

⁶ASST Papa Giovanni XXIII, Bergamo, Italy

⁷Regina Margherita, Children Hospital, University of Turin, Turin, Italy

⁸Vilnius University Hospital, Santaros Klinikos, Vilnius Lithuania and
Vilnius University, Vilnius

⁹Sheffield Teaching Hospital NHS Foundation Trust, Sheffield, UK

¹⁰Sahlgrenska University Hospital, Gothenburg, Sweden

¹¹Hopital Robert Debré, Paris, France

¹²Nottingham University Hospital, Nottingham

¹³AORN San Giuseppe Moscati, Avellino, Italy

¹⁴Federico II, University of Naples, Naples, Italy

¹⁵French reference Center for Aplastic Anemia and PNH, BMT unit,
Saint-Louis Hospital, Paris, France

¹⁶Hematology Unit, IRCCS, Gaslini Institute Genova, Italy

Correspondence

Audrey Françoise Petit, CHU de la Timone, Hématologie Pédiatrique,
264 rue Saint Pierre, 13005 Marseille, France.

Email: audrey.petit@ap-hm.fr

ORCID

Audrey Françoise Petit  <https://orcid.org/0000-0001-7231-7881>

Austin G. Kulasekararaj  <https://orcid.org/0000-0003-3180-3570>

Alexander Kulagin  <https://orcid.org/0000-0002-9589-4136>

Antonio M. Risitano  <https://orcid.org/0000-0002-4744-5109>

Régis Peffault de Latour  <https://orcid.org/0000-0001-6222-4753>

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

Received: 20 September 2021 | Accepted: 14 October 2021

DOI: 10.1002/ajh.26380

Distinct neutralization profile of spike variants by antibodies induced upon SARS-CoV-2 infection or vaccination

To The Editor:

Emerging SARS-CoV-2 variants have raised concerns about levels of immunity to variant Spike proteins after prior SARS-CoV-2 infection or vaccination. Here, we report distinct cross-reactivity of Wuhan-HA-1 (WA1)-induced antibodies upon BNT162b2 mRNA vaccination and SARS-CoV-2 infection. We show that neutralizing antibodies against WA1 strongly correlate with Delta neutralization, and that SARS-CoV-2 infection-induced antibodies have better neutralization capability for the Delta variant compared to vaccination.

We measured the magnitude and breadth of Spike antibody responses against the original WA1 and three variants (Alpha [B.1.1.7], Beta [B.1.351], and Delta [B.1.617.2]) of SARS-CoV-2 in three cohorts: (i) SARS-CoV-2 naïve recipients of two doses of BNT162b2 mRNA (Pfizer/BioNTech) vaccine (day 90 post first dose; $n = 55$); (ii) COVID-19 convalescent patients who received the first dose of the BNT162b2 mRNA vaccine at 6–10 months postsymptom onset (day 90 post first dose; $n = 5$); (iii) COVID-19 convalescent patients ($n = 23$) at peak postinfection at a median of 2 months postsymptom onset. We examined the levels and breadth of anti-Spike antibodies and their neutralizing ability against WA1 and the variants Alpha, Beta, and Delta, which differ by one to three amino acids (AA) within the receptor-binding domain (RBD) (Figure 1A).

The study participants including patients and volunteers are described in NCT04408209 and NCT04743388. CoV-2 naïve volunteers and COVID-19 convalescent patients received two doses of BNT162b2 mRNA vaccine at day 1 and 21, respectively.¹ The convalescent vaccine recipients received first dose at 6–10 months postsymptom onset.¹ SARS-CoV-2-infected patients analyzed at a median of 2 months postsymptom onset have been described.^{2,3} In-house ELISA using a panel of purified Spike-RBD proteins (AA 319–525) were detailed elsewhere.^{2–4} Neutralization was performed using a pseudotyped HIV_{NLΔEnv}-Nanoluc assay^{5,6} carrying a panel of Spike (AA 1–1254) proteins as described.^{2–4} Statistical analyses were performed using analysis of variance and Spearman correlation (GraphPad Prism Version 9.0.2 X; GraphPad Software, Inc.).

