

# Long-term analysis of antibodies elicited by SPUTNIK V: A prospective cohort study in Tucumán, Argentina

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## Abstract

**Background** Gam-COVID-Vac (SPUTNIK V) has been granted emergency use authorization in 70 nations and has been administered to millions worldwide. However, there are very few peer-reviewed studies describing its effects. Independent reports regarding safety and effectiveness could accelerate the final approval by the WHO. We aimed to study the long-term humoral immune response in naïve and previously infected volunteers who received SPUTNIK V.

**Methods** Humoral immune responses, assayed by anti-SARS-CoV-2-spike-RBD IgG ELISA and neutralization assays, were measured in 602 healthcare workers at 0, 14, 28, 60 and 180 days after receiving SPUTNIK V between December 2020 and July 2021 in Tucumán, Argentina.

**Findings** Seroconversion was detected in 97% of individuals after 28 days post-vaccination (dpv) ( $N = 405$ ). Anti-RBD titers began to decrease after 60 dpv ( $N = 328$ ), but remained detectable in 94% at 90 dpv ( $N = 224$ ). At 180 dpv, anti-RBD titers persisted in 31% ( $N = 146$ ). Previous infection triggered an increased immune response to the first dose and increased neutralization activity against variants of concern (VOC). Second doses in previously infected individuals further increased titers, even 90 dpv ( $N = 75$ ). Basal antibody titers had more influence on post-vaccination anti-RBD responses than the time elapsed between diagnosis and vaccination ( $N = 274$ ).

**Interpretation** Data presented herein provides essential knowledge regarding the kinetics of antibodies induced by SPUTNIK V up to six months after immunization, and suggests that when considering one-dose vaccination policies for individuals with previous SARS-CoV-2 infection, serological studies to determine basal titers may be important, independent of when diagnosis occurred.

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**Keywords:** SPUTNIK V; Gam-COVID-Vac; COVID-19; SARS-CoV-2; Humoral immune response; Seroconversion; Long term immunity; RBD spike

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## Introduction

The need to control the transmissibility and mortality associated with SARS-CoV-2 has transformed vaccines

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## Research in context

### *Evidence before this study*

The Moscow-based Gamaleya Research Institute of Epidemiology and Microbiology developed SPUTNIK V based on their experience with Ebola and MERS vaccines. This vaccine was registered on August 11, 2020, based on phase I–II results which were later published in *The Lancet*. In this context, Argentina granted emergency use authorization on December 23, 2020, and became the third country worldwide to begin vaccinating its citizens against COVID-19 with SPUTNIK V. Phase III Clinical Trial, later published, showed 91.6% efficacy without unusual side effects. In Tucumán, as in the rest of Argentina, SPUTNIK V has been the most-used immunization platform. Of the 4 vaccines available, it represents 37% of the total doses inoculated, with more than 320,000 jabs administered as of July 1st 2021. Despite being approved in 70 nations, there are very few published studies on the effects of SPUTNIK V compared to other vaccines. The question of whether two doses of mRNA or adenovirus vaccines are needed for individuals who recovered from COVID-19 is being debated. Recently, Rossi and colleagues evaluated a transversal study of 288 healthcare workers vaccinated with SPUTNIK V in Buenos Aires, Argentina. Data obtained allowed the authors to suggest that since one dose in individuals who were seropositive at baseline elicited higher titers than two doses in seronegative volunteers, a second dose for these previously infected individuals would provide no evident benefit. This would carry the added value of increasing the number of vaccinated individuals. In the U.K., the decision to delay the second dose of the ChAdOx1 nCoV-19 (AZD1222) vaccine has been effective in providing partial protection to more people. The Argentinean health authority has also proposed delaying the administration of second doses to immunize more individuals. In other countries, and with other vaccine platforms, the same strategy is being proposed.

### *Added value of this study*

Approval of SPUTNIK V by the WHO is crucial for inclusion in the COVID-19 Vaccines Global Access (COVAX), a program that is essential to diminish SARS-CoV-2 transmission in low-income countries. Results presented herein add knowledge on SPUTNIK V, as we provide the first evaluation of antibody persistence up to 6 months after immunization with this platform. Our data suggest that only seropositive individuals with a baseline titer above a specific threshold could reach, with a single dose, a humoral immune response similar to those with the complete vaccination scheme. Additionally, antibodies elicited after the first dose in previously infected individuals possessed increased neutralization ability against various VOC, compared to immune responses in uninfected volunteers who received both doses. The time elapsed between diagnosis and vaccination (up to 120 days), however, did not influence titers elicited. These findings could become important when

formulating immunization guidelines like one-dose vaccination for individuals with previous SARS-CoV-2 infection. The current study provides readers, especially those from countries where SPUTNIK V has been authorized, a sense of seroconversion rates and persistence of titers related to this vaccine.

### *Implications of all the available evidence*

Individuals with previous SARS-CoV-2 infection show increased humoral immune responses to the first dose of SPUTNIK V. Moreover, second doses in these individuals further increased antibody titers, suggesting that second doses may still be beneficial to this population. This increase persisted in time up to three months post-vaccination. One-dose vaccination policies for the previously infected would benefit from basal titer determination to identify those individuals in which one dose would elicit strong and consistent responses.

into a critical resource to mitigate the devastating effects of the current pandemic.<sup>1</sup> The appearance of new variants reinforces the need to accelerate vaccination rates in all countries.<sup>2-3</sup> However, despite the unprecedented speed of research, development and application of different vaccines around the world, their availability remains limited in developing countries.

In March 2020, Argentina reported its first case of SARS-CoV-2 infection.<sup>4</sup> Eight months later it was the first of the Americas, and the 3rd in the world, to grant emergency use authorization for the heterologous prime-boost recombinant adenoviral-based SPUTNIK V (Gam-COVID-Vac) vaccine.<sup>5-7</sup> As of August 2021 a total of 70 countries, mostly in Latin America, Asia and Africa, have granted similar approvals. There is limited peer-reviewed published information on SPUTNIK V, which is unique in that each dose is based on the combination of two different human adenoviral vectors (rAd26 and rAd5).<sup>6</sup> Determining how local populations respond to government-led vaccination programs is essential for an effective public healthcare response to the pandemic.

