as previously described[16], coverage shown in Supplementary
3 Table 1, and variants of concern (VOC) described in Supplementary Table 2.

4 IFN γ ELISpot kit (Mabtech) was used following manufacture instructions. PBMCs
5 were thawed, rested for 4 hours at 37°C and 5% of CO₂, plated at 1.25 –
6 2·10⁵ cells/well, stimulated with peptides (5 μ g/ml), and cultured for 20 hours at 37°C
7 and 5% of CO₂. PHA (15 μ g/ml, Sigma-Aldrich) was used as positive control, and no
8 stimuli as negative control. Plates were revealed using BCIP/NBT-plus substrate
9 solution (BioRad) and IFN γ -secreting cells were quantified under ImmunoCapture and
10 ImmunoSpot software[17]. Background responses were defined as mean number of
11 spots of the negative control wells plus three times the standard deviation, and
12 subtracted from each well containing peptides.

13 14 **Statistical analysis**

15 Sample size was based on the eligible number of adult PLWH with <200 CD4⁺ T-
16 cells/mm³ who accepted to participate. Differences of medians between baseline and w8
17 were compared using Wilcoxon matched-pairs signed rank test and for comparisons
18 between 3 timepoints Friedman test was used. Differences of medians among groups
19 were compared using Mann-Whitney or Kruskal-Wallis tests. Fisher's exact test was
20 used to compare proportion among groups. Spearman's rank test was used for
21 correlations. All tests were two-sided, unadjusted for multiple comparisons, with
22 $\alpha=0.05$. GraphPad Prism v9.1 was used for analysis and graphs.

23

24

1 RESULTS

2 Between April 21st and July 5th, 2021, 58 individuals were included in the HIV<200, 36
3 individuals in the HIV>500 and 33 as HIV-1-negative control group. Nine individuals
4 received BNT162b2 and 118 individuals were vaccinated with mRNA-1273. The
5 demographics are summarized in Table 1. Participants were mainly males with a
6 median (IQR) age of 51 years (35; 57). Median (IQR) CD4⁺ T-cell count and CD4/CD8
7 ratio at baseline were 173 (117; 257) cells/mm³ and 0.23 (0.15; 0.42) in the HIV<200
8 group, and 785 (655; 966) cells/mm³ and 1.21 (0.92-1.47) in the HIV>500 group,
9 respectively ($p<0.001$). Additionally, the percentage of individuals with plasma viral
10 load below 50 copies/ml within the year prior to enrollment was 81% in the HIV<200
11 group and 100% in the HIV>500 group ($P=0.0058$).

13 Vaccine-induced humoral immune responses against SARS-CoV-2

14 To investigate the magnitude of vaccine-induced antibody responses in PLWH, we
15 assessed anti-S IgG and anti-RBD IgG at enrollment, week 4 and week 8 in all
16 participants to identify potential differences among groups. All groups showed a
17 significant increase of anti-S and anti-RBD IgG titers after first and second dose (Fig. 2,
18 $P<0.0001$). However, anti-S and anti-RBD IgG in the HIV<200 group were
19 significantly lower than in HIV>500 and control groups, at w4 and w8 (Fig 2A-B,
20 $P<0.05$). Specifically, the median (IQR) anti-S IgG titer at w8 was 153.6 (26.4; 654.9)
21 in the HIV<200; 245.6 (145; 824) in the HIV>500 and 274.7 (193.7; 680.4) BAU/mL in
22 the control group. Similarly, median (IQR) anti-RBD IgG titer was 171.9 (61.8; 425.8),
23 555.8 (166.4; 1751) and 281.6 (181; 831.8) BAU/mL, respectively. Notably, there were
24 no statistically significant differences between the HIV>500 and control group (Fig.2).

1 Next, we classified all individuals into 'low S-IgG' and 'high S-IgG' according to
2 whether the magnitude of S-specific IgG response at w8 was either below or above
3 193.7 BAU/mL, a value that corresponds to the 25th percentile of the anti-S IgG levels
4 seen in the control group. The proportion of 'low S-IgG' among PLWH was
5 significantly higher compared to the control group (Fig.2 $P=0.0084$). In terms of
6 clinical variables, 'Low S-IgG' individuals were virologically suppressed for a
7 significantly shorter period compared to 'high S-IgG' (5 *versus* 11 years respectively,
8 $P=0.0520$, Table 2).

9
10 In a subgroup of individuals, we also evaluated the functional avidity of anti-S IgG and
11 quantified the levels of anti-S IgM and IgA. We did not find any statistically
12 significant differences between groups ($P=0.0640$; supplementary Fig.1), even after
13 comparing individuals with 'Low S-IgG' and 'High S-IgG' antibodies (Supplementary
14 Fig.1). SARS-CoV-2 vaccination elicited similar anti-S IgA responses among all groups
15 that were successfully boosted with each vaccine dose. However, individuals from the
16 HIV<200 group showed higher IgM responses at w8 than HIV>500 and control groups
17 (Supplementary Fig2).

