RESEARCH LETTER

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TO THE EDITOR:

Third-dose SARS-CoV-2 mRNA vaccine increases Omicron variant neutralization in patients with chronic myeloid disorders

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Concerns remain over the response to vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in patients with hematological malignancy. We and others have reported high serological and T-cell response rates to vaccination in patients with chronic myeloproliferative neoplasms (MPNs).[1-3] However, some patient groups were identified with a suboptimal response, necessitating further evaluation of the effects of additional vaccine doses.[4] Moreover, the degree of immune response offered by current vaccines against variants of concern also requires further evaluation. To address these concerns, we performed a comprehensive immunological evaluation of the response to SARS-CoV-2 vaccination in patients with chronic MPNs after 3 doses of vaccine, including neutralizing titers against the Omicron variant and T-cell responses.

Antibody response was assessed using anti-spike (anti-S) immunoglobulin G (IgG) with anti-nucleocapsid (anti-N) IgG used to determine previous infection, as described previously.[2] Neutralizing antibody analysis was performed by assessing the inhibitory effect of plasma on entry of HIV-1 particles pseudotyped with Wuhan or Omicron BA.1 spike proteins into cells expressing the ACE2 receptor.[2] T-cell response was evaluated using a FluoroSpot assay assessing interferon gamma (IFN-γ)/interleukin 2 (IL-2) secretion upon reexposure to S peptides (Mabtech, supplemental Methods). Plates were analyzed using the IRIS reader providing both spot-forming unit (SFU) frequency and relative spot volume (RSV).

To date, samples have been collected from 104 patients with MPN or after allogeneic stem cell transplantation in total. Testing was performed in 61 patients after 2 doses and 33 patients after 3 doses. Generalized mixed linear model, regular *t* test, Fisher exact test, and Pearson correlation coefficients were used as appropriate models for hypothesis testing. Samples were collected at a median of 49 days after a second dose of vaccine (range, 22-88) and 43 days after a third dose (range, 27-72). This study received ethical approval from the Edgbaston Research and Ethics Committee, IRAS identification 285396 - 20/WM/0187/AM04, and was conducted in accordance with the Declaration of Helsinki.

Serological analysis was performed after a third dose of messenger RNA (mRNA) vaccine in 33 patients, including chronic myeloid leukemia (CML, n = 13), essential thrombocythemia (ET, n = 4), polycythemia vera (PV, n = 6), and myelofibrosis (MF, n = 10). Of the MPN patient cohort, treatments included ruxolitinib (n = 9), hydroxycarbamide (n = 4), pegylated interferon alpha (n = 3), and active surveillance (n = 4). Of the CML cohort, tyrosine kinase inhibitor therapy included nilotinib (n = 5), dasatinib (n = 2), bosutinib (n = 1), and ponatinib (n = 5). Patient characteristics of those analyzed after a third dose are summarized in supplemental Table 1.

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The data that support the findings of this study are available upon reasonable request from the corresponding author, Hugues de Lavallade (hugues.2.de_lavallade@kcl.ac.uk).

The full-text version of this article contains a data supplement.

In 24 paired samples and excluding those with elevated anti-N optical density (n = 4), a statistically significant increase was seen after 3 doses in mean anti-S IgG 50% effective concentration (4094 vs 1265 after 2 doses; $P = .006$). Similarly, mean neutralizing antibodies against Wuhan spike pseudotypes were 231 after 2 doses and 1461 after 3 doses in paired samples (n = 19, $P = .05$).

Mean neutralizing antibody titers against the Omicron pseudotype from 19 paired samples increased from 82 after 2 doses to 365 after a third dose ($P = .026$, Figure 1A). After 3 doses of vaccine, patients from the initial cohort were more likely to have detectable neutralizing antibodies against Omicron, with 95.7% having a detectable response (50% infective dose > 25) compared with 70% after 2 doses (18 of 19 vs 13 of 19, $P = .062$, Figure 1B). However, the mean level of Wuhan spike neutralizing antibodies was significantly higher than the mean level of Omicron neutralizing antibodies after 2 doses of vaccine (241 vs 116, $P = .026$, Figure 1C). Similarly, after 3 doses, mean neutralizing antibody titers were 1168 against the Wuhan spike compared with 357 against Omicron ($P = .075$, Figure 1D).