The northwestern province of Tucumán, located in the Andean foothills and the second-most densely populated in the country, has been one of the cities hardest hit by the pandemic, with more than 143,000 infections and 2400 COVID-deaths in a population of 1.5 million. The fact that Argentina was the second country beyond Russia to grant emergency approval for the use of SPUTNIK V, provided us with the opportunity to independently assess anti-RBD titers elicited in a different population and up to six months post-vaccination. Although only 20% of antibodies induced by the spike glycoprotein are directed against the RBD domain, 60% of these possess neutralizing activity, in comparison to non-RBD antibodies, of which only 5% neutralize the virus.<sup>8</sup>

Here, we followed anti-RBD antibody responses to one or two doses of SPUTNIK V in a cohort of 602 public healthcare personnel (HCP) volunteers from Tucumán, with or without previous SARS-CoV-2 infection, at different time-points and up to 6 months post-vaccination. As a secondary objective, we determined how vaccination affected antibody titer and neutralization ability in individuals previously infected with SARS-CoV-2. With this, we hope to contribute to an incipient body of knowledge regarding this vaccine, used to immunize tens of millions worldwide.

## Methods

### Population studied

This study was approved by the Tucumán Public Health System Research Committee: <https://msptucuman.gov.ar/wordpress/wp-content/uploads/2021/03/Expediente-N3929-410-P2020-Seroconversion-post-vacunacion-para-SARS-CoV-2-immune-response-after-a-single-vaccine-dose.pdf>. The Ethics Committee of the Ministry of Health of the Province of Tucumán evaluated and approved the protocol of the present study (Ex. N° 3929-410-P-2020), (<https://msptucuman.gov.ar/wordpress/wp-content/uploads/2021/03/03.Serocconversion-DICTAMEN-19-1-2021.pdf>), following the Declaration of Helsinki.

All 12,502 Tucumán Public Healthcare (Sistema Provincial de Salud - SIPROSA) personnel (HCP) from the districts of San Miguel de Tucumán, Banda del Río Salí, Yerba Buena and Tafi Viejo, who were the first group to be vaccinated with SPUTNIK V between December 2020 and February 2021 in 12 different vaccination nodes, were invited to participate in the study. All enrolled volunteers were properly informed regarding their choice to participate in the study and provided a signed informed consent (forms can be found at <https://msptucuman.gov.ar/wordpress/wp-content/uploads/2021/03/FCI-SeroconversionRCSfinal.pdf>). Personal data from all volunteers were encrypted. The inclusion criteria were: 1- HCP from 18 to 60 years old, asymptomatic for COVID-19, enlisted to receive the SPUTNIK V vaccine against COVID-19. 2- Individuals who had signed the Informed Consent to participate in this study. The exclusion criteria were: Individuals with current COVID-19 symptoms (dry cough, fever, dyspnea) or current COVID-19 diagnosis at the time of enrollment.

The of the total number of HCP that were programmed to receive vaccination with SPUTNIK V at the time and worked in the districts included in the study ( $N = 2088$ ) were invited to participate. The first 602 that met the inclusion criteria were included. After reaching this number the study was closed. This corresponds to a 29% of response rate.

Vaccination side effects in HCP were screened by and documented on-site by local physicians and nurses at the Public Health System Hospitals and Clinics where the HCP work. Pain at the injection site, fever, fatigue, headache, muscle pain, chills and diarrhea, were considered mild side effects while severe side effects were considered as any effect requiring hospitalization.

Our prospective cohort study was performed with 602 previously enrolled HCP, however, different subgroups were used according to the number of individuals that provided serum samples for those time points. Of the total cohort of  $N = 602$  of participants included, the number of volunteers who provided serum samples for each time point are indicated in parenthesis: on 0 dpv ( $N = 253$ ), 14 dpv (594), 28 dpv ( $N = 405$ ), 60 dpv ( $N = 328$ ), 90 dpv ( $N = 224$ ), 180 dpv ( $N = 146$ ). For comparing titers obtained post-vaccination with those measured by us with the same ELISA platform and reported in Tomas-Grau et al. true positive SARS-CoV-2 convalescent individuals ( $N = 309$ ) (as defined by diagnosis with RT-PCR and serology with both anti-RBD IgG ELISA and anti-N CMIA) who provided serum samples 28–42 days-post diagnosis with SARS-CoV-2 infection were also included.<sup>9</sup> For evaluating the effect of previous SARS-CoV-2 infection on titers elicited at different time points post-vaccination in the same group of individuals, 4 groups were used in which individuals were required to have provided serum at 0 dpv and at the time point-indicated. In this way,  $N = 107$  individuals in provided samples at 0, 14, and 28 dpv,  $N = 98$  individuals provided samples at 0 and 60 dpv,  $N = 75$  individuals provided samples at 0 and 90 dpv, and  $N = 35$  provided samples at 0 and 180 dpv. To determine the importance of basal titer thresholds and time elapsed between diagnosis and vaccination with the first dose, the subgroup of individuals with previous SARS-CoV-2 infection who provided samples at 0, 14, and 28 dpv ( $N = 42$ ) were further analyzed.

### Anti-RBD IgG antibody ELISA assay

Anti-RBD IgG titers were determined by an “In-House” ELISA developed and validated with more than 758 samples in our laboratory,<sup>9</sup> following a protocol modified from Stadlbauer and colleagues which presents high sensitivity (92.2%) and specificity (100%).<sup>10</sup> Briefly, recombinant RBD from SARS-CoV-2 was obtained from HEK293 cells, which were previously transduced with a pHAGE2 lentivirus (pHAGE2-RBD-His).<sup>11</sup> The transgenic cell line generated constitutively secretes a his-tagged RBD. This protein domain was subsequently purified from the conditioning media by affinity chromatography and purity was confirmed by SDS-PAGE. Purified RBD was immobilized in each well (0.1  $\mu\text{g}$ ) of 96-well flat polystyrene bottom plates (High Binding, Half-Area, Greiner #675,061). The

antibody titer value representing the highest accuracy (Sensitivity + Specificity) was calculated using the receiver operating curve (ROC) to establish the cutoff value. Titers were calculated as the dilution in which the optical density (OD<sub>450 nm</sub>) obtained was equal to the cutoff. Blood samples for IgG anti-RBD detection were collected at (a) the same day before the first dose application (0-day post-vaccine; dpv), (b) 14 ± 1 days after the first dose (14 dpv), (c) 28 ± 2 days after the first dose (28 dpv) (which corresponds to 7 days after the second dose), (d) 60 ± 3 days after the first dose (60 dpv), (e) 90 ± 3 days after the first dose (90 dpv), and (f) 180 ± 3 days after the first dose (180 dpv). After peripheral blood collection (5 ml), serum was obtained by spontaneous coagulation after two hours and the samples were stored at -20°C until use.

