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Letter

# Augmentation of humoral and cellular immune responses after third-dose SARS-CoV-2 vaccination and viral neutralization in myeloma patients

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Despite the efficacy of COVID-19 vaccines in healthy individuals, multiple myeloma (MM) patients are immunocompromised and mount suboptimal humoral and cellular responses after two doses of mRNA vaccine (Addeo et al., 2021; Aleman et al., 2021; Van Oekelen et al., 2021). A broader observation of limited vaccine responses in cancer patients, particularly those with hematologic malignancies (Thakkar et al., 2021), has led to the implementation of additional (i.e., third-dose) vaccine administration as a way to increase protection for patients with immune suppression. A third dose of BNT162b2 (Pfizer-BioNTech) COVID-19 vaccine has shown to be effective in preventing severe COVID-19 caused by the SARS-CoV-2 B.1.617.2 (Delta) variant in the general population (Bar-On et al., 2021; Barda et al., 2021). Furthermore, third-dose administration of either the BNT162b2 (Pfizer-BioNTech) or mRNA-1273 (Moderna) COVID-19 vaccine was associated with augmented immune responses in a diverse cohort of cancer patients (Shapiro et al., 2022). However, the real-world effectiveness of additional dosing in myeloma patients and viral neutralization have not been reported. Additionally, the impact of the currently dominant SARS-CoV-2 B.1.1.529 (Omicron) variant on efficacy of the third dose is largely unknown in patients with hematologic malignancies (Zeng et al., 2022).

We studied the humoral and cellular immune response to COVID-19 vaccination longitudinally in a real-world cohort of 476 MM patients and compared it with data of age-matched vaccinated healthcare workers. Of the full cohort, 354 patients (74%) had anti-SARS-CoV-2 spike (S) IgG levels collected at least 6 months after two doses of mRNA vaccine, and 261 (55%) had anti-S IgG measured at least 1 week after the third dose administration. Summarized demographic characteristics of the cohort are shown in Table S1. The study cohort was predominantly male (57%), with a median age of 67 years (range 38–96 years). Forty patients (8%) were included with a diagnosis of smoldering MM. Patients included had received a median of two lines of treatment (range 0–16) at the time of initial vaccination. Of note, documented COVID-19 infection occurred in 124 patients (26%) at any time during the pandemic.

The serologic effect of the third dose is illustrated in Figure S1A. Patients were split by COVID-19 infection status (i.e., whether they developed COVID-19 before or at any time after the initial vaccination) to separate the effect of natural infection. Anti-S IgG level increased significantly after administration of the third dose, both in patients with COVID-19 (median 110 AU/mL after dose 2 to 381 AU/mL after dose 3, $p < 0.001$) and in patients without COVID-19 (median 27 AU/mL after dose 2 to 161 AU/mL after dose 3, $p < 0.001$). To better characterize the benefit of the third vaccine dose, we specifically looked at the 241 MM patients for whom anti-S IgG levels were available at time points both before and after the third dose (i.e., paired samples). Sixty-eight patients (28%) were seronegative (i.e., they had no detectable anti-S IgG) at the last time point collected prior to the third dose (median 183 days post dose 2, range 15–336 days). Of these, 60/68 (88%) developed detectable anti-S IgG after dose 3 (median 0 AU/mL after dose 2 to 45.5 AU/mL after dose 3) (Figure S1B, sero-conversion). Of 173 patients who had measurable anti-S IgG after two doses, anti-S IgG increased in 158 patients (91%) after dose 3 (median 43 AU/mL after dose 2 to 300 AU/mL after dose 3) (Figure S1B, sero-elevation). Although the third dose provided a robust

boost to serological status, MM patients that were in both the sero-conversion and the sero-elevation group had significantly lower serological levels than age-matched healthy donors (HDs) after three doses (Figure S1B, $p < 0.001$).