T-cell FluoroSpot analysis was performed in 30 patients. A positive response was observed in 90% (27 of 30) after a third dose, with a similar response rate as observed after 2 doses (88.3%, 53 of 60). Significantly higher mean RSV was observed for IFN-γ in polyfunctional cells compared with monofunctional (21 885 vs 9184, $P \leq .001$). After a third vaccine dose, IFN-γ SFUs and RSV were, however, significantly higher at 242 and 9184 vs 72 and 5031, respectively, after the second dose ($P \leq .001/<.001$, Figure 2A-B). Of note, we observed an association in the time between the third dose of vaccine and sampling and reduction in T-cell reactivity, as indicated by SFUs for IL-2 ($r = -0.5$, $P = .026$; supplemental Figure 1). There was no significant trend observed for other indicators of vaccine response and time between vaccine dose and sampling.

We next assessed the impact of therapy on T-cell responses. After 3 doses, patients on ruxolitinib (a JAK1/2 inhibitor) had lower SFU and RSV of IFN-γ than other patients at 79 and 7690 vs 302 and 9727, respectively ($P = .004/.015$, Figure 2C-D). Patients taking ruxolitinib also had reduced polyfunctional IFN-γ and IL-2 RSV compared with other patients with 9425 vs 22 437 IFN-γ RSV and 1858 vs 4771 IL-2 RSV, respectively ($P = .047/.022$). Finally, patients on ruxolitinib were also more likely to have a negative T-cell response after 3 doses than other patients (4 of 8 vs 2 of 22, $P = .029$). Patients with MF had lower SFU for IFN-γ than patients with CML, PV, and ET at 93 vs 294, 224, and 446, $P = .075/.349/.024$, respectively (Figure 2E). Similarly, patients with MF had lower RSV than those with CML, PV, and ET at 7532 vs 9528, 10 499, and 10 630, $P = .035/.017/.021$, respectively (Figure 2F). We observed a significant correlation between humoral and T-cell response, for both anti-S IgG and Wuhan spike neutralizing antibody titers, and SFU for IL-2–secreting T cells ($r = 0.4$, $P = .03$ and $r = 0.6$, $P = .003$, respectively, supplemental Figure 2i-ii). We also observed a significant correlation between neutralizing titers for Omicron pseudotype and SFU for IFN-γ–secreting T cells ($r = 0.4$, $P = .049$; supplemental Figure 2iii).

Of the 29 patients assessed, 7 (24.1%) reported confirmed SARS-CoV-2 breakthrough infection after a third dose with 2 requiring hospitalization and high dependency unit admission, both of whom were treated with ruxolitinib. We previously reported that after 2 doses of vaccine, only 1 of 55 patients completing a post-vaccination survey developed confirmed COVID-19 infection, although this was before the emergence of the Omicron variant and the related surge in cases.[4] After a third dose, multivariate analysis did not identify neutralizing antibody levels toward Omicron or T-cell response as significantly associated with SARS-CoV-2 infection; however, there was a trend toward association between breakthrough infection and ruxolitinib treatment (12.31; 95% confidence interval, 0.54-770.87; $P = .1$).

