LETTER TO THE EDITOR

Longitudinal dynamics and clinically available predictors of poor response to COVID-19 vaccination in multiple myeloma

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Supplementary Table 1: Baseline patient, COVID and myeloma disease characteristics.
MM patients were invited to provide peripheral blood samples ≥3 weeks following doses 2-4 of COVID-19 vaccination. Patient demographics are displayed for cohorts providing a sample at individual time points, following doses (*N*=241), three (*N*=240) or four (*N*=229) of COVID-19 vaccination, as well as patients who provided three serial samples across all three time points (*N*=141).

Factor	Single time points				Three serial samples (n=141) *
	post-2nd (n=241)	post-3rd (n=240)	post-4th (n=229)	*p*	
Demographics					
Female (%)	109 (45.2%)	110 (45.8%)	103 (45.0%)	0.9817[a]	64 (45.4%)
Median age [SD]	66.2 [9.2]	65.8 [9.3]	66.3 [9.1]	0.8792[b]	66.3 [8.9]
White-UK	214 (88.8%)	213 (88.8%)	202 (88.2%)	0.7979[a]	130 (92.2%)
Other	17 (7.1%)	13 (5.4%)	17 (7.4%)		9 (6.4%)
Unknown	10 (4.1%)	14 (5.8%)	10 (4.4%)		2 (1.4%)
COVID-19 and vaccination history					
Positive Anti-N serology, n (%)	7 (2.9%)	11 (4.6%)	29 (12.7%)	<0.0001[a]	15 (10.6%)
Median days since last dose [range]	66 [21-216]	70 [24-156]	105 [25-233]	<0.0001[b]	104 [25-205]
Adenoviral vector-based (%)	116 (48.1%)	3 (1.2%)	2 (0.9%)	<0.0001[a]	0 (0.0%)
mRNA-based (%)	85 (35.3%)	224 (93.3%)	219 (95.6%)		138 (97.9%)
Unknown (%)	40 (16.6%)	13 (5.4%)	8 (3.5%)		3 (2.1%)
Myeloma disease and treatment factors					
Median months since myeloma diagnosis [IQR]	49.4 [24.2-87.8]	52.9 [29.4-95.7]	55.1 [32.4-94.7]	0.1301[b]	60.2 [36.6-96.8]
IMWG response group **					
CR/VGPR (%)	90 (37.3%)	102 (42.5%)	96 (41.9%)	0.3331[a]	63 (44.7%)
PR/stable (%)	29 (12.0%)	34 (14.2%)	36 (15.7%)		19 (13.5%)
Progressive/relapse (%)	44 (18.3%)	39 (16.2%)	45 (19.7%)		32 (22.7%)
Unknown, n (%)	78 (32.4%)	65 (27.1%)	52 (22.7%)		27 (19.1%)
Current treatment **					
Anti-CD38/BCMA-based	41 (17.0%)	41 (17.1%)	40 (17.5%)	0.9666[a]	30 (21.3%)
Other Treatments	85 (35.3%)	92 (38.3%)	85 (37.1%)		53 (37.6%)
No Treatment	71 (29.5%)	72 (30.0%)	68 (29.7%)		43 (30.5%)
Unknown	44 (18.3%)	35 (14.6%)	36 (15.7%)		15 (10.6%)

[a] Chi-squared test.
[b] Kruskal-Wallis test.
* n=141 patients providing post-2nd, post-3rd and post-4th serial samples; COVID-19 history, vaccination history and myeloma disease and treatment factors stated for at the time of fourth dose for this cohort.
** Last recorded International Myeloma Working Group (IMWG) response classification or treatment at time of sample collection for each time point. CR = complete response; VGPR = very good partial response; PR = partial response; BCMA = B-cell maturation antigen targeting agents.

Supplementary Table 2: Extended data sheet of clinical factors associated with variable vaccination response.

Raw data from Figure 2, displaying vaccination response stratified by clinical variables and dose, or correlations with peripheral blood immunoglobulin levels and lymphocyte subsets. Humoral response measured by COVID-19 anti-spike antibody titre and cellular response by T-cell IGRA to S-antigen.