18
19 Overall, these results indicate that PLWH are able to elicit immunogen-specific humoral
20 immune responses after two doses of mRNA vaccination, even though the magnitude of
21 this response is significantly weaker in individuals with <200 CD4⁺ T-cell counts
22 compared to PLWH with >500 CD4⁺ T-cell counts, specially if they have been
23 virologically suppressed for a shorter period of time.

24

25

1 **Vaccine-induced neutralizing activity against SARS-CoV-2**

2 To determine whether SARS-CoV-2-specific antibodies generated after vaccination
3 were able to block the virus, we tested the neutralization capacity of the plasma of a
4 subgroup of individuals at baseline and at week 8, using an assay based on wild-type
5 SARS-CoV-2[5]. At baseline, five participants already had detectable neutralizing
6 activity at baseline (Fig.2), most likely due to previous asymptomatic SARS-CoV-2
7 infection, but they were not excluded from the analysis. There was a significant increase
8 in neutralization titers from baseline to w8 in all groups ($P<0.0001$), showing similar
9 neutralization levels (Fig.2). No clear cutoff for a neutralizing activity that
10 unambiguously protects against SARS-CoV-2 infection has been established.
11 Nevertheless, based on data gathered from an outbreak, neutralizing activities above
12 1:250 is a cut-off that might be strong enough to prevent infection[13,18]. We identified
13 33.3% individuals in the HIV<200 (n=13) compared to 3.7% in the HIV >500 (n=1)
14 and none in the control group showing $ID_{50}<1:250$ (referred to as 'low neutralizers',
15 $P=0.0003$, Fig.2).

16 Levels of neutralizing activity positively correlated with anti-S and anti-RBD antibody
17 titers in all groups at w8 ($P<0.0001$, Spearman's correlation; $\rho=0.7715$; $\rho=0.6276$,
18 respectively, Fig.3).

19 Overall, these data suggest that, in the context of PLWH with poor immune
20 reconstitution, one out of three individuals showed low levels of neutralizing antibodies
21 that might be below the protective cut-off against SARS-CoV-2 after two doses of
22 mRNA vaccines.

23

24

1 **Vaccine-induced cellular immune responses against SARS-CoV-2**

2 To assess whether PLWH with <200 CD4⁺ T-cell counts were able to mount SARS-
3 CoV-2-specific T-cell responses despite their impaired humoral responses after
4 vaccination, we measured IFN γ -secreting cells by ELISpot in a subgroup of individuals
5 selected according to their neutralization activity. ‘High neutralizers’ showed a
6 tendency towards an increase in SARS-CoV-2-specific T-cell responses directed against
7 S1 subunit (more than 4-fold increase, $P=0.0673$) from baseline to w8. Notably, we
8 identified statistically significant differences in peptide pools B50-B51 corresponding to
9 the C-half of the S1 subunit, and B53-B54 which covers part of RBD region (Fig. 4).
10 However, we did not observe statistically significant increases in cellular responses
11 against S2 subunit (Fig. 4; $P=0.3864$), even after exclusion of B82 and B83 peptide
12 pools which cover the fusion peptide and the most cross-reactive regions to other human
13 coronaviruses[19–21]. By contrast, “low neutralizers” were unable to induce SARS-
14 CoV-2-specific T-cell responses to any specific pool of peptides tested for the S1 and
15 S2 subunits, except for the B82 peptide (Fig. 4). Thus, the overall magnitude of cellular
16 responses against S and the RBD regions at w8 were significantly higher in “high
17 neutralizers” when compared to “low neutralizers” (Fig.4, $P=0.0158$, 2 versus 0.7-fold
18 increase, respectively).

19 Regarding SARS-CoV-2 specific T-cell responses against VOCs at w8, we observed a
20 significant loss of IFN γ -secreting cells against Alpha ($P=0.0010$) and Kappa, Delta and
21 B.1.617.3 VOC ($P=0.0100$) in ‘high neutralizers’ (Supplementary Fig.3).

22 Taken together, these data suggest a lack of vaccine-induced T-cell immunity against
23 SARS-CoV-2 after two doses of mRNA vaccines in the context of PLWH with CD4⁺ T-
24 cell counts <200 cell/mm³.

25

1 **DISCUSSION**

2 We conducted a comprehensive analysis of a large cohort of PLWH with poor immune
3 reconstitution. Here, beyond anti-S IgG measurements already reported[22–25], we
4 analyzed functional avidity of IgG, neutralization capacity using replicative SARS-
5 CoV-2 and the cellular immunity of these individuals. Overall, these data reveal a
6 complete picture of this high-risk population, where approximately one third of
7 individuals induce low IgG levels, reduced in vitro neutralization activity and no
8 vaccine-induced T-cells after four weeks of the second mRNA vaccine dose.

