Cancer Cell, Volume *40*

Supplemental information

Attenuated response to SARS-CoV-2 vaccine in patients with asymptomatic precursor stages of multiple myeloma and Waldenstrom macroglobulinemia

Yoshinobu Konishi, Romanos Sklavenitis-Pistofidis, Hong Yue, Federico Ferrari, Robert A. Redd, Elizabeth D. Lightbody, Massimiliano Russo, Jacqueline Perry, Erica Horowitz, Anna V. Justis, Nader A. Shayegh, Alexandra Savell, Julia Prescott, Shohreh Varmeh, Radosław P. Nowak, Mark Hamilton, Daniel Auclair, Catherine R. Marinac, Lorenzo Trippa, Eric S. Fischer, and Irene M. Ghobrial

Supplemental Information

Table S1. Patient demographics and results of multiple variate analysis

(A) Patient demographics of 3,005 individuals enrolled in the IMPACT study. (B) Patient demographics of 628 individuals analyzed by ELISA. (C) Treatment history of 58 SMM patients. (D, E) Results of multivariate analysis using multiple linear regression models. Association between vaccine-induced antibody titer and clinical variables among patients with plasma cell dyscrasias (D) or patients with SMM (E) was examined. Data of healthy individuals or low-risk SMM patients were used as a control, respectively. Data represented as β coefficient +/- 95% confidence interval.

Table S1A: Characteristics of 3,005 individuals enrolled in this study

		Diagnosis					
	Total n=3,005 (%)	**Healthy n=1054 (%)**	**MGUS n=547 (%)**	**SMM n=346 (%)**	**SWM n=81 (%)**	**MM n=82 (%)**	**Others[#] n=895 (%)**
Age							
Median (range)	60 (18–93)	58 (25–78)	64 (22–93)	64 (30–83)	68 (51–87)	67 (30–86)	53 (18-80)
Gender							
Male	975 (32)	289 (27)	194 (35)	149 (43)	39 (48)	45 (55)	259 (29)
Female	1992 (66)	765 (73)	353 (65)	197 (57)	42 (52)	37 (45)	598 (67)
Not answered	38 (2)	-	-	-	-	-	38 (4)
Race							
White or Caucasian	2689 (90)	940 (89)	464 (85)	317 (92)	78 (96)	81 (99)	809 (91)
Black or African American	162 (5)	69 (7)	32 (6)	13 (4)	-	1 (1)	47 (5)
Others	154 (5)	45 (4)	51 (9)	16 (4)	3 (4)	-	39 (4)
Vaccine							
Received full vaccination	2771 (92)	988 (94)	499 (91)	328 (95)	78 (96)	80 (98)	798 (89)
BNT162b (Pfizer)	1385 (46)	496 (47)	239 (44)	173 (50)	39 (48)	42 (52)	396 (44)
mRNA-1273 (Moderna)	1090 (36)	429 (41)	200 (36)	119 (35)	33 (41)	31 (38)	278 (31)
Ad26.COV2.S (J&J)	145 (5)	57 (5)	22 (4)	11 (3)	1 (1)	6 (7)	48 (5)
Unknown	151 (5)	6 (1)	38 (7)	25 (7)	5 (6)	1 (1)	76 (9)
Never received vaccination	234 (8)	66 (6)	48 (9)	18 (5)	3 (4)	2 (2)	97 (11)
3rd dose vaccine							
Received 3rd dose vaccine	269 (9)	25 (2)	62 (11)	71 (21)	20 (25)	59 (72)	32 (3)
SARS-CoV-2 PCR positive							
Before full vaccination	176 (6)	56 (5)	36 (7)	18 (5)	3 (4)	3 (4)	60 (7)
After full vaccination	33 (1)	8 (1)	9 (2)	6 (2)	-	-	10 (1)
Never received vaccination	44 (1)	12 (1)	9 (2)	6 (2)	-	1 (1)	16 (2)

[#]There were 836 participants from the PROMISE study who had pending diagnostic studies and therefore, we had no information at the time of writing this manuscript on their status for precursor myeloma. In addition, PCROWD enrolled other hematological malignancies (n = 59) and these included the following cases that also participated in the vaccine questionnaire: myeloproliferative neoplasms n = 15, monoclonal B cell lymphocytosis n =8, early MDS n = 6, solitary plasmacytoma n =2, and 28 other cases of precursor hematological conditions including early-stage asymptomatic low-grade lymphoma and POEMS. Of note, those cases were not included in the plasma samples analyzed in the manuscript.