#### Virus neutralization assays

Virus neutralization assays were performed using recombinant VSV expressing the indicated wild-type, B.1.1.7 (alpha), B.1.351 (beta), or E484K mutant SARS-CoV-2 spikes on 293T-ACE2-TMPRSS2 (F8-2 clone) cells exactly as described previously.<sup>12</sup>

#### Statistical analysis

Statistical analyses were performed using the Prism 8.0 software (GraphPad, San Diego, CA). Normality of the data distribution was performed with the Shapiro-Wilk test. Data that did not show a normal distribution were depicted as medians. Differences among the distribution between two groups were analyzed with the Mann-Whitney U test. This test was used to analyze the behavior of the anti-RBD titers when comparing two consecutive time-points, detailed with asterisks according to the significance level provided by the statistical test. For neutralization assays, neutralization curves were performed with a serial 4-fold dilution of serum (1:10–1:40,960) and normalized against the no-serum control set at 100% using nonlinear regression. Data points were fitted using the standard log(inhibitor) vs normalized response – variable slope nonlinear regression equation in PRISM v9.12. Data points represent mean ± s.d.. Each neutralization curve was performed in triplicate. For analyzing the effect of the time elapsed between previous COVID diagnosis and vaccination on anti-RBD titers elicited by SPUTNIK V, the median anti-RBD titers of all groups were compared using Kruskal-Wallis with a correction for multiple comparisons by controlling the false discovery rate using the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli. Statistical significances are indicated in the figures by asterisks as follows \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001. In all cases, a *p*-value of less than 0.05 was assumed as statistically significant.

#### Role of the funding source

Ministry of Public Health of Tucumán (Argentina): provided supplies and trained personnel for ELISA test development. Argentinean Research Council-CONICET (PIP 722 and 806): provided supplies. Argentinean Research Agency-MINCYT grants (PICT-2018–3379 and PICT2018–02,989): provided supplies. National University of Tucuman (PIUNT-UNT D644/1 and D624): provided supplies. Fundación Florencio Fiorini: provided supplies. Mr. Claude Burgio (SkyBio LLC): provided supplies. Funders had no role in study design, data analysis, interpretation, writing of the report or decision to submit.

#### Results

Humoral immune responses against the receptor-binding domain (RBD) of the SARS-CoV-2 spike glycoprotein were evaluated in 602 healthcare personnel (HCP) volunteers vaccinated with SPUTNIK V in Tucumán, Argentina. The basic demographics of the volunteer population studied are shown in Table 1. Immune responses were analyzed using an anti-SARS-CoV-2-RBD ELISA (see Methods),<sup>9,13,14</sup> which have demonstrated an excellent correlation with virus-neutralizing activity.<sup>15–19</sup> The SPUTNIK V vaccination scheme and the days post-vaccination (dpv) in which serum samples were collected from volunteers are depicted in Fig. 1a.

In a prospective cohort study of the 602 volunteers, basal anti-RBD IgG antibody titers were found in 44% of individuals before vaccination (dark and light purple, Fig. 1b), of which more than half (66%) lacked previous COVID diagnosis (either by RT-PCR or rapid antigen tests) (light purple, Fig. 1b), implying they underwent asymptomatic or unrecognized infections (Fig. 1b). After vaccination, 75% of volunteers reported no adverse effects, while the remaining 25% reported only mild side effects (Table 1). Fourteen days after the first dose (14 dpv), anti-RBD IgG antibody titers were detected in only 48% of volunteers (Fig. 1c) and displayed a geometric mean titer (GMT) of 302.9. However, one week after completing the vaccination scheme (28 dpv), antibody titers were present in 97.6% of volunteers, corroborating previous reports,<sup>6,20,21</sup> and the GMT value rose to 1111.0 (Fig. 1c). Two months after initiating immunizations (60 dpv), median anti-RBD levels began to decrease, reaching a median titer of 493.7 and remained without significant variation at 90 dpv (GMT = 584.4) (Fig. 1c). Importantly anti-RBD titers were still detectable in 31% of volunteers six months after vaccination (GMT = 193.9) (Fig. 1c). Anti-RBD titers elicited by 309 true positive convalescent individuals (previous positive RT-PCR and both positive anti-RBD IgG ELISA and anti-N IgG CMIA), measured by our group with the same ELISA platform but for a different study, are also displayed for comparison and showed a GMT of 391.7

Characteristics	Group	Totals per group
<b>Number of study participants</b>		602
<b>Days Post Vaccination</b>	0	253 (42.0)
	14	594 (98.8)
	28	405 (67.3)
	60	328 (54.5)
	90	224 (37.2)
	180	146 (24.3)
<b>Sex</b>	Male	191 (31.7)
	Female	410 (68.1)
	No answer	1 (0.2)
	Sex Ratio [M/F]	0.47
<b>Age- categories</b>		43.19 ± 8.57
	≤20–29 years	39 (6.5)
	30–39 years	168 (27.9)
	40–49 years	235 (39.0)
	≥50 years	160 (26.6)
	No answer	0 (0)
<b>Blood group</b>	0	240 (39.9)
	A	140 (23.3)
	B	47 (7.8)
	AB	11 (1.8)
	No answer	164 (27.2)
<b>Close contact<sup>1</sup></b>	Yes	193 (32.1)
	No	224 (37.2)
	No answer	185 (30.9)
<b>Previous SARS-CoV-2 detection (TR-PCR or Rapid Ag Test) and primary outcomes</b>	<b>Yes</b>	180 (30.0)
	Yes, symptomatic	47 (7.8)
	Yes, asymptomatic	33 (5.5)
	No answer	100 (16.6)
	COVID hospitalization	5 (3.7)
	Outpatients	129 (96.3)
	No answer	46 (7.6)
	<b>No</b>	422 (70.0)
<b>Health condition at baseline</b>	Auto-immune diseases <sup>2</sup>	14 (2.3)
	Asthma	15 (2.5)
	Cancer	3 (0.5)
	Diabetes	12 (2.0)
	Hypertension	44 (7.3)
	Hypothyroidism	31 (5.2)
	Metabolic syndrome	11 (1.8)
	Obesity	11 (1.8)
	Smoking	3 (0.5)
	Others <sup>3</sup>	6 (1.0)
	None	306 (50.8)
	No answer	185 (30.7)
<b>Infections post vaccination</b>		7 (1.2)
	Age	41 ± 8.2
	Sex Ratio [M/F]	0.75
	Hospitalized	0 (0)

(continued)

Table 1. (Continued)

Characteristics	Group	Totals per group
<b>Side effect after vaccination</b>	Mild <sup>4</sup>	149 (24.8)
	Severe	0 (0)
	No symptoms	453 (75.2)

Table 1.: Basic demographics of the volunteer HCP population studied.

