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ANTI-RBD IgG RESPONSE AFTER A PRIMARY ANTI-SARS-COV-2 VACCINATION COURSE WITH BNT162B2 AND AFTER A THIRD DOSE WITH A FIRST-GENERATION mRNA VACCINE IN A COHORT OF PEOPLE LIVING WITH HIV

S.S.D. MED/17

Coordinatore: Prof. Simone Accordini

Signature _____

Tutor: Prof.ssa Evelina Tacconelli

Signature_____

Student: Dott.ssa Anna Maria Maddalena Azzini

Signature _____

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Anna Maria Maddalena Azzini

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SOMMARIO

Introduzione

I dati sull'immunogenicità dei vaccini contro SARS-CoV-2 documentano la riduzione del titolo anticorpale sei mesi dopo la fine del ciclo di vaccinazione primaria e suggeriscono la somministrazione di una dose aggiuntiva di vaccino, soprattutto nei soggetti fragili e/o immunodepressi. I dati sull'immunogenicità dei vaccini ad m-RNA anti-SARS-CoV-2 nelle persone con infezione da HIV (PLWH) sono attualmente limitati, non solo relativamente al ciclo primario di vaccinazione ma anche relativamente alla dose di richiamo.

Metodi

Una coorte di pazienti con infezione da HIV ed in terapia antiretrovirale sottoposti a un programma vaccinale anti-SARS-CoV-2, sono stati seguiti prospetticamente per valutare la risposta umorale anti-RBD IgG dopo un ciclo di vaccinazione primaria con BNT162b2 e dopo la terza dose di richiamo con un vaccino a m-RNA di prima generazione. I partecipanti sono stati stratificati in base alla conta dei linfociti T CD4 al baseline in tre categorie: scarso recupero di CD4, PCDR: <200/mm^3; recupero intermedio di CD4, ICDR: 200–500/mm^3; alto recupero di CD4, HCDR: >500/mm^3. Le IgG anti-RBD sono state misurate utilizzando un test anticorpale commerciale per microparticelle a chemiluminescenza (Roche Elecsys® Anti-SARS-CoV-2 S) e il titolo anticorpale è stato stratificato in cinque categorie: non reattivo (< 5,58 BAU/mL); non conclusivo (≥5,58 BAU/mL e < 45 BAU/mL); positivo basso (≥45 BAU/mL e < 205 BAU/mL); positivo intermedio (≥205 BAU/mL e < 817 BAU/mL) e positivo alto (≥817 BAU/mL).

I pazienti inclusi sono stati valutati in quattro momenti successivi: prima dell'inizio della vaccinazione (T1), 3 settimane dopo la prima dose (T2), 3 mesi ± 1 mese dopo la prima dose (T3) e 1 mese ± 1 mese dopo la terza dose (T4). L'insorgenza di infezione break-through è stata valutata fino a 6 mesi dopo la somministrazione della terza dose di vaccino.

Risultati

Complessivamente sono stati arruolati 304 soggetti. Tre mesi dopo il completamento di un ciclo di vaccinazione primaria con BNT162b2, il 97% di 214 PLWH naive per SARS-CoV-2 ha sviluppato una risposta sierologica rilevabile, principalmente con titoli anticorpali intermedi (51%) e alti (35%). Il titolo elicitato è stato prevalentemente

positivo-intermedio, sia per i soggetti con conta linfocitaria pre-vaccinale bassa sia per i soggetti con conta linfocitaria pre-vaccinale più alta (56% vs 50%). È stato raggiunto un titolo positivo-alto di IgG anti-RBD soprattutto nei soggetti con conte basali di linfociti T CD4-positivi superiori a 500/mm^3 rispetto a coloro con conte basali inferiori a 500/mm^3 (39% vs 21%). L'età avanzata e il sesso maschile si sono associati alla probabilità di sviluppare un titolo anticorpale medio-basso. La presenza di due o più comorbidità è sembrata associarsi a una maggiore probabilità di sviluppare una risposta sierologica medio-bassa. Una conta basale di linfociti T CD4 positivi superiore a 500/mm^3 si è associata a una maggiore probabilità di sviluppare un'elevata risposta sierologica ma solo all'analisi univariata. La dose booster di vaccino ad m-RNA, ha portato le IgG anti-RBD a titoli notevolmente più elevati rispetto a quelli pre-booster, in soggetti con una conta basale di linfociti T CD4 sia superiore che inferiore a 500/mm^3 (97% vs 83%). Il 27% di 301 soggetti valutabili ha contratto almeno un'infezione break-through, generalmente asintomatica o pauci-sintomatica. Nell'80% dei pazienti con infezione break-through, l'episodio infettivo si è verificato dopo la terza dose di vaccino, in media 4,2 ± 3,2 mesi dopo la somministrazione del richiamo. La probabilità di infezione post-booster è risultata maggiore fra gli individui naive per SARS-CoV-2 (P .001).

Conclusioni

Il 97% dei PLWH ha sviluppato una risposta umorale contro SARS-CoV-2 nei tre mesi successivi all'aver ricevuto un ciclo di vaccinazione primaria con BNT162b2. I titoli anticorpali sono risultati più bassi fra i soggetti con conta basale dei linfociti T CD4 positivi <500/mm^3 rispetto ai soggetti con conta >500 cellule/mm^3.

La dose booster con un vaccino a m-RNA, ha sviluppato titoli IgG anti-RBD significativamente più elevati rispetto al periodo pre-richiamo vaccinale, sia in soggetti con una conta basale di linfociti T CD4 superiore che inferiore a 500/mm^3 (97% vs 83%). I vaccini a m-RNA di prima generazione, compresa la dose di richiamo, non hanno prevenuto l'infezione da SARS-CoV-2 nel 27% degli arruolati, ma gli episodi infettivi sono stati asintomatici o paucisintomatici, suggerendo la loro capacità di prevenire forme gravi di COVID-19 sostenute anche da Omicron.

Sono necessari ulteriori studi per monitorare nel tempo l'immunogenicità delle dosi vaccinali di richiamo nei pazienti con infezione da HIV, in particolare l'immunogenicità dei nuovi vaccini bivalenti specifici per la variante Omicron.

ABSTRACT

Background

Data on the immunogenicity of vaccines against SARS-CoV-2 document the reduction of the antibody titer six months after the end of the primary vaccination course and suggest the benefit of an additional vaccine dose, especially in frail and/or immunosuppressed subjects. Data on SARS-CoV-2 m-RNA vaccines immunogenicity in people living with HIV (PLWH) are currently limited, in relation to both the primary vaccination course and the boosting dose.

Methods

A cohort of PLWH on antiretroviral therapy attending a SARS-CoV-2 vaccination program were included prospectively after receiving a primary vaccine course with BNT162b2 and after a boosting dose with a first-generation m-RNA vaccine. Participants were stratified by baseline CD4 T-lymphocytes count (poor CD4 recovery, PCDR: <200/mm^3; intermediate CD4 recovery, ICDR: 200–500/mm^3; high CD4 recovery, HCDR: >500/mm^3). RBD-binding IgG were measured using a commercial chemiluminescence microparticle antibody assay (Roche Elecsys® Anti-SARS-CoV-2 S) and titers were stratified in five categories: non-reactive (<5.58 BAU/mL); inconclusive (≥5.58 BAU/mL and <45 BAU/mL); positive low (≥45 BAU/mL and <205 BAU/mL); positive intermediate (≥205 BAU/mL and <817 BAU/mL), and positive high (≥817 BAU/mL). PLWH were evaluated at four successive time points: before the start of vaccination (T1), 3 weeks after the first dose (T2), 3 months ± 1 month after the first dose (T3) and 1 month ± 1 month after the third dose (T4). The onset of break-through infection was evaluated up to 6 months after the third vaccine dose.

Results

A total of 304 subjects were enrolled. Three months after the primary vaccination course with BNT162b2, 97% of 214 PLWH naïve for SARS-CoV-2 infection had a detectable antibody response, mainly at intermediate (51%) and high (35%) titers. Elicited titer was mainly positive-intermediate, for subjects both with low and with high pre-vaccinal CD4 T-lymphocyte counts (56% vs 50%). A positive-high anti-RBD IgG titer was reached especially in subjects with more than 500/mm^3 CD4 T-lymphocytes at baseline in comparison to those with less than 500/mm^3 (39% vs 21%). Advanced age and male gender were associated with the probability of developing a

medium-low antibody titer. The presence of two or more comorbidities also seemed to be associated with a higher probability of developing a low-medium serological response. Current CD4 T-lymphocytes count greater than 500/mm^3 before the administration of the first vaccine dose, was associated with an increased likelihood of developing a high serological response (OR 2.39; CI95% 1.04-5.53), but only at univariable analysis. An additional dose of SARS-CoV-2 mRNA vaccine after the initial two-dose vaccination, resulted in higher levels of immunity, both in PLWH with a baseline CD4 T-lymphocytes count higher and lower than 500/mm^3 (97% vs 83%). 27% of 301 evaluable PLWH experienced at least one SARS-CoV-2 infection, mainly asymptomatic or pauci-symptomatic and at an average of 4.2 ± 3.2 months after the third booster dose (80% of infectious episodes). The probability of becoming infected after the booster was higher among SARS-CoV-2 naïve individuals (P .001).

Conclusions

97% of PLWHs developed a humoral response against SARS-CoV-2 within three months after receiving a primary course with BNT162b2, albeit poorer in those with CD4 T-cell <500/mm^3 versus those with >500 cell/mm^3 at baseline.

An additional dose of a m-RNA vaccine resulted in higher levels of immunity, independently from the baseline CD4 T-lymphocyte counts.

First generation m-RNA vaccines, including booster doses, did not prevent SARS-CoV-2 infection in 27% of subjects but infective episodes were asymptomatic or pauci-symptomatic, suggesting the ability to prevent severe COVID-19 also caused by Omicron.

Studies to monitor over time the humoral responses after boosting vaccination and on the immunogenicity of new bivalent omicron-containing vaccine are needed, also in PLWH.

INDEX

BACKGROUND

At the end of 2019, an unknown disease emerged, responsible for pneumonia-like symptoms and lung fibrosis (1). It was described for the first time in the city of Wuhan, Hubei Province, China (2, 3, 4). China reported this pneumonia of unidentified cause first to the World Health Organization (WHO) country office on December 31, 2019 (5). The Chinese Center for Disease Control and Prevention identified a virus, a novel coronavirus (CoV2), from the throat swab sample of an infected patient on January 7, 2020 (3).

The WHO declared the disease as Public Health Emergency of International Concern in January 2020 and officially named the disease caused by the novel CoV2 as coronavirus disease 2019 (COVID-19) on February 12, 2020 (1, 6).

1. Origin and evolution of coronaviruses

In the mid-1930s, a severe respiratory infection of chicken was the earliest known disease by coronaviruses; this infection is presently known as avian infectious bronchitis and is caused by the avian infectious bronchitis virus (IBV).

The era of human coronaviruses began in 1965, when Tyrrell and Bynoe demonstrated that common colds could be transmitted by nasal secretion that did not contain rhinoviruses. They could serially sub-passage a virus in tissue culture while doing research on human participants at the Common Cold Unit close to Salisbury (United Kingdom) and named this virus as B814 (7). Thanks to further *in vitro* experiments of nasal swabs from these participants inoculated onto organ cultures obtained from respiratory tract cell lines, they discovered the presence of enveloped RNA viruses with the feature morphology of coronaviruses like the previously defined IBV. They were unable to grow the virus in tissue culture at that time, but in 1966, Hamre and Procknow succeeded in growing a new virus named 229E (8).

Both B814 and 229E were ether sensitive and required a lipid-containing coat for infectivity. These viruses were designated OC as they were grown in the organ culture of respiratory tract. One year later, Almeida and Tyrrell were able to demonstrate the similar morphology of B814 and IBV under the electron microscopy of the fluid obtained from the inoculated organ culture. The virus particles were of size around 80–150 nm, pleomorphic, enveloped with membrane coating, and multiple club-shaped surface projections (9). All of these viruses (229E, B814, and IBV) along with

the virus causing mouse hepatitis and transmissible gastroenteritis of swine, had a similar morphology under electron microscopy (10). This new group of viruses was named coronavirus in 1968, reflecting their morphology in the electron microscope, and further *Coronaviridae* was accepted as their family name in 1975 (11).

229E and OC43 were the only two human coronaviruses discovered at the beginning, and both were recognized as scarcely pathogenic for human beings, causing mild flu-like infection.

A new human coronavirus was associated with a severe acute respiratory syndrome outbreak in 2002–2003 with the epicenter in China and spread expeditiously all over the world (12, 13). Named SARS-CoV (severe acute respiratory syndrome coronavirus), it was found to be more pathogenic and cause serious respiratory complications (14,15). Ten years after the SARS outbreak another human coronavirus, the Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in the Middle East nations (16). Both SARS-CoV and MERS-CoV were transmitted directly to humans from feline animals (civet) and dromedary camels, respectively (17, 18).

SARS-CoV-2 is the most recent coronavirus that appeared in China, and which is responsible for the COVID-19 pandemic.

2. Coronaviruses morphology, genome organization, replication

Coronaviruses belong to the order *Nidovirales*, family *Coronaviridae*, and subfamily *Orthocoronavirinae*. *Orthocoronavirinae* consists of four genera: *Alphacoronaviruses* (αCoV), *Betacoronaviruses* (βCoV), *Gammacoronaviruses* (γCoV), and *Deltacoronaviruses* (δCoV). αCoV and βCoV exclusively infect mammals, while γCoV and δCoV have a wider range of hosts, including birds (19). SARS-CoV, MERS-CoV, HCoV-OC43, and SARS-CoV-2 are βCoV (figure 1). Evolutionary trend analysis of coronaviruses has revealed that αCoV and βCoV originated from bats and rodents, while γCoV and δCoV were found to have originated from avian species (20).

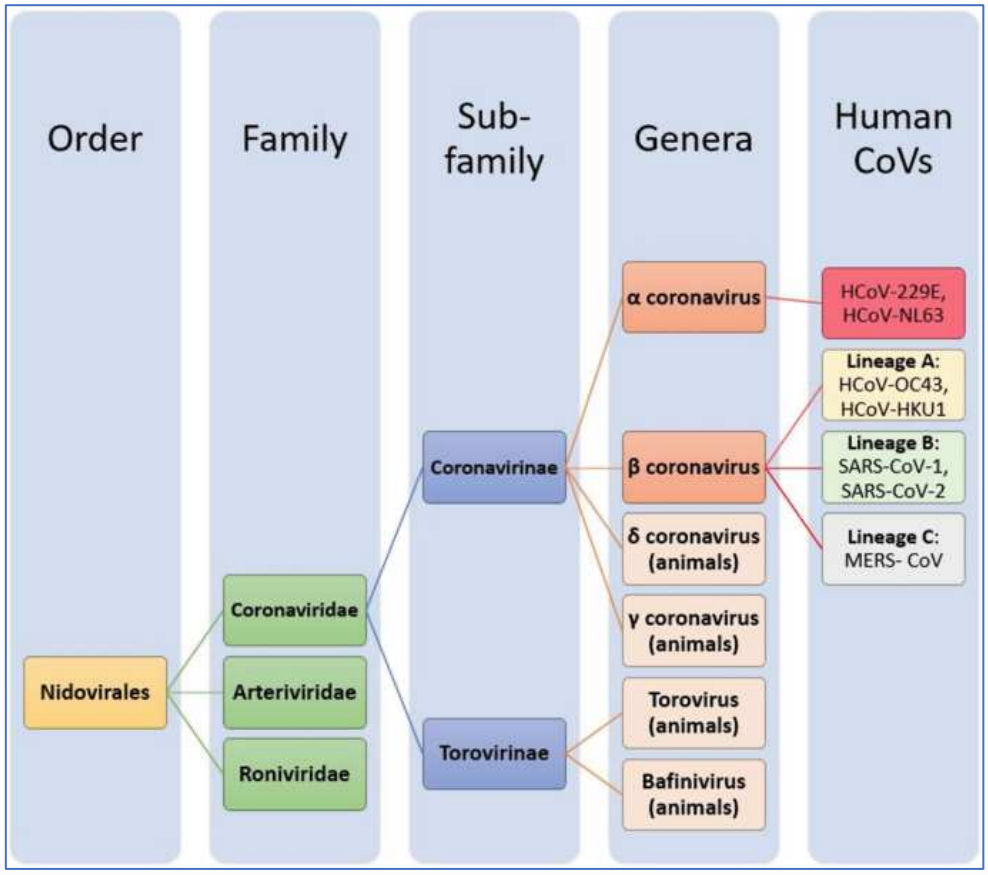

Figure 1: coronaviruses family tree

Coronaviruses have a spherical shape of the virion with a diameter of 80 to 120 nm, framed by the so-called "spikes", trimers of the Spike protein (S) (figure 2). The viral envelope is supported by the membrane protein (M) and contains small inclusions of the envelope protein (E). Under the virion envelope is a helically symmetrical nucleocapsid formed by a single-stranded genomic RNA coated with the nucleocapsid protein (N) (21).