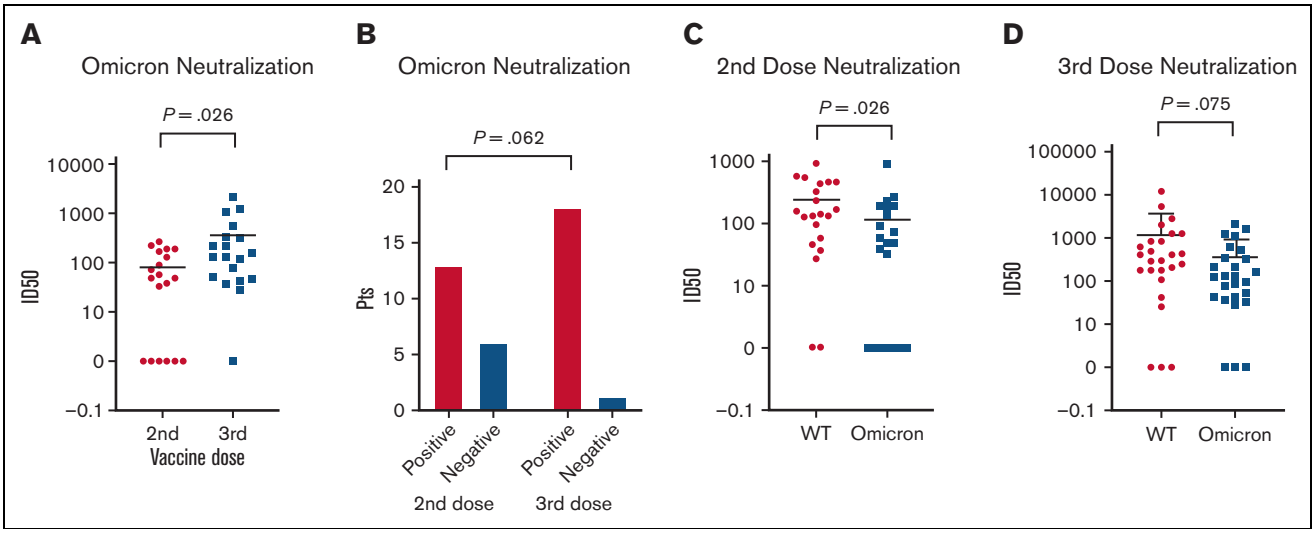

Figure 1. Humoral response to third dose of SARS-CoV-2 mRNA vaccination in patients with chronic myeloid disorders. (A) Increased neutralization of Omicron variant pseudotypes after third vaccine dose compared with after second dose. (B) Reduced frequency of patients with detectable Omicron neutralizing antibody response after second vaccine dose compared with after third dose. (C) Neutralizing antibody response against Wuhan and Omicron pseudotype after 2 doses of vaccine showing reduced response against Omicron. (D) Neutralizing antibody response against Wuhan and Omicron pseudotype after 3 doses of vaccine showing reduced.