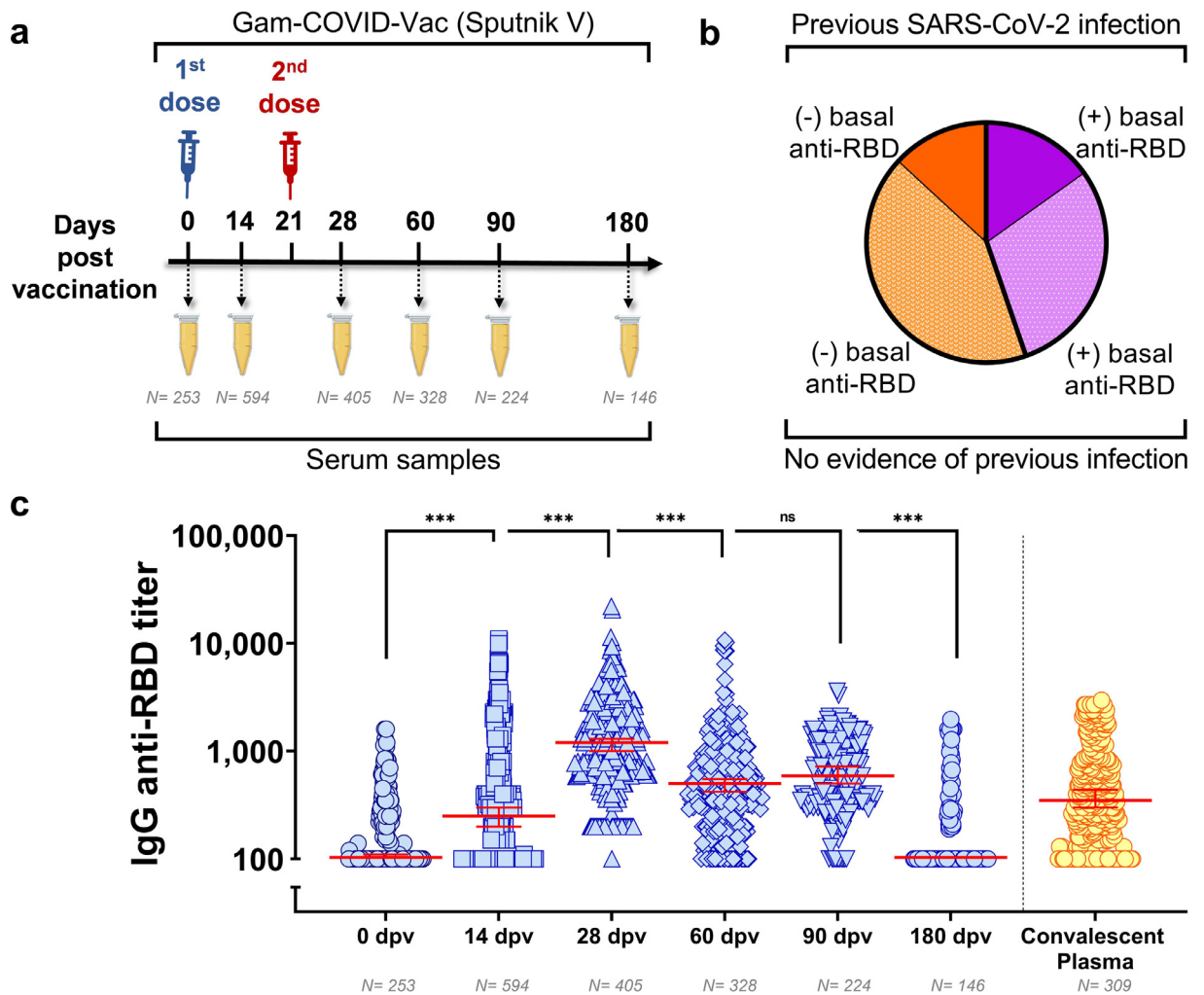
Variables are expressed as n (%) or mean ± standard deviation.

<sup>1</sup> Close Contact: Someone who was within 6 feet of an infected person (laboratory-confirmed or a clinically compatible illness) for a cumulative total of 15 min or more over a 24 h period.

<sup>2</sup> Arthropathies (4), Celiac disease (2), Hashimoto thyroiditis (5), Lupus (1), Multiple sclerosis (1), Psoriasis (1).

<sup>3</sup> COPD (1), Dyslipidemia (1), Fatty liver (1), Mitral insufficiency (1), Ulcerative colitis (1), Membranous nephropathy (1).

<sup>4</sup> Mild: side effects include pain at the injection site, fever, fatigue, headache, muscle pain, chills and diarrhea.



**Fig. 1.** Humoral immune response to the SPUTNIK V vaccine. (a) Diagram depicting SPUTNIK V vaccination scheme, the days when serum samples were extracted for antibody determination, and the number of volunteers (N) for each time-point. (b) Distribution of the vaccinated population according to the presence of basal IgG anti-RBD and documented previous SARS-CoV-2 infection. (c) A prospective cohort study of 602 HCP volunteer anti-RBD titers, as measured by ELISA at 0, 14, 28, 60, 90 and 180 days post-vaccination (dpv), and comparison with true convalescent individuals (N = 309) measured with the same ELISA platform [8]. Statistical analyses were performed with Mann-Whitney U test. \*\*\*  $p < 0.0001$  and ns = not-significant.

(Fig. 1c).<sup>9</sup> For more information regarding this subset of individuals refer to the Methods section.

For mRNA vaccine platforms, it has been reported that first doses may act as boosters in individuals previously exposed to SARS-CoV-2.<sup>22–24</sup> To determine if this is also the case in our cohort, a subset of 107 individuals who had samples taken at all 0, 14 and 28 dpv were selected for further analyzes. Interestingly, the group with previous SARS-CoV-2 infection showed a median anti-RBD titer 4.6-fold higher after the first dose (14 dpv) in comparison to individuals in the unexposed cohort (460 vs 100) (Fig. 2a). Nevertheless, the second SPUTNIK V dose further increased the median anti-RBD titer in previously infected individuals compared to the control group (1300 vs 755) at 28 dpv. This suggests that the first dose of SPUTNIK V in individuals pre-exposed to SARS-CoV-2 elicited a secondary immune response. Because high dropout rates were observed at different stages of the study, a full analysis with the entire cohort for all time points was not possible. Therefore, we evaluated the effect of previous SARS-CoV-2 infection on the levels of anti-RBD elicited by SPUTNIK V in 60, 90, and 180 dpv separately. Upon examining antibody levels at 60 dpv (prospective cohort study,  $N = 98$ ), the median titer elicited in the group with previous infection was still significantly higher than the control group (795 vs 400) (Fig. 2b). This difference, while still significant, was slightly reduced after 90 dpv ( $N = 75$ ) (Fig. 2c). However, 6 months after vaccination, anti-RBD titers were diminished and no significant difference was observed between median titers elicited by the two groups ( $N = 35$ ) (Fig. 2d). The volunteer characteristics for these two groups, naïve and previously infected, are reported in Supplementary Table 1 (Table S1).