9
10 In our cohort, 20% of individuals in the HIV<200 group had detectable HIV-1 viraemia
11 and, importantly, time under ART-suppression was associated with higher anti-S IgG
12 levels after vaccination. This suggests that chronic immune activation/exhaustion
13 associated with incompletely HIV suppression could impact vaccine-induced humoral
14 responses as seen for other vaccinations[26]. However, at the beginning of 2021, SARS-
15 CoV-2 vaccination was prioritized in these individuals rather than delaying vaccination
16 until they were undetectable. Since there is a significant proportion of PLWH
17 worldwide with low CD4⁺ T-cell counts and persistent viraemia, often suffering from
18 several comorbidities and more severe outcomes for infectious diseases[27], our data
19 might advise clinicians to make an informed risk/benefit decision upon deferring
20 vaccination depending on individual characteristics and incidence status of the
21 pandemic.

22
23 Impaired immune responses to pneumococcus[26], influenza[28] and hepatitis A and
24 B[29], are widely described in PLWH. Previous studies have shown that primary
25 responses and the maintenance of long-term serologic memory are affected during HIV-

1 1 infection[30,31]. The inability of CD4⁺ T-cells to effectively activate B-cells[32] and
2 the HIV-associated premature exhaustion of B-cells could cause a suboptimal humoral
3 response to vaccination[33]. Importantly, vaccine responsiveness remain often
4 diminished despite optimal ART-suppression[26]. Although PLWH with lower CD4⁺
5 T-cell counts have received priority access to primary SARS-CoV-2 vaccination, future
6 studies should address different adjuvants or additional/heterologous vaccinations in
7 this population.

8
9 In contrast to S-specific IgG, the role of anti-S IgM and S-IgA is less well characterized.
10 Interestingly, it has been shown that the coexistence of anti-S IgG and IgM was
11 associated with improved anti-SARS-CoV-2 humoral response and with higher
12 neutralizing activity in plasma[34]. It has been documented that after natural infection,
13 IgA dominate the early neutralizing humoral response to SARS-CoV-2[35]. In addition,
14 mRNA SARS-CoV-2 vaccination successfully boosted mucosal IgA response in
15 convalescent individuals[36]. Here HIV<200 individuals showed comparable titers of
16 IgA but higher IgM responses than HIV>500 and control groups. If the increase of anti-
17 S IgM responses observed in HIV<200 group is a consequence of an impaired anti-S
18 IgG response needs further investigation.

19
20 In our study, using a virus isolated during the first COVID-19 wave in Spain -March
21 2020 harboring the S D614G mutation, 33% of individuals from the HIV<200 group
22 showed reduced levels of neutralization at w8 after vaccination. These results confirm
23 the findings recently reported in an Italian cohort of PLWH with different degrees of
24 immune recovery[37]. Thus, it is likely that in our cohort, an even higher proportion of
25 immunosuppressed individuals would have showed reduced neutralizing activity in vivo

1 against more divergent and contemporaneous VOC, such as the Beta, Delta and/or
2 Omicron variants.

3
4 In addition to the role of neutralizing antibodies in SARS-CoV-2 viral control, there is
5 growing evidence for an important contribution of virus-specific T-cell responses to
6 limit disease progression towards severe COVID-19[19,38–41]. We showed here an
7 impaired vaccine-induced T-cell immunity against SARS-CoV-2 in PLWH that were
8 both “low S-IgG” and “low neutralizers”. These results are further supported by a recent
9 study showing that the magnitude of SARS-CoV-2-specific T-cell responses after
10 natural infection in PLWH positively correlates with naive CD4⁺ T-cells and the
11 CD4/CD8 ratio[42]. These findings could be explained in part by the reduced
12 production of IL-2 from antigen-specific CD8⁺ T-cells in PLWH, which could hinder
13 their proliferative potential and long-term immune memory following natural infection
14 and/or immunization[43]. Importantly, ‘high neutralizers’ showed reduced T-cell
15 responses against some VOC, which probably can be more relevant with
16 contemporaneous VOC such as delta and omicron.

17
18 Given the impaired humoral and cellular immune responses after two SARS-CoV-2
19 vaccine doses in a significant proportion of PLWH with less than 200 CD4⁺ T-cells, our
20 data suggest that these individuals could benefit from close monitoring to prioritize
21 them for alternative strategies aimed at achieving better immunity against SARS-CoV-2
22 but especially, limiting severe disease outcomes. These strategies could consist on
23 additional doses (homologous or heterologous boosters) together with a close
24 monitoring of antibody levels to detect suboptimal responsiveness, such as what is
25 contemplated in the ongoing European EU funded project RBDCOV (101046118). As

1 we identified a positive correlation between neutralization activity and anti-S IgG
2 levels, these individuals might be easily identified using quick antibody detection
3 methods.