Table S1B: Characteristics of 628 individuals analyzed using ELISA

		Diagnosis				
	Total n=628 (%)	**Healthy n=100 (%)**	**MGUS n=201 (%)**	**SMM n=221 (%)**	**SWM n=40 (%)**	**MM n=66 (%)**
Age						
Median (range)	64 (30–90)	59 (41–74)	64 (34–90)	65 (39–83)	69 (51–87)	64 (30–86)
Gender						
Male	242 (39)	26 (26)	67 (33)	99 (45)	19 (48)	31 (47)
Female	386 (61)	74 (74)	134 (67)	122 (55)	21 (52)	35 (53)
Race						
White or Caucasian	584 (93)	89 (89)	182 (91)	209 (94)	39 (98)	65 (98)
Black or African American	25 (4)	7 (7)	14 (7)	4 (2)	-	-
Others	19 (3)	4 (4)	5 (2)	8 (4)	1 (2)	1 (2)
Vaccine Type						
BNT162b (Pfizer)	334 (53)	45 (45)	102 (51)	130 (59)	19 (48)	38 (58)
mRNA-1273 (Moderna)	262 (42)	47 (47)	88 (44)	84 (38)	19 (48)	24 (36)
Ad26.COV2.S (J&J)	32 (5)	8 (8)	11 (5)	7 (3)	2 (4)	4 (6)
SARS-CoV-2 PCR positive test						
Before full vaccination	34 (5)	7 (7)	12 (6)	12 (5)	3 (8)	-
After full vaccination	10 (2)	1 (1)	3 (1)	6 (3)	-	-
Blood collection						
Both pre-and post-vaccination	272 (43)	28 (28)	74 (37)	136 (62)	18 (45)	16 (24)
Only pre-vaccination	81 (13)	25 (25)	32 (16)	17 (7)	5 (12)	2 (3)
Only post-vaccination	275 (44)	47 (47)	95 (47)	68 (31)	17 (43)	48 (73)
3rd dose vaccine						
Received 3rd dose vaccine	124 (20)	3 (3)	28 (14)	38 (17)	10 (25)	45 (68)
Blood collection after 3rd dose	59 (9)	1 (1)	13 (6)	12 (5)	2 (5)	31 (47)

Table S1C. Treatment history in SMM patients

	Total n=58 (%)	**On therapy n=17 (%)**	**Post-treatment n=41 (%)**
Single-agent daratumumab	24 (41)	5 (29)	19 (46)
Daratumumab/Bortezomib/Lenalidomide/Dexamethasone	7 (12)	7 (41)	0 (0)
Daratumumab/Carfizomib/Lenalidomide/Dexamethasone	1 (2)	1 (6)	0 (0)
Ixazomib/Lenalidomide/Dexamethasone	25 (43)	4 (24)	21 (51)
Lenalidomide/Dexamethasone	1 (2)	0	1 (3)

Table S1D. Model of the association between antibody titer and clinical variables in patients with plasma cell dyscrasias

Predictors	**Coefficient**	**95% CI**	***p-values***
MGUS	-0.13	-0.28 – 0.03	0.103
Low-risk SMM	-0.22	-0.42 – -0.03	**0.027**
Intermediate-risk SMM	-0.40	-0.61 – -0.19	**<0.001**
High-risk SMM	-0.53	-0.88 – -0.18	**0.003**
SWM	-0.15	-0.36 – 0.07	0.182
MM	-0.44	-0.67 – -0.21	**<0.001**
Days after full vaccination	-0.00	-0.01 – -0.00	**<0.001**
Age	-0.04	-0.08 – 0.01	0.163
Male	-0.12	-0.22 – -0.02	**0.019**
Self-reported race White	-0.29	-0.58 – 0.01	0.056
SARS-CoV-2 infection prior to vaccination	0.78	0.58 – 0.98	**<0.001**
Receiving BNT162b2	-0.38	-0.48 - -0.29	**<0.001**
Treatment status (active treatment)	-0.21	-0.44 – 0.02	0.078
Treatment status (post-treatment)	0.09	-0.09 – 0.28	0.311