Despite the decrease in anti-RBD titers after 6 months, the anamnestic anti-RBD binding response elicited by the SPUTNIK V in previously infected individuals resulted in qualitatively different neutralizing responses compared with that from naïve vaccinees. This can be seen when we compared pooled sera from the 28 dpv group of uninfected volunteers ('Group 1', basal anti-RBD IgG negative) with the 14 dpv group of previously infected individuals ('Group 2', basal anti-RBD IgG positive) (Fig. 3). Due to the wide dispersion of anti-RBD titers in both groups, we pooled samples with the highest titers (>1200) in both groups and assessed their neutralizing activity against recombinant VSV engineered to express wild-type, B.1.1.7 (alpha), B.1.351 (beta), or E484K mutant SARS-CoV-2 spike.<sup>12</sup> Group 1 sera effectively neutralized all SARS-CoV-2 spikes except B.1.351 (Fig. 3a–c) as we and others have previously observed.<sup>12,25,26</sup> Remarkably, Group 2 sera not only inhibited wild-type, B.1.1.7 and E484K viruses 5–20 fold better than Group 1 sera, they also potently neutralized B.1.351 (Fig. 3d,e). This marked increase in neutralization potency and breadth can be seen clearly

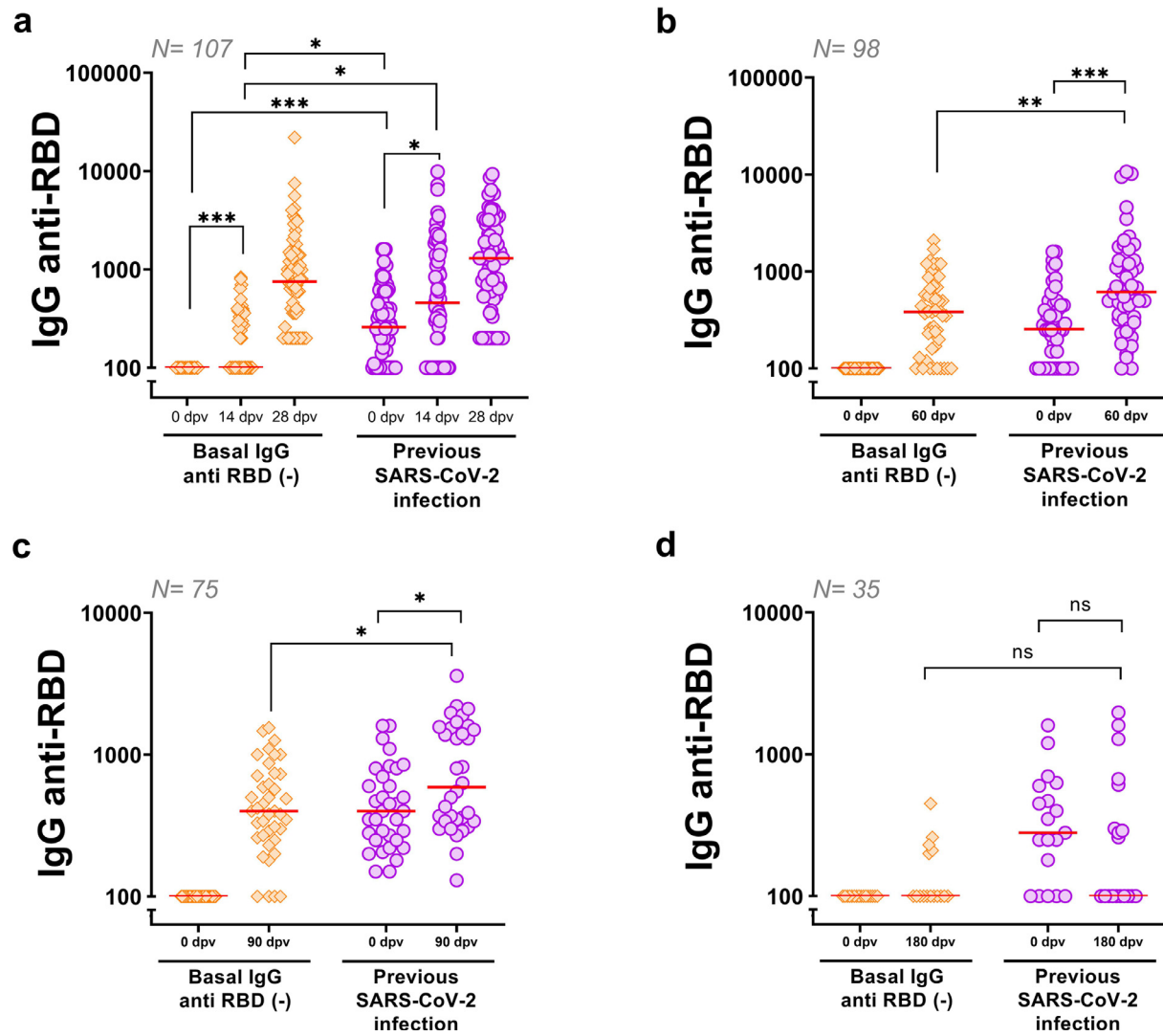
in the aggregated comparison of  $IC_{50s}$  across all the viruses examined (Fig. 3f).

Within the population of volunteers that presented detectable anti-RBD antibodies before vaccination, we observed a wide distribution of antibody responses to the first and second doses of SPUTNIK V. However, we noted that individuals with titers 400 or above elicited a stronger and uniform (where median and mean titers are equal) response to both the first (14 dpv) and second (28 dpv) doses of SPUTNIK V, compared to individuals with basal titers between 100 and 399 (Fig. 4a). Notably, in individuals previously infected with SARS-CoV-2, the time elapsed between diagnosis (RT-PCR or rapid antigen test) and vaccination with the first dose (at least up to 120 days) had no influence on antibody titers elicited either 14 or 28 dpv (Fig. 4b).