4 Although vaccination remains the most important intervention available to lower the
5 risk of severe disease, the recent demonstration of the use of neutralizing monoclonal
6 antibodies, such as the combination of Tixagevimab and Cilgavimab, for preventing
7 SARS-CoV-2 acquisition (Evusheld)[44] suggests that passive antibody prophylaxis
8 could be an approach to consider in PLWH who do not have an adequate immune
9 response to vaccination or cannot be vaccinated against COVID-19 because of severe
10 allergies. Since we identified that PLWH with less than 200 CD4⁺ T-cells have an
11 impaired vaccine responsiveness, which is in line with previous studies[37], we propose
12 the use of the recently approved Evusheld in this particular population, which is not
13 currently eligible for this treatment.

14
15 This study has some limitations. First, we did not assess immune responses in PLWH
16 with CD4⁺ T-cells counts between 200 and 500 cells/mm³. Second, the latest time-point
17 analyzed in our study was one month after the second vaccine dose. Thus, it is unclear
18 how fast the S-IgG levels wane in these individuals and consequently, long-term data
19 are needed to better guide needs and timings for booster vaccinations in this population.
20 Finally, other functions of antibodies such as ADCC that might also contribute to
21 control of COVID-19 outcomes or activity against more contemporaneous VOC has not
22 been assessed.

23
24 In conclusion, our study demonstrated that one out of three PLWH with CD4⁺ T-cell
25 counts <200 cells/mm³ reached low levels of anti-S and anti-RBD IgG together with

1 weak neutralization activity and absence of cellular responses. These individuals would
2 benefit from monitoring vaccine responsiveness to prioritize them for additional booster
3 vaccinations and/or alternative preventive approaches against SARS-CoV-2.

4

5 **NOTES**

6 **Authors contribution**

7 Study conception, design, and funding: BC, RP, NI-U, JC, JGP, JM, BM. Collected
8 data: CM, SG, SB. Performed humoral experiments, analyzed and interpreted data: EA-
9 E, NP-L, JM-B, DR-R, DP-Z, MLRC, CA, YA-S, NI-U, SB, BM. Performed T-cell
10 experiments, analyzed and interpreted data: OB-L, RP, EJ, SC, TE, LR-M, YA-S, GFR-
11 L, JGP, SB, BM. Manuscript editing: SB, OB-L, EA-E, NP-L, JM-B, DR-R, DP-Z, RP,
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14 authors reviewed and approved the final draft of the paper.

15 **FOOTNOTE PAGE:**

16 **Conflicts of interest**

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1 TABLES

2 **Table 1:** Baseline characteristics of the study cohort at inclusion.

	HIV <200 group n=58	HIV >500 group n=36	Control group n=33	p-value
Age, years	52 (40; 56)	51 (40; 56)	53 (35; 57)	0.8631 ^ℓ
Male gender, n (%)	46 (79.3%)	29 (80.6%)	18 (54.5%)	0.0187 [†]
HIV route of acquisition, n (%)				0.3771 [§]
Sexual contact	39 (67.2%)	25 (69.4%)	N/A	
UDVP	13 (22.4%)	10 (27.8%)	N/A	
Other	6 (10.3%)	1 (2.8%)	N/A	
pVL (log10 copies/ml)	1.30 (1.30; 1.52)	1.30 (1.30; 1.30)	N/A	
Undetectable pVL, n (%)	47 (81%)	36 (100%)	N/A	0.0058 [§]
Time on virological suppression, years	3.75 (1.09; 14.88)	10.88 (6.63; 11.42)	N/A	0.1248 [§]
Time from HIV diagnosis to first COVID-19 vaccine, years	14.08 (1.69; 28.31)	17.79 (3.50; 28.32)	N/A	0.1013 [§]
Absolute CD4, cells/mm ³	173 (117; 257)	785 (655; 966)	N/A	<0.0001 [§]
CD4/CD8 ratio	0.23 (0.15; 0.42)	1.21 (0.92; 1.47)	N/A	<0.0001 [§]
CD4 nadir, cells/mm ³	64 (22; 110)	259 (146; 375)	N/A	<0.0001 [§]

3 N/A: Not applicable

4 UDVP: intravenous drug users

5 pVL: plasma viral load

6 Virological suppression was considered below 50 copies/ml

7 All values were expressed as median (interquartile range) except when noted

8 ℓ Kruskal-Wallis test was used for comparison between the three groups

9 † Chi-squared test was used for comparison between groups

10 § Mann-Whitney test was used for comparison between groups

1 **Table 2:** Baseline characteristics of PLWH with low or high anti-S IgG antibody levels.
 2 Median and IQR from individuals are shown, except for categorical data in which
 3 frequency and percentage are presented.