S protein (128-160 kDa) determines tropism of the virus; it binds to the receptors localized on the surface of the host cell. N protein determines architecture of the virus genome by forming a nucleocapsid with genomic RNA. Localized in the region of endoplasmic reticulum and Golgi apparatus, it is involved in the assembly and budding of the viral particles. N protein is also believed to be involved in regulation and modulation of replication and transcription. It has been shown that it could not only nonspecifically bind RNA, but also specifically interact with some sequences, including the transcription-regulating sequences (TRS). The N protein of SARS-CoV-2 promotes the association of RNA with the complex of non-structural proteins, nsp-7

- nsp8 - nsp12, and likely the initiation of replication and transcription (22). M protein is a core membrane protein. It is embedded in the lipid bilayer by three transmembrane domains; glycosylated ectodomain of the protein protrudes outwards. M protein maintains the viral envelope and determines the shape and size of the viral capsid interacting with other structural proteins. Interaction of M and S proteins is necessary to retain S protein in the intermediate compartment between endoplasmic reticulum (ER) and Golgi apparatus, the so-called endoplasmic reticulum-Golgi intermediate compartment (ERGIC), and its inclusion in new virions. Binding of M and N proteins stabilizes nucleocapsid as well as inner core of the virion and ultimately contributes to the completion of the virus assembly. E protein is a small integral protein (8-12 kDa), it is anchored into the membrane by the transmembrane domain, has been shown to form homopentamers that act as ion channels. Such structures modulate the process of virion release, taking an active part in the cell infection (23, 24).

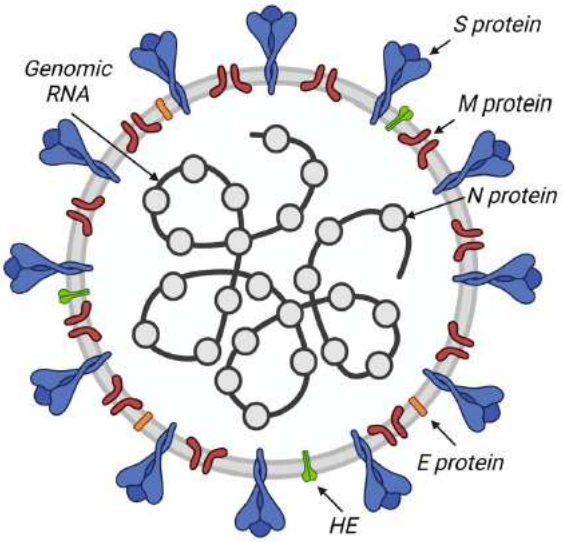

Figure 2: Coronavirus structure: N, S, M, E structural proteins and genomic RNA are indicated. In the HCoV-OC43 and HCoV-HKU1 coronaviruses, HE (hemagglutinin esterase) acts with S protein to penetrate the target cells (24).

The SARS-CoV-2 genome is 26 to 32 kb, it encodes 16 non-structural (nsp1-16), 4 structural (S, M, N, and E), and 11 accessory proteins (ORF3a, ORF3b, ORF3c, ORF3d, ORF6, ORF7a, ORF7b, ORF8, ORF9b, ORF9c, and ORF10) (25). The carrier of genetic information of coronaviruses is the positive-sense single-stranded RNA. Each viral transcript, like genomic RNA, is capped at the 5'-end and polyadenylated at the 3'-end. The presence of the 5'-cap and a 3'-polyA tail at the ends

of the genomic RNA allows immediate translation of nonstructural proteins from the ORF1a and ORF1b (figure 3). Reading frames are separated from each other by the reading frameshift site (slippery sequence). Translation results in two polyprotein chains, pp1a and pp1ab. Nonstructural proteins are formed because of proteolytic processing of pp1a and pp1ab by two cysteine proteases: papain-like proteinase (PLpro) and 3-chymotrypsin-like proteases (Mpro, major protease). Genes of structural and accessory proteins are transcribed into a set of sub-genomic m-RNAs. Genomic RNA and all sub-genomic m-RNAs contain the same leader sequence at their 5'-ends.

This structure allows the cell ribosome to recognize genomic RNA as m-RNA and immediately include it in the process of translation of viral proteins without adding transcription complex into the virion. In addition, all viral RNAs have a special leader sequence at their 5'-end, which distinguishes viral and cellular RNAs (figure 3) (26).

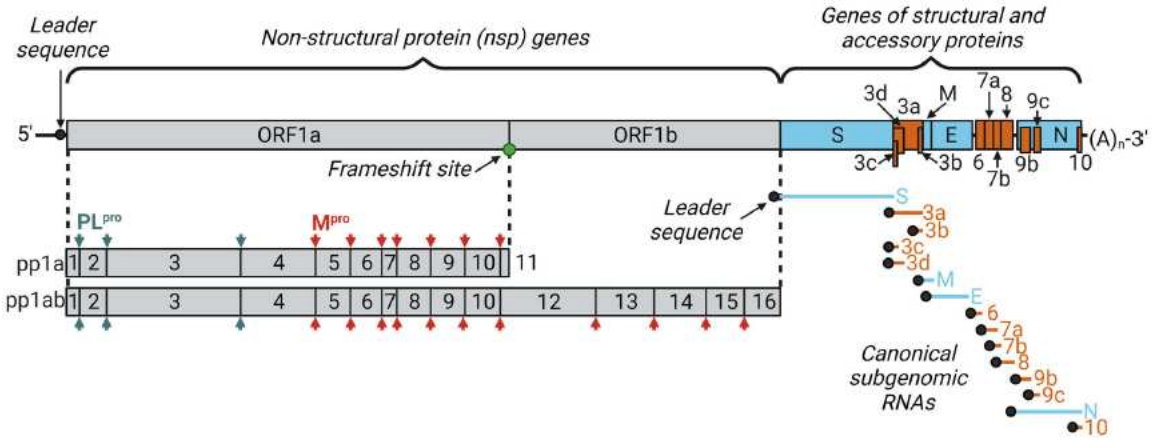

Figure 3: Schematic representation of SARS-CoV-2 genome organization (24).

In the life cycle of coronavirus (figure 4), surface of the virus is coated with the S protein, which interacts with the receptor and activates fusion of the virus with the cell membrane after being cleaved by the cell surface protease. Genomic RNA, getting inside the cell, is immediately recognized by the ribosome, and translation of polyproteins and their processing to individual non-structural proteins occurs. For transcription of structural and accessory proteins, coronaviruses form special replication organelles, double-membrane vesicles (DMV), which are a reticulo-vesicular network of double-membrane vesicles with interconnected outer membranes (figure 4) (27). Formation of the DMV occurs in the membranes of endoplasmic reticulum; the processes of replication and transcription of sub-genomic RNA of

coronaviruses take place in DMV replication–transcription complexes (RTCs) (28). Genomic sense RNA is first converted into the antisense form to form genomic and sub-genomic RNAs, and then into the sense form of the genomic RNA and sub-genomic m-RNAs. Sub-genomic m-RNAs are translated inside the ER into structural and accessory proteins. Structural and accessory proteins are synthesized and moved first to the ERGIC. Genomic RNA interacts with N protein, forming a nucleocapsid, which combines with the structural proteins to form a virion in the Golgi apparatus. The mature virion is released from the cell by constitutive exocytosis (figure 4) (29, 30).

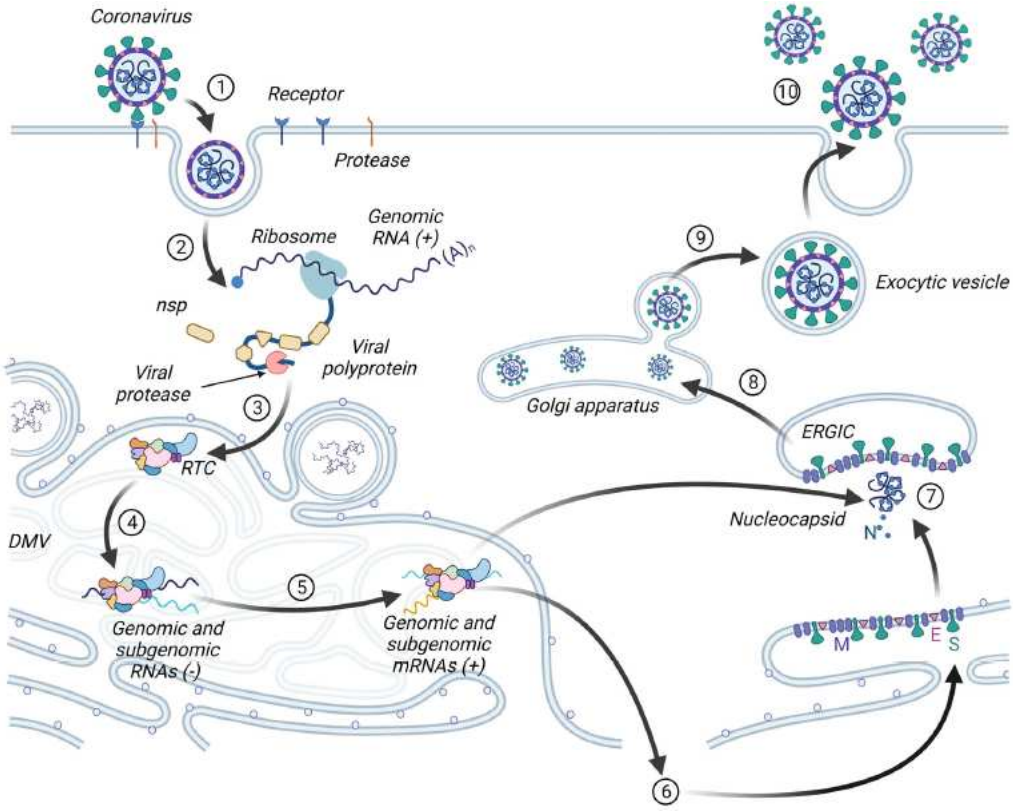

Figure 4: Life cycle of coronaviruses (24).

3. Mechanism of the virus entry into the target cell

SARS-CoV-2 enters the cell following two successive events: (i) viral S protein interacts with its receptor, the angiotensin-converting enzyme ACE2, on the cell surface, and next (ii) S protein is transformed into its active form, capable of stimulating fusion of the viral and cell membranes by cleavage with the intracellular protease.

S protein is located on the surface of the virion in the form of a trimer, forming a convex "head" and a "stem" (figure 5). There are two subunits in the S protein: S1 is responsible for binding to the receptor, and S2 is responsible for fusion of the virus with the host cell membrane. Main domains of the S1 subunit are the N-terminal domain (NTD) and the receptor-binding domain (RBD), which directly binds to the receptor through its inner receptor-binding motif (RBM). Fusion peptide (FP), two domains (HR1 and HR2), transmembrane (TMD), and cytoplasmic (CTD) domains are identified in the S2 subunit. The S1 subunit "wraps" the S2 subunit, which forms the center of the protein.

S protein contains two regions, sequential cleavage of which leads to its activation on the cell surface. The first site, S1/S2, is located at the junction of two subunits, the second site is located within the S2 subunit and is called S2'. Cleavage first at the S1/S2 site and next at the S2' site by extracellular and cellular transmembrane proteases, allows the FP exposition to the outside and triggers fusion with the host cell membrane. Hydrophobic interactions then occur between the HR1 and HR2 domains of the S2 subunit, resulting in formation of a six-chain structure that causes the membranes of the virus and the cell to approach each other forming a pore. The pore size increases until genetic material of the virus enters the cell (31, 32).

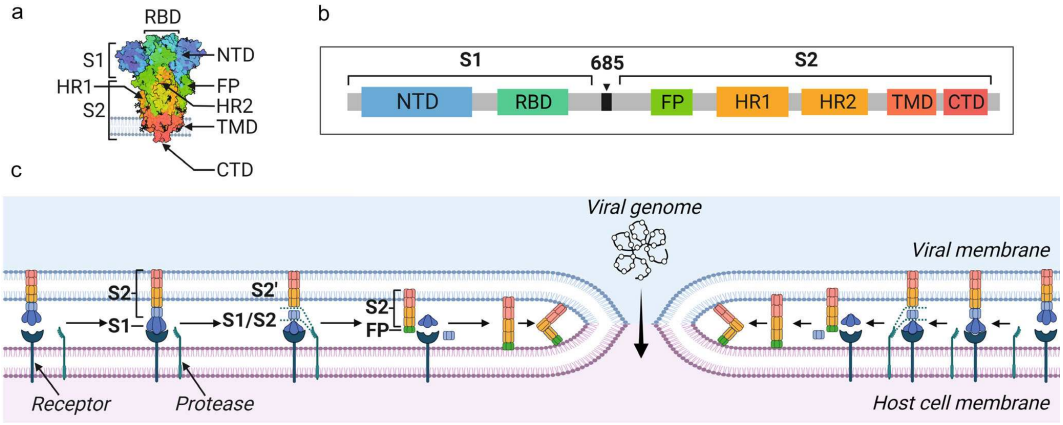

Figure 5: Domain organization of S protein and its participation in the fusion of a viral particle with a target cell. a) 3D structure of the S protein of SARS-CoV-2; b) primary structure of the S protein of SARS-CoV-2; c) scheme of interaction between a cell and a coronavirus particle. S1 subunit: N-terminal domain, NTD; receptor-binding domain, RBD; S2 subunit: fusion peptide, FP; HR1 and HR2 domains; transmembrane domain, TMD; cytoplasmic domains, CTD (24).

4. Viral variants

SARS-CoV-2 change over time. At the beginning of emergence of SARS-CoV-2 variants, the D614G mutation in the S protein gene (33) contributed to the rapid

spread of the virus and became the first mutation that was preserved in all variants that appeared after its first emergence in Wuhan (34). Presence of this mutation is associated with high viral load in the infected patients and high infectivity in the *in vitro* model. At the same time, presence of the D614G mutation does not correlate with severity of the disease (35).

Most mutations have little to no impact on the virus' properties. However, some changes may affect the virus's properties, such as how easily it spreads, the associated disease severity, or the performance of vaccines, therapeutic medicines, diagnostic tools, or other public health and social measures.

WHO has been monitoring and assessing the evolution of SARS-CoV-2 since January 2020. During late 2020, the emergence of variants that posed an increased risk to global public health prompted the characterization of specific Variants of Interest (VOIs) and Variants of Concern (VOCs), to prioritize global monitoring and research, and ultimately to inform the ongoing response to the COVID-19 pandemic. The established nomenclature systems for naming and tracking SARS-CoV-2 lineages follows GISAID (https://gisaid.org/), Nextstrain (https://nextstrain.org/) and Pango (https://cov-lineages.org/) definitions.

As indicated by WHO, VOI is defined as a SARS-CoV-2 variant

- with genetic changes that are predicted or known to affect virus characteristics such as transmissibility, disease severity, immune escape, diagnostic or therapeutic escape.

and

- identified to cause significant community transmission or multiple COVID-19 clusters, in multiple countries with increasing relative prevalence alongside increasing number over time, or other apparent epidemiological impacts to suggest an emerging risk to global public health.

A VOC is defined as a SARS-CoV-2 variant that meets the definition of a VOI and, through a comparative assessment, has been demonstrated to be associated with one or more of the following changes at a degree of global public health significance:

- increase in transmissibility or detrimental change in COVID-19 epidemiology

or

- increase in virulence or change in clinical disease presentation

or

- decrease in effectiveness of public health and social measures or available diagnostics, vaccines, therapeutics

For WHO there is not currently circulating VOIs; previous VOIs were, as per WHO label, Epsilon, Zeta, Eta, Theta, Iota, Kappa, Lambda, and Mu.

Omicron, B1.1.529 Pango lineage, currently is the only and dominant VOC circulating globally, accounting for more than 98% of viral sequencies shared on GISAID after February 2022. WHO also includes BA.1, BA.2, BA.3, BA.4, BA.5 and descendent lineages and BA.1/BA.2 recombinant forms. Table 1 summarizes previously circulating VOCs.