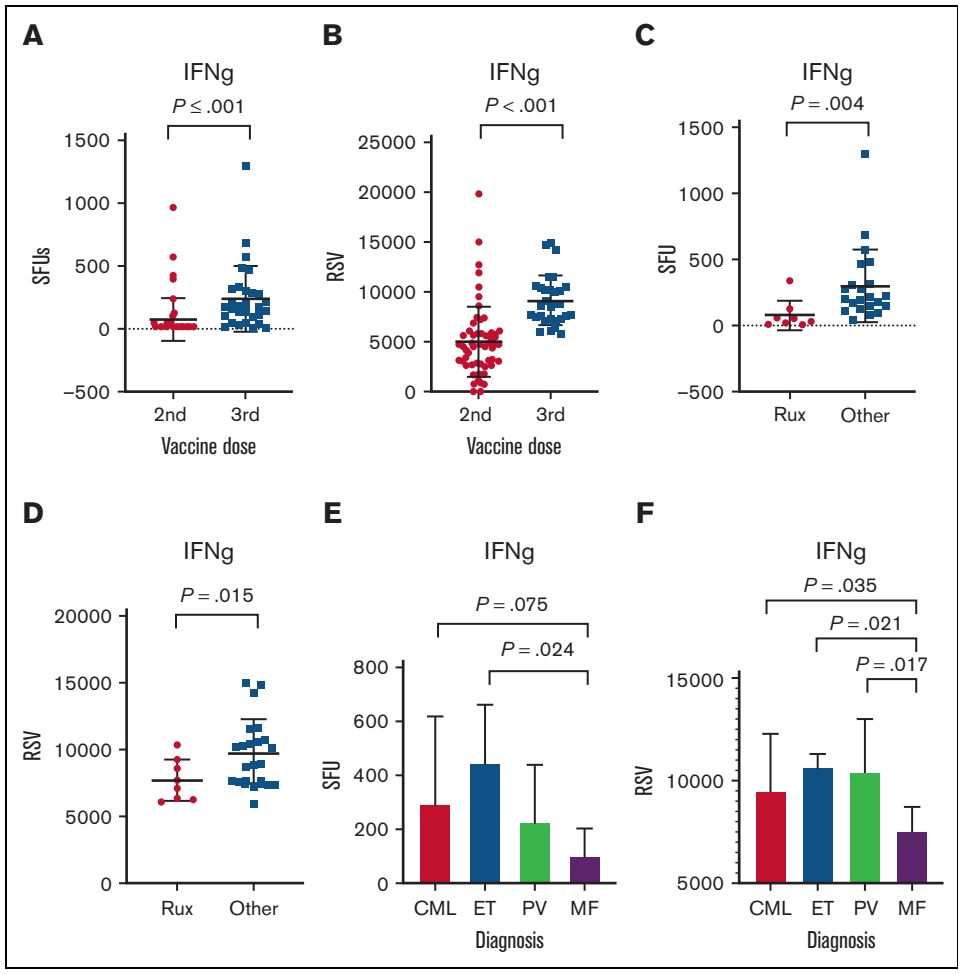

Figure 2. T-cell response to second and third doses of SARS-CoV-2 mRNA vaccination in patients with chronic myeloid disorders. (A) Frequency of IFN-γ SFUs after 2 and 3 doses of vaccine showing increased T-cell response after third dose. (B) Increased RSV of IFN-γ SFUs after 3 doses of vaccine compared with after 2 doses. (C) Reduced frequency of SFUs for IFN-γ in patients taking ruxolitinib after 3 doses of vaccine. (D) Reduced RSV of IFN-γ SFUs in patients taking ruxolitinib after a third dose of vaccine. (E) Reduced frequency of SFUs for IFN-γ in patients with diagnosis of MF when compared with CML and ET diagnosis. (F) Reduced RSV of SFUs for IFN-γ in patients with diagnosis of MF when compared with CML, PV, and ET diagnosis.

Patients with hematological malignancy have been identified as a particularly vulnerable group with reduced response to vaccination when compared with patients with other types of cancer.[5] This is increasingly relevant due to the poor outcomes observed with SARS-CoV-2 infection, including in patients with CML. However, most studies evaluating immunological vaccine response have reported only on serology, which underestimates the frequency of responders when compared with studies also evaluating cellular response.[6] Moreover, patients with hematological malignancy have been shown to frequently have discordant serological and cellular responses, although we do show a moderate correlation between immune responses in our analysis.[7-9]

The Omicron variant is capable of immune escape and impaired efficacy of current vaccines, particularly after 2 doses of vaccine. A T-cell response, however, can be induced by a wide range of epitopes, and vaccine-induced memory T cells retain activity against variants in comparison with neutralizing antibodies.[10] As such, the increase in T-cell reactivity after 3 doses observed in our analysis is of particular significance in view of the reduced Omicron neutralization. Our data, however, demonstrate that cellular response to vaccination continues to be impaired in patients taking ruxolitinib, which is known to have pleiotropic effects on the immune system. This may also be reflective of reduced immune function in patients with more advanced MF, typically associated with inflammation, who frequently require JAK inhibitor therapy for symptomatic control. Taken together, these findings highlight the need for additional approaches for these patients.[11] Finally, further longitudinal studies with larger cohorts are required to elucidate the long-term efficacy of vaccination against SARS-CoV-2 in this population.

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