Initial two-dose vaccination was associated with a significantly weaker responses among MM patients treated with anti-CD38 monoclonal antibodies (mAb) or BCMA-targeted therapy (Aleman et al., 2021; Van Oekelen et al., 2021). In patients who did not develop COVID-19, the third dose resulted in significant increases of anti-S IgG across all treatment groups (Figure S1C), including in patients receiving an anti-CD38 mAb ($p < 0.001$) or a BCMA-targeted therapy (chimeric antigen receptor (CAR) T cell therapy, bispecific antibody therapy, or antibody-drug conjugate) ($p < 0.01$), although the level of anti-S IgG after dose 3 in patients on anti-CD38 mAb remained significantly lower in comparison to MM patients that did not receive active treatment (median 121 versus 312 AU/mL, $p < 0.01$).

In a subset of 31 patients, we analyzed cellular and neutralizing responses. We characterized the cellular responses in a subset of 14 sero-conversion MM patients, 17 sero-elevation MM patients, and 13 seropositive HDs, before and after third mRNA vaccination, using high-dimensional flow cytometry. The third vaccination dose resulted in a significant increase in spike-reactive B cells in MM patients in both the sero-elevation and sero-conversion groups ($p < 0.05$, Figure S1D). The presence of spike-reactive memory B cells also strongly correlated with the magnitude of detectable anti-S IgG antibody titers ($r = 0.6$, $p < 0.001$). Spike-specific T cell responses were measured by stimulating peripheral blood mononuclear cells (PBMC) with a pool of spike peptides (15-mer sequences with an 11 amino acid overlap spanning the entire spike protein) and quantifying cytokine-producing cells in $CD4^+$ T cells expressing CD154 and CD69. Total cytokine-expressing $CD4^+$ T cells were estimated by aggregating activated $CD4^+$ T cells producing GM-CSF, IFN-$\gamma$, IL-2, IL-4, IL-17, and TNF-$\alpha$. In sero-conversion and sero-elevation MM patients, we observed a significant increase in spike-specific $CD4^+$ T cell-mediated cytokine responses after the third dose ($p < 0.05$, Figure S1E). In HD, however, B and T cell responses were not significantly augmented after the administration of the third vaccination.

To better characterize the protection against infection, we compared the effect of a third-dose vaccination on the neutralizing capacity to WA1, the wild-type virus, across MM patients and HD (Figure S1F). The sero-conversion group of MM patients was most vulnerable, with no subjects having detectable neutralization capacity prior to third dose. Only half (7/13, 54%) of the MM patients in the sero-elevation group had neutralizing titers, compared to 80% (8/10) of HD prior to third vaccination. Although the third vaccination dose increased neutralizing capacity against WA1, only 40% (2/5) of sero-conversion MM patients had neutralizing titers, which was strikingly lower than the 92% (12/13) of sero-elevation MM patients and 100% of HD (n = 10/10) achieving detectable neutralizing titers (Figure S1G).

An important outstanding question remains as to whether the mRNA vaccine-induced immune response offers adequate protection against SARS-CoV-2 variants. For the Omicron variant specifically, evasion of (humoral) immunity from vaccination or infection with earlier variants has been reported due to the accumulation of mutations in the spike protein gene (McCallum et al., 2022; Zeng et al., 2022). This is especially relevant for patients with pre-existing immune deficiency (e.g., hematologic malignancy), who could be at higher risk of severe infection. In our cohort, we observed a peak with 40 cases of COVID-19 diagnosed after December 1, 2021 (Figure S1H), coinciding with the Omicron variant becoming dominant locally. Seventeen of these patients had already received a third dose. In these patients, anti-S IgG levels collected within 90 days prior to developing COVID-19 in the Omicron-dominant period were highly variable (median 51 AU/mL; range 0–2,511 AU/mL) and were non-significantly ($p = 0.3$) lower when compared to anti-S IgG levels collected in the same time period for subjects after three doses of vaccine who did not develop COVID-19 (median 201 AU/mL; range 0–4,078 AU/mL) (Figure S1I).