	Low S-IgG n=50	High S-IgG n=43	p-value
Age, years	51 (40; 57)	52 (43; 56)	0.7544
Undetectable pVL, n (%)	42 (84%)	40 (93%)	0.2133 ^T
Time on virological suppression, years	5 (1; 22)	11 (3; 13)	0.0520
Time from HIV diagnosis to first COVID-19 vaccine, years	14 (3; 29)	19 (7; 29)	0.3323
Absolute CD4, cells/mm ³	254 (132; 663)	346 (171; 780)	0.1918
CD4/CD8 ratio	0.83 (0.21; 1.44)	0.69 (0.25; 1.4)	0.6267
CD4 nadir, cells/mm ³	101 (60; 553)	115 (23; 257)	0.7142

4
 5 *All values were expressed as median (interquartile range) except when noted*
 6 *Virological suppression was considered below 50 copies/ml*
 7 *Mann-Whitney test was used for comparison between groups, except in (^T) where Fisher Exact test was used.*
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10

11 **FIGURE LEGENDS**

12

13 **Figure 1.** Flow diagram for the prospective observational cohort study according to
 14 STROBE statement.

15

16 **Figure 2. Vaccine-induced humoral immune responses in individuals from our**
 17 **cohort.** Antibody binding titers against SARS-CoV-2 S protein (A)- or RBD (B) in
 18 individuals in the HIV<200 group, the HIV>500 group and control group at baseline
 19 (BSL), at w4 (w4) and at w8 (w8) after vaccination. Median titer of antibodies and IQR
 20 shown are quantified in normalized Binding Antibody Units (BAU). Friedman test was
 21 used to compare antibody titers at baseline and w8 for each study group and Mann-
 22 Whitney test was used to compare median levels of antibody at w8 between groups. C.
 23 Proportion of ‘low S-IgG’ individuals according to the magnitude of anti-S IgG
 24 response at w8. The percentage of ‘low S-IgG’ (defined as anti-S IgG <193.7 BAU/mL)
 25 at w8 is indicated. Fisher’s exact test is used. D. SARS-CoV-2 neutralization titers (ID₅₀

1 expressed as reciprocal dilution) from individuals belonging to either the HIV<200
2 group (n=42), the HIV>500 group (n=27) and the control group (n=27) at baseline and
3 at w8. Median titer of neutralization antibodies and IQR are depicted. Individuals with
4 neutralizing capacity at baseline are represented with diamond dots. Grey shaded area
5 shows titers below 250, which are considered as reduced neutralizing activity.
6 Percentage of vaccinated individuals that do not achieve high neutralization capacity
7 (i.e have titers <250) is highlighted in red boxes. Wilcoxon matched-pairs signed rank
8 test was used to compare neutralization titers at BSL and w8 for each study group and
9 Mann-Whitney test was used to compare median levels at w8 between groups.

10 ns: not significant.

11 All undetectable levels are represented as 1.

12

13 **Figure 3. Association between SARS-CoV-2 neutralization titers and levels of binding**
14 **antibodies at w8.**

15 A. Association between SARS-CoV-2 neutralization titers and levels of binding anti-S
16 IgG antibodies against the virus from individuals analyzed at w8. Spearman`s
17 correlation was used. B. Association between SARS-CoV-2 neutralization titers and
18 levels of binding anti-RBD IgG antibodies against the virus from individuals analyzed
19 at w8. Spearman`s correlation was used.

20 ns: not significant.

21 All undetectable levels are represented as 1.

22

23 **Figure 4. Vaccine-induced T-cell immune responses against the S1 and S2 subunits**
24 **from the SARS-CoV-2 Spike protein in ‘High and Low neutralizers’ from HIV<200**
25 **group.** Magnitude of IFN- γ -SFC per million of PBMCs to SARS-CoV-2 peptide pools

1 covering S1 (A) and S2 (B) subunits in ‘High neutralizers’ individuals at baseline
2 (BSL) and w8 (w8) post-vaccination. Magnitude of IFN- γ -SFC per million of PBMCs
3 to SARS-CoV-2 peptide pools covering S1 (C) and S2 subunits (D) in ‘low
4 neutralizers’ at baseline and at w8 post-vaccination.

5 ns: not significant.

6

7 **Figure 5. Comparison of vaccine-induced T-cell responses at w8 between ‘High
8 and Low neutralizers’ from HIV<200 group.**

9 Total magnitude of IFN- γ -SFC per million of PBMCs to S (A) and RBD peptide pools
10 (B) in Low and High neutralizers at w8 post-vaccination. Scatter plots show the median
11 and interquartile ranges. We used the Mann-Whitney test in unpaired samples and the
12 Wilcoxon test in paired samples for statistical analysis.

13 ns: not significant.