WHO label	**Pango Lineage**	**GISAID clade**	**Nextstrain clade**	**Earliest documented samples**	**Date of designation**
Alpha	B.1.1.7	GRY	20I (V1)	United Kingdom, Sep-2020	VOC: 18-Dec-2020 Previous VOC: 09-Mar-2022
Beta	B.1.351	GH/501Y.V2	20H (V2)	South Africa, May-2020	VOC: 18-Dec-2020 Previous VOC: 09-Mar-2022
Gamma	P.1	GR/501Y.V3	20J (V3)	Brazil, Nov-2020	VOC: 11-Jan-2021 Previous VOC: 09-Mar-2022
Delta	B.1.617.2	G/478K.V1	21A, 21I, 21J	India, Oct-2020	VOI: 04-Apr-2021 VOC: 11-May-2021 Previous VOC: 07-Jun-2022

Table 1: previously circulating VOCs (https://www.who.int/activities/tracking-SARS-CoV-2-variants assessed on 9th February)

For the Delta variant, four critical mutations which affect characteristics of the virus have been described: D614G and G142D increase affinity for ACE2, as well as L452R and T478K provide high affinity and inability of antibodies to neutralize the virus (36, 37, 38). These mutations are not unique and occur in other variants, but their combination in the Delta variant could determine long and severe course of the disease. Firstly, even at low levels of ACE2, the Delta variant S protein fuses efficiently with the cell. Secondly, infection of the target cells with this variant occurs much faster than with the viruses of another variant. Thus, with a relatively short exposure, Delta variant can quickly infect many more cells, resulting in a short incubation period and a higher viral load during infection (39).

In the S protein of Omicron variant, the number of mutations is more than 30 (40) with more than 10 located in the RBD domain. Among mutations found in all Omicron's sub-variant, the N501Y and Q498R are thought to enhance binding to the ACE2 receptor, and the H655Y, N679K, and P681H to increase S protein cleavage and facilitate virus transmission (41). Substitutions in the S protein at positions 452, 486, and 493 are thought to alter ACE2 binding and affect antibody interactions. Mutation at L452 position impairs the ability of antibodies to neutralize the virus (42). The amino acid at position F486 is involved in the binding of the virus to the ACE2 receptor, mutation at this position results in the decrease of neutralizing activity of the class I and II antibodies and polyclonal serum. It is believed that F486 gives the virus a great advantage in avoiding the action of neutralizing antibodies, including antibodies that neutralize BA.1. The BA.4 and BA.5 sub-variants acquired even more effective antibody evasion mechanism than the previous sub-variants (42). The ability to avoid neutralizing antibodies could be the reason for easier spread of this variant between people: the Omicron variant B.1.1.529 is 3.3-fold more transmissive than the Delta variant (43).

As transmission of Omicron has been sustained, this has led to significant intra-variant evolution: the viruses part of the Omicron complex have continued to evolve, leading to descendent lineages with different genetic mutations. Considering the widespread transmission of the Omicron VOC across the globe and the subsequent expected increased viral diversity, WHO has added the new category "Omicron subvariant under monitoring". A variant under monitoring (VUM) is a viral variant that, according to phylogenetic analysis, belongs to a currently circulating VOC and shows signals of transmission advantage compared to other circulating VOC lineages and has additional amino acid changes that are known or suspected to confer the observed change in epidemiology and fitness advantage as compared to other circulating variants.

If any of these Omicron sublineages is proven to have distinct characteristics as compared to the original VOC and could pose an additional threat to global public health, it could become a new VOC with a new WHO-label. Table 2 describes Omicron VUMs.

Pango lineage (+ mutation)	**GISAID clade**	**Nextstrain clade**	**Relationship to circulating VOC lineages**	**Spike genetic features**	**Earliest documented samples**
BF.7*****	GRA	22B	BA.5 sublineage	BA.5 + S:R346T	24-01-2022
BQ.1•	GRA	22E	BA.5 sublineage	BQ.1 and BQ.1.1: BA.5 + S:R346T, S:K444T, S:N460K	07-02-2022
BA.2.75º	GRA	22D	BA.2 sublineage	BA.2.75: BA.2 + S:K147E, S:W152R, S:F157L, S:I210V, S:G257S, S:D339H, S:G446S, S:N460K, S:Q493R reversion	31-12-2021
CH.1.1 º	GRA	22D	BA.2 sublineage	BA.2.75 + S:L452R, S:F486S	27-07-2022
XBB #	GRA	22F	Recombinant of BA.2.10.1 and BA.2.75 sublineages. i.e. BJ1 and BM.1.1.1, with a breakpoint in S1	BA.2 + S:V83A, S:Y144-, S:H146Q, S:Q183E, S:V213E, S:G252V, S:G339H, S:R346T, S:L368I, S:V445P, S:G446S, S:N460K, S:F486S, S:F490S	13-08-2022
XBB.1.5	GRA	23A	Recombinant of BA.2.10.1 and BA.2.75 sublineages. i.e. BJ1 and BM.1.1.1, with a breakpoint in S1	XBB + S:F486P	05-01-2022
XBF	GRA		Recombinant of BA.5.2.3 and CJ.1 (BA.2.75.3 sublineage)	BA.5 + S:K147E. S:W152R, S:F157L, S:I210V, S:G257S, S:G339H, S:R346T, S:G446S, S:N460K, S:F486P, S:F490S	27-07-2022

Table 2: Omicron sub variant under monitoring (as of 9th February 2023). * additional mutations outside the spike protein: N:G30-S33F, ORF9b: M26-, A29I, V30L**;** • additional mutations outside the spike protein: ORF1a:Q556k, L3829F, ORF1b: Y264H, M1156I, N1191S, N: E136D, ORF9b:P10F; º additional mutations outside the spike protein: ORF1a:S1221L, P1640S, N4060S, ORF1b: G662S, E:T11A; # additional mutations outside the spike protein: ORF1a: K47R, ORF1b: G662S, S959P, E: T11A, ORF8: G8*****. (https://www.who.int/activities/tracking-SARS-CoV-2-variants assessed on 9th February)

On 23 January 2023, the WHO has revised the confidence level of the risk assessment for XBB.1.5 from "Low" (assessed on 11 January 2023) to "Moderate" (25 January 2023). XBB.1.5 is descendent lineage of XBB, which is a recombinant of two BA.2 descendent lineages. In the United States of America, XBB1.5 is increasing in many regions (the prevalence of XBB.1.5 in some regions is predicted to be 80%, while in others, 20-50%) (43); also, the European Centre for Disease Prevention and Control (ECDC) has reported growth of XBB.1.5 in several countries, including Iceland where it has increased to 8.7% in week 2 of 2023 (44).

The amino acid change to 486P contributes to higher ACE-2 binding affinity of this variant and suggests a mechanism for XBB.1.5 to have a higher growth advantage as compared to its parent lineage XBB.1 (45). Severity assessments in human populations are ongoing; the number of cases associated with XBB.1.5 is still low and thus clinical severity cannot yet be confidently assessed. XBB.1.5 does not carry any known mutation(s) associated with potential changes in severity (such as S:P681R) (46, 47). Using pseudotyped virus neutralization assays, XBB.1.5 is shown to be as immune evasive as XBB.1, one of the Omicron subvariants with the highest immune escape to date (45, 48, 49, 50, 51). Antibody titers against XBB.1 are mostly absent in individuals with a history of vaccination with the index vaccine (2-4 doses), are higher in those who recently received a bivalent (BA.5) vaccine booster, and highest in individuals with hybrid immunity (48,49). There are currently no data on real world vaccine effectiveness against severe disease or death.

Latest ECDC assessment on SARS-CoV-2 variants, other than as indicated by WHO, consider Omicron BA.2, BA.4 and BA.5 as VOCs; Omicron BA.2.75, BQ.1, XBB and XBB.1.5 as VOIs; and finally Omicron BA.2.3.20, BF.7, XBC, BN.1, CH.1.1 and the new Pango lineage XAY as VUMs (https://www.ecdc.europa.eu/en/covid-19/variants-concern assessed on 10th February 2023).

5. SARS-CoV-2 epidemiological update

Globally, as of 10 February 2023, there have been 755385709 confirmed cases of COVID-19, including 6833388 deaths, reported to WHO.

Figure 6 and 7 describes the number of confirmed cases and deaths reported by WHO regions from the very beginning of the COVID-19 pandemic.

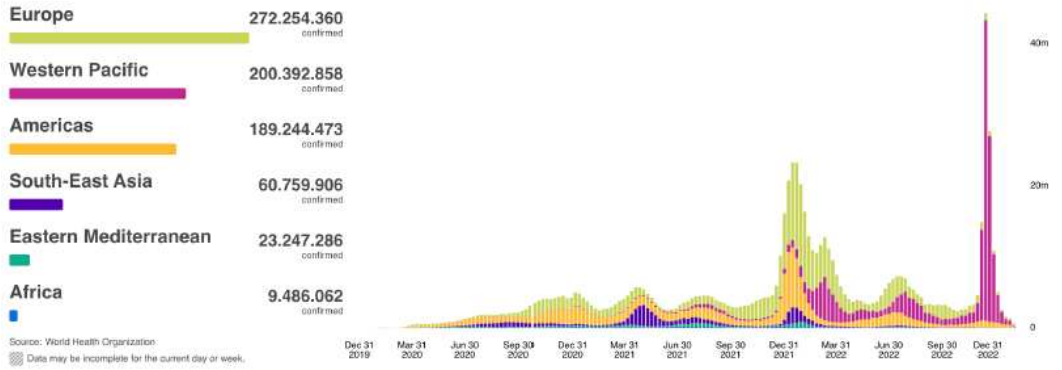

Figure 6: situation by WHO regions of weekly confirmed cases of COVID-19. (https://covid19.who.int/)

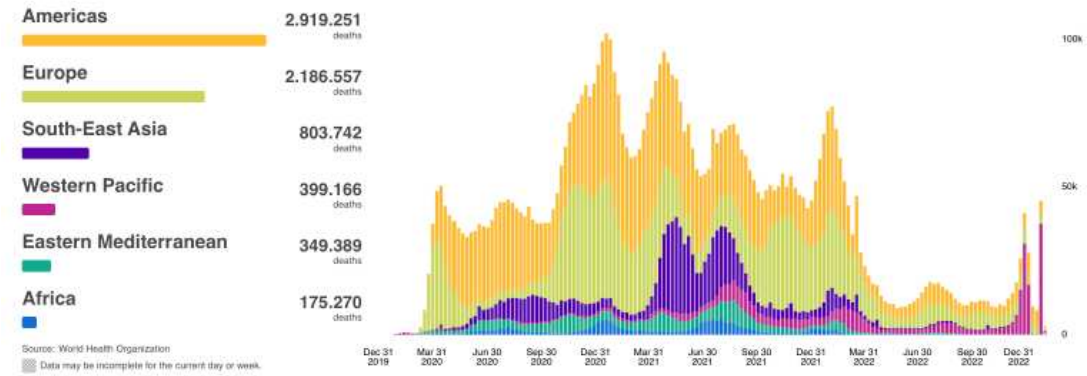

Figure 7: situation by WHO regions of weekly deaths for COVID-19. ((https://covid19.who.int/)

Several epidemic waves have alternated in Italy since the beginning of the pandemic: the first lasted from February to the end of May 2020 and was sustained by a virus that had already undergone significant mutations compared to the wild-type virus sequenced in Wuhan at the end 2019. This variant, in fact, already presented the D614G mutation, also called primary mutation, not present in the wild-type (52). The second wave, mainly supported by VOC Alpha, occurred between September 2020 and February 2021, and was quickly followed by the third, during spring 2021, mainly caused by VOC Alpha and Delta. The fourth wave began in September 2021, largely supported by the Delta variant then replaced by Omicron, which to date has become the most common variant both in Italy and worldwide (https://coronavirus.jhu.edu/region/italy ; https://www.ecdc.europa.eu/en/covid-19/country-overviews) (figure 8 and 9).

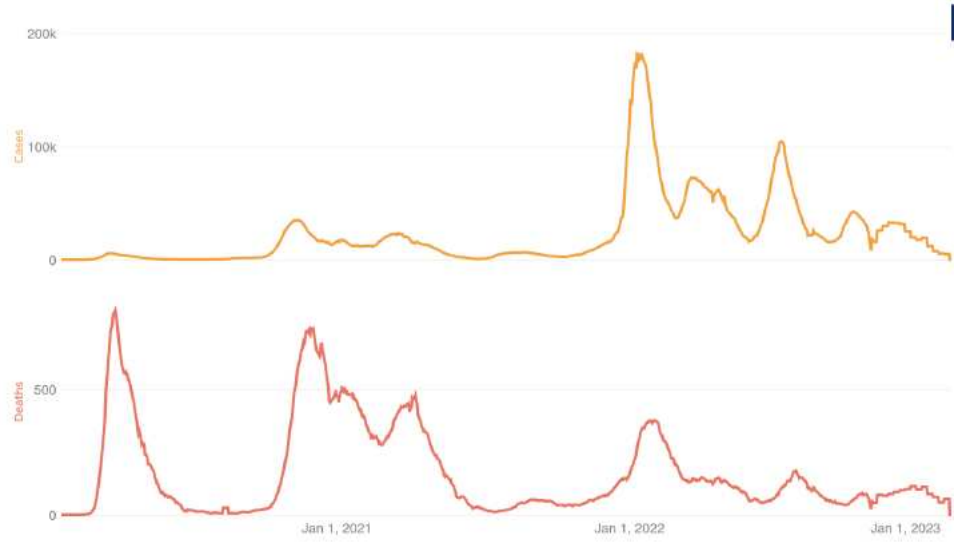

Figure 8: timeline comparisons of SARS-CoV-2 cases and deaths by COVID-19 in Italy (https://covid19.who.int/)

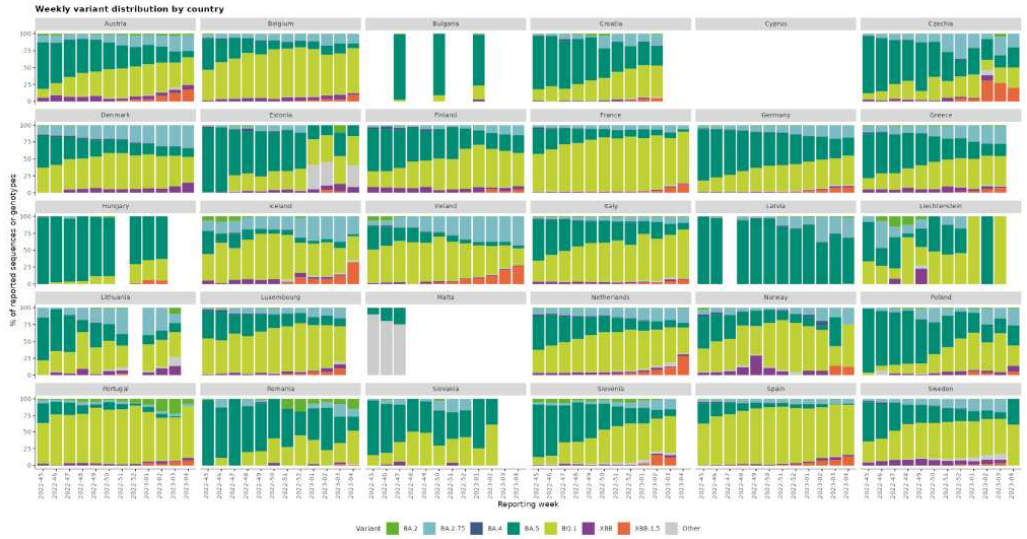

Figure 9: weekly variant distribution by European countries from 7 November 2022 to 29 January 2023. (https://covid19.who.int/)

6. SARS-CoV-2 infection and COVID-19 outcome in PLWH

Concerns arose in the beginning of the COVID-19 pandemic that immunocompromised patients are at higher risk of more severe SARS-CoV-2 infection because evidence of other respiratory viral infections suggested an association between immunosuppressive condition and high frequencies of superinfection, pneumonia, and death (53).

Due to abnormal humoral and cellular immunity, PLWH were considered to be at an increased risk of severe COVID-19 outcomes once infected (54; 55), and the WHO Global Clinical Platform for COVID-19 affirmed that HIV appeared to be a significant

independent risk factor for severe or critical illness at hospital admission and in-hospital mortality, regardless of the varying characteristics of HIV status (56).

There is still no clarity as to the impact of HIV infection on the risk of contracting SARS-CoV-2, nor on the outcome of COVID-19. PLWH have generally not been found to have increased incidence rates of respiratory viral infections such as influenza when compared to the general population. However, SARS-CoV-2 transmission is much more widespread than influenza and infection is generally determined by social determinants of health, such as unstable housing (57) and racial/ethnic disparities (58). Moreover, comorbidities and behaviors among PLWH, such as higher rates of underlying pulmonary disease (59), inhalational drug use (60), increased tobacco use (61), and hazardous alcohol use (62, 63), could lead to increased susceptibility to SARS-CoV-2 infection (64). Furthermore, PLWH have increased rates of comorbidities which can raise the risk of severe COVID-19 disease (65) if exposed to SARS-CoV-2, including cardiovascular disease (66), pulmonary disease (59), cancer (67), and obesity and diabetes (68).