N = 628. CI = confidence interval. Coefficient, CI, and p-values from multiple regression model. α=0.05.

Table S1E. Model of the association between antibody titer and clinical variables in SMM patients			
Predictors	**Coefficient**	**95% CI**	***p-values***
Days after full vaccination	-0.01	-0.01 – -0.00	**<0.001**
Intermediate-risk based on 2/20/20	0.08	-0.21 – 0.37	0.583
High-risk based on 2/20/20	0.59	-0.04 – 1.22	0.065
Age	0.00	-0.09 – 0.10	0.944
Male	-0.20	-0.42 – 0.01	0.064
Self-reported race White	-0.35	-0.83 – 0.13	0.156
SARS-CoV-2 infection prior to vaccination	0.92	0.57 – 1.27	**<0.001**
Receiving BNT162b2	-0.38	-0.58 – -0.18	**<0.001**
Immunofixation (IgG subtype)	-0.17	-0.48 – 0.14	0.272
Hemoglobin (g/dL)	-0.00	-0.13 – 0.13	0.987
Albumin (g/dL)	0.02	-0.10 – 0.13	0.805
Creatinine (mg/dL)	0.03	-0.09 – 0.15	0.570
M spike protein (g/dL)	-0.11	-0.25 – 0.04	0.141
Kappa to lambda free light chain ration	-0.16	-0.32 – 0.01	0.060
Plasma cell percentage on BM biopsy (%)	-0.20	-0.37 – -0.03	**0.018**
Treatment status (active treatment)	-0.26	-0.54 – 0.02	0.068
Treatment status (post-treatment)	-0.02	-0.24 – 0.19	0.825

N = 193. CI = confidence interval. Coefficient, CI, and p-values from multiple regression model. α=0.05.

