Cancer Cell, Volume *39*

Supplemental information

Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma

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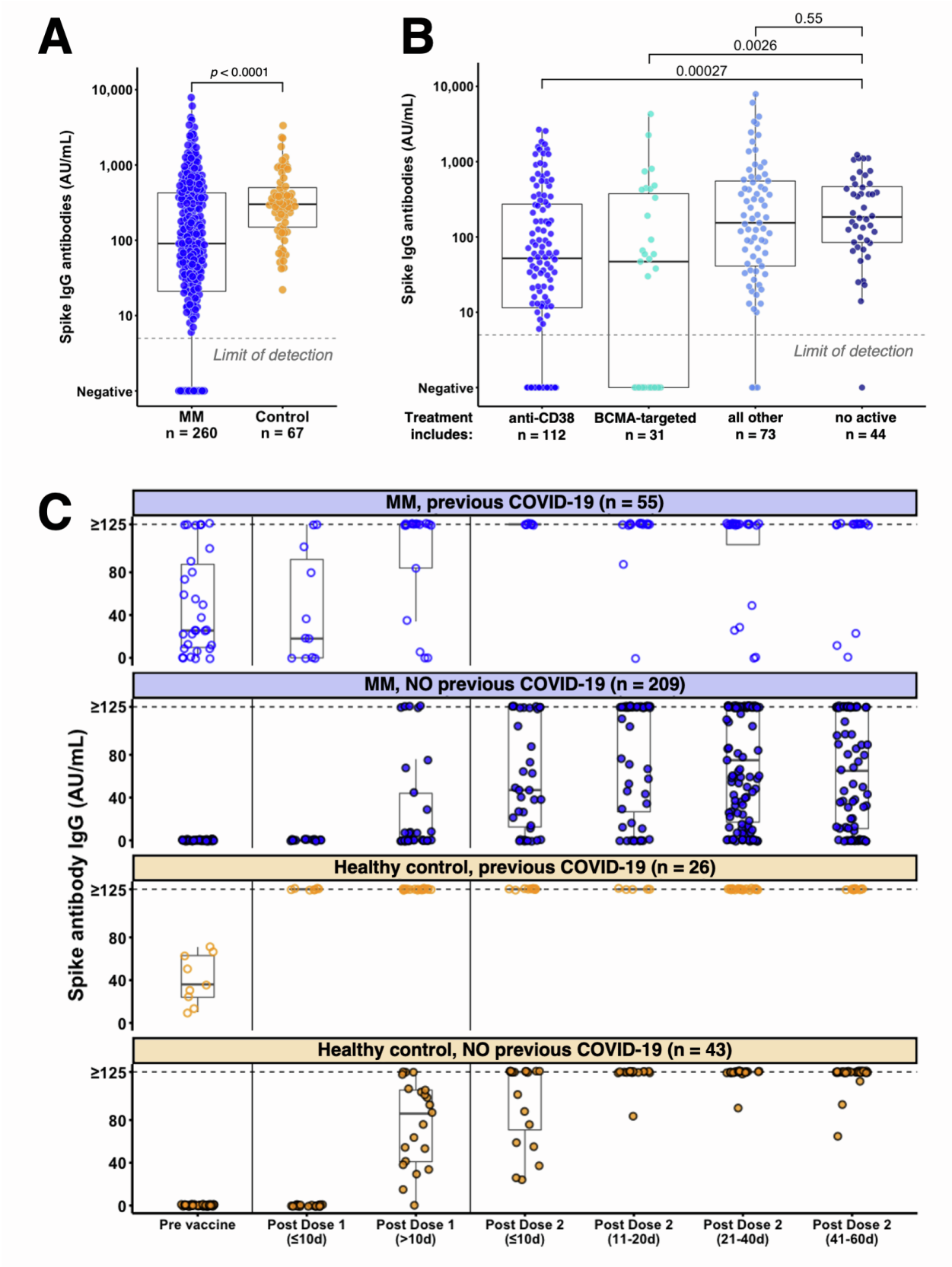
A
p < 0.0001
Spike IgG antibodies (AU/mL)
10,000
1,000
100
10
Negative
Limit of detection
MM
n = 260
Control
n = 67
B
0.55
0.0026
0.00027
Spike IgG antibodies (AU/mL)
Limit of detection
Treatment includes:
anti-CD38
n = 112
BCMA-targeted
n = 31
all other
n = 73
no active
n = 44
C
MM, previous COVID-19 (n = 55)
MM, NO previous COVID-19 (n = 209)
Healthy control, previous COVID-19 (n = 26)
Healthy control, NO previous COVID-19 (n = 43)
Spike antibody IgG (AU/mL)
≥125
80
40
0
Pre vaccine
Post Dose 1 (≤10d)
Post Dose 1 (>10d)
Post Dose 2 (≤10d)
Post Dose 2 (11-20d)
Post Dose 2 (21-40d)
Post Dose 2 (41-60d)

Figure S1. SARS-CoV-2 anti-spike IgG antibody responses in patients with multiple myeloma (MM) before and after COVID-19 RNA vaccination.

(**A**) Anti-SARS-CoV-2 spike IgG antibody level at least 10 days after receiving two doses of COVID-19 mRNA vaccine in MM patients versus healthy controls. Antibody concentrations measured in artificial units per mL (AU/mL) are depicted on a log-10 scale to better capture the full range of responses.

(**B**) Anti-SARS-CoV-2 spike IgG antibody levels at least 10 days after receiving two doses of SARS-CoV-2 mRNA vaccine in MM patients are split according to major treatment groups.