6.1. Susceptibility to SARS-CoV-2 infection among PLWH

Throughout the pandemic, large, population-based studies have mostly demonstrated lower or similar incidence of SARS-CoV-2 infection among PLWH when compared to those without HIV in the general population (69, 70, 71, 72, 73, 74, 75). The first reported population-based studies from the early months of the pandemic (through April 2020) generally demonstrated lower SARS-CoV-2 incidence among PLWH.

A prospective cohort study among HIV clinics in 60 hospitals that serve 77,590 PLWH in Madrid observed lower age and sex-standardized risk of infection among PLWH on anti-retroviral therapy (ART) compared to the general population (30 per 10,000 among PLWH vs. 41.7 per 10,000 among the general population), although the risk in the general population attenuated to 33 per 10,000 when health care workers were excluded (69). A population-based cohort in Wuhan, similarly, found lower incidence among PLWH compared to the general population early in the pandemic (0.38%, 95% confidence interval (CI): 0.24–0.53% vs. 0.45%, 95% CI: 0.45–0.46%) (70). The findings from these early studies may have resulted from greater social distancing among PLWH soon after the pandemic was declared (76).

As the pandemic matured, later population-based studies have generally demonstrated similar SARS-CoV-2 incidence among PLWH and the general population A population based study within New York State through June 2020, which included 2988 PLWH who were diagnosed with SARS-CoV-2, demonstrated a similar incidence of infection to the general population after standardization (adjusted rate ratio (ARR) 0.94 (95% CI, 0.91–0.97)) (71). US National COVID Cohort Collaborative data, which included 54 sites and 13,170 PLWH, demonstrated lower rates of mild-moderate infection among PLWH compared to those without HIV (adjusted odds ratio (AOR) 0.53 (95% CI: 0.51–0.55)) (72). In a population-based study from the Western Cape Province, South Africa, which included 540,552 PLWH, the cumulative incidence of SARS-CoV-2 infection through June 2020, was similar by HIV status (0.74% vs. 0.63%) (73).

Cohorts from large health systems have instead been more mixed, with higher incidence of COVID-19 with the University of California, San Diego health system (77), and Kaiser Permanente Southern California (78), but similar incidence within the San Francisco Department of Public Health Clinics (74).

In conclusion, large population-based studies support a generally similar incidence of SARS-CoV-2 among PLWH compared to the general population.

6.2 Risk of severe COVID-19 among PLWH and its risk factors

Initial data from earlier in the pandemic did not suggest high rates of severe COVID-19 among PLWH, however, these data were predominantly from single center studies (hospitalized cohorts or case series) including mostly individuals on antiretroviral therapy and with a well-controlled HIV infection (79, 80, 81, 82, 83, 84, 85, 86, 87).

On the contrary, other observational retrospective (88, 89) and prospective (90) cohort studies described more severe COVID-19 clinical course and higher risk of death in PLWH. The study by Spence et al., involving 281 PLWH and 1124 matched HIV-seronegative patients affected by COVID-19, described an overall mortality of 6% (n = 18/281) in PLWH versus 3% (n = 33/1124) in HIV-seronegative individuals ($p < 0.0001$) (89). Similarly, the study by Fleischer et al., including 31 PLWH and 155 non-HIV controls with similar baseline characteristics, showed a close to threefold higher odds of having ICU consultation (odds ratio [OR]: 2.9, 95% CI: 1.2–6.9, $p = 0.015$), and a trend toward having a severe or critical COVID-19 illness among PLWH

compared to controls (OR: 1.9, 95% CI: 0.8–4.7,p = 0.164). Furthermore, 14-day survival was 50% (95% CI: 28%–69%) among PLWH and 65% (95% CI: 55%–73%) among controls (p = 0.280), with a trend toward having 1.7-fold higher odds of mortality among PLWH compared to controls (OR: 1.7, 95% CI: 0.8–3.8, p = 0.181) (88).

The largest published population-based cohort from the Western Cape Providence, South Africa, included 3978 cases of COVID-19 among 540,552 PLWH out of a cohort of 3460932 patients served by the public sector health care system. PLWH had an increased hazard rate for COVID-19 death after adjustment for age and sex when compared to those without HIV (ARR 2.14; 95% CI: 1.70–2.70) (73). Some aspects of this population included a low rate of confirmed virologic suppression within the prior 15 months (45%) and high rates of co-occurring tuberculosis and uncontrolled diabetes. However, elevated mortality was similarly demonstrated in the OpenSAFELY platform from the UK primary care clinic system, which included 27480 PLWH within a population of 17282905 individuals. After adjusting for age and sex, PLWH had a higher risk of COVID-19 death (AHR 2.59 (95% CI 1.74–3.84)) (91) than those without HIV. In the US National COVID Cohort Collaborative, which included 13170 cases of COVID-19 among PLWH, there were an increased adjusted odds of mortality (AOR 1.29 (95% CI 1.16–1.44)) (72) among PLWH. Similarly, among 2988 PLWH diagnosed with COVID-19 in New York State, the mortality rate was higher compared to those without HIV (standardized RR, 1.23 (95% CI, 1.07–1.40)) (71).

Also systematic reviews and meta-analyses have been published to understand the relationship between COVID-19 and HIV infection. Oyelade et al. (92) considered 43 studies, thirteen of which presenting data on the severity of COVID-19 in PLWH and non-HIV patients. These studies included a total of 485540 COVID-19 cases, of whom 7768 (1.6%) were PLWH. The pooled global risk ratio was not significant and showed that PLWH may not be at risk of developing severe COVID-19, being the result very close to significance (RR (95% CI) = 1.21 (0.99–1.48); p = 0.477). The lack of significance was true for both Europe and USA, regions associated with better prevention and management of HIV infections. However, the risk for severe COVID-19 among PLWH from Africa was found to increase by 14% (RR (95% CI) = 1.14 (1.05–1.24)) compared with non-HIV COVID-19 patients. Interestingly, 60% (3/5) of

the studies analyzed for the risk of severe COVID-19 in Africa were conducted in South Africa, and all studies originated from sub-Saharan Africa, a region associated with a high HIV infection rate and poorer antiretroviral treatment availability.

17 studies were included in the assessment of the risk of mortality from COVID-19 in PLWH compared with non-HIV COVID-19 patients (588960 COVID-19 cases, including 8013 (1.4%) PLWH). The meta-analysis results showed that HIV infection increased the risk of death from COVID-19 by 2.3-fold globally (RR (95% CI): 2.29 (1.51–3.46)). On the regional level, there was no significantly increased risk of COVID-19 mortality in PLWH in Africa or Europe. However, a twofold increase in risk of mortality was observed in the USA. Most studies within the USA were conducted in Georgia and New York, both of which were among the top 10 states with the highest HIV infections and hardest hit by the COVID-19 pandemic.

These results are consistent with the systematic review by Ssentongo et al. (93), involving 22 reports from Africa, Asia, Europe and North America, but are in contrast with Gao et al. (94), which reported no significant increase in the risk of severe COVID-19 or related death due to HIV infection, and with Lee et al., involving 643018 PLWH, which reported no significant increase in the risk of adverse outcomes of COVID-19 in PLWH (95).

Finally, Wang et al. included a total of 32 studies and 71779737 study samples, of whom 797564 (1.11%) were PLWH. Compared with COVID-19 patients without HIV infection, PLWH had comparable risk of SARS-CoV-2 infection (ARR=1.07, 95% CI:0.53-2.16, I2 = 96%, study n=6, n=20,199,805) and risk of developing severe COVID-19 symptoms (ARR=1.06, 95% CI: 0.97-1.16, I2 = 75%, n=10, n=2,243,370). PLWH, if infected with SARS-CoV-2, were found to have an increased risk of mortality compared with people without HIV (ARR=1.30, 95% CI: 1.09-1.56, I2 = 76%, study n=16, n=71,032,659) (96).

Several HIV-specific risk factors among PLWH have been identified for severe COVID-19, in addition to traditional risk factor. PLWH with CD4 positive T-lymphocyte counts < 200 cells/mm3 had elevated mortality risk in the US National COVID Cohort Collaborative (AOR 2.73; 95%CI 1.80–4.14 vs. > 500) (72), within the US TriNetX database (97), as well as in the Western Cape Province, South Africa (AHR 2.36; 95% CI: 1.47–3.78) (73) analysis. Having a CD4 positive T-lymphocyte count nadir < 200 cells/mm3 was additionally associated with hospitalization in a

multi-center cohort of PLWH in the USA which included 649 cases of COVID-19 (ARR 1.67; 95% CI 1.18–2.36) (98). In this cohort, a current CD4 positive T-lymphocyte count < 350 cells/mm3 was also associated with hospitalization (ARR 2.68; 95% CI 1.93–3.71). The data on virologic suppression are more limited and mixed, with the US National COVID Cohort Collaborative demonstrating an increased risk of hospitalization, but not death (72) in those without virologic suppression, although viremia was significantly associated with disease severity within the multi-center cohort of PLWH in the USA (98). Tuberculosis co-infection was additionally associated with mortality in the Western Cape Province Cohort (AHR 2.70; 95% CI, 1.81–4.04). Mazzitelli et al. (99) conducted a prospective observational cohort study including PLWH not vaccinated for SARS-CoV-2, with plasma HIV-RNA < 40 cp/mL for at least six months, and who were diagnosed with SARS-CoV-2 infection from 20 February 2020 to 31 March 2021. At multivariate analysis, after including univariate significant variables (age, ethnicity, osteoporosis, polypharmacy, length of HIV infection and previous AIDS episodes), only polypharmacy was independently associated with an increased risk of more severe SARSCoV-2 infections (AOR 9.4 [1.4–64.7], p = 0.023 for mild COVID-19 versus asymptomatic infections and AOR 12.9 [1.8–94.4], p = 0.011 for moderate/severe COVID-19 versus COVID-19 not requiring hospitalization). Polypharmacy could be considered a proxy of multimorbidity, which is a highly represented clinical issue in PLWH, mainly due to ageing and effective antiretrovirals (100). In the same study the length of HIV was significantly associated with the risk of severe COVID-19 at univariate, but not confirmed at multivariate analysis, making reasonable to think that PLWH with a longer history of disease, even if with suppressed HIV viral load and a good CD4 positive T-lymphocyte count, may have higher levels of inflammation and immune dysfunction (101, 102) that may lead patients to develop more severe COVID-19.

Understanding whether susceptibility to SARS-CoV-2 infection or propensity to develop severe disease is increased among PLWH are crucial questions for both these individuals and their providers, but to date, no conclusive evidence about the relationship between COVID-19 and HIV infection is still available.

7. COVID-19 vaccines

Since the genetic sequence of the SARS-CoV-2 virus was published on January 11, 2020, scientists, industries, and other organizations around the world have collaborated to develop safe and effective vaccines against COVID-19. Vaccines are the most promising approach for curbing the COVID-19 pandemic, providing strong protection against serious illness, hospitalization, and death from COVID-19 (103). High vaccination coverage of the general population, also helps reduce the likelihood of new variants emerging (104, 105, 106).

Several different types of potential vaccines for COVID-19 have been developed, including:

- Inactivated or weakened virus vaccines
- Protein-based vaccines
- Viral vector vaccines
- RNA and DNA vaccines

Vaccines authorized in the European Union (EU) to prevent COVID-19, following evaluation by the European Medicines Agency (EMA) are reported in figure 10. In Italy SARS-CoV-2 vaccination became available between December 2020 (Comirnaty®, 22/12/2020) and January 2021 (Spikevax®, 07/01/2021; Vaxzevria®, 29/01/2021). The vaccination campaign began following a priority criterion based on the risk of infection and severe disease for COVID-19 in different population groups. The priority categories were 1) health and social health workers, 2) elderly residents and staff of residential and long-term care facilities, and 3) people older than 80 years. With the increase of authorized vaccines and available doses, the vaccination was extended to other population groups such as people with at least one chronic comorbidity, school staff, or law enforcement. Subsequently, vaccination was made available to all age groups in descending order. In 2021 it is estimated that in Italy, 2828366 COVID-19 cases (2365390-3418612), 290044 related hospitalizations (218436-399436), 37706 (27375-53964) related intensive care unit admissions, and 77671 (54467-113700) deaths were avoided thanks to vaccination, equal to 43% (39%-48%), 58% (51%-65%), 57% (49%-66%) and 64% (55%-72%) respectively of expected events (observed plus avoided) (103).

Figure 10: EU authorized COVID-19 vaccines (https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-vaccines).

8. m-RNA vaccines

The new vaccines platform based on the use of messenger-RNA (m-RNA) has been first used during the COVID-19 pandemic. These vaccines are based on the use of m-RNA coding for the Spike (S) protein and inserted into nano-lipid particles which preserve its integrity. The m-RNA presents two point-mutations artificially induced and aimed at stabilizing the S protein. These nanoparticles, once injected intramuscularly, enter the cytoplasm of the cells, whose ribosomes translate the m-RNA into S proteins which are then presented on the cell membrane both by MHC-

1, expressed by all nucleated cells, and by MHC-2, expressed by macrophages, dendritic cells, and B cells.

CD4 T-lymphocytes, through the TCR receptor are activated by the S protein presented by MHC-2 and produce various cytokines, including IL-2, IL-4, and IL-5. These in turn stimulate B-lymphocytes to produce neutralizing antibodies and promote the maturation of memory T-lymphocytes. CD8 T-lymphocytes, on the other hand, interact with proteins expressed on MHC-1 complexes and stimulate both a cytotoxic response and the amplification of other immune pathways (figure 11) (107).

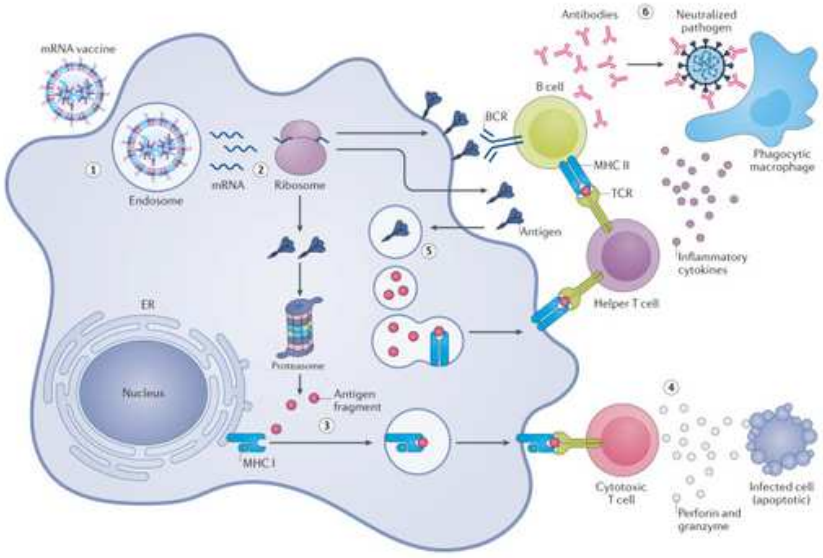

Figure 11: schematic representation of m-RNA vaccines' mechanism of action

Extensive data supporting the use of m-RNA vaccines have accumulated since their availability; less data on safety and efficacy of protein-based vaccines are available (108, 109). The preference for m-RNA vaccines over viral vector vaccines, is based on a more favorable risk-benefit profile with the m-RNA vaccines. Adenoviral vector vaccines have been associated with thrombosis with thrombocytopenia (110, 111) and possibly Guillain-Barrè syndrome (112, 113), and the m-RNA vaccines have been associated with myocarditis (114). The risks of these events are extremely small, and the benefits of all the vaccines outweigh them. However, cases of vaccine-associated thrombosis with thrombocytopenia and Guillain-Barrè syndrome have been more severe with greater morbidity compared with cases of vaccine-associated myocarditis. Additionally, although precise comparative efficacy is uncertain because the different vaccines have not been compared directly in trials, limited evidence suggests that m-

RNA vaccines may be more effective than Ad26.COV2.S (Jcovden ®), including against severe infection. In several observational studies, vaccine effectiveness associated with two doses of the m-RNA vaccines was higher than that with one dose of Ad26.COV2.S (115, 116).

9. BNT162b2 (Comirnaty®) immunogenicity and efficacy in the general population

BNT162b2 was the first SARS-CoV-2 vaccine to be approved by EMA.