14

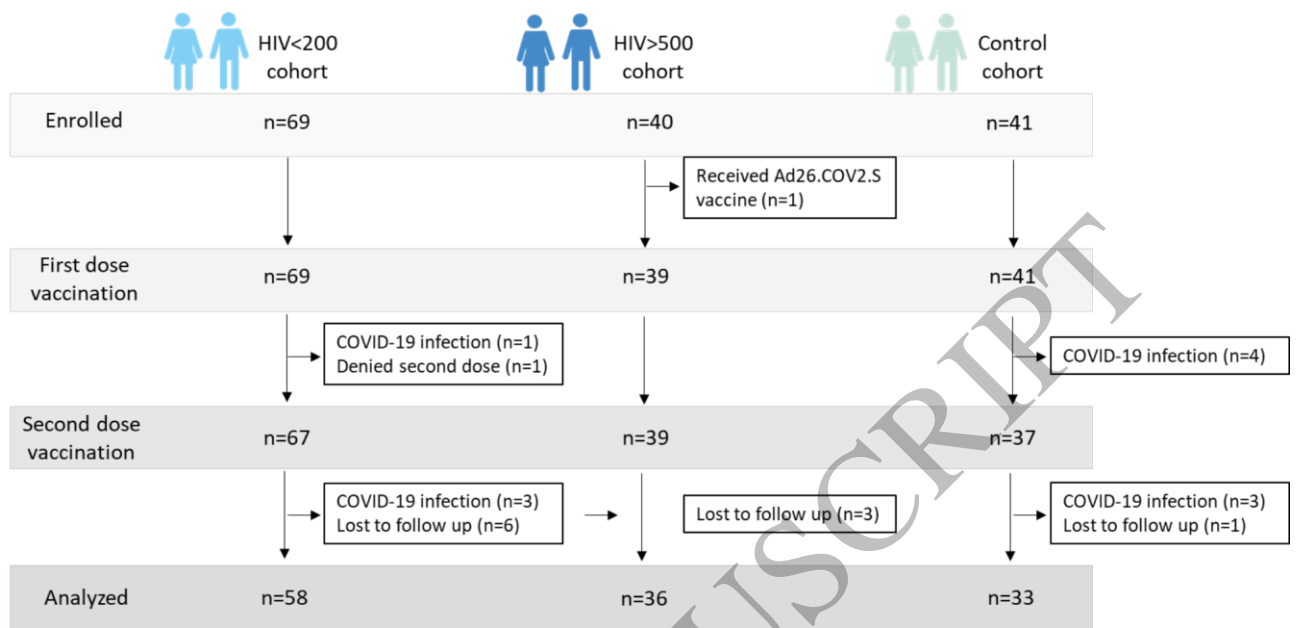


Figure 1
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