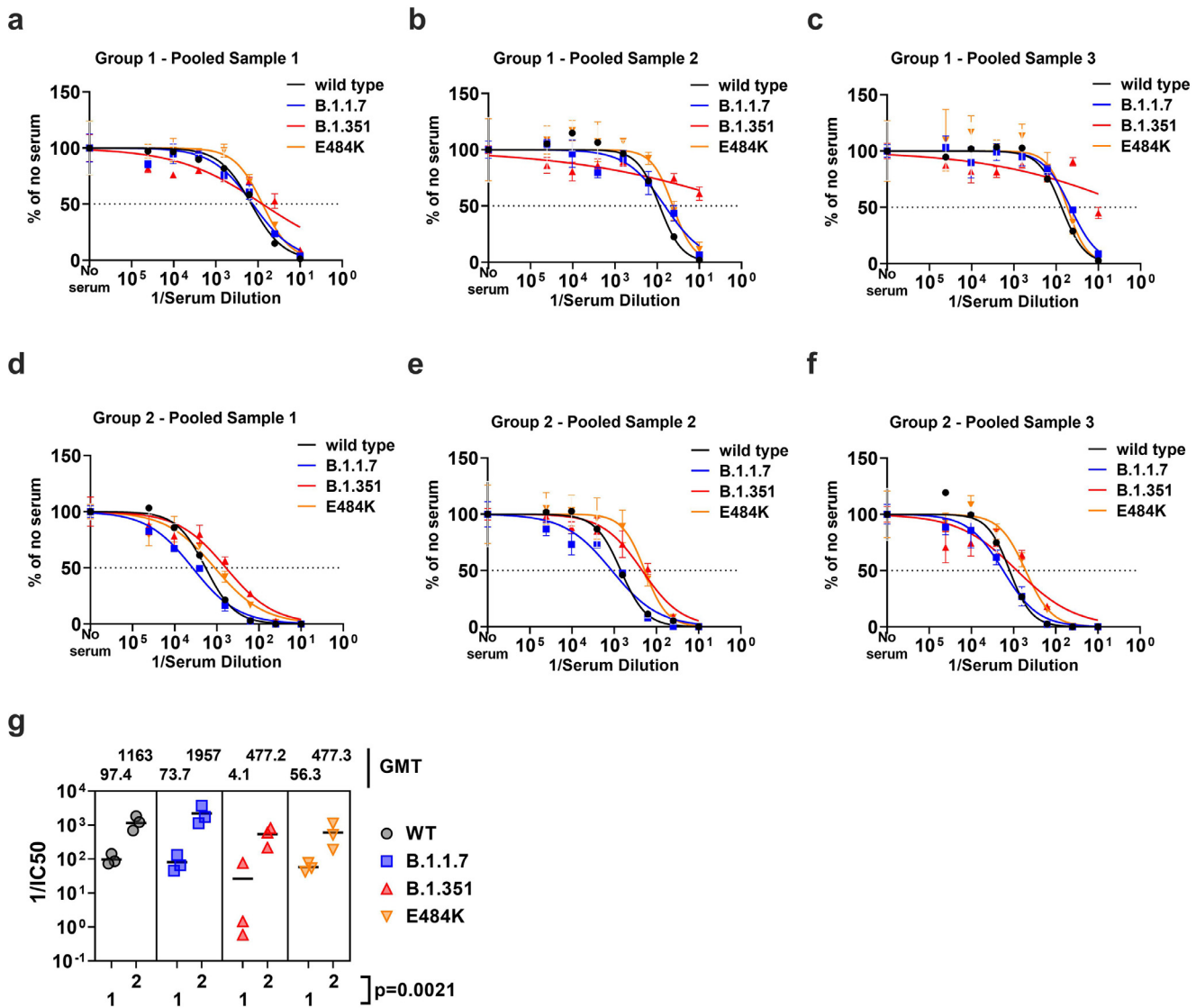
## Discussion

We have evaluated the long-term humoral anti-RBD response of the SPUTNIK V vaccine against COVID-19 in Tucumán, Argentina in a prospective cohort study with individuals with and without previous SARS-CoV-2 infection. The high seroconversion rates found are in agreement with what has been previously published for SPUTNIK V and other COVID-19 vaccines.<sup>6,20,27–30</sup> We show here that this high seropositivity remained 90 dpv but dropped after 180 dpv. Individuals with previous SARS-CoV-2 infection elicited higher titers to both the first and second doses, an increase that remained detectable up to 90 dpv. Serum from previously infected individuals showed increased neutralization ability against different VOCs, and their basal titers had more influence on post-vaccination anti-RBD levels than the time elapsed between diagnosis and vaccination. A great number of studies have explored the waning or persistence of antibodies elicited to other vaccination platforms.<sup>31–36</sup> However, to our knowledge, this is the first report to show the immune response persistence 180 dpv with SPUTNIK V.

Vaccination against SARS-CoV-2 has accelerated worldwide. One of the currently available vaccines is SPUTNIK V, developed and produced by the Gamaleya National Research Center of Epidemiology and Microbiology in Moscow, Russia.<sup>6,7</sup> Even though more than 70 countries, mainly in South America, Africa and Asia, have currently issued emergency approval for this vaccine, very few reports have been published describing its effects and efficacy outside of Russia.<sup>21</sup> The few available studies, pre-prints and official reports from ministries of health of different nations have confirmed in large cohorts the safety and efficacy of SPUTNIK V originally reported in Phase I/II and III studies.<sup>21,37,38</sup> Nevertheless, SPUTNIK V has yet to be approved for emergency use by the World Health Organization and the European Medicines Agency, the former of which is critical for this vaccine to be deployed to low-income