Supplemental figure 1

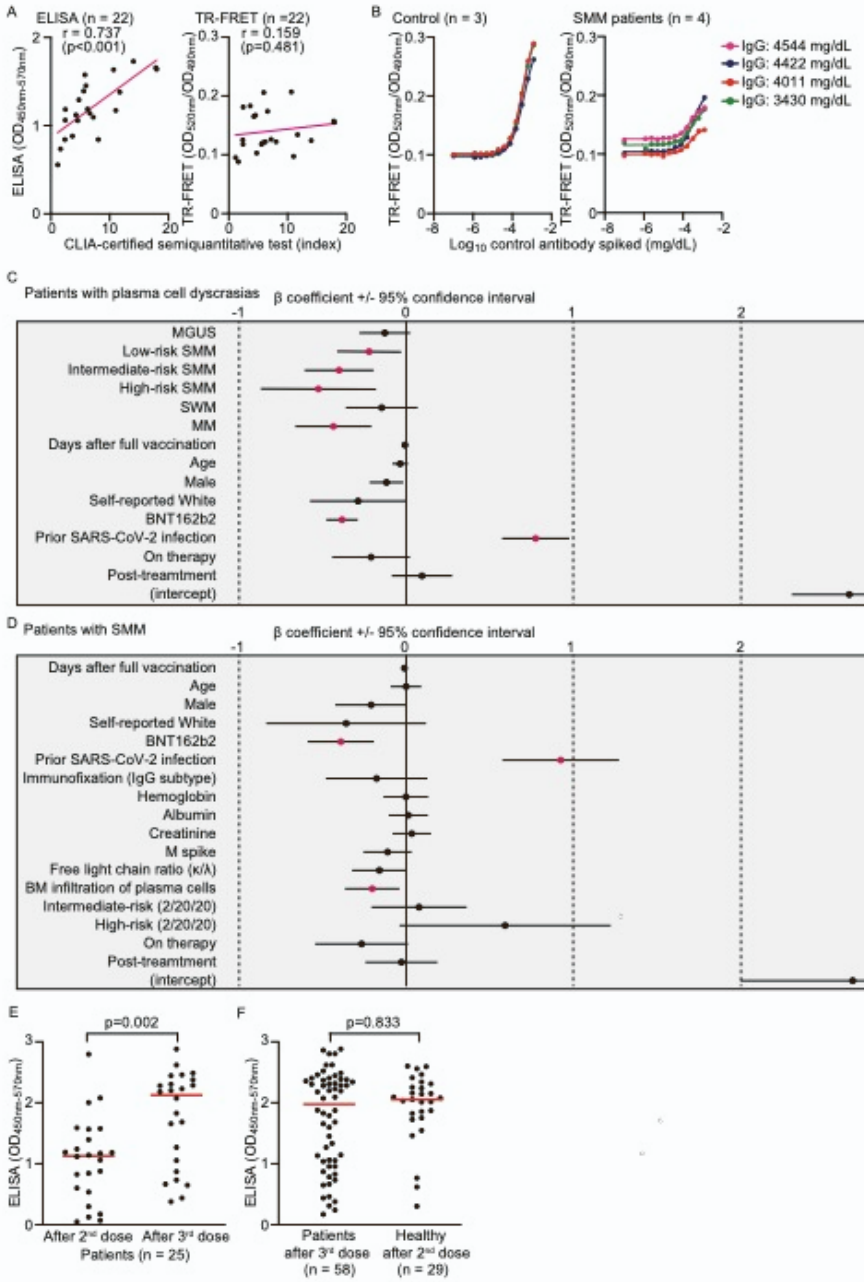

Figure S1. SARS-CoV-2 anti-spike protein (S) IgG antibody response in patients with a spectrum of plasma cell dyscrasia after full vaccination

(A) The correlation between clinical-grade and research-level antibody tests. Each dot indicates an individual test result (n = 22). The x-axis represents the CLIA-certified semiquantitative test results within its quantitative range (1 – 20 index) and Y-axis represents ELISA measurements ($OD_{450nm-570nm}$) (left) or TR-FRET measurements (OD_{520nm}/OD_{490nm}) (right), respectively. Pearson correlation was applied (r; correlation coefficient); $\alpha = 0.05$. Magenta line represents the regression line. (B) TR-FRET measurements using blood samples taken from individuals without plasma cell dyscrasias (left, n = 3) or SMM patients (right, n = 4) spiked with various concentrations of positive control antibody (CR3022 IgG). Each dot represents an individual test result, and each line represents the dose-response curves based on a non-linear variable slope model. The increase in TR-FRET measurements observed in control samples after adding the positive control antibody was masked in samples obtained from SMM patients. The IgG level (mg/dL) of each sample is labeled on the right. (C, D) Multivariate analysis of vaccine-induced antibody response among patients with plasma cell dyscrasias (C) or in patients with SMM (D). Data of healthy individuals or low-risk SMM patients was used as the control, respectively. A multiple linear regression model was applied. Data represented as β coefficient +/- 95% confidence interval. Magenta circles indicate statically significant results. All tests were two-sided, with $\alpha = 0.05$. (E) Anti-SARS-CoV-2 IgG antibody levels before and after receiving a 3rd vaccine dose in patients with plasma cell dyscrasia (n = 25; 6 MGUS, 10 SMM, 2 SWM, and 7 MM). P-value from Wilcoxon matched-pairs signed rank test is displayed; $\alpha = 0.05$. (E) Antibody titers in patients with plasma cell dyscrasias after the 3rd dose (n = 58; 13 MGUS, 12 SMM, 2 SWM, and 31 MM) and healthy individuals after the 2nd dose (n =29). P-value from Mann-Whitney test is displayed; $\alpha = 0.05$.