(**C**) Time course of anti-SARS-CoV-2 spike IgG antibody levels in MM patients with COVID-19 (112 antibody measurements in 55 patients), in MM patients without prior COVID-19 (349 antibody measurements in 209 patients), in healthy controls that were seropositive (78 antibody measurements in 26 individuals) and seronegative (172 antibody measurements in 43 individuals) prior to vaccination. Sample collection time points are binned into seven distinct time intervals. Antibody concentrations shown are capped at 125 AU/mL (any value >40 AU/mL is categorized as strong positive). For each panel, the lower and upper hinges of the boxplot correspond to the first and third quartiles (the 25th and 75th percentiles) with a bold horizontal line indicating the median. Vertical whiskers are extended up to 1.5 times the interquartile range (IQR). Values above the brackets correspond to the *p* value of a Mann-Whitney *U* test for comparison of 2 independent samples.

Table S1A. Demographics, vaccine type, disease and treatment characteristics of the study participants included in this report with univariate and multivariate statistical analysis (i.e. multivariate logistic regression model with absence of detectable IgG antibody levels > 10 days after full vaccination as dichotomized outcome).

Table S1B. Characteristics of patients that developed COVID-19 at some time after receiving at least one mRNA vaccine dose.

Supplemental Materials and Methods

Study information and patient selection

Patients with multiple myeloma (MM): The study cohort consisted of 320 patients with and without previously documented COVID-19 pooled from two different non-interventional Institutional Review Board (IRB) approved study protocols at The Icahn School of Medicine at Mount Sinai.

A total of 112 MM patients were enrolled after obtaining written informed consent for an ongoing longitudinal study at our institution (IRB-16-00791). Patients had blood taken for analysis at multiple time points before or after administration of the SARS-CoV-2 mRNA vaccine. All specimens were coded prior to processing and antibody testing for all serum specimen was performed in a blinded manner. All participants with, at least, one post vaccine antibody data point available at the time of writing this report were included in the analysis.

The remaining 208 MM patients were identified under a retrospective study (IRB: GCO#: 11-1433) by conducting a chart review for patients at our MM clinic who had SARS-CoV-2 spike IgG results at various time points around SARS-CoV-2 mRNA vaccine administration. Chart review was conducted to retrieve patient clinical characteristics. The cohort may contain an overrepresentation of patients on BCMA-targeted treatments as we are a tertiary care center for MM where patients are routinely referred for treatment on clinical trials.

Control group: The PARIS (***P****rotection* ***A****ssociated with* ***R****apid* ***I****mmunity to* ***S****ARS-CoV-2*) study seeks to investigate the durability and effectiveness of SARS-CoV-2 immune responses in health care workers over time. The study was reviewed and approved by the Mount Sinai Hospital Institutional Review Board (IRB-20-03374). All participants provided informed consent prior to collection of data and specimen. All specimens were coded prior to processing and antibody testing for all serum specimen was performed in a blinded manner. The current report includes serological data from a subgroup selected to best match the demographics and age of the MM patient population. 69 participants over the age of 50 years without known underlying immune modulatory co-morbidities and, at least, one post vaccine dose 2 antibody test were included.

SARS-CoV-2 antibody ELISA: Anti-SARS-CoV-2 spike protein IgG antibody testing was performed using an anti-IgG assay developed at Mount Sinai Health System Department of Pathology in collaboration with the Icahn School of Medicine at Mount Sinai Department of Microbiology under a Food and Drug Administration (FDA) Emergency Use Authorization. The antibodies to SARS-CoV-2 spike protein were detected using an established two-step ELISA (COVID-SeroKlir Kantaro SARS-CoV-2 IgG Ab Kit 100% Prev, Cat. Nr. COV219). The assay shows a performance of 100% specificity and 95% sensitivity in in-house evaluation. The qualitative results are reported out as negative (less than 5 AU/mL), weak positive (5-15 AU/mL), moderate positive (16-39 AU/mL) and strong positive (40 AU/mL and above) as described in the FDA emergency authorization. In addition, quantitative results are reported in artificial Units/ml (AU/mL). Starting Mid-February 2021, the dynamic range of the spike binding antibody test was extended in order to better capture the high antibody titers mounted by many individuals upon receiving SARS-CoV-2 vaccination (standard range up to 125 AU/ml; extended range: no upper limit). Thus, while the antibody test is the same, the upper limit of detection changed depending on when the antibody test was performed (before or after February 2021). Figure 1A and 1B show antibody data generated with the extended dynamic range with the exception of two MM patients who had values of >125 AU/mL. The data shown in Fig. 1C summarizing the longitudinal antibody responses upon COVID-19 vaccination were capped at 125 AU/mL because for several of the MM patients we had antibody values of >125 AU/mL.

Statistical analysis: Continuous variables are presented throughout the manuscript as median with the range of values being shown by disclosing minimum and maximum values. In addition, individual antibody data points are shown. Categorical variables are shown as a percentage and the absolute number of subjects. Wherever two outcome groups are compared, Fisher's exact test was used to determine significance and where applicable odds ratios (ORs) were reported.

The Mann-Whitney U test was used to determine significance for continuous variables. A two-sided alpha < 0.05 was considered statistically significant. All statistical analyses were done using R (v4.0.2). Multivariate statistical analysis was done using the presence or absence of measurable anti-spike IgG response > 10 days after dose 2 as a binary outcome variable and conducted using the glm(…, family = “binomial”) function in R (v4.0.2).