All three cohorts showed robust humoral response to WA1 Spike-RBD. Similar antibody levels were detected in the naïve BNT162b2 mRNA vaccine recipients at day 90 and in the COVID-19

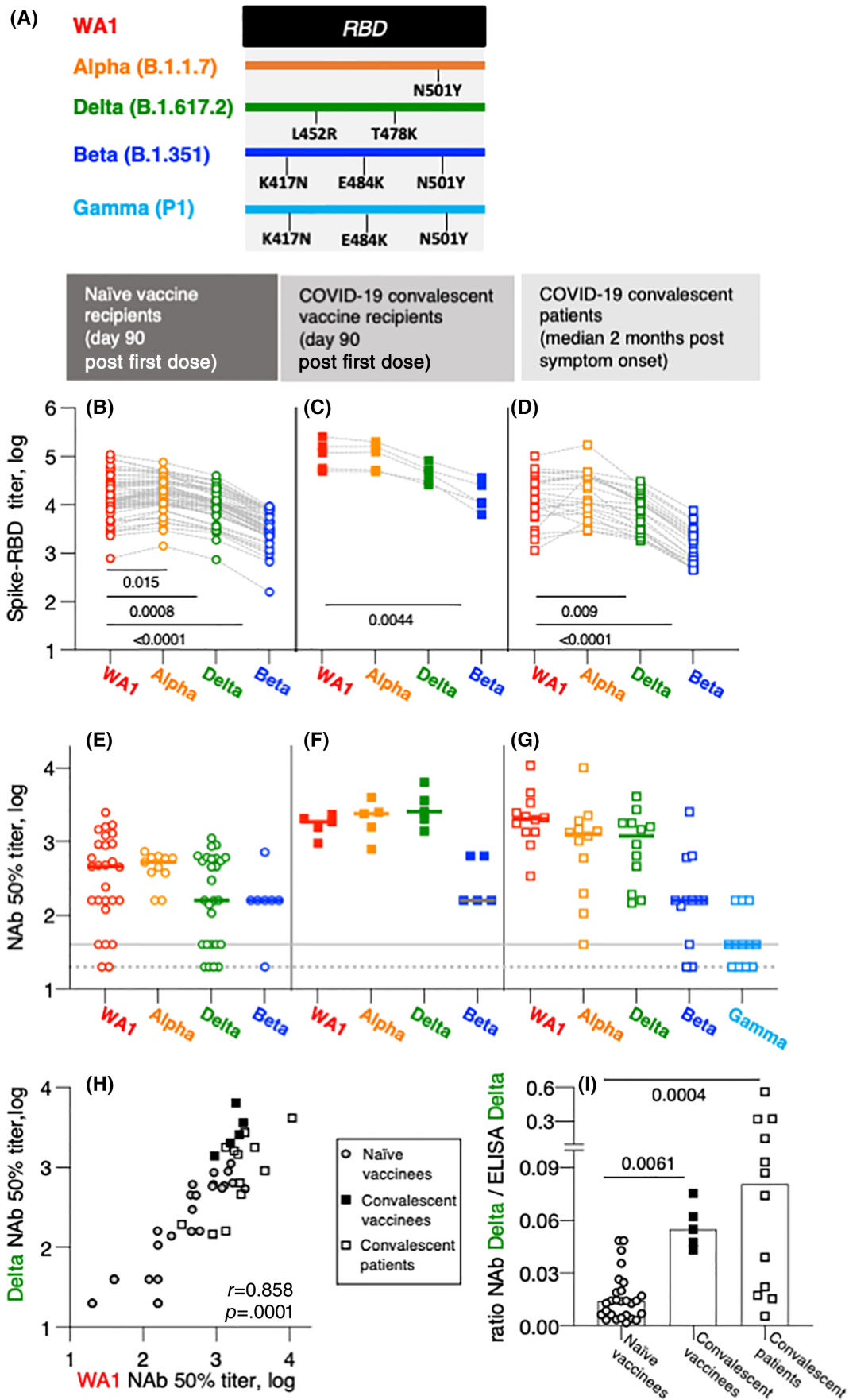


FIGURE 1 Legend on next page.