Supplemental methods

Study information and patient selection

The IMPACT study (Dana-Farber Cancer Institute IRB #20-332) is a prospective observational cohort study in collaboration with Multiple Myeloma Research Foundation (MMRF), which enrolled individuals nationwide who have been diagnosed with a spectrum of plasma cell dyscrasias and healthy individuals with risk factors for MM. These risk factors are age (40 – 75 years), self-identified Black or African American race, or having family relatives with hematologic malignancies. These participants were enrolled on other studies that allowed them to participate in the IMPACT study including the PROMISE study (a study to screen high-risk

individuals for myeloma specifically focusing on Black Americans, NCT03689595), the PCROWD study (an observational prospective cohort study of MGUS and SMM, NCT02269592), and the CureCloud study (an MMRF study recruiting SMM and active myeloma patients for direct-to-patient access of liquid biopsy genomic results, NCT03657251). A questionnaire regarding prior SARS-CoV-2 infection or vaccination (vaccine type and dates of administration) was sent out to all participants in IMPACT. All participants enrolled in one of these studies who answered the questionnaire were included in this study [913 IMPACT study (435 PROMISE, 396 PCROWD, and 82 CureCloud), 1558 PROMISE study, and 534 PCROWD]. Of 913 participants enrolled in IMPACT, 514 individuals received clinically approved SARS-CoV-2 IgG antibody test provided by Quest Diagnostics (Secaucus, NJ). All participants provided informed consent prior to the collection of data and specimens. Self-reported data were collected on demographic variables (age, sex, race), diagnosis, vaccine type and dates, prior SARS-CoV-2 infections confirmed by PCR test, past medical history of malignancies or autoimmune diseases, and family history of malignancies. Chart review was conducted to retrieve patient clinical variables when available (diagnosis, prior history of treatment, and clinical laboratory test results). The clinical laboratory test results closest to the date of 1st dose of vaccine were adapted. Individuals who received two doses of mRNA vaccine (BNT162b2 or mRNA-1273) and a single dose of adenovirus-vector vaccine (Ad26.COV2.S) were considered as fully vaccinated. For patients who experienced disease progression during the time of sample collection, the diagnosis at the time of 1st dose of vaccination was adopted. For SMM patients with treatment history (summarized in Table S1C), the regimen applied closest to the date of 1st dose of vaccine was adopted as the regimen for each patient. SMM patients with the treatment withdrawal date more than 2 years ago were considered "untreated". All specimens were de-identified prior to processing and antibody testing for all plasma specimens was performed in a blinded manner.

ELISA assay to detect IgG antibodies against SARS-CoV-2

ELISA assay to detect anti-SARS-CoV-2 antibodies was performed as previously described with some modifications (Yue et al., 2020). In brief, 384-well ELISA plates (ThermoFisher #464718) were coated with 50 µl/well of 500 ng/ml SARS-CoV-2 S protein in coating buffer (1 capsule of carbonate-bicarbonate buffer (Sigma #C3041100CAP) per 100 mL Milli-Q H_2O) for 30 minutes at room temperature. Plates were then washed 3 times with 100 µl/well of wash buffer (0.05% Tween-20, 400 mM NaCl, 50 mM Tris pH 8.0 in Milli-Q H_2O) using a Tecan automated plate washer, followed by blocking using 100 µl/well of blocking buffer (1% BSA,

140 mM NaCl, 50 mM Tris pH 8.0 in Milli-Q H_2O) for 30 minutes at room temperature. After washing 3 times as described above, 50 µl of diluted plasma sample (in dilution buffer; 1% BSA, 0.05% Tween-20, 140 mM NaCl, 50 mM Tris (pH 8.0) in Milli-Q H_2O) were added to each well and incubated for 30 minutes at 37°C. After washing 5 times, 50 µl of diluted detection antibody solution (HRP anti-human IgG; Bethyl Laboratory #A80-104P) was added to each well and incubated for 30 minutes at room temperature. Following an additional 5 washes, 40 µl of TMB peroxidase substrate (Thermo Fisher #34029) was added to each well and incubated at room temperature for 3 minutes. Then, the reaction was stopped by adding 40 µl of stop solution (1 M H_2SO_4 in Milli-Q H_2O) to each well. OD was read at 450 nm and 570 nm on a PHERAstar FSX plate reader. The final data used in the analysis was calculated by subtracting 570 nm background from 450 nm signal.