According to the study by Lustig et al. (117), vaccination with two doses of BNT162b2 elicited the production of anti-RBD IgG in 99.9% of the general population and that of neutralizing antibodies in 96% of them within two weeks of the second dose. The administration of the second dose was crucial in the elderly, in immunocompromised patients and in those with other comorbidities (including diabetes, hypertension, heart disease, autoimmune diseases), who had significantly lower antibody levels than the general population after the first dose, but only slightly lower after the second dose. Despite this, in the population considered to be at risk of serious complications by COVID-19 (males, elderly, immunocompromised, diabetics, hypertensives, heart patients and patients with autoimmune diseases), the concentration of anti-RBD IgG and neutralizing antibodies after a time interval between one and two weeks after the second dose remained significantly lower than in the non-at-risk population (117). The same authors evaluated the anti-RBD IgG titer and neutralizing antibodies in health care workers during the six months following the second dose of BNT162b2 vaccine: after six months the humoral response was reduced, especially in males, subjects older than 65 years, and in immunocompromised subjects. Both anti-RBD IgG and neutralizing antibodies' peaks were reached between 4 and 30 days after the second dose; subsequently the IgG progressively decreased reaching, after six months, a level 18.3 times lower than the peak values. During the six-month period, a strong correlation between anti-RBD IgG titers and neutralizing antibodies was maintained, with neutralizing antibodies undergoing a considerable drop as anti-RBD IgG (118).

In line with studies on the BNT162b2's immunogenicity, the pivotal study (119) involving 43548 patients 16 years of age or older, showed an efficacy of 95% (95% CI: 90.3-97.6) in preventing infection after a median time of two months following vaccination. The same research group reevaluated efficacy and safety six months after

vaccination in 44165 patients aged 16 years or older and in 2264 patients between 12 and 15 years, highlighting a 91.3% efficacy (95% CI: 89-93.2) in preventing infection, a 96.7% efficacy (95% CI: 80.3-99.9) in preventing severe disease and an excellent safety profile, since no long-term adverse reactions were reported (120).

The study by Dagan et al. (121), involving almost 600000 patients, confirmed previous results, finding 90% of efficacy (95% CI: 83-94) in the prevention of asymptomatic infection, 94 % of efficacy (95% CI: 87-98) in the prevention of symptomatic infection, 87% of efficacy (95% CI: 55-100) in preventing hospitalization, and of 92% (95% CI: 75-100) in preventing severe disease. No significant differences were found between males and females or according to age, while it seemed that the efficacy was slightly reduced in patients with multiple comorbidities (121). Also in the study of Saciuk and colleagues, involving 1.6 million patients, it emerged that vaccination with two doses of BNT162b2 had an efficacy of 93% (CI: 92.6-93.4) in preventing infection, 93.4% (CI:91.9-94.7) in preventing hospitalization and 91.1% (CI:86.5-94.1) in preventing death, with no difference between males and females (122). In the same study, a decreased effectiveness in preventing the infection was found in patients over 75 years of age, suffering from arterial hypertension, diabetes mellitus and obese, without however detecting differences as regards hospitalization and death (122). Furthermore, subjects vaccinated with two doses of BNT162b2 had a lower viral load than the unvaccinated population in the case of subsequent infection (123).

Multiple observational studies have suggested that vaccine protection against SARS-CoV-2 infection wanes over time in children and adults (124, 125, 126, 127). This is a consequence both of waning immunity and immune evasion by certain circulating SARS-CoV-2 variants. As an example, in vitro the Delta variant is eight times less sensitive to neutralizing antibodies elicited by a complete course with two doses of m-RNA vaccine than the variant only expressing the D614G mutation (126, 46). In another study, sera from the individuals who received both doses of the vaccine exhibited a neutralizing effect against the Delta variant, however, were about three to five times less effective than against the Alpha variant (37). Protection against hospitalization and death also wanes somewhat, but less than protection against infection (128, 129, 130, 131). In a study of statewide data in North Carolina that included over 10 million adults, adjusted m-RNA vaccine effectiveness at seven months following the primary series was 54%-70% against infection, 86%-90% against

hospitalization, and 90%-93% percent against death (132). At 12 months, the same measures were 38%-47%, 60%-65%, and 70%-65% respectively (132).

A primary vaccination with two BNT162b2 doses resulted in a weak immune response in solid organ transplant recipient and hematological patients (133, 134), as clinically documented by the emergence of severe cases of COVID-19 also after the completion of a primary vaccination course in these populations (135).

Observation of waning immunity some months after the second dose in the general population, and weak immunogenicity in fragile subjects after two vaccine's doses, together with the early evidence that a third booster dose could increase both the prevalence and titers of anti-SARS-CoV-2 antibodies (136), strongly suggested a booster dose at least in elderly people, and immunocompromised subjects.

Recently it has emerged that in health care workers the median anti-RBD IgG level and neutralizing antibodies increased significantly after the third vaccination dose and that humoral and cellular immunity induced by the third dose was more durable than that induced by the second dose (137, 138). Similar results have been observed in patients with solid cancer after third dose with BNT162b2 (139).

In an observational study from Israel of over four million individuals aged 16 years or older who had received two doses of BNT162b2 at least five months previously, receipt of a booster dose was associated with a 10-times lower rate of infection in all age groups compared with those who did not receive a booster (adjusted rate difference of 57 to 90 infections per 100000 days, depending on age group), and among individuals 60 years or older, an 18-times lower rate of severe illness (absolute difference 5.4 cases per 100000 days) (140).

An additional booster dose appears to improve relative vaccine effectiveness against both infection and severe disease, at least in the short term. In a retrospective study from Israel that included over 1 million individuals aged 60 years or older who had received a primary series and an initial booster dose with BNT162b2, receipt of a second booster dose at least four months after the last was associated with twofold lower risk of confirmed infection and a 3.5-fold lower risk of severe infection. The reduction of risk for confirmed infection waned by eight weeks after the second booster dose, whereas the reduction of risk for severe infection remained stable over the study period (141).

10. mRNA-1273 (Spikevax®) immunogenicity and efficacy in the general population

Randomized trials in adults demonstrate a substantially reduced risk of symptomatic and severe COVID-19 in the first several months after mRNA-1273 vaccination. In a large placebo-controlled trial, vaccine efficacy of the two-dose primary series in preventing symptomatic COVID-19 at a median of two-months follow-up was 94.1% (95% CI 89.3-96.8) among adults 18 years or older (142). Among adults 65 years or older, vaccine efficacy was 86.4% (95% CI 61.4-95.5). After a median follow-up of 5.2 months, vaccine efficacy was 93.2% for symptomatic infection (9.6 versus 136.6 cases/100 person-years with placebo) and 98.2 % for severe disease (i.e., with hypoxia, organ dysfunction, or critical illness; 2 versus 106 cases with placebo) (143). Observational data evaluating vaccine effectiveness also support the trial findings in adults (144, 145): mRNA-1273 has been associated with approximately 90% or higher vaccine effectiveness in preventing COVID-19-related emergency visits, hospitalization, intensive care unit admission, and death.

Like BNT162b2, also mRNA-1273 effectiveness wanes over time and may be decreased in protecting against infection with certain SARS-CoV-2 variants, although protection against severe disease due to variants remains substantial.

These efficacy data are consistent with evidence from immunogenicity studies that demonstrated robust binding and neutralizing antibody responses with mRNA-1273 in adults of all ages (146, 147). Over six months, antibody titers decline slightly but remain high and neutralizing activity persists (148).

Vaccination with mRNA-1273 compared with BNT162b2 is associated with higher antibody titers after the second dose (149).

11. m-RNA vaccines and Omicron variant

For the Omicron variant and its sublineages (BA.1, BA.2, BA.2.12.1, BA.4, and BA.5), COVID-19 vaccines remain effective in preventing severe disease, but observational studies consistently suggest that vaccination has substantially reduced effectiveness against symptomatic infection and wanes after several months. As an example, in an observational study from the United States, vaccine effectiveness against hospitalization within five months of receiving the last of three m-RNA COVID-19 vaccine doses was 79% and 60% during the BA.1/BA.2 and BA.4/BA.5 periods

respectively, but decreased to 41% and 29% more than five months after vaccination (150).

Consistent with the observed attenuated vaccine effectiveness against infection with the Omicron subvariant, neutralizing activity of sera from vaccinated individuals is reduced against Omicron compared with the original Wuhan strain virus and the Delta variant, and most infection-naïve individuals who received a primary vaccine series have no detectable neutralizing activity against Omicron (151, 152, 153, 154). However, previously infected individuals who received a primary series and individuals who receive booster vaccination, retain adequate neutralizing titers against Omicron sublineages BA.1 and BA.2. Neutralizing titers from such individuals are usually lower for BA.4 and BA.5, but in most cases still above baseline (155, 156).

The discrepancy between the lack of neutralizing activity against Omicron in sera from vaccinated individuals and the persistence of protection against severe disease with vaccination may be in part because neutralizing activity is not the only immune measure of vaccine protection. Vaccine- or infection-induced cellular immunity appears robust against Omicron (157, 158, 159).

To address issues of both waning efficacy since the last vaccine dose and the attenuated efficacy of m-RNA vaccines against viral variants that escape the immune response directed against S protein targeted by the original vaccines, some countries have introduced bivalent m-RNA COVID-19 vaccine boosters that encode S protein from the original SARS-CoV-2 strain and from the Omicron variants (BA.1 or BA.4/BA.5). Evidence for the effectiveness of bivalent booster vaccines is limited to observational data but suggests modest to moderate protection against infection depending on time since the last vaccine dose. In a study in the United States of over 250000 symptomatic individuals who were tested for SARS-CoV-2 and had received two to four doses of monovalent vaccines, estimated vaccine effectiveness of an additional bivalent booster dose was 28%-31% among those who had received the last dose two to three months previously and 43%-56% among those last vaccinated more than eight months previously (160). Limited data also suggest bivalent booster effectiveness against symptomatic infection with the Omicron subvariants XBB/XBB.1.5 despite reduced binding by anti-SARS-CoV-2 neutralizing antibodies (161).

Data from immunogenicity studies evaluating bivalent vaccines suggest that bivalent boosters that include the BA.4/BA.5 S protein induce higher antibody levels against

BA.4/BA.5 virus compared with pre-booster levels and compared with monovalent boosters (162, 163). Some of these studies also suggest that the antibody response elicited by the bivalent boosters sufficiently neutralizes other Omicron subvariants, such as BQ.1.1 and XBB. In other studies, the antibody response to the bivalent booster was like that with the monovalent booster and had minimal neutralizing activity against other Omicron subvariants (49).

12. Vaccination in people living with HIV

There are few controlled studies on the clinical efficacy and effectiveness of vaccination in HIV-infected adults receiving highly active antiretroviral therapy. PLWH experience more rapidly waning neutralizing antibody titers in response to yellow fever vaccination than those without HIV, an effect that may be predicted by lower CD4/CD8 ratio or higher plasma kynurenine/tryptophan ratio (164). PLWH also respond less well to hepatitis B vaccination (165, 166). In one study, after three doses of anti-HBV vaccine, HBsAb titers were much lower in PLWH compared to controls. Furthermore, although an increase in memory cells was observed in HIV positive patients, HBsAb levels were significantly lower than controls ($P < 0.05$) indicating a functional defect of memory cells in HIV/AIDS patients.

Finally, depending on CD4 count, they mount attenuated immune responses to other vaccines such as diphtheria, tetanus, and poliomyelitis vaccines (167), influenza (168, 169), and *S.pneumoniae* (170).

Published data indicate that antiretroviral therapy restores vaccine immunogenicity, improving the rates and persistence of immune responses, while reducing the risk of vaccine-related adverse events. Despite effective therapy responses remain often suboptimal relative to HIV-negative individuals, they improve with larger and more frequent vaccine doses (171).

13. SARS-CoV-2 vaccination in people living with HIV

HIV infection is characterized by a profound disruption of the adaptive immune system, in both its cellular and humoral components, with destruction of CD4 T-lymphocytes, increased CD8 T-lymphocytes, T-lymphocytes activation/exhaustion, defective T follicular helper (Tfh) lymphocytes activity, and dysfunction and polyclonal activation of B-lymphocytes (172, 173). Early combination antiretroviral therapy

(ART) (174) reduces but does not fully eliminate HIV-induced inflammation and immune activation, leading to a chronic immune dysregulation (175), and incomplete restoration of immune health (176).

Large studies suggest that people living with HIV (PLWHs), particularly those with low CD4 T-lymphocytes counts or untreated HIV infection, might have a more severe clinical course of COVID-19 compared to HIV-negative individuals (177). An increased risk of mortality for these subjects has been reported in different meta-analyses (178, 179, 93). In addition, a large multi-country analysis has recently shown that HIV infection is independently associated with increased odds of severe/critical COVID-19 and increased likelihood of in-hospital mortality (56).

On these bases, vaccination is one of the strategies recommended by national and international HIV societies for PLWHs to protect them from severe illness.

Previous reports showing that certain vaccines against pandemic H1N1 influenza (168) and hepatitis B (165) induce suboptimal responses in PLWHs raise concern over the immunogenicity of SARS-CoV-2 vaccines (168).

In general, immune responses to m-RNA vaccines (mRNA-1273 and BNT162b2) have been reported as being similar to those of the general population in people with well controlled HIV infection (180, 181). However, other studies found that humoral and cellular immune response correlates with the CD4 T-lymphocyte levels with response being significantly weaker in PLWHs with less than 200/mm^3 cells (182). In a small prospective study of PLWHs with a median CD4 T-lymphocytes count of 913 cells/mm^3 receiving BNT162b2, a robust humoral and cellular immune response that was comparable to that observed in healthy donors was observed (183). In another small size prospective study enrolling PLWHs receiving BNT162b2 or mRNA-1273, 86% of them had more than 200/mm^3 CD4 T-lymphocytes and developed high titers of anti-RBD antibodies (184).

Antinori and colleagues (181) found that one month after the second dose of an m-RNA vaccine, a detectable RBD-binding IgG response was elicited in 86.7% of PLWHs with less than 200/mm^3 CD4 T-cells, in 100% of subjects with a CD4 T-lymphocyte count between 200 and 500/mm^3, and in 98.7% of subjects with more than 500/mm^3 CD4 T-lymphocytes. They also found a significant correlation between CD4 T-lymphocytes counts and the magnitude of RBD-binding IgG, because the level of RBD-binding IgG response after the second vaccine's dose was lower in subjects

with less than 200/mm^3 CD4 T-cell counts (507 BAU/mL median) if compared to subjects with CD4 T-cells counts between 200 and 500/mm^3 (1477 BAU/mL) and with more than 500/mm^3 CD4 T-cells counts (1782 BAU/mL). After the second vaccine's dose they did not find any difference in the magnitude of RBD-binding IgG titer between subjects with intermediate and high CD4 T-cells counts. These results were in line with those of other authors (118,185, 186, 187, 188, 189), strongly supporting the hypothesis that m-RNA vaccination would be able to elicit robust humoral and cellular immune responses against SARS-CoV-2 in most PLWHs receiving ART, particularly in those with full immune recovery after suppressive therapy.

In addition to low CD4 T-lymphocytes counts, other predictors of weak seroconversion after SARS-CoV-2 immunization in PLWHs are older age (190), comorbidities (191) and, with lower strong evidence, also the nadir CD4 T-lymphocytes counts and plasma HIV-RNA before vaccination (187). Possibly also the type of vaccine can influence the magnitude of the serological response: two recently published case reports in 14 and 12 virologically suppressed PLWHs found high antibody titers after the second vaccination with mRNA-1273 (183, 184). Current research indicates that the immune response is stronger in immunocompetent individuals when applying the mRNA-1273 vaccine compared with BNT162b2 because of its higher m-RNA content and the longer interval between the first and the second dose (4 vs 3 weeks) (149). Speich and colleagues (192) found more PLWHs with neutralization activity after receiving mRNA-1273 compared with BNT162b2; in addition, the assessed titer levels were somewhat higher with mRNA-1273, but because of the large variance in the data, they could not be conclusive.

PLWHs with low CD4 T-cell counts, detectable viremia, and/or previous AIDS were found to have weaker and less durable humoral and T-cell responses to m-RNA vaccines (180, 181, 193, 194, 195, 196), suggesting that they may benefit from additional vaccine doses.

Also the evidence of waning immunity (118) and reducing vaccine efficacy/effectiveness against infection and symptomatic disease by 6 months after the two-dose primary vaccination (124), together with the emergence and circulation of new immune-escaping variants, strongly recommended a vaccine booster dose. In this respect, a third dose of an m-RNA vaccine following the primary cycle has been shown

to strongly boost humoral albeit not T-lymphocytes responses in PLWHs with advanced disease at the time of HIV diagnosis (CD4 + T-cells < 200/μL and/ or AIDS), irrespective of the current CD4 T-lymphocytes count (197). Furthermore, studies that used a primary m-RNA vaccination, followed by a booster dose with an m-RNA-based vaccine, reported that antibody levels after the third dose substantially exceeded those mounted after the primary series (198).