convalescent patients (2 months postinfection). A 12-fold higher Ab level was detected in the convalescent vaccinees,¹ as a result of a strong anamnestic response in this cohort^{1,7-9} (Figure 1B-D). Compared to the responses against WA1, the vaccine-induced antibodies showed significantly lower recognition of Alpha and Delta Spike-RBD and greatly reduced binding of Beta Spike-RBD in the naïve vaccinees (Figure 1B). In contrast, the Spike-RBD antibodies in the SARS-CoV-2 convalescent vaccine recipients (Figure 1C) showed similar strong binding to Beta and Delta indicating some improved breadth. Despite the increased humoral responses, recognition of Beta Spike-RBD was significantly reduced (Figure 1C). We further compared the anti-Spike-antibody breadth in a cohort of COVID-19 convalescent patients (Figure 1D). We noted a similar ranking of responses as in the cohort of naïve vaccine recipients with reduced recognition of Delta and Beta Spike-RBD. Together, our data suggest a strong benefit of vaccination for COVID-19 convalescent patients. Our data further point to two AA changes (K417N and E484K; Figure 1A), which play a key role for RBD recognition. These data mirror our recent findings from nonhuman primates which received WA1 Spike-based DNA vaccines.⁴ Importantly, increased Spike-RBD antibody magnitude cannot compensate for this. Thus, a booster vaccination including different variants may be beneficial and should be considered.

Next, we tested the neutralizing capability of the Spike antibodies in the different groups (Figure 1E-G). Sera were tested for their ability to neutralize infection by reporter viruses pseudotyped with different Spike variants. Analysis of both the naïve vaccine recipients (Figure 1E; $n = 27$) and the convalescent vaccine recipients (Figure 1F; $n = 5$) showed that WA1, Alpha and Delta Spike pseudotyped viruses were most susceptible. Similar data were found in the convalescent cohort (Figure 1G; $n = 12$). A drastic reduction in the ability to neutralize Beta was found in all the groups. These data were further supported by the testing of neutralization of Gamma which shares with Beta the same AA changes in RBD but differs in several AA in Spike (Figure 1A). The convalescent cohort (Figure 1G) also showed a strong reduction in the neutralization ability of Gamma. These data support the key role of the AA K417N and E484K (Figure 1A) for both binding and neutralization by WA1-induced antibodies. Our data on the cross-reactive recognition of Spike variants are in overall agreement with other recent reports analyzing the Moderna mRNA-1273, Pfizer BNT162b2, or AstraZeneca vaccines in healthy vaccine recipients or in COVID-19

convalescent vaccine recipients¹⁰⁻¹⁵ and in our DNA vaccine study in nonhuman primates.⁴

Because of the practical importance of the ability of WA1-induced antibodies to control infection by the Delta variant, we compared neutralization abilities of antibodies against WA1 and Delta in the three cohorts (Figure 1H). We found a strong direct correlation (Spearman $r = 0.8586$, $p < .0001$), supporting the notion that individuals with robust NAb against WA1 also strongly neutralize Delta. Recent findings of breakthrough infections in individuals with vaccine- or infection-induced SARS-CoV-2 immunity^{16,17} by the circulating Delta strain may occur in individuals with low anti-WA1 antibodies. Our data indicate that a WA1-based booster vaccination will greatly improve anti-Delta immune response.

To further characterize the WA1 vaccine-induced and the infection-induced Delta-specific antibodies, we compared the ratio of the respective NAb and binding antibodies in the three cohorts (Figure 1I). Interestingly, compared to the naïve vaccine recipients, we found significant higher ratios in the convalescent cohort and in the convalescent vaccine recipients. These data support the conclusion that antibodies induced by SARS-CoV-2 infection have better Delta-specific neutralization function. The subsequent vaccination of the convalescent patients was able to increase the level and maintain the breadth of anti-Delta neutralizing Ab.

The report presented here shows a side-by-side comparison of characteristics of Spike antibodies induced upon BNT162b2 mRNA vaccination, upon CoV-2 infection or a combination thereof using the same methodologies. We found that anti-Spike antibodies from the vaccine recipients (naïve and convalescent) and SARS-CoV-2 convalescent patients show a strong ability to recognize and neutralize the autologous WA1, as well as the Alpha and Delta Spike variants but that they show greatly impaired recognition of Beta, in agreement with others,¹⁰⁻¹⁵ pointing to the importance of AA changes within RBD. Recently emerging variants of concern show a multitude of such changes which will likely affect NAb function of the WA1-induced antibodies. Interestingly, our data further showed that SARS-CoV-2 infection-induced antibodies show better neutralization capability of the Delta variant.¹⁰⁻¹⁵ This finding points to subtle but critical differences in antibody development perhaps guided through continuous antigen exposure upon infection, whereas vaccination provides a limited number of short-lived exposures.