TR-FRET assay to detect IgG antibodies against SARS-CoV-2

TR-FRET assay to detect anti-SARS-CoV-2 antibodies was performed as previously described (Yue et al., 2020). As a positive control, we used recombinantly expressed SARS-CoV-1 IgG antibody CR3022 which has been shown to cross-react with spike protein of SARS-CoV-2 (ter Meulen et al., 2006; Tian et al., 2020). Plasmids encoding CR3022 was a gift from Galit Alter, MGH, Boston, MA. Titration of CR3022 IgG antibody or dilution of tested human plasma samples was added to assay mix with final concentrations of 7.5 nM Terbium-labeled SARS-CoV-2 Spike protein, 250 nM BODIPY-labeled αIgG in a buffer containing PBS, 0.05% Tween-20 (Sigma Aldrich #P9416). Plasma samples were diluted in the buffer containing 50 mM Tris pH 8.0, 140 mM NaCl, 0.05% Tween-20, and 1% BSA (Cell Signaling Technology #9998S). TR-FRET assays were performed in a 384-well microplate (Corning, #4514) with 15 µL final assay volume. Before TR-FRET measurements were conducted, the reactions were incubated for 1 h at room temperature. After excitation of terbium fluorescence at 337 nm, emission at 490 nm (terbium) and 520 nm (BODIPY) were recorded with a 70 µs delay over 130 µs to reduce background fluorescence and the reaction was followed by >20 or > 100-second cycles of each data point using a PHERAstar FS microplate reader (BMG Labtech). The TR-FRET signal of each data point was extracted by calculating the 520/490 nm ratio.

TR-FRET assay to examine the immunoglobulin interference

To examine the immunoglobulin interference in TR-FRET assay, we prepared two groups of samples; samples from individuals without plasma cell dyscrasias (n = 3; provided from the

Fischer Lab) and samples from SMM patients (n = 4) with high serum IgG titers (**Figure S1B**). We added CR3022 IgG, to these samples with several concentration (range from 1 x 10^{-2} mg/dL to 1 x 10^{-8} mg/dL). If there is any interference due to serum IgG, the TR-FRET measurements using patient's samples were expected to come back negative or unexpectedly low values compared to control samples.

Statistical analysis

Continuous variables are presented as medians with the range. Categorical variables are shown as a percent and number of subjects. We applied multiple linear regression in our multivariate analyses. We reported β coefficient, 95% CIs, and p-values, where applicable. The Wilcoxon matched-pairs signed rank test or Mann-Whitney test were used to compare groups. A two-sided alpha <0.05 was considered statistically significant. All statistical analysis was done using either Graph Pad Prism (version 9.0.1) or R software (version 4.1.1).

Supplemental References

Ter Meulen, J., van den Brink, E. N., Poon, L. L. M., Marissen, W. E., Leung, C. S. W., Cox, F., Cheung, C. Y., Bakker, A. Q., Bogaards, J. A., van Deventer, E., *et al.* (2006). Human Monoclonal Antibody Combination against SARS Coronavirus: Synergy and Coverage of Escape Mutants. PLOS Medicine *3*, e237.

Tian, X., Li, C., Huang, A., Xia, S., Lu, S., Shi, Z., Lu, L., Jiang, S., Yang, Z., Wu, Y., and Ying, T. (2020). Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect *9*, 382-385.

Yue, H., Nowak, R. P., Overwijn, D., Payne, N. C., Fischinger, S., Atyeo, C., Baden, L. R., Nilles, E. J., Karlson, E. W., Yu, X. G., *et al.* (2020). Rapid "mix and read" assay for scalable detection of SARS-CoV-2 antibodies in patient plasma. medRxiv, 2020.2009.2001.20184101.