Evidence by Lapointe (198) describes that third vaccine dose boosted antibody concentrations to an average of 0.4-0.5 $\log_{10}$ U/mL higher than peak post-second dose levels, with similar results for PLWHs and controls. They also did not find a significant relationship between most recent or nadir CD4 T-lymphocytes count in PLWHs and antibody concentrations one month after the third dose. Similar results have been described by Gianserra (191) and Vergori (197), they also observed that neither age, sex, body max index (BMI), nadir and baseline CD4 T-lymphocytes, nor current ART regimen significantly affected the humoral response after the third dose.

Lapointe (198) also observed the third dose significantly boosted anti-Omicron IgG concentrations to an average of 0.3-0.5 $\log_{10}$ U/mL higher than after the second dose levels in both PLWHs and controls. In particular, the third dose boosted all antibody levels above two-dose levels, but BA.1 specific responses remained significantly lower than wild-type specific ones, with BA.5 specific responses lower still. Having received mRNA-1273 for the third dose, and a longer interval between second and third doses, were significantly associated with higher Omicron-specific anti-RBD IgG responses. Consistent with the accumulating evidence (199, 200, 201, 202), Omicron-specific antibody responses remained universally weaker than wild-type-specific ones at all times tested. Nevertheless, after three doses, antibody concentrations in PLWHs were equivalent to controls, while neutralization activities (including against Omicron) were slightly higher.

AIMS OF THE STUDY

The present study is part of the ORCHESTRA project, a three-year international research project aimed at tackling the coronavirus pandemic. The primary aim of ORCHESTRA was the creation of a new pan-European cohort including SARS-CoV-2 infected and non-infected individuals of all ages and conditions. To answer different research question and vaccine strategies, four different populations have been involved: general population, COVID-19 patients, fragile individuals (children, solid organ transplanted, oncological, hematological, HIV infected, rheumatological, patients with cystic fibrosis and those with Parkinson disease), and health-care workers.
This study, namely the HIV-COVID VERONA study, is part of the ORCHESTRA project focused on fragile cohorts and is a prospective longitudinal cohort study to describe the humoral immune response directed against SARS-CoV-2 vaccination in people living with HIV (PLWH) undergoing a complete primary vaccination course with BNT162b2, and a subsequent third (booster) dose with an m-RNA vaccine.
The primary objective of the study is the evaluation of the anti-RBD IgG titers after 3 weeks (T2) and after 3 months +/- 1 month (T3) from the administration of the first dose of BNT162b2 (T1), and after 1 month +/- 1 month (T4) from the administration of the first booster dose with an m-RNA vaccine (BNT162b2 or mRNA-1273).
Secondary objectives are:

- the identification of possible determinants of vaccine immunogenicity after a complete primary vaccination course with two doses of BNT162b2
- the evaluation of the frequency and risk factors of breakthrough infection (BI) acquired within 6 months +/- 2 months of receiving the vaccine booster dose.

METHODS

1. Study design and population

The HIV-COVID VERONA is a prospective, longitudinal protocol.

On 7 April 2021, as part of the Italian nationwide mass vaccination program, the Azienda Ospedaliera Universitaria Integrata-Verona (AOUI-Verona) in Verona, started the vaccination campaign against SARS-CoV-2 in PLWH. Regardless of the previous AIDS diagnosis and/or the actual CD4 T-lymphocyte count, vaccination was offered to all adult PLWH attending the outpatient clinic of the Infectious Diseases Unit. All those who joined the vaccination campaign were offered participation in the HIV-COVID VERONA project and subjects who signed the informed consent for the specific study were enrolled on the day of the administration of the first vaccine dose (T1). Every enrolled patient was pseudo-anonymized at the enrollment.

BNT162b2 was offered to all PLWH; the vaccination schedule consisted of two doses of 30μg each, administered intramuscularly 21 days apart. The vaccine doses were prepared and stored by the hospital pharmacy until their administration following the manufacturer's instructions.

Vaccination was not administered, and patients were not enrolled, in case of a SARS-CoV-2 infection microbiologically documented within the previous three months. In case of SARS-CoV-2 infection contracted between the previous three and six months, a single dose of vaccine was administered; if the SARS-CoV-2 infection dated back more than six months, both doses were administered. In case of SARS-CoV-2 infection within 14 days of the first dose, the second vaccine dose administration was indicated within six months of the documented infection; in case of infection contracted between 14 and 21 days after the first dose, it was not possible to administer the second dose earlier than six months after the onset of the infection.

According to the protocol, demographic, epidemiologic, clinical, and laboratory characteristics of vaccinated PLWH were collected on T1. Data regarding the year of HIV infection diagnosis, previous AIDS diagnosis (if applicable), the CD4 T-lymphocyte nadir count and the HIV-RNA zenith, the time of ART's initiation, current ART, and the risk category for HIV were collected as well.

The time-points taken into consideration after T1 to evaluate the anti-RBD IgG titers were:

- T2: 21 days after the first dose of vaccine, before the administration of the second vaccine's dose.
- T3: 3 months +/- 1 month from the first vaccine's dose
- T3a: 6 months +/- 2 months from the first vaccine's dose
- T3b: 12 months +/- 3 months from the first vaccine's dose

Patients who underwent the third vaccine dose before completing the follow-up described above were no longer followed according to the remaining time points, but according to the following new follow-up:

- T4: 1 month +/- 1 month from the third dose (booster dose)
- T4a: 6 months +/-2 months from the third dose

At each time point current CD4 T-lymphocyte count, CD8 T-lymphocyte count, CD4/CD8 ratio, HIV-RNA, comorbidities, current ART and its start date, compliance to current ART and if concomitant use of immunosuppressant drugs were collected. Blood samples were collected to detect anti-Spike and anti-Nucleocapsid antibodies as well as to perform sero-neutralization assays in sera samples. Blood samples were also collected to assess the cytokines and cell-mediated immune response to vaccination.

At each scheduled visit, the patient was asked whether they had contracted a microbiologically documented SARS-CoV-2 infection after the previous visit, documented by an antigenic or molecular test performed on nasopharyngeal swab. The date of collection of the positive diagnostic sample was registered. Finally, each enrolled patient underwent a molecular nasopharyngeal swab to check for SARS-CoV-2 infection on the day of the scheduled visit.

According to the Centre for Disease Control and Prevention (CDC), a BI was a SARS-CoV-2 infection, microbiologically documented by a molecular or an antigenic assay performed on a respiratory sample, occurring after at least 14 days from the end of a primary vaccine schedule of an approved vaccine. The severity of the infectious episode was defined by applying the WHO clinical progression score (203):

- 0: uninfected; no viral RNA detected
- 1: ambulatory mild disease, asymptomatic; viral RNA detected
- 2: ambulatory mild disease, symptomatic; independent
- 4: hospitalized: moderate disease, no oxygen therapy
- 5: hospitalized: moderate disease, oxygen by mask or nasal pongs
- 6: hospitalized: severe disease, oxygen by non-invasive ventilation or high flow

- 7: hospitalized: severe disease, intubation and mechanical ventilation, pO2/FiO2 < 150 mmHg, or SpO2/FiO2 < 200 mmHg
- 8: hospitalized: severe disease, mechanical ventilation, pO2/FiO2 < 150 mmHg (SpO2/FiO2 < 200 mmHg) or vasopressors
- 9: hospitalized: severe disease, mechanical ventilation, pO2/FiO2 < 150 mmHg and vasopressors, dialysis or ECMO
- 10: dead

The protocol was approved by the local Etic Committee: CESC VR_RO, protocol N° 41258 (21/07/2021).

2. Laboratory procedure

A commercial chemiluminescence microparticle antibody assay (Roche Elecsys® Anti-SARS-CoV-2 S) was performed and used according to the manufacturer's instruction. It allows the in vitro quantification of anti-RBD IgG using a double antigen sandwich test and a recombinant protein that constitutes the RBD domain. The test has a sensitivity of 98.8% (95% CI: 98.1-99.3%) and a specificity of 99.98% (95% CI: 99.91-100%) for serum samples collected after at least 14 days from the first molecular test positive for SARS-CoV-2. Moreover, it shows a concordance of 92.3% (95% CI: 63.97-99.81%) between the serum IgG positivity and its neutralizing capacity evaluated by a pseudo-neutralization test (204).

As specified by the manufacturer, the result is expressed in Units (U) per mL. In this study the serological results were then converted into Binding Antibody Units (BAU) per mL according to what indicated by the first WHO's International Standard for anti-SARS-CoV-2 immunoglobulins: 1 U equal to 0.972 BAU.

As specified by the manufacturer, the result can be:

- non-reactive if IgG titer < 0.4 BAU/mL
- reactive if IgG titer ≥ 0.4 BAU/mL

The test quantifies a maximum of 2500 BAU/mL, above this value the quantitative result provided is > 2500 BAU/mL.

For the purposes of qualitative interpretation of the anti-RBD IgG response, the following five categories were identified:

• Non-reactive: less than 5.58 BAU/mL

• Inconclusive: equal or more than 5.58 BAU/mL and less than 45 BAU/mL

• Positive low: equal or more than 45 BAU/mL and less than 205 BAU/mL
• Positive intermediate: equal or more than 205 BAU/mL and less than 817 BAU/mL
• Positive high: equal or more than 817 BAU/mL

The upper limit for "non-reactive" was determined as the average plus one standard deviation of anti-Spike measurements in 50 serum samples collected before 2019. The lower limit for "low", "medium" and "high" were based on the BAU/mL concentrations of "low" (NIBSC code 20/140), "mild" (NIBSC code 20/148) and "high" (NIBSC code 20/150) WHO's International Standards for anti-SARS-CoV-2 immunoglobulins (204, 205).

Determination of anti-Nucleocapsid antibodies was not performed by the laboratory, as well as the sero-neutralization assay.

Samples collected at T3a, T3b and T4a were not processed by the laboratory in time to include the corresponding serological results in this work. Analysis on cellular immunity were performed on a sub-group of PLWH and are not included in this work.

3. Statistical Analyses

PLWH included in the present analysis were stratified into the following 3 groups according to the degree of immune recovery: patients with current CD4 T-lymphocytes count <200 cells/mm^3 (poor CD4 recovery - PCDR), patients with current CD4 T-lymphocytes count between 200 and 500 cells/mm^3 (intermediate CD4 recovery - ICDR), and patients with current CD4 T-lymphocyte count >500 cells/mm^3 (high CD4 recovery - HCDR).

Descriptive statistics were presented as median with interquartile range (IQR) for continuous variables and frequency with proportion for categorical variables.

From the initial cohort of 304 subjects, 214 individuals were analyzed respecting the following characteristics: 1) two vaccine doses; 2) SARS-CoV-2 infection diagnosis excluded before T1. Individuals with a reverse-transcription polymerase chain reaction positive result on a respiratory sample collected before/at T1 or with a positivity to anti-spike RBD antibodies at T1, and individuals with a reverse-transcription polymerase chain reaction positive result before completing the primary vaccine cycle were not included. Also subjects without a collected blood sample at T1 and for which it was not possible to define the serological status before the start of vaccination were

not included. Subjects without the serological result available both at T1 and T3 were also excluded.

Included individuals were stratified in five groups based on the anti-RBD IgG serological titer: i) not reactive, if less than 5.58 BAU/mL; ii) inconclusive, if greater than or equal to 5.58 and less than 45 BAU/mL; iii) positive low, if greater than or equal to 45 and less than 205 BAU/mL; iiii) positive intermediate, if greater than or equal to 205 and less than 817 BAU/mL; iiiii) positive high, if greater or equal to 817 BAU/mL.

The analysis was carried out with the aim of identifying which factors could influence a positive-high titer compared with an intermediate-low titer, 3 months after the primary vaccine cycle. Since at T3 only three subjects were not reactive/inconclusive among the 214 subjects included, it was not possible to compare non-responders and responders, so the analysis was carried out only considering 211 patients with a positive serological test at T3. Age, gender, length of HIV infection, CD4 T-lymphocytes count at T1 ($\leq$ 500 vs $>$ 500 cells/mm^3), CD4 T-lymphocytes nadir, previous AIDS diagnosis, presence of comorbidities at T1 (none; one; more than one), and HIV-RNA at T1 ($<$20 vs $\geq$ 20 copies/mL) were evaluated as risk factors with a logistic regression model, both individually and simultaneously in order to correct any unbalanced to internal groups. In addition, CD8 T-lymphocytes count and CD4/CD8 ratio at T1 were tested individually, as possible risk factors. The comorbidities taken into consideration were hypertension, chronic heart disease, myocardial infarction, chronic kidney/liver disease, connective tissue disease, diabetes, chronic obstructive pulmonary disease (COPD), liver disease (not hepatitis), lymphoma, leukemia, cancer in last 5 years, metastatic solid cancer, rheumatological/autoimmune disease, transfusions in the last year. A second analysis was carried out with the aim of identifying risk factors for breakthrough infection after the third vaccine dose, considering all individuals with the third vaccine dose and comparing those without a subsequent breakthrough infection, and subjects with at least one subsequent breakthrough infection. Overall, 249 subjects were included, satisfying the following criteria: 1) having three doses of vaccine, and 2) having T1 serology to verify the pre-vaccinal anti-RBD IgG title. Variables considered were age, gender, body max index (BMI), smoking status, length of HIV infection, previous diagnosis of AIDS, CD4 T-lymphocytes nadir, zenith of HIV-RNA, HIV-RNA at T1, CD4 and CD8 T-

lymphocytes and CD4/CD8 ratio at T1, presence of comorbidity (none, one, more than one) at T1, and previous COVID-19 infection. Pearson's χ^2 tests, Fisher's exact test or Wilcoxon test were used for comparison. A P value < .05 was considered statistically significant. Analyses were performed using STATA v17.

RESULTS

1. Characteristics of the study population

At the beginning of the vaccine campaign (T1) 304 PLWH were enrolled, 76% males and 24% females with a median age of 53 years (IQR: 44-59) (table 3), and a median duration of HIV infection of 15 years (IQR: 8-24). About half of the patients (45.6%) presented a previous AIDS diagnosis. The median CD4 T-lymphocytes nadir was 234/mm^3 (IQR: 95-365), and the median HIV-RNA zenith was 108157 copies/mL (IQR: 35221-254450). Overall, the study population documented an optimal immune recovery, with a median CD4 T-lymphocytes count equal to 708/mm^3 (IQR 523-951) before the SARS-CoV-2 vaccination. The median CD8 T-lymphocytes count was 839/mm^3 (IQR: 632-1126) and the median CD4/CD8 ratio was 0.84 (IQR: 0.6-1.2). All patients were on ART; HIV-RNA tested undetectable (lower than 20 copies/mL) in 80.7% of subjects, while in the remaining 19.3% the HIV-RNA was detectable with a median of 44 copies/mL (IQR: 25-92).

Approximately half of the enrolled patients did not present any comorbidity (55.6%): 28% presented only one and 16.5% more than one. The most frequently reported comorbidity was hypertension (26.3% of subjects), followed by chronic cardiac disease, including congenital heart disease, and any tumor within the last 5 years (7.9% both), and chronic renal/kidney disease (6.6 %). None was affected by ongoing hematological malignancy, and only 2.3% had COPD.

Characteristics	**N**	**Value**
Age, median (IQR), years	304	53 (44 - 59)
Gender, male, n (%)	304	231 (76)
Ethnicity, Caucasian, n (%)	304	279 (91.8)
African, n (%)		10 (3.3)
Hispanic, n (%)		15 (4.9)
BMI, median (IQR), Kg/m^2	268	24.6 (22.7 - 27.3)
Smoking status, Non-smoker, n (%)	282	100 (35.5)
Active- smoker, n (%)		104 (36.9)
Previous smoker, n (%)		78 (27.7)
HIV risk category, Drug user, n (%)	304	52 (17.1)
MSM, n (%)		113 (37.2)
Heterosexual contacts, n (%)		42 (13.8)
Vertical transmission, n (%)		3 (1.0)
Other, n (%)		5 (1.6)
Unknown, n (%)		94 (30.9)

Years living with HIV, median (IQR)	289	15 (8 - 24)
Previous AIDS diagnosis, yes, n (%)	298	136 (45.6)
Nadir CD4 T-lymphocytes, cells/mm^3, median (IQR)	201	234 (95 - 365)
Zenith HIV-RNA, copies/mL, median (IQR)	172	108157.5 (35221 - 254450)
Current HIV-RNA < 20 copies/mL, n (%)	296	239 (80.7)
≥ 20 copies/mL, n (%)		57 (19.3)
HIV-RNA detectable, median (IQR)	57	44 (25 – 92)
Current CD4 T-lymphocytes, cells/mm^3, median (IQR)	303	708 (523 - 951)
PCDR (CD4 T-cells ≤ 200/mm^3), n (%)	303	8 (2.6)
ICDR (CD4 T-cells between 201-500/mm^3), n (%)		60 (19.8)
HCDR, (CD4 T-cells > 500/mm^3), n (%)		235 (77.6)
Current CD8 T-lymphocytes, cells/mm^3, median (IQR)	303	839 (632 - 1126)
CD4/CD8 ratio, median (IQR)	303	0.84 (0.6 - 1.2)
Comorbidity, none, n (%)	304	169 (55.6)
one, n (%)		85 (28)
more than one, n (%)		50 (16.5)
Hypertension, n (%)		80 (26.3)
Chronic cardiac disease, including congenital heart disease (not hypertension), n (%)		24 (7.9)
Tumor within last 5 years, n (%)		24 (7.9)
Chronic kidney disease, n (%)		20 (6.6)
Myocardial infarction, n (%)		11 (3.6)
Connective tissue disease, n (%)		-
Diabetes, n (%)		16 (5.3)
Chronic Obstructive Pulmonary Disease (not asthma), n (%)		7 (2.3)
Liver disease (not hepatitis), n (%)		14 (4.6)
Lymphoma, n (%)		-
Leukemia, n (%)		-
Metastatic solid tumor, n (%)		1 (0.3)
Any rheumatological / autoimmune disease, n (%)		14 (4.6)
Blood transfusion in the last year, n (%)	304	-
Type of vaccine administered	304	
BNT162b2, n (%)		304 (100)

Table 3: main characteristics of people living with HIV at time of the firts dose of SARS-CoV-2 vaccine (T1) according with current CD4 T-lymphocytes count (cells/mm^3). Abbreviation: BMI, body max index; MSM, men who have sex with men; IQR, interquartile range; PCDR, poor CD4 recovery; ICDR, intermediate CD4 recovery; HCDR, high CD4 recovery.