We compared the effect of a third-dose vaccination on the neutralizing activity against the Omicron variant using sera from MM patients and HD collected before and after the third vaccine dose (Figure S1J). Neutralizing titers against the Omicron variant were detectable after third-dose vaccination in all HDs (100%, 10/10), in contrast to only 54% (7/13) of sero-elevation MM patients and none of the sero-conversion MM patients (0%, 0/5, Figure S1K). Omicron-neutralizing antibody titers correlated with anti-S IgG antibody levels ($r = 0.68$, $p < 0.001$, Figure S1L) as well as the magnitude of cellular spike-reactive B cells ($r = 0.55$, $p < 0.001$, Figure S1M).

In our data, a high fraction of MM patients (28%) had undetectable anti-S IgG prior to dose 3, suggesting that the initial humoral response to two vaccine doses is not only suboptimal (Terpos et al., 2021; Van Oekelen et al., 2021) but also decreases and, in some cases, disappears over time. We here show that the third dose induces sero-conversion in more than 80% of the MM patients with undetectable anti-S IgG. However, this population may remain vulnerable, as shown by the lack of neutralization capacity of ancestral (e.g., WA1) as well as emerging viral variants of concern (e.g., Omicron). Our findings indicate that a third mRNA vaccine dose significantly augments cellular and humoral immune responses against SARS-CoV-2, including the antigenically distinct Omicron variant, in MM patients. Therefore, patients with MM should be encouraged to receive the third dose when eligible. Sera from less than half of the MM patients in our study were able to neutralize the Omicron variant, although it should be noted that prior to the third dose virtually all MM patients had an undetectable neutralizing titer. These findings underscore the need for continued monitoring of immune responses and further research around measures such as additional vaccine doses or passive immunization for individual MM patients that may remain vulnerable after third-dose vaccination, especially as COVID-19 restrictions are being lifted worldwide and new waves of viral variants are emerging.

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2022.03.013.

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## AUTHOR CONTRIBUTIONS

V.S., A.W., S.P., and PVI study group provided conceptualization, methodology, analysis, and resources for this work. A.A., O.V.O., K.K., K.B., K. Serebryakova, S.A., C.R.G., K. Srivastava, and PVI were involved in organizational aspects of the clinical studies, patient recruitment, data collection, and analysis. A.A., O.V.O., S.A., C.R.G., C.C.-C., and F.K. were involved in design, data collection, analysis, visualization, and interpretation of serological data. A.A., B.U., K.T., and A.K.Z. were involved in design, execution, analysis, visualization, and interpretation of T and B and T cell assays. S.J., A.W., S.P., V.S., and MM Clinical Group were involved in different aspects of patient care. A.A., O.V.O., B.U., M.M., S.J., A.W., V.S., and S.P. provided interpretation of the data and conceptualization of the first manuscript draft. A.A., O.V.O., B.U., A.W., V.S., and S.P. contributed to the writing of the first manuscript draft. H.A., V.S., C.R.G., K. Srivastava, K.B., and PVI study group were involved in neutralization assays and serological quantification. The Almo lab made recombinant spike protein used in spike-reactive B cell identification. All coauthors provided critical edits to the initial manuscript draft and approved the final version.

## DECLARATION OF INTERESTS

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines which list Florian Krammer as co-inventor. Viviana Simon and Carlos Cardon-Cordo are listed on the serological assay patent application as co-inventors. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. Ajai Chari reports grants and personal fees from Janssen, Bristol Myers Squibb (Celgene), Amgen, Seattle Genetics, and Millennium Pharmaceuticals/Takeda and personal fees from Karyopharm, Sanofi, Oncopeptides, Antengene, Glaxo Smith Kline, Secura Bio, Shattuck Labs, Genentech, and Abbvie. Florian Krammer reports grants and personal fees from Pfizer and personal fees from Seqirus and Avimex. The Krammer laboratory is collaborating with Pfizer on animal models of SARS-CoV-2. Sundar Jagannath reports consulting fees for Bristol Myers Squibb (Celgene), Janssen, Karyopharm Therapeutics, Merck, Sanofi, and Takeda Pharmaceuticals. Samir Parekh reports consulting fees from Foundation Medicine and research funding from Bristol Myers Squibb (Celgene), Karyopharm, and Amgen. The other authors reported no relevant conflicts of interest.

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