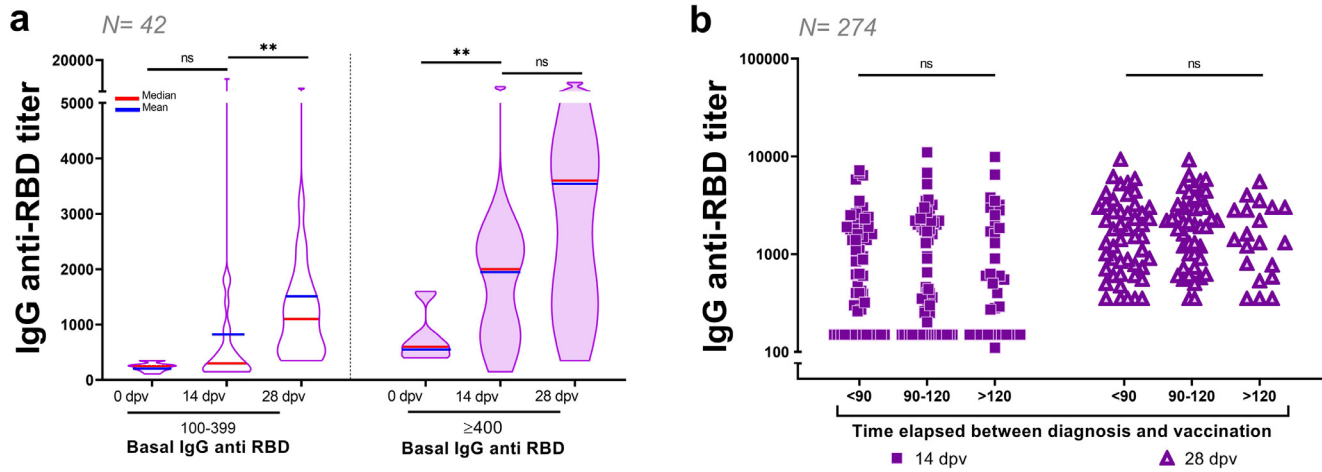




**Fig. 3.** Neutralization activity of antibody responses elicited by the SPUTNIK V vaccine in naïve versus previously infected individuals. Neutralizing activity of pooled serum samples taken at 28 dpv (a–c) or 14 dpv (d,e) from naïve (Group 1) versus previously infected (Group 2) individuals, respectively. Each pooled serum sample comprises of three serum samples from each group (see Fig. 1a) with anti-RBD IgG titers > 1200. Nine and six serum samples from Group 1 and 2 were tested as three (a–c) and two (d,e) pooled samples, respectively. Neutralization was performed against recombinant VSV engineered to express wild-type, B.1.1.7 (alpha), B.1.351 (beta), or E484K mutant SARS-CoV-2 spike. Neutralization curves were generated using a serial four-fold dilution of serum (1:10–1:40,960) and normalized against the no-serum control set at 100% using nonlinear regression. Data points represent mean  $\pm$  s.d. with each neutralization curve performed in triplicates. The dotted line represents the normalized 50% infection mark. (f) The reciprocal  $IC_{50}$  (1/ $IC_{50}$ ) values from the nonlinear regression curves shown in (a–e) are plotted for comparison. The colored symbols correspond to the viruses indicated. The open and filled symbols represent the reciprocal  $IC_{50}$  values from Group 1 and Group 2 pooled sera, respectively.

nations through the COVID-19 Global Access (COVAX) initiative.<sup>21</sup> This shortage of peer-reviewed published data is in stark contrast to the plethora of studies and wealth of information regarding the BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna/NIAID) and AZD1222 (AstraZeneca/University of Oxford) vaccines.<sup>1,21,39–41</sup>

In most countries, HCP were among the first group to receive COVID-19 vaccines due to their increased exposure to the virus and the strategic tasks they perform during a pandemic.<sup>42,43</sup> For this reason, our study focused on HCP volunteers from the public health care system, between 18 and 60 years of age and comprised mostly of female volunteers (68%) (Table 1). The



**Fig. 4.** Influence of basal titer and time elapsed after SARS-CoV-2 detection on SPUTNIK V humoral immune response. (a) Distribution of IgG anti-RBD antibody titers at 0, 14 and 28 dpv in individuals previously infected with SARS-CoV-2, segregated according to basal titers of 100–399 or  $\geq 400$  ( $N = 42$ ). Statistical analysis was performed with Mann-Whitney U test (b) Anti-RBD titers triggered by the first (14 dpv) and second (28 dpv) doses when administered less than 90, between 90 and 120, and more than 120 days post-diagnosis of SARS-CoV-2 infection ( $N = 274$ ). Statistical analyses were performed with Kruskal-Wallis test. ns= not-significant, \*\*  $p < 0.01$ .

present study has several limitations and the results should be interpreted in light of them. First, the study lacks an unvaccinated control group, given the high percentage of HCP enlisted for vaccination in our public healthcare system (near 100%). Secondly, the response rate was low (29%), which can be explained by the fact that only a fraction of the entire HCP workforce was attending the workplace during the study enrolment period.

Surprisingly, 66% of HCP that presented basal anti-RBD titers lacked a previous COVID diagnosis, suggesting they underwent asymptomatic or unrecognized infections and highlighting their high level of viral exposure. Our study also shows that only one week after receiving the second dose of SPUTNIK V, anti-RBD IgG antibodies were elicited in 97.3% of volunteers; a percentage of seroconversion that is in agreement with the original phase I/II study and other reports.<sup>6,20</sup> Likewise, we also found no major safety issues related to vaccination, as only mild side-effects were reported in 25% of participants (Table 1), consistent with what has been reported previously.<sup>6,7,37</sup> Although the phase I/II studies of SPUTNIK V report higher anti-RBD titers measured by ELISA at 28 dpv than what we have found, this could be because the anti-RBD ELISA platforms used were not the same, as convalescent individuals who recovered from SARS-CoV-2 infection also showed lower anti-RBD titers with our platform.<sup>6,9</sup> Most studies on the effect of vaccination-induced immune-boosting include a 42 dpv in addition to a 28 dpv time-point. Although our report skips the former, it includes the latter, as well as three additional subsequent time points.

A major question regarding vaccination with different COVID-19 vaccine platforms around the globe has been what type of humoral immune response would individuals who recovered from previous SARS-CoV-2 infection have, compared to individuals without prior contact.<sup>44</sup> For mRNA vaccines, it has been shown that in previously infected individuals, first doses act essentially as boosters.<sup>22–24</sup> This phenomenon has also been reported for adenoviral-based platforms.<sup>20,27,45</sup> In a context of high viral circulation, with millions of patients recovering from severe, mild or asymptomatic COVID-19, this information could be important for designing future health policies in regions facing vaccine shortages, such as developing nations. Here, we confirm that SPUTNIK V vaccine behaves similarly, as those individuals that underwent previous SARS-CoV-2 infection elicited higher antibody titers than naïve individuals (Fig. 2). Lack of cellular immunity assays is a limitation of our study, as it is for most SPUTNIK V-related research reports. Recently, the neutralizing activity of post-SPUTNIK V vaccination sera against alpha (B.1.1.7) and beta (B.1.351) VOC has been characterized by the use of *de novo* generated replication-competent vesicular stomatitis virus expressing various SARS-CoV-2 spike mutations.<sup>12</sup> We show here that antibodies induced by

the first dose of SPUTNIK V in previously infected individuals (14 dpv) were above 10-fold more potent in neutralization assays against these same variants compared to sera from uninfected volunteers who received the complete vaccination scheme (28 dpv) (Fig. 3).