2. SARS-CoV-2 vaccination and humoral response in PLWH

All the 304 PLWH received first vaccine dose at T1. 4.3% of enrolled patients reported microbiologically documented SARS-CoV-2 infection before vaccination: 12 patients reported it in their medical history and one subject had a positive molecular nasal swab performed at T1, but still took the dose of vaccine as the swab result arrived the day

after the vaccination session. One subject contracted SARS-CoV-2 infection within three weeks following administration of the first dose of vaccine.

A total of 302 subjects (99%) received the second dose of vaccine (T2): 292 subjects after three weeks from the first one and 10 more than 3 weeks after the first dose, having reported a SARS-CoV-2 infection in the 3-6 months preceding T1. It was a homologous vaccination for 301 individuals, in one case the second dose was carried out with mRNA-1273. Two subjects immediately dropped out of follow-up after T1.

275 subjects over 301 (91.4%) underwent the third vaccine dose: 67% with BNT-162b2 and 33% with mRNA-1273. One further subject was lost to follow-up, and 26 subjects did not receive the third dose. 12 individuals contracted the SARS-CoV-2 infection close to the booster vaccination campaign and subsequently never received the vaccine again; in 14 cases the third dose was not carried out by choice and regardless of a recent history of infection. After 6 months +/- 2 months from the third dose, only 45 subjects (16.3%) underwent the fourth vaccine dose, all of them with BNT-162b2.

Table 4 describes anti-RBD IgG titers at each time point for the overall population, independent of a previous, microbiologically documented, SARS-CoV-2 infection.

	Patients N	Non reactive n (%)	Inconclus. n (%)	Positive-low n (%)	Positive-intermed. n (%)	Positive-high n (%)
T1	273	240 (87.9)	14 (5.1)	14 (5.1)	4 (1.4)	1 (0.5)
Anti-RBD IgG, BAU/mL, median (IQR)		0.4 (0.4 - 0.4)	17.8 (12.7 - 23)	109 (86.5 - 159)	328.5 (253 - 446)	1623
T2	282	70 (25%)	96 (34%)	75 (26.6%)	13 (4.6%)	28 (9.8%)
Anti-RBD IgG, BAU/mL, median (IQR)		1.1 (0.4 - 2.9)	18.6 (11.2 - 27.8)	95.4 (65.8 - 140)	248 (212- 349)	2500 (2500 - 2500)
T3	268	2 (0.7%)	3 (1%)	28 (10.5%)	129 (48.3%)	106 (39.5%)
Anti-RBD IgG, BAU/mL, median (IQR)		0.4 (0.4 - 0.4)	30.5 (16.7 - 40.4)	131.5 (91 - 178.5)	431 (322 - 576)	1614.5 (1173 - 2500)
T4	126	1 (0.8%)	1 (0.8%)	3 (2.6%)	1 (0.8%)	120 (95%)
Anti-RBD IgG, BAU/mL, median (IQR)		0.4	43	74 (52.5 - 122)	607	2500 (2500 - 2500)

Table 4: qualitative and quantitative anti-RBD IgG titer at each time point (overall population). Abbreviations: IQR, interquartile range; T1, before vaccination; T2, day of second dose administration; T3, 3 months +/- 1 month from the first vaccine's dose; T4, 1 month +/-1 month after the third vaccine's dose.

10% of the enrolled patients not reporting a microbiologically documented SARS-CoV-2 infection in their history did not provide a blood sample at T1 and therefore it was not possible to evaluate the presence of pre-vaccination anti-RBD IgG antibodies. 93% of subjects for whom a blood sample was available at baseline presented a negative or inconclusive antibody titer before vaccination. Serology therefore identified 19 subjects with detectable anti-RBD IgG, more than those who had reported SARS-CoV-2 infection in their previous history. It is noteworthy that only 3 out of 13 subjects with a pre-T1 microbiologically documented SARS-CoV-2 infection, presented a positive anti-RBD IgG titer at T1: one subject with a low-positive titer and two subjects with a high-positive titer.

Table 5 describes the anti-RBD IgG response at T2 and T3 in subjects who completed the two-doses primary vaccine course. Only individuals without a microbiologically

documented SARS-CoV-2 infection at any previous time, without positivity to anti-spike RBD IgG antibodies at T1, and for whom a blood-sample was available both at T2 and T3, were included.

	Population N	**Non reactive n (%)**	**Inconcl. n (%)**	**Positive-low n (%)**	**Positive-intermediate n (%)**	**Positive-high n (%)**
T2 (before II dose)	207	58 (28)	77 (37.2)	56 (27.1)	9 (4,3)	7 (3.4)
Anti-RBD IgG, BAU/mL, median (IQR)		1.2 (0.4 – 3.0)	17.3 (11.1–28.1)	95.7 (71.4–141)	218 (210-248)	>2500
T3 (3 months +/- 1 month after I dose)	214	2 (1)	1 (0.5)	26 (12)	110 (51.5)	75 (35)
Anti-RBD IgG, BAU/mL, median (IQR)		0.4 (0.4-0.4)	30.5	131.5 (93.4-177)	431.5 (322-560)	1369 (1125-2317)

Table 5: qualitative and quantitative anti-RBD IgG response at T2 and T3; individuals who completed the primary vaccination cycle, without a previously reported microbiologically documented SARS-CoV-2 infection (any time) and without detectable IgG anti-RBD titer at T1. Abbreviation: IQR, interquartile range.

At T2 about one third of subjects still had a negative anti-RBD IgG titer, and 37% of subjects had an inconclusive titer. Only 34% of subjects had a positive serological result, but in 27% of cases with a low titer. After 3 months +/- 1 month from the first dose (T3), only two subjects did not have yet a detectable anti-RBD IgG titer, while 97% had a positive antibody response, mainly at intermediate (51%) and high (35%) titers.

The serological response at T4 was analyzed in 96 subjects who had taken three doses of vaccine and for whom neither a microbiologically documented SARS-CoV-2 infection was reported at any previous time, nor a positive anti-RBD IgG titer at T1was detected. In addition, a blood sample from each of these subjects was collected at each previous time point. Almost all of them had a positive anti-RBD IgG titer (98%) with median values higher than those at T3 (table 6).

Figure 12 represents the dispersion of anti-RBD IgG titers at each time-point considering individuals without a history of microbiologically documented SARS-

CoV2 infection at any time: independently from the baseline CD4 T-lymphocytes count, the number of subjects with a positive serological response and with a high anti-RBD IgG titer increased with increasing number of administered vaccine doses. Furthermore, in most of the subjects the magnitude of the antibody titer one month after the third dose was greater than that detected three months after the second vaccine dose.

	Population N	**Non reactive n (%)**	**Inconcl. n (%)**	**Positive low n (%)**	**Positive intermediate n (%)**	**Positive high n (%)**
T4 (1 month +/-1 month from the III dose)	96	1 (1)	1 (1)	2 (2)	1 (1)	91 (95)
Anti-RBD IgG, BAU/mL, median (IQR)		0.4	43	98 (74–122)	607	2500 (2500-2500)

Table 6: qualitative and quantitative anti-RBD IgG response at T4; individuals who completed the primary vaccination cycle and received also a third dose, without a previously reported microbiologically documented SARS-CoV-2 infection (any time) and without detectable IgG anti-RBD titer at T1. Abbreviation: IQR, interquartile range.

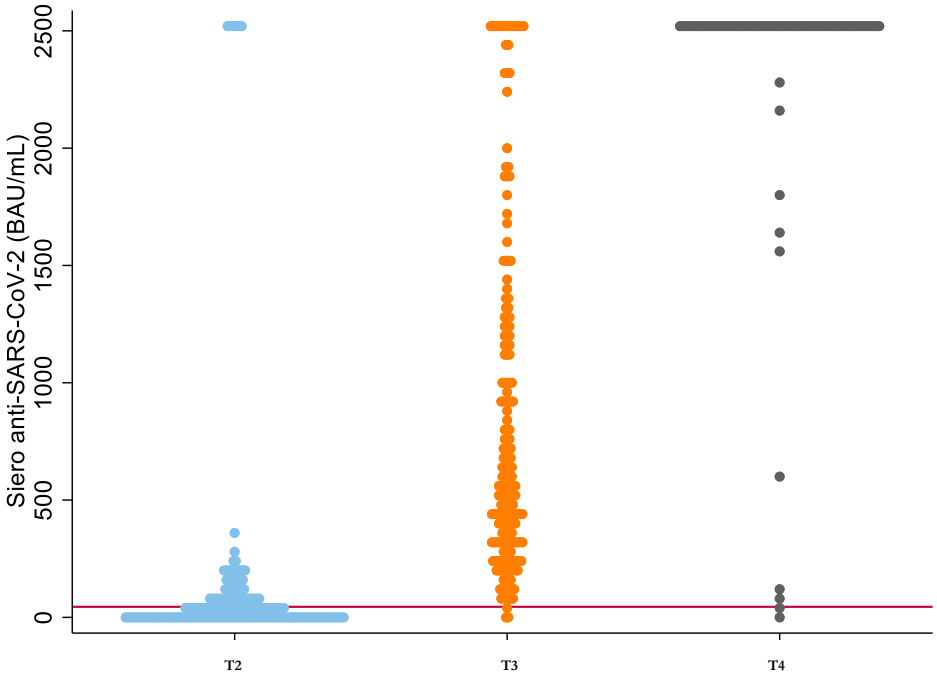

Figure 12: dispersion of anti-RBD IgG titers at T2, T3 and T4 in subjects without a history of microbiologically documented SARS-CoV-2 infection. Red line indicates 45 BAU/mL, the lower limit of the "positive-low" serological category.

Figure 13 shows the ant-RBD IgG response at T2, T3, and T4 stratifying patients into 2 categories based on patients' CD4 T-lymphocyte counts before starting SARS-CoV-2 vaccination: less or equal to 500/mm^3 and more than 500/mm^3.

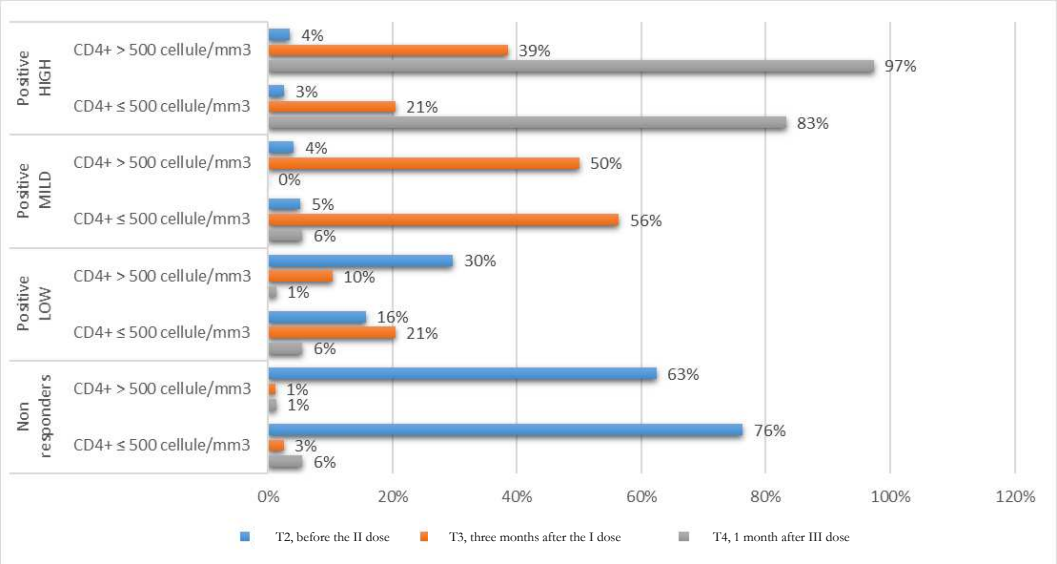

Figure 13: comparison of anti-RBD IgG titers at T2, T3 and T4 stratifying patients by CD4 T lymphocytes count before BNT-162b2 first dose administration.

After the first dose, the antibody response remained negative or inconclusive in most of the population analyzed and regardless of the CD4 T-lymphocytes count prior to vaccination. After the first vaccine's dose, 38% of patients with CD4 T-lymphocytes count more than 500/mm^3 and 24% of those with less than 500/mm^3 presented a positive serological response. After the second dose, on the contrary, almost all the subjects elicited a positive serological response. In most of included individuals, the antibody elicited titer was positive-intermediate, both for subjects with a low pre-vaccinal lymphocyte counts and for subjects with higher pre-vaccinal lymphocyte counts (56% vs 50%). The serological response was positive-high especially for patients with more than 500/mm^3 CD4 T-lymphocytes count before vaccination in comparison to those with less than 500/mm^3 (39% vs 21%).

After 1 month from the third dose, 97% of subjects with more than 500/mm^3 CD4 T-lymphocytes and 83% of subjects with less than 500/mm^3 CD4 T-lymphocytes at baseline developed a high positive antibody response. At the same time point, an antibody response was not elicited by 6% of subjects with low pre-vaccinal lymphocyte counts compared with 1% of subjects with high pre-vaccinal lymphocyte counts. A

low-positive or intermediate-positive antibody titer was elicited by 12% of subjects with low pre-vaccinal counts compared with 1% of those with higher counts.

3. Predictors of seroconversion after SARS-CoV-2 vaccination among PLWH

In evaluating predictors of serological response at T3, it was not possible to compare patients with positive serological response and patients with negative/inconclusive serological response since only three subjects fell into the latter category. The subjects with high-positive serology versus those with low-medium serology were then compared (table 7).

Variables	Univariable analysis		Multivariable analysis*	
	Odds ratio (CI 95%)	**P-value**	**Odds ratio (CI 95%)**	**P-value**
Reference: positive low-intermediate anti-RBD IgG titer	1.00	-	1.00	-
Age	0.96 (0.94 – 0.99)	**0.008**	0.94 (0.90 – 0.98)	**0.008**
Gender	2.69 (1.44 – 5.03)	**0.002**	3.33 (1.35 – 8.24)	**0.009**
Years living with HIV	1.00 (0.97 – 1.03)	0.867	0.99 (0.95 – 1.04)	0.732
CD4 T-lymphocytes > 500/mm^3 at T1	2.39 (1.04 – 5.53)	**0.041**	1.75 (0.53 – 5.79)	0.361
CD8 T-lymphocytes at T1	1.00 (0.999 – 1.001)	0.890	-	-
CD4/CD8 ratio at T1	1.29 (0.79 – 2.09)	0.306	-	-
Nadir CD4 T-lymphocytes	1.00 (0.998 – 1.002)	0.831	1.00 (0.997 – 1.003)	0.904
Previous AIDS diagnosis	0.95 (0.54 – 1.68)	0.860	2.02 (0.65 – 6.31)	0.224
One comorbidity at T1	0.68 (0.35 – 1.31)	0.248	1.19 (0.47 – 2.99)	0.712
Two or more comorbidity at T1	0.44 (0.19 – 1.02)	**0.056**	0.86 (0.26 – 2.84)	0.803
HIV-RNA ≥ 20 copies/mL at T1	0.70 (0.31 – 1.55)	0.376	0.58 (0.21 – 1.63)	0.305
* by age, gender, length of HIV infection, CD4 T-lymphocytes count at T1 (≤ 500 vs > 500 cells/mm^3), nadir CD4 T-lymphocytes count, previous AIDS diagnosis, HIV-RNA at T1 (<20 vs ≥ 20 copies/mL), comorbidities at T1				

Table 7: Predictors of antibody response to BNT-162b2 primary course among people living with HIV (N 214)

The univariable analysis identified advanced age and male gender as variables associated with the greater probability of developing a medium-low antibody titer. The presence of two or more comorbidities also seemed to be associated with a higher probability of developing a low-medium serological response, although the association was at the limits of statistical significance. Current CD4 T-lymphocytes count greater than 500/mm^3 before the administration of the first vaccine dose was associated with an increased likelihood of developing a high serological response, but again the association remained slightly below statistical significance.