SARS-CoV-2 booster vaccination using WA1 is being implemented in many countries to increase the anti-Spike antibody titers, and our

FIGURE 1 Anti-Spike antibody characterization in BNT162b2 mRNA vaccinated and CoV-2 infected persons. (A) The cartoon depicts AA changes in RBD (AA 332-532). Beta and Gamma share the identical RBD but differ in several AA within Spike used in neutralization assay. (B-D) Wuhan-Spike induced antibodies were measured by in-house ELISA using a panel of purified Spike-RBD proteins. (B and C) CoV-2 naïve volunteers¹ and COVID-19 convalescent patients¹ received the BNT162b2 mRNA vaccine and were analyzed at day 90. The convalescent vaccine recipients¹ had detectable Spike-RBD antibodies (median titer 2.8 log, range 2.6-3.9) at the day of the first dose. (D) SARS-CoV-2-infected patients^{2,3} were analyzed at a median of 2 months postsymptom onset. Comparison between the groups were made using ANOVA Friedman's multiple comparison test. (E-G) Neutralization was performed with the samples shown in panels (B-D) using a pseudotyped HIV_{NL}ΔEnv-Nanoluc assay carrying a panel of Spike (AA 1-1254) proteins. Threshold of detection in gray solid line; threshold of quantification in gray dotted line. (H) Correlation of NAb to WA1 and Delta in the three cohorts described in panels (E-G). Spearman r and p value are given. (I) Ratios of Delta NAb (from panels E-G) and Delta antibody titers (panel B-D) for the different cohorts were calculated using linear values. The p values are from ANOVA Kruskal-Wallis test. ANOVA, analysis of variance

data support its merit to improve anti-WA1 antibody magnitude and thereby increased ability to recognize Delta. However, our data also show that the magnitude alone is not sufficient to neutralize effectively current SARS-CoV-2 variants of concern. Thus, future booster vaccinations with variant Spike vaccines, including Delta and Beta, should be considered to increase the breadth of the immune responses. The nucleic acid vaccine platform offers the necessary versatility to rapidly adapt to emerging variants of concern, which need to be considered in the future to improve vaccine efficacy.

ACKNOWLEDGMENTS











The authors thank D. Esposito (Protein Expression lab; NCI) for SARS-CoV-2 Spike-RBD proteins; members of the Felber and Pavlakis labs for discussion, and T. Jones for assistance. This work was supported by funding from the Intramural Research Program, National Institutes of Health, National Cancer Institute, Center for Cancer Research to George N. Pavlakis and Barbara K. Felber. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Margherita Rosati¹ , Evangelos Terpos² , Mahesh Agarwal³, Vangelis Karalis⁴, Jenifer Bear³ , Robert Burns³, Xintao Hu³ , Demetrios Papademetriou¹ , Ioannis Ntanasis-Stathopoulos² , Ioannis P. Trougakos⁵ , Meletios-Athanasios Dimopoulos² , George N. Pavlakis¹ , Barbara K. Felber³ 

¹Human Retrovirus Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

²Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

³Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA

⁴Department of Pharmacy, School of Health Sciences, National and Kapodistrian University of Athens, Athens, Greece

⁵Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

Correspondence

Evangelos Terpos, Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, 11528 Athens, Greece.

Email: eterpos@med.uoa.gr

Barbara K. Felber, Human Retrovirus Pathogenesis Section, Vaccine Branch, Center for Cancer Research, National Cancer Institute, Frederick, MD 21702, USA.
Email: barbara.felber@nih.gov

Margherita Rosati and Evangelos Terpos contributed equally as first authors.