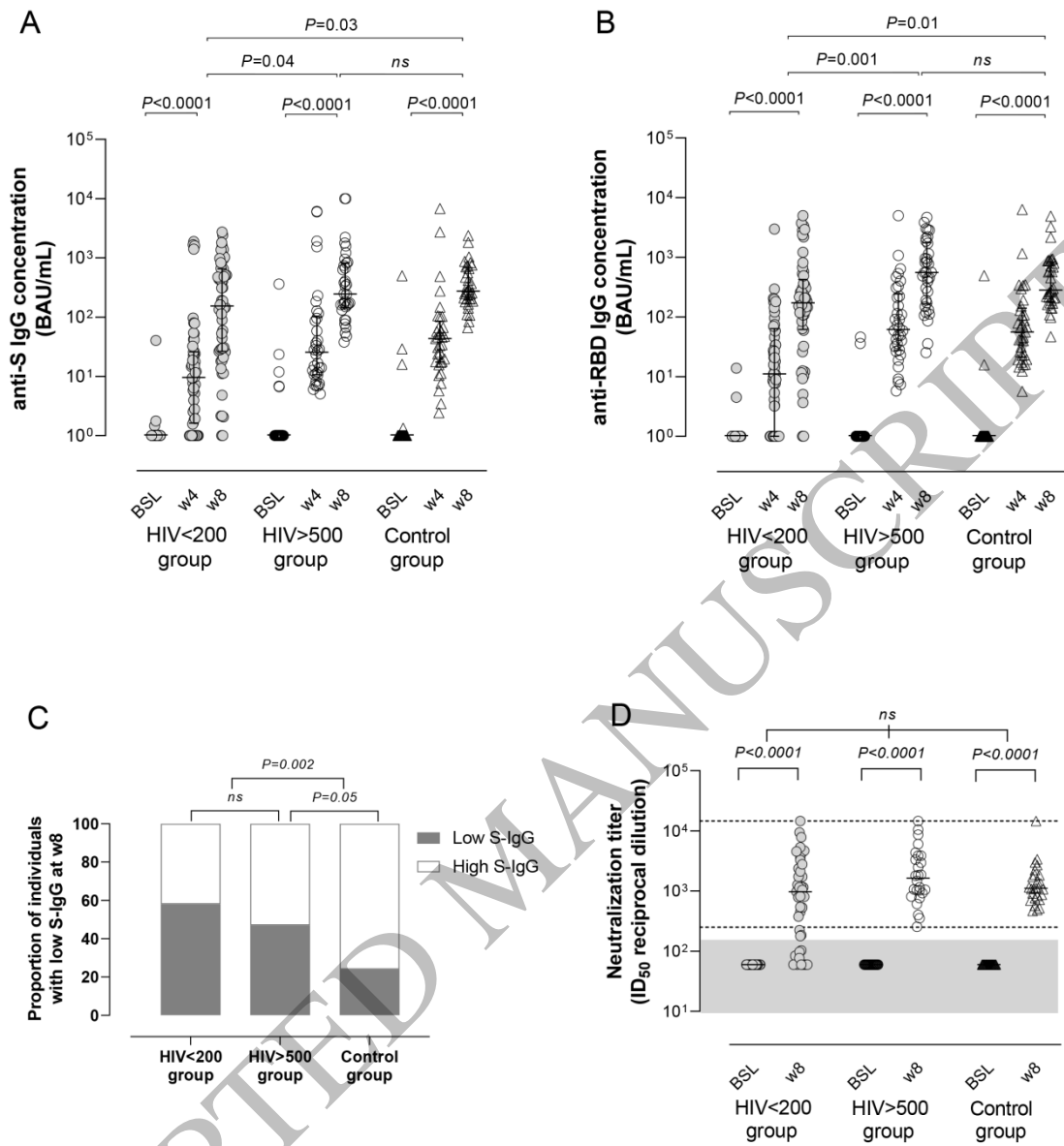
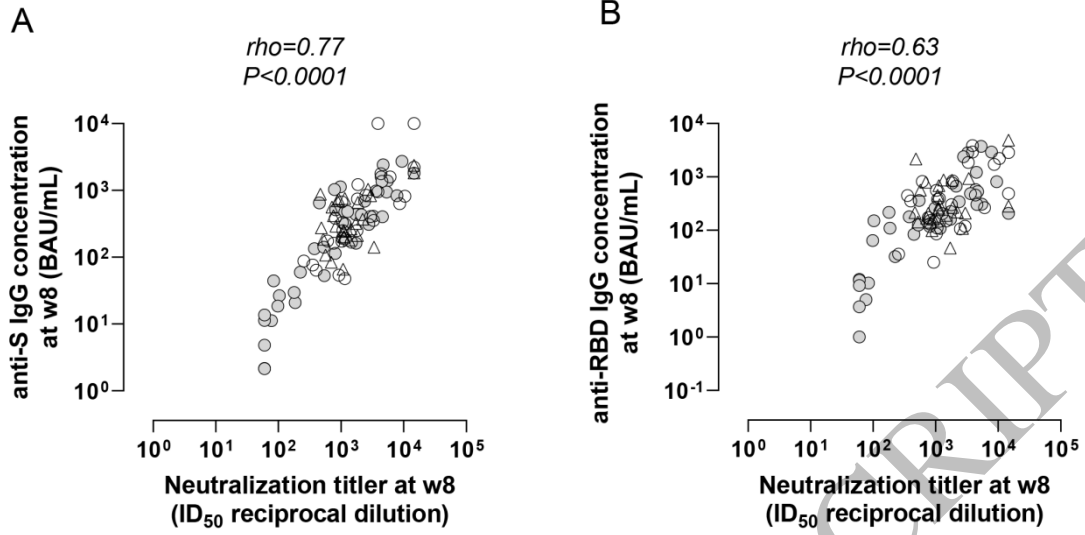