Although previously infected individuals elicited higher anti-RBD titers post-vaccination with SPUTNIK V, not all responded equally. We found that only volunteers that possessed basal anti-RBD IgG levels above 400 consistently reached high titers after the first dose (median of 2000), which further increased after the second dose (median of 3600). Therefore, basal titer levels, not only seropositivity or negativity, could be crucial when considering previous SARS-CoV-2 infection status as a parameter to guide vaccination strategies or assess potential benefits (or harms) of administering second doses. Importantly, we also show that the time elapsed between previous COVID diagnosis and vaccination, at least up to 120 days, did not affect antibody titers (Fig. 4b), data that also could be useful for future public health guidelines.

For many infections, humoral immune kinetics show that antibody levels peak and then begin to drop after a certain number of weeks.<sup>46</sup> Following infection with SARS-CoV-2, antibodies begin to wane after 3 to 8 months.<sup>47,48</sup> Although the half-life of antibodies elicited by COVID-19 vaccines is still being analyzed, it is suggested that they may last as long as 6–8 months.<sup>49–52</sup> We have studied the humoral immune kinetics of anti-RBD antibodies elicited by SPUTNIK V up to 180 days post-vaccination and found that although anti-RBD titers begin to drop 60 days after vaccination, antibodies are still present in 94.6% of volunteers at 90 dpv (Fig. 1c). One limitation of our study was high participant dropout due to the effects of the second wave of COVID-19 in Argentina, with subsequent restrictions and lockdowns. Nevertheless, we report that even 3 months after vaccination (90 dpv), individuals with previous SARS-CoV-2 infection still presented with higher anti-RBD titers than uninfected individuals, although the difference was less significant than in earlier time-points (Fig. 2).

Notably, 7 of the 602 (1.2%) fully vaccinated HCP volunteers, who are constantly exposed to high viral loads and new VOC, contracted SARS-CoV-2 within 6 months post-vaccination and developed mild COVID-19 symptomatology, not requiring hospitalization (Table 1). Our results, although obtained in a smaller cohort, are in agreement with the recently published paper by Moriah Bergwerk and colleagues.<sup>53</sup> In this study, among 1497 fully-vaccinated healthcare workers, 39 SARS-CoV-2 (2.6%) breakthrough infections were documented. Neutralizing antibody titers for case patients during the peri-infection period were lower than those in matched uninfected controls, and higher peri-infection neutralizing antibody titers were associated with lower infectivity. Most breakthrough cases were mild or

asymptomatic, although 19% had persistent symptoms (> 6 weeks). These findings may suggest that peri-infection neutralizing antibody titers correlated with the viral load and thus with the infectivity of breakthrough cases. Clearly, vaccine-induced immunity has been shown greatly protective against clinical disease but somewhat less protective against both infection and infectivity. Both studies confirm that, while both the SPUTNIK V and the BNT162b2 vaccine are extremely effective, rare breakthrough infections carry an infectious potential and create a special challenge, since such infections are often asymptomatic and may pose a risk to vulnerable populations.

Many countries are considering postponing the second dose of two-dose vaccination regimens to allow for the vaccination of more people.<sup>54</sup> Our results with SPUTNIK V highlight the importance of evaluating specific basal antibody titers in previously exposed individuals to better guide the optimization of vaccination strategies. In a context where combining different vaccine platforms is under evaluation to counteract shortages or increase efficiency, here we provide the longest-term study describing anti-RBD levels elicited after vaccination with SPUTNIK V, which consists of 2 different adenoviral vector platforms and has been granted emergency approval in more than 70 countries worldwide.<sup>55,56</sup>

### Data availability

Raw data acquired during this study is available from the corresponding author upon reasonable request.

### CRedit authorship contribution statement

**Rossana Elena Chahla:** Conceptualization, Funding acquisition, Project administration. **Rodrigo Hernán Tomas-Grau:** Methodology, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Silvia Inés Cazorla:** Methodology, Formal analysis, Visualization. **Diego Ploper:** Methodology, Writing – original draft, Formal analysis, Visualization, Funding acquisition, Writing – review & editing. **Esteban Vera Pingitore:** Methodology, Supervision, Writing – review & editing. **Mónica Aguilar López:** project administration. **Patricia Aznar:** Data curation. **María Elena Alcorta:** Data curation. **Eva María del Mar Vélez:** Data curation. **Agustín Stagnetto:** Data curation, Visualization. **César Luis Ávila:** Methodology, Data curation, Funding acquisition. **Carolina Maldonado-Galdeano:** Methodology, Data curation, Funding acquisition. **Sergio Benjamín Socías:** Data curation, Funding acquisition. **Dar Heinze:** methodology. **Silvia Adriana Navarro:** methodology. **Conrado Juan Llapur:** Conceptualization. **Dardo Costas:** supervision. **Isolina Flores:** supervision. **Alexis Edelstein:** Methodology, Data curation. **Shreyas Kowdle:** Methodology, Data curation. **Claudia Perandones:**

Formal analysis, Visualization, Writing – review & editing. **Benhur Lee:** Formal analysis, Writing – review & editing, Visualization. **Gabriela Apfelbaum:** Conceptualization, Formal analysis, Visualization, Writing – review & editing. **Raúl Mostoslavsky:** Formal analysis, Visualization, Writing – review & editing. **Gustavo Mostoslavsky:** Formal analysis, Visualization, Writing – review & editing. **Gabriela Perdígón:** Conceptualization, Formal analysis, Visualization, Writing – original draft. **Rosana Nieves Chehín:** Conceptualization, Formal analysis, Visualization, Funding acquisition, Supervision, Writing – original draft, Writing – review & editing.

### Declaration of Interests

B. L. is a named inventor on a patent filed by the Icahn School of Medicine, which includes the 293T-ACE2-TMPRSS2 (F8–2) cells used for the virus neutralization assay. REC declares being involved in the vaccination process for the Ministry of Health of the Province of Tucumán, Argentina. All other authors report no competing interests.

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### Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:[10.1016/j.lana.2021.100123](https://doi.org/10.1016/j.lana.2021.100123).

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