Analysis	Units	Dose	Subgroup	Humoral Response (Anti-S)			Cellular Response (T-cell IGRA, S antigen)		
				n	Value	*p*	n	Value (SD)	*p*
Previous COVID-19 Exposure [a]	Titre / count [median (SD)]	2	Anti-N Negative	232	902 (7,487)	<0.0001	183	10.0 (18.3)	0.0006
			Anti-N Positive	7	10,269 (11,578)		8	50.0 (15.7)	
		3	Anti-N Negative	227	5,739 (13,784)	<0.0001	140	8.5 (17.4)	0.0021
			Anti-N Positive	11	40,000 (12,576)		5	50.0 (17.0)	
		4	Anti-N Negative	196	11,121 (14,409)	<0.0001	146	13.0 (20.0)	0.0008
			Anti-N Positive	29	40,000 (13,148)		20	32.5 (13.2)	
Anti-S x T-cell IGRA positivity	Titre [median (SD)]	2	Negative T-IGRA	77	471 (5,079)	<0.0001			
			Positive T-IGRA	112	1,470 (8,742)				
		3	Negative T-IGRA	64	3,376 (13,016)	<0.0001			
			Positive T-IGRA	79	10,904 (14,209)				
		4	Negative T-IGRA	48	6,457 (13,263)	<0.0001			
			Positive T-IGRA	115	20,636 (15,151)				
Vaccine Platform [b]	Titre / count [mean (SD)]	2	A-A	113	985 (5,441)	0.0221	94	23.0 (20.1)	<0.0001
			M-M	81	2,016 (10,002)		65	10.0 (13.0)	
		3	A-A-M	115	5,975 (13,469)	0.9151	72	22.3 (18.9)	<0.0001
			M-M-M	81	6,131 (13,963)		51	6.6 (12.8)	
		4	A-A-M-M	116	12,738 (14,764)	0.6349	88	28.5 (20.0)	0.0001
			M-M-M-M	74	13,828 (15,496)		49	14.8 (17.1)	
Peripheral blood immune marker [c]	Spearman *r* (95% CI upper)	4	IgG	225	-0.05 (-0.18, 0.09)	0.4614	165	0.33 (0.18, 0.46)	<0.0001
			IgA	225	0.36 (0.24, 0.47)	<0.0001	165	0.16 (0.00, 0.31)	0.0424
			IgM	225	0.39 (0.27, 0.50)	<0.0001	165	0.26 (0.10, 0.40)	0.0009
			Lymphocyte count	189	0.05 (-0.10, 0.19)	0.5148	162	0.35 (0.20, 0.48)	<0.0001
			T-cell count	189	0.00 (-0.15, 0.15)	0.9841	162	0.36 (0.21, 0.49)	<0.0001
			CD4 count	189	-0.05 (-0.19, 0.10)	0.5324	162	0.33 (0.18, 0.46)	<0.0001
			CD8 count	189	0.05 (-0.09, 0.20)	0.4586	162	0.32 (0.17, 0.45)	<0.0001
			B cell count	189	0.11 (-0.04, 0.25)	0.1274	162	0.12 (-0.04, 0.28)	0.1164
			NK count	189	0.13 (-0.01, 0.28)	0.0667	162	0.27 (0.11, 0.41)	0.0006
IMWG Disease Control / Therapy [d]	Anti-S: titre [median (SD)] T-IGRA: # positive [n (%)]	4	Prog/Relapse	45	3,530 (14,479)	<0.0001	32	17 (53.1%)	0.0477
			PR/Stable	35	9,669 (12,602)		23	14 (60.9%)	
			VGPR/CR	95	24,278 (14,514)		72	55 (76.4%)	
			Anti-CD38/BCMA	40	6,157 (11,691)	0.0113	25	13 (52.0%)	0.0433
			Other Treatment	83	16,102 (14,867)		58	43 (74.1%)	
			No Treatment	67	17,578 (15,539)		52	41 (78.8%)	

[a] Previous COVID-19 exposure defined by concurrently positive antibody titre to COVID-19 nucleocapsid antigen (≥1.4 IU/mL = Anti-N Positive), as per assay manufacturers.

[b] A-A-M-M [two adenoviral vector-based followed by two mRNA-based vaccines] regimen, compared to M-M-M-M [four mRNA-based vaccines] regimen.

[c] Correlation between peripheral blood immunoglobulin counts / lymphocyte subsets and vaccination response in post-4th samples.

[d] Concurrent myeloma disease control (defined by International Myeloma Working Group (IMWG) classification of therapy response) or antimyeloma therapy at time of 4th dose. CR = complete response; VGPR = very good partial response; PR = partial response; BCMA = B-cell maturation antigen targeting agents.

