Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Augmentation of humoral and cellular immune responses after third-dose SARS-CoV-2 vaccination and viral neutralization in myeloma patients

Adolfo Alemán,1,2,3,8 Oliver Van Oeckelen,1,2,3,8 Bhaskar Upadhyaya,2,3 Katherine Beach,4,5 Ariel Kogan Zajdman,2,3 Hala Alishammery,4,5 Kseniya Serbryakova,2,3 Sarita Agte,4,5 Katerina Kappes,2,3 Charles R. Gleason,7* Komal Srivastava,4,5 PV1/MM/Seronet Study Group, Steve Almo,6 Carlos Cordon-Cardo,1,7,8 Florian Kramer,4,5 Miriam Merad,2,3,15 Sundar Jagannath,2,3 Ania Wajnberg,1,11 Viviana Simon,4,5,13,14,* and Samir Parekh2,3,8,10,*

1Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
2Department of Medicine, Hematology, and Medical Oncology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
3Tisch Cancer Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
4Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, NY, USA
5Department of Pathology, Molecular and Cell-Based Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
6Department of Biochemistry, Albert Einstein College of Medicine, New York, NY, USA
7Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, NY, USA
8Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA
9Human Immune Monitoring Center, Icahn School of Medicine at Mount Sinai, New York, NY, USA
10Precision Immunology Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
11Division of Infectious Disease, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
12Department of Geriatrics and Palliative Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
13Division of Infectious Disease, Department of Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA
14Global Health and Emerging Pathogen Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA
15These authors contributed equally

*Correspondence: viviana.simon@mssm.edu (V.S.), samir.parekh@mssm.edu (S.P.)

https://doi.org/10.1016/j.ccell.2022.03.013

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 (Adddeo et al., 2021; Alemán et al., 2021; Van Oeckelen 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 in 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-γ, IL-2, IL-4, IL-17, and TNF-α. 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 without detectable 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.

ACKNOWLEDGMENTS
We thank participants for their generosity and willingness to participate in longitudinal COVID-19
research studies. None of this work would be possible without their contributions. We acknowledge the clinical and research staff at the Center of Excellence for Multiple Myeloma at Mount Sinai. S.P. is supported by National Cancer Institute (NCI) R01 CA244893, CA202222 and receives research funding from Amgen, Celgene/BMS, and Karyopharm. This work was partially funded by the NIADC Collaborative Influenza Vaccine Innovation Centers (CIVIC) contract 75N93019C00051, NIADC Center of Excellence for Influenza Research and Surveillance (CEIRS, contracts HHSN272M000008C and HHSN272M000006C), and NIADC grants U01AI141990 and U01AI150747; by the generous support of the JPB Foundation and the Open Philanthropy Project (research grant 2020-215611 [5384]); and by anonymous donors. This effort was supported by the Serological Sciences Network (SeroNet) in part with federal funds from the National Cancer Institute, National Institutes of Health, under contract 75N91019D00024, task order 75N91021F00001. This work was supported by The Price Family Foundation (SCA), the Wollwock Family Foundation Chair in Multiple Sclerosis and Immunology (SCA), the Einstein-Rockefeller-CUNY Center for AIDS Research (P30AI22441-4), and the Einstein Macromolecular Therapeutics Development Facility supported by the Albert Einstein Cancer Center (P30CA13330). 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. We acknowledge support of the Center of Excellence for Multiple Myeloma Philanthropy.

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., PVI were involved in design, execution, analysis, visualization, and interpretation of T and B cell assays. S.J., A.W., S.P., 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 re-combinant 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 Ichac 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. Aji Chari reports grants and personal fees from Janssen, Bristol Myers Squibb (Celgene), Amgen, Seattle Genetics, and Millenium Pharmaceuticals/Takeda and personal fees from Karyopharm, Sanofi, Oncoproteptides, Apte- ngene, Glaxo Smith Kline, Secura Bio, Shattuck Labs, Genentech, and Abbvie. Florian Krammer reports grants and personal fees from Pfizer and personal fees from Sequiries 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 Par- ehk 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.

REFERENCES

Addeo, A., Shah, P.K., Bordy, N., Hudson, R.D., Abracht, B., Di Marco, M., Kaklamani, V., Dietrich, P.-Y., Taylor, B.S., Simand, P.-F., et al. (2021). Immunogenicity of SARS-CoV-2 messenger RNA vaccines in patients with cancer. Cancer Cell 39, 1091–1098.e2.

Alieman, A., Upadhayya, B., Tuballes, K., Kappes, K., Gleason, C.R., Beach, K., Agte, S., Srivastava, K., Van Oekelen, O., Barcessat, V., et al.; PVI/Seronet Study Group (2021). Variable cellular responses to SARS-CoV-2 in fully vaccinated patients with multiple myeloma. Cancer Cell 39, 1442–1444.

Bar-On, Y.M., Goldberg, Y., Mandel, M., Bodenheimer, O., Freedman, L., Kalkstein, N., Mizrahi, B., Alroy-Reis, S., Ash, N., Milo, R., and Huppert, A. (2021). Protection of BNT162b2 vaccine booster against Covid-19 in Israel. N. Engl. J. Med. 385, 1393–1400.

Barda, N., Dagan, N., Cohen, C., Hernán, M.A., Lipstitch, M., Kohane, I.S., Reis, B.Y., and Balicer, R.D. (2021). Effectiveness of a third dose of the BNT162b2 mRNA COVID-19 vaccine for preventing severe outcomes in Israel: an observational study. Lancet 398, 2093–2100.

McCallum, M., Czudnochowski, N., Rosen, L.E., Zepeida, S.K., Bowen, J.E., Walls, A.C., Hauser, K., Joshi, A., Stewart, C., Dillen, J.R., et al. (2022). Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. Science 375, 864–868.

Shapiro, L.C., Thakkar, A., Campbell, S.T., Forest, S.K., Pradhan, K., Gonzalez-Lugo, J.D., Quinn, R., Bhagat, T.D., Choudhary, G.S., McCort, M., et al. (2022). Efficacy of booster doses in augmenting waning immune responses to COVID-19 vaccine in patients with cancer. Cancer Cell 40, 3–5.

Terpos, E., Gaviatopoulou, M., Ntanasis-Stathopoulos, I., Briassoulis, A., Gumeni, S., Malandrakis, P., Fotiou, D., Papagnanou, E.-D., Migkou, M., Theodorakakou, F., et al. (2021). The neutralizing antibody response post COVID-19 vaccination in patients with myeloma is highly dependent on the type of anti-myeloma treatment. Blood Cancer J. 11, 138.

Thakkar, A., Gonzalez-Lugo, J.D., Goradia, N., Gali, R., Shapiro, L.C., Pradhan, K., Rahman, S., Kim, S.Y., Ko, B., Sica, R.A., et al. (2021). Seroconversion rates following COVID-19 vaccination among patients with cancer. Cancer Cell 39, 1081–1090.e2.

Van Oekelen, O., Gleason, C.R., Agte, S., Srivastava, K., Beach, K.F., Alieman, A., Kappes, K., Mouhieddine, T.H., Wang, B., Chari, A., et al.; PVI/Seronet team (2021). Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma. Cancer Cell 39, 1028–1030.

Zeng, C., Evans, J.P., Chakravartty, K., Qu, P., Reisinger, S., Song, N.J., Rubinstein, M.P., Shields, P.G., Li, Z., and Liu, S.L. (2022). COVID-19 mRNA booster vaccines elicit strong protection against SARS-CoV-2 Omicron variant in patients with cancer. Cancer Cell 40, 117–119.