ORCID

Margherita Rosati  <https://orcid.org/0000-0003-3173-9716>

Evangelos Terpos  <https://orcid.org/0000-0001-5133-1422>

Jenifer Bear  <https://orcid.org/0000-0001-5065-6081>

Xintao Hu  <https://orcid.org/0000-0002-6552-9357>

Demetrios Papademetriou  <https://orcid.org/0000-0001-6228-4318>

Ioannis Ntanasis-Stathopoulos  <https://orcid.org/0000-0002-6328-9783>

Ioannis P. Trougakos  <https://orcid.org/0000-0002-6179-2772>

Meletios-Athanasios Dimopoulos  <https://orcid.org/0000-0001-8990-3254>

George N. Pavlakis  <https://orcid.org/0000-0002-4027-4036>

Barbara K. Felber  <https://orcid.org/0000-0001-8925-8128>

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Received: 29 June 2021 | Revised: 7 October 2021 | Accepted: 17 October 2021

DOI: 10.1002/ajh.26381

Multicenter evaluation of efficacy and toxicity of venetoclax-based combinations in patients with accelerated and blast phase myeloproliferative neoplasms

To the Editor:

BCR-*Abl*-negative myeloproliferative neoplasms (MPNs), including essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) carry a risk for disease progression to an accelerated phase (AP-MPN) and/or blast phase (BP-MPN). AP-MPN is defined as the presence of 10%–19% peripheral or bone marrow blasts while BP-MPN is defined by $\geq 20\%$ blasts in peripheral blood or bone marrow. AP/BP-MPN outcomes are poor, with median overall survival (OS) estimated at 3–4 months from the time of diagnosis. Traditional treatment strategies, including intensive chemotherapy and hypomethylating agents (HMAs), elicit inferior response rates in patients with AP/BP-MPN compared de novo acute myeloid leukemia (AML).¹

The B-cell lymphoma 2 inhibitor, venetoclax (VEN) is approved in combination with either a HMA or low-dose cytarabine (LDAC) for initial therapy of AML in unfit or elderly patients. A recent Phase III study comparing VEN + azacitidine (AZA) versus AZA monotherapy for treatment-naïve (TN) AML patients ineligible for intensive therapy demonstrated that VEN + AZA significantly improved median OS (14.7 vs. 9.6 months). Notably, patients with a history of prior MPN were largely excluded, thus raising the question of applicability of these data to AP/BP-MPN patients.²

Retrospective series have assessed the clinical utility of VEN-based therapy in MPN patients. Gangat et al. conducted a retrospective, multicenter evaluation of 32 patients with treatment naïve ($n = 23$) and previously treated ($n = 9$) BP-MPN who received HMA + VEN. The rate of composite CR/CRi was 44% and median OS was 8 months. The authors suggested VEN + HMA produces a superior rate of response with minimal incremental toxicity when compared to historical controls treated with HMA.³ By contrast, a single-center retrospective review of 31 BP-MPN patients evaluated VEN in combination with HMA (58%) or a variety of other agents. Both relapsed or refractory (R/R) ($n = 17$) and TN ($n = 14$) patients were included. No responses were observed in the R/R setting and the median OS was three months. Significant treatment-related toxicity was observed and the 8-week mortality rate was 32%.⁴

Given the rarity of AP/BP-MPN and limited and conflicting data to-date with VEN-based therapies for AP/BP-MPN, we performed an IRB-approved, multicenter, retrospective analysis of treatment outcomes of 27 patients across three academic cancer centers in the United States (Memorial Sloan Kettering Cancer Center $n = 12$, Moffitt Cancer Center $n = 10$, Roswell Park Comprehensive Cancer Center $n = 5$).

VEN-naïve patients with a diagnosis of either R/R or TN AP-MPN or BP-MPN treated with at least seven days of VEN in combination with LDAC, decitabine (DEC), or AZA between January 1, 2016 and January 7, 2020 were included. Best response was determined by retrospective review of bone marrow biopsy reports and peripheral blood studies by the local investigators. Responses were confirmed by two independent reviewers. Patients were evaluable for a response within 42 days of initial VEN dose. Overall response rate (ORR) was considered attainment of a complete molecular response, complete cytogenetic response (CCR), acute leukemia response-complete (ALR-C), or acute leukemia response-partial (ALR-P) by MPN-BP Consensus Criteria.⁵ EFS was defined as the time between VEN initiation and date of disease progression or death. OS was defined as the time between VEN initiation and date of death. OS and EFS were evaluated by Kaplan–Meier method and the difference between groups was determined by log rank test. Associations between ORR and patient and disease characteristics were tested by Fisher's exact test and odds ratios (ORs) were estimated by logistic regression. The effects of patient and disease characteristics on OS and EFS were estimated by univariate Cox proportional hazard model with $p < .05$ being considered significant. All statistical analyses were performed using R.