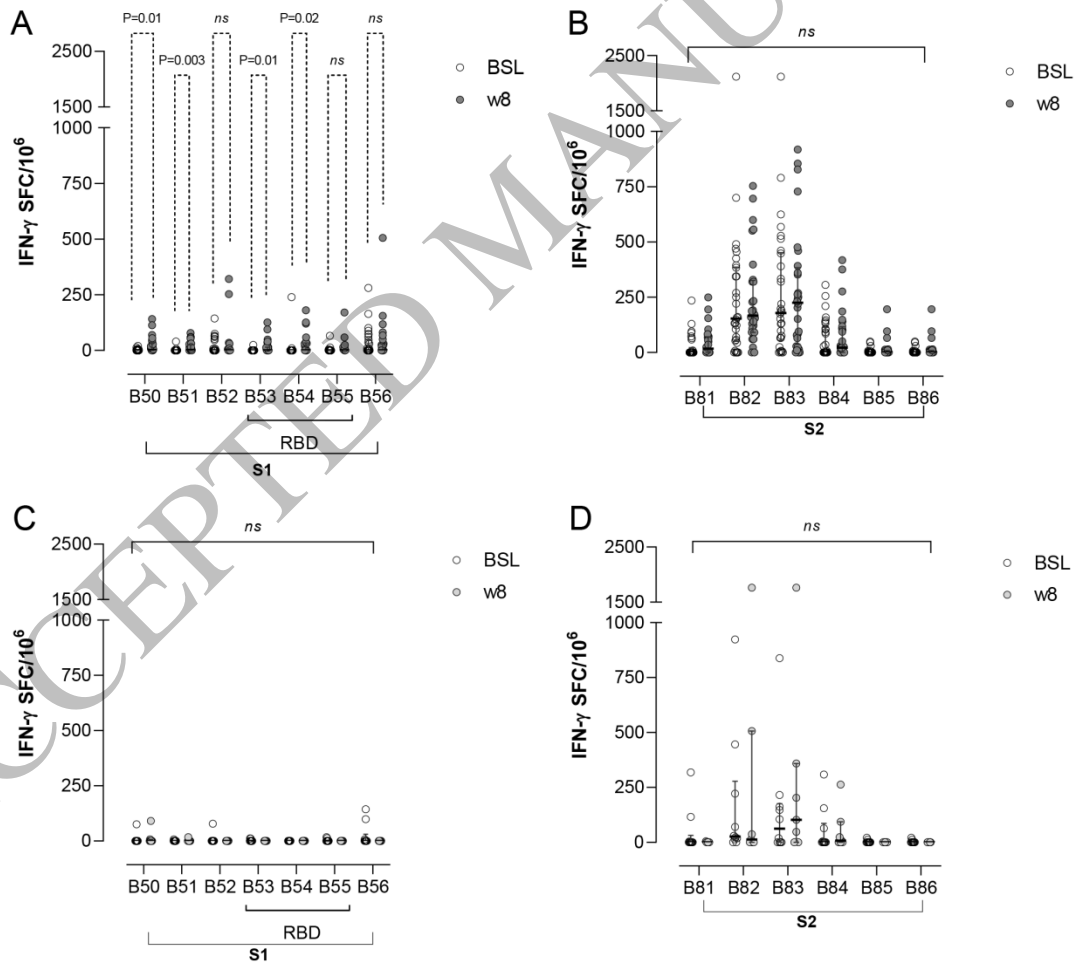
Figure 2
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Figure 3
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Figure 4
192x165 mm (x DPI)

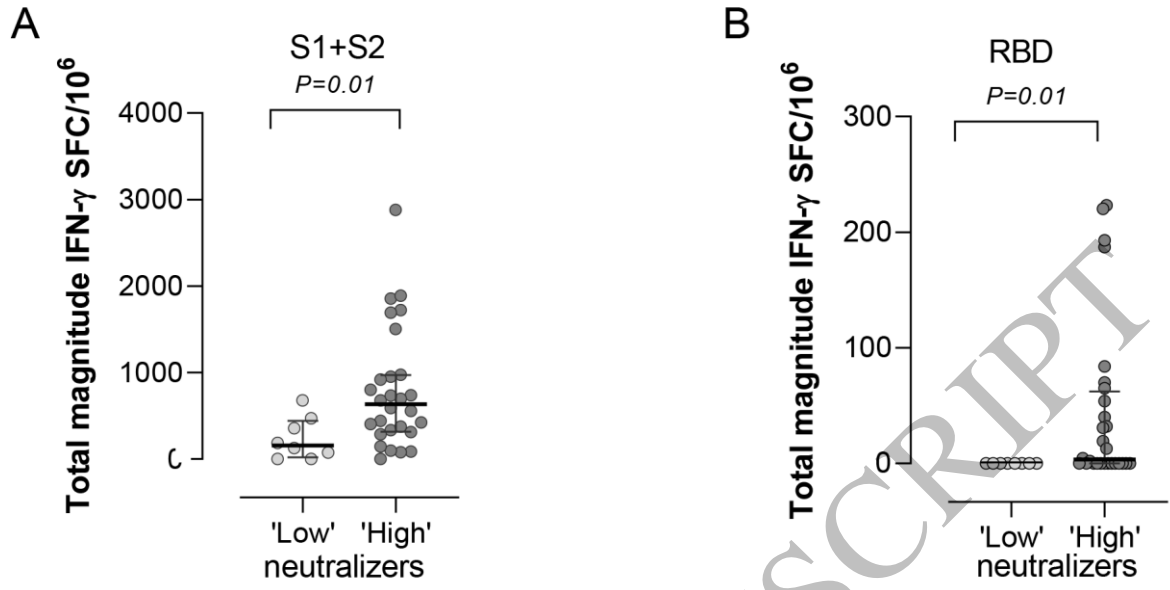


Figure 5
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