Supplementary Table 3: Cohort comparison to selection of previous reports internationally.
MM = multiple myeloma; SMM = smouldering multiple myeloma.

Study	Population	Doses	≥1 AAV vector-based vaccine	Multivariate predictors of poor humoral/cellular response
Aleman 2022 (Sinai, USA) [1]	MM (n=436) SMM (n=40)	2,3	0% (mRNA-only)	[no multivariate analysis]
Azeem 2023 (Emory, USA) [2]	MM (n=331)	2,3	0% (mRNA-only)	Lack of prior COVID-19 exposure; low total IgG; >2 prior lines of therapy; anti-BCMA therapy
Terpos 2022 (Greece) [3]	MM (n=167)	3	0% (mRNA-only)	Low post-2nd titre; anti-BCMA therapy
Keppler-Hafkemeyer 2023 (Germany) [4]	MM (n=22) Lymphoma (n=38)	1,2,3	13%	[no multivariate analysis]
Mancuso 2023 (Italy) [5]	MM=102	1,2,3	0% (mRNA-only)	Lack of T-cell response; not achieving complete response; anti-CD38 or proteasomal inhibitor therapy
Agarwal 2023, present study (UK)	**MM (n=330)**	**2,3,4**	**59%**	**Lack of prior COVID-19 exposure; progressive/relapsed disease; anti-CD38/BCMA therapy; low total lymphocyte count; low serum IgM**

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5. Mancuso K, Zamagni E, Solli V, et al. Long term follow-up of humoral and cellular response to mRNA-based vaccines for SARS-CoV-2 in patients with active multiple myeloma. Front Oncol. 2023;13.

Legend for myeloma datasheet file:

- **Sex**: participant-reported sex
- **Ethnicity**: participant-reported ethnicity
- **Dose{2,3,4}_imwg**: Participant-reported International Myeloma Working Group (IMWG) therapy response group at time of each dose; CR=complete response; VGPR = very good partial response; PR = partial response
- **Dose{2,3,4}_chemo**: Participant-reported concurrent chemotherapy at time of each dose; Dara/BCMA = daratumumab or B-cell maturation antigen targeting agent; other Tx = all other treatment; None = no treatment
- **Vacc{1,2,3,4}_brand**: Participant-reported vaccination platform at time of each dose; AZ = AstraZeneca adenoviral vector based; PZ = Pfizer mRNA-based; MD = Moderna mRNA-based
- **Vacc{2,3,4}_cohort**: Indicates whether participant provided peripheral blood sample after each dose
- **Sample{2,3,4}_date**: Date of blood sample at dose {2,3,4}
- **Dose{2,3,4}_antiS**: COVID-19 Anti-Spike antibody titre [IgG serology only], measured by turbimetry (Abbott); assay value: 0 - 40,000 IU/mL
- **Dose{2,3,4}_antiN**: COVID-19 Anti-Nucleocapsid antibody titre [IgG serology only], measured by turbimetry (Abbott); value >1.4 IU/mL taken as indicative of prior natural COVID-19 exposure, as per kit manufacturer's instructions.
- **Dose{2,3,4}_tspot_S**: COVID-19 spike antigen specific effector T-cells (interferon gamma-releasing cells/106 PBMCs) [Oxford Immotec T IGRA]; assay value: 0 = negative response; 8-50 = positive response.
- **Dose{2,3,4}_tspot_N**: COVID-19 nucleocapsid antigen specific effector T-cells (interferon gamma-releasing cells/106 PBMCs) [Oxford Immotec T IGRA]; assay value: 0 = negative response; 8-50 = positive response.
- **Dose4_{IgG,IgA,IgM}**: Peripheral blood immunoglobulin G, A and M measurements at time of the post-4th dose sample
- **Dose4_{lymph,Tcell,CD4,CD8,Bcell,NKcell}_count**: Peripheral blood immunoglobulin G, A and M measurements at time of the post-4th dose sample
- **Dose{2,3,4}_age**: Participant-reported age at time of dose 2,3,4
- **Sample{2,3,4}_days_lastvacc**: Days between peripheral blood sample and previous COVID-19 vaccination date (as reported by participants).
- **Dose{2,3,4}_months_diagnosis**: Months between multiple myeloma diagnosis date and dose 2,3,4 of COVID-19 vaccination