Multivariable analysis confirmed advanced age and female gender as associated with the statistically significant probability of developing a medium-low and high serological response respectively (table 7).

At T4, 2 of the 96 subjects included in the analysis presented a negative antibody titer and 91 subjects presented a high-titer serological response, therefore the analysis of the determinants of a negative serological response after the third dose of vaccine was not carried out. Both subjects with a negative serological titer after the third dose of vaccine had already previously received the diagnosis of AIDS, had detectable HIV-RNA at T1, and received a homologous booster dose with BNT162b2; one subject had only 5/mm^3 CD4 T-lymphocytes at T1, and the other 949/mm^3.

4. Break-through SARS-CoV-2 infection after the primary cycle and after the first booster dose of m-RNA SARS-CoV-2 vaccines in PLWH

Only one subject contracted SARS-CoV-2 infection within 3 weeks of the first dose. Overall, 82 out of 301 evaluable subjects (27% - 3 lost to follow-up) experienced at least one break-through infection for a total of 85 infective episodes (3 subjects contracted two microbiologically confirmed infections). Most of them were pauci-symptomatic as described by a WHO clinical progression score with a median of 2. In one fifth of cases (17 subjects), the infection was contracted after the second vaccine dose: 11 subjects no longer received the booster dose, while 6 took it in the months following the infective episode. For these infections the mean time between the first vaccine dose and the diagnostic swab for SARS-CoV-2 infection was 7.6 months (SD $\pm$ 1.7). 66 individuals (80.5%) became infected after the third dose, on average after 4.2 months (SD $\pm$ 3.2) from the booster administration (table 8). Only one subject had SARS-CoV-2 infections both before and after the third vaccine dose.

Variable	N	n (%)
Patients with at least one COVID-19 breakthrough infection	301	82 (27.2)
Patients with one COVID-19 breakthrough infection after the second vaccine dose (no third dose performed)	82	11 (13.4)
Patients with one COVID-19 breakthrough infection between the second vaccine dose and the third dose	82	6 (7.3)
Patients with at least one COVID-19 breakthrough infection after the third vaccine dose #	82	66 (80.5)**
Time between third dose and SARS-CoV-2 infection †, months, mean ± SD, (first infective episode considered)	66	4.2 ± 3.2
Time between third dose and SARS-CoV-2 infection †, months, median (IQR), (first infective episode considered)	66	3 (1 - 7)
Time between first dose and breakthrough infection, months, mean ± SD‡	17	7.6 ± 1.7
Time between first dose and breakthrough infection, months, median (IQR)‡	17	8 (8 - 9)
WHO clinical progression score**, median	54	2
WHO clinical progression score – infections after II dose‡		
1	10	1 (10)
2	10	7 (70)
3	10	2 (20)
WHO clinical progression score – infections after III dose **		
1	44	9 (20.5)
2	44	33 (75)
3	44	1 (2.3)
6	44	1 (2.2)

Table 8: breakthrough SARS-CoV-2 infections frequency and severity; one subject with one SARS-CoV-2 infection after the second vaccine's dose and a second SARS-CoV-2 infection after the third vaccine dose. Abbreviations: SD, standard deviation; IQR, interquartile range. # Two individuals with two SARS-CoV-2 infections each after the third vaccine's dose; ** only the first infective episode considered; † only infections after third dose; ‡ only infections between II and III dose or occurred in patients without third dose.

5. Predictors of SARS-CoV-2 infections after SARS-CoV-2 booster vaccine dose among PLWH

Considering subjects with third vaccine dose and comparing those without SARS-CoV-2 infection after the booster dose (group A) and those with SARS-CoV-2 after the booster dose (group B), it emerged that group B had a lower CD8 T-lymphocytes count at T1 and never had a microbiologically or serologically documented SARS-CoV-2 infection (table 9). 26 individuals without serology at T1 were excluded since, while not reporting SARS-CoV-2 infection before vaccination, it was not possible to certify their pre-vaccination immune status at least with anti-RBD IgG, as an anti-N titration was not performed in this study.

Variable		No SARS-CoV-2 after the first booster dose (group A) N 186	SARS-CoV-2 after the first booster dose (group B) N 63	P-value
Age, years, median (IQR)		54 (46 - 59)	51 (45 - 58)	0.308
Gender, %	Male	74.2	79.4	0.409
	Female	25.8	20.6	
BMI, kg/m^2, median (IQR)		24.9 (22.8 - 27.4)	24.0 (22.1 - 26.8)	0.135
Smoking status, %	no-smoker	34.5	43.9	0.187
	actual smoker	39.6	26.3	
	previous-smoker	26.0	29.8	
Length of HIV infection, years, median (IQR)		16 (8 – 24.5)	13 (8 - 24)	0.504
Previous AIDS diagnosis, %	No	56.0	50.8	0.483
	yes	44.0	49.2	
Nadir CD4 T-lymphocyte count, cells/mm^3, median (IQR)		229 (76 - 335)	265 (104 - 423)	0.225
HIV-RNA zenith, copies/mL, median (IQR)		107326 (29000 - 271300)	118136 (56239 - 317890)	0.190
HIV-RNA at T1, copies/mL, %	< 20	81.1	82.0	0.882
	≥ 20	18.9	18.0	
HIV-RNA at T1, median (IQR)		< 20	< 20	0.893
CD4 T-lymphocytes at T1, cells/mm^3, median (IQR)		714 (566 - 950)	651 (512 - 988)	0.205
CD4 T-lymphocytes at T1, cells/mm^3, %	≤ 200	2.2	4.8	0.456
	201 - 500	17.3	19.1	
	> 500	80.5	76.2	
CD8 T-lymphocytes at T1, cells/mm^3, median (IQR)		894 (648 - 1193)	774 (604 - 929)	**0.013**
CD4/CD8 ratio at T1, median (IQR)		0.8 (0.6 - 1.2)	0.9 (0.6 - 1.2)	0.220
Comorbidity at T1, %	None	54.3	60.3	0.667
	One	27.4	22.2	
	More than one	18.3	17.5	
SARS-CoV-2 infection before III-dose, %	No	85.5	100.0	**0.001**
	Yes	14.5	0.0	

Table 9: comparison of individuals with and without SARS-CoV-2 infection post-third dose. Abbreviations: IQR, interquartile range; BMI, body max index.

DISCUSSION

In PLWH cellular immunity is mostly affected, CD4 T-lymphocytes being the target of this virus. CD4 T-lymphocytes are central in influencing the humoral and cellular immune responses to vaccination and have an important impact on antibody production. In the past, it has been shown that PLWH have lower responses to some types of vaccine and that these responses are dependent on the level of CD4 T-lymphocytes. With the development of more potent and well tolerated ART to treat HIV infection, many people on treatment achieve an immune recovery with normalization of CD4 T-lymphocytes counts. However, even in this population, subtle defects in immune function persist (175) and may impair vaccine response. Furthermore, a proportion of PLWH have very advanced disease with low CD4T-lymphocytes counts and are at higher risk of not responding to vaccine (206).

According to our findings, SARS-CoV-2 vaccination with BNT162b2 primary course induced a robust humoral immune response in most PLWH receiving ART and with a stable viral suppression and robust CD4 T-cell count. Three months after the second vaccine dose, 97% of our population elicited a positive anti-RBD IgG titer, in line with the results of Speich and Milano who described 98.6% and 94.3% of seroconversion respectively after a primary course with a m-RNA vaccine in SARS-CoV-2 naïve PLWH (192, 185). This result is also aligned to that found one month after the end of the primary vaccine's course with BNT162b2 or mRNA-1273 by Antinori (86.7%-100% of seroconversion) and Corma-Gomez (94% of seroconversion) (181, 187).

Three months after the second vaccine dose, about half of PLWH (51.5%) elicited a positive-intermediate anti-RBD IgG response with a median titer of 431.5 BAU/mL (IQR 322-560), and 35% a positive-high serological response with a median titer of 1369 BAU/mL (IQR 1125-2317). Stratifying patients by baseline CD4 T-cell counts, intermediate positive antibody titer was elicited mostly by PCDR/LCDR vs HCDR (56% vs 50%), conversely high antibody titer was mostly elicited by HCDR vs PCDR/LCDR (39% vs 21%). These results are in line with those of other studies and underline the impact that current CD4 T-lymphocyte counts before m-RNA anti-SARS-CoV-2 vaccination have on the proportion and magnitude of seroconversion in PLWHs (181, 187, 207), being more robust in individuals with a CD4 T-cells count higher than 500/mm^3. Also in our population the median values of RBD-binding IgG titers three months after the second dose of m-RNA vaccine were significantly lower

for all PLWHs groups (LCDR, ICDR and HCDR), if compared with those reported by several studies in health care workers or in HIV-negative individuals (181, 207).
In the multivariable analysis, male gender and advanced age were predictors of poor seroconversion, in line with Corma-Gomes (187) and Tuan (190). Current CD4 T-lymphocytes counts higher than 500/mm^3 at the time of administering the first dose of vaccine, were associated with an increased likelihood of developing a high serological response only at the univariable analysis and at the limits of statistical significance, in contrast with other studies in which the association tested statistically significant also at multivariable analysis (187, 207). In our study also the presence of two or more comorbidities were found to be associated with a higher probability to develop a low-intermediate serological response, in line with Søgaard and colleagues (208), but only at univariable analysis and at the limits of statistical significance.
The third vaccine dose elicited a positive serological response in 98% of subjects, reaching the highest antibody level (more than 2500 BAU/mL) in 95% of evaluated individuals and independent of age, gender, CD4 T-lymphocyte counts before the first vaccine's dose and type of m-RNA vaccine used for boosting. Of note, the titer of anti-RBD IgG achieved after the third dose was even higher than that observed three months after the second dose of the primary cycle, in line with other studies (191, 197, 198). As suggested elsewhere (197), the observed increase in humoral response could be consistent with the hypothesis that the third dose induces a robust B cell memory response, previously elicited by the primary vaccination series, and highlights the fact that the SARS-CoV-2 m-RNA vaccines are able to stimulate a satisfactory humoral response even in immunocompromised patients such as those with low CD4 T-cells count and a previous or current diagnosis of AIDS. In particular, the achieved humoral responses were significant, considering the chronic immune dysregulation, as well as the reduction of viral specific T and B cell clones even in PLWH on effective ART (209, 210). This finding could imply that effective ART is able to restore a good immune response, also in patients with low CD4 T-cells count or who experienced AIDS. However, the impact of HIV-related immunosuppression on the duration of the vaccine-induced immunity remains to be elucidated. CD4 T-lymphocyte count is a determinant to achieve an optimal vaccine induced immune response (195, 211), and in chronic PLWH the CD4 T-cells as well as the persistent inflammatory environment could weaken the differentiation of effective memory B and T cell immunity (212).

27% of our PLWH experienced at least one SARS-CoV-2 infection, mainly after the third booster dose (80% of infected subjects). In our population the vaccination booster campaign was carried out with monovalent m-RNA vaccines, BNT162b2 or mRNA-1273, and was conducted between November 2021 and February 2022, in a period in which Delta, from mainly circulating VOC, was rapidly replaced by Omicron (https://coronavirus.jhu.edu/region/italy ; https://www.ecdc.europa.eu/en/covid-19/country-overviews). At the beginning of that period Omicron BA.1 rapidly outcompeted the Delta variant, which dominated the COVID-19 landscape across all continents at the time (213), and rapidly was followed by the genetically distinct BA.2 sublineage, generating two overlapping peaks in most countries in the winter and early spring 2022. Typically, BA.1 and BA.2 show a dramatic drop in susceptibility to neutralization compared to the ancestral B.1 strain containing the Spike D614G mutation (214). Sera from vaccinees who received two vaccine doses do not cross-neutralize Omicron sublineages BA.1 and BA.2 (155, 214, 215) and this, together with the waning immunity after 6 months from the primary course described in the general population, could explain the breakthrough infections that occurred in patients without the booster vaccine's dose. Our study did not evaluate the neutralizing activity of sera collected after vaccine's third dose administration, however it is likely that the high frequency of infections despite the administration of the booster dose was a consequence of the immunological escape documented in Omicron (42, 43), the dominant VOC in that period. Four months after the third dose, antibodies elicited by first generation vaccines partially retain neutralizing ability against Omicron, mainly BA.2, despite a significant drop compared to the ancestral B.1 infection (216). Convalescent sera from ancestral B.1 infection are unable to cross-neutralize BA.1 but similarly neutralized Omicron BA.2, BA.4 and BA.5. This could explain the high frequency (80%) of breakthrough infections on average 4 months (±SD 3.2) after the third vaccine's dose, and the higher probability of PLWH naïve for SARS-CoV-2 natural infection to contract the infection despite the third dose.

Breakthrough infections after third vaccine dose were predominantly pauci-symptomatic (20% of infectious episodes with a 1 point WHO clinical progression score and 75% with a 2 point WHO clinical progression score). This could be in part a consequence of lower pathogenicity of Omicron strains than Delta, in particular of the early BA.1 and BA.2 strains that were dominant in that period (217). It could also

reflect that, also in this PLWH population, an additional booster dose may improve relative vaccine effectiveness against severe disease, similarly to that described in the general population (140, 141) and as a consequence that not only neutralizing humoral activity but also cellular immunity plays a role against Omicron (157, 158, 159).

This study has limitations. First, due to the absence of a control group of HIV-negative subjects, uncontrolled sources of unmeasured confounding bias may exist. Second, the present analysis concerns only the short-term serological response (up to 1 month after the third dose) and therefore we are unable to provide estimates of the durability of immune response and waning of immunogenicity after the booster dose in PLWH. Analysis of blood samples collected 6 months after the third dose is ongoing and maybe will answer this question.

Third, we did not evaluate the anti-Nucleocapsid antibodies to identify with confidence subjects with natural infection, however aliquots of sera are stored and could be used for this purpose. Moreover, cellular immune response was not assessed and it is known that coordination between humoral and cellular responses is pivotal to control and clear SARS-CoV-2 (218). T-cell responses may also play a critical role, particularly against variants (159, 158).

Finally, our results may not be generalizable to PLWH who are not receiving antiretroviral therapy, or who have CD4 T-cell counts lower than 200 cells/mmm^3. Further data should be provided regarding the immune response in the subset of PLWH with severe depletion of CD4 T-cell (e.g., below 200 cells/mm^3), which are also those who may benefit the most from vaccination, and those with uncontrolled viral infection (i.e., those viremic).

The strength of this study is that we collected data on the frequency and severity of breakthrough infection, so we can evaluate both the immunogenicity and clinical efficacy of m-RNA vaccines in PLWH.

CONCLUSIONS

Three months after the completion of a primary vaccination course with BNT162b2, 97% of PLWH had a positive antibody response, mainly at intermediate (51%) and high (35%) titers. Elicited titer was positive-intermediate, both for subjects with a low pre-vaccinal lymphocyte counts and for subjects with higher pre-vaccinal lymphocyte counts (56% vs 50%). A positive-high anti-RBD IgG titer was reached especially in subjects with more than 500/mm^3 CD4 T-lymphocytes count before vaccination in comparison to those with less than 500/mm^3 (39% vs 21%). Advanced age and male gender were associated with a greater probability of developing a medium-low antibody titer. The presence of two or more comorbidities also seemed to be associated with a higher probability of developing a low-medium serological response. Current CD4 T-lymphocytes count greater than 500/mm^3 before administering the first vaccine dose, was associated with an increased likelihood of developing a high serological response.

An additional dose of SARS-CoV-2 m-RNA vaccine after the initial two-dose vaccination, resulted in markedly higher levels of boosted immunity, both in PLWH with a baseline CD4 T-lymphocytes count higher and lower than 500/mL (97% vs 83%). It will be important to monitor these responses over time.

First generation m-RNA vaccines, including booster doses, do not prevent SARS-CoV-2 infection but reduce the risk of severe disease outcomes, including against Omicron.

Studies on the immunogenicity of new bivalent omicron-containing vaccine are needed, also in PLWH.

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A thought for Atropina (meow).

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