haematologica
Journal of the Ferrata Storti Foundation

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

by Gaurav Agarwal, Sally Moore, Ross Sadler, Sherin Varghese, Alison Turner, Lucia Y. Chen, Jemma Larham, Nathanael Gray, Oluremi Carty, Joe Barrett, Constantinos Koshiaris, Jaimal Kothari, Stella Bowcock, Udo Oppermann, Vicky Gamble, Gordon Cook, Chara Kyriakou, Mark Drayson, Supratik Basu, Sarah McDonald, Shelagh McKinley, Sarah Gooding, Muhammad K. Javaid, and Karthik Ramasamy

Received: September 17, 2023.
Accepted: January 17, 2024.

Citation: Gaurav Agarwal, Sally Moore, Ross Sadler, Sherin Varghese, Alison Turner, Lucia Y. Chen, Jemma Larham, Nathanael Gray, Oluremi Carty, Joe Barrett, Constantinos Koshiaris, Jaimal Kothari, Stella Bowcock, Udo Oppermann, Vicky Gamble, Gordon Cook, Chara Kyriakou, Mark Drayson, Supratik Basu, Sarah McDonald, Shelagh McKinley, Sarah Gooding, Muhammad K. Javaid, and Karthik Ramasamy. Longitudinal dynamics and clinically available predictors of poor response to COVID-19 vaccination in multiple myeloma.
Haematologica. 2024 Jan 25. doi: 10.3324/haematol.2023.284286 [Epub ahead of print]

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

Running Title: Longitudinal COVID-19 vaccine response in myeloma

Gaurav Agarwal*[1], Sally Moore*[2], Ross Sadler[3], Sherin Varghese[3], Alison Turner[4], Lucia Y Chen[3], Jemma Larham[3], Nathanael Gray[4], Oluremi Carty[3], Joe Barrett[4], Constantinos Koshiaris[5], Jaimal Kothari[3], Stella Bowcock[6], Udo Oppermann[4], Vicky Gamble[4], Gordon Cook[7], Chara Kyriakou[8], Mark Drayson[9], Supratik Basu[10,11], Sarah McDonald[12], Shelagh McKinley[13], Sarah Gooding[3,14], Muhammad K Javaid[#4] and Karthik Ramasamy[#3]

* GA and SM contributed equally as co-first authors
# MKJ and KR contributed equally as co-senior authors

[1]Division of Haematology/Oncology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA
[2]Bath Royal United Hospitals, Bath, UK
[3]Oxford University Hospitals NHS Trust, Oxford, UK
[4]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[5]Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, UK
[6]King's College Hospital NHS Trust, London, UK
[7]Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, UK
[8]University College London Hospitals NHS Trust, London, UK
[9]University of Birmingham, Birmingham, UK
[10]The Royal Wolverhampton NHS Trust, Wolverhampton, UK
[11]University of Wolverhampton, Wolverhampton, UK
[12]Blood Cancer UK, London
[13]Myeloma UK, Edinburgh, UK
[14]MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine

Keywords: myeloma, COVID-19, vaccination, longitudinal, response, predictors

Corresponding Author:
Karthik Ramasamy
Email: karthik.ramasamy@ndorms.ox.ac.uk
Tel: +441865235882

Acknowledgements / Sources of Funding

The work was supported by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC). Funding for this study has been received from Blood Cancer Vaccine Consortium, Myeloma UK and Janssen UK. RUDY platform has been funded by NIHR.

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Data sharing statement: Data are available on request to the corresponding author.

Author Contributions

All listed authors made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; and drafting the work or revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. KR is the guarantor and accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

Concept and design: MKJ, KR, RS, SG, SM, SMcD and SMcK
Acquisition, analysis, or interpretation of data: All authors
Drafting of the manuscript: GA, SM, RS, LYC, SV, StB, ChK, MD, SuB, SG, MKJ and KR
Critical revision of the manuscript for important intellectual content: All authors
Statistical analysis: GA, CoK and MKJ
Obtained funding: KR and MKJ
Administrative, technical, or material support: VG, JB, OC, SV, AT and NG
Supervision: MKJ and KR

Disclosures

All authors completed the ICMJE disclosure form. The following personal or financial relationships relevant to this manuscript existed during the conduct of the study. LYC reports funding from International Myeloma Society Career Development Award. NG reports grant from Kyowa Kirin, EU commission, MRC and NIHR and is salaried by Jansen and Amgen for this project. UO reports grant from GSK and BMS. ChK reports non restricted Educational Grant for research project in QOL from Celgene/BMS. MD reports shares in Abingdon Health. SMcK report being salaried by Myeloma UK. SG reports grant from Cancer Research UK, Innovate UK (UKRI) and Bristol Myers Squibb; SG also reports honoraria from American Society of Haematology. KMJ reports institutional grant support from Amgen. KR reports honoraria, research grant from Janssen, Celgene, Takeda and Amgen. He also reports advisory board from Celgene, Takeda, Janssen, Amgen, Abbvie, Sanofi, Oncopeptides, Karyopharm, GSK, Adaptive biotech, Pfizer and speaker's bureau from Celgene, Takeda and Adaptive Biotech. GA, SM, RS, SV, AT, JL, OC, JB, CoK, JK, StB, VG, GC, SuB and SMcD report no conflict of interests.

MM patients suffered from high mortality during the initial waves of the COVID-19 pandemic[1]. Functional studies revealed an attenuated immune response to COVID-19 infection and vaccination in MM[2], with many patients remaining seronegative and at elevated risk of breakthrough infections and severe COVID-19[3,4]. Waning of immune response is well documented, but little is known about the evolution of vaccination response following successive doses and predictors of persistently poor response after four doses. Here, we report results of a longitudinal prospective observational study that measured COVID-19 vaccination responses after doses two, three and four in a UK population of MM patients.

The study was based on the Rare UK Diseases Study (RUDY) platform (LREC 14/SC/0126 & RUDY LREC 17/SC/0501) - an established online rare disease platform with dynamic consent and participant entered data - and approved by South Central / Berkshire B Research Ethics Committee. MM patients were recruited between May 2021 to September 2022. Participants self-reported clinical details, including COVID-19 vaccination doses and dates, MM disease control [by International Myeloma Working Group (IMWG) response classification) and anti-myeloma therapy at time of each dose. Participants provided serum, EDTA and heparin blood samples ≥3 weeks following dose two, three and four. Collected serum samples were analysed for COVID-19 spike (S) and nucleocapsid (N) antibodies [IgG serology only] by turbidimetry (Abbott), as previously described[2,5]. Samples producing values >50 IU/mL and >1.4 IU/mL were considered a positive result, respectively; the assay was bound by a maximum value of 40,000 IU/mL. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinised samples; lymphocyte subsets were determined by immunophenotyping, and an interferon gamma release assay (Oxford Immunotec T IGRA) was used to quantify COVID-19 specific effector T cells (separately against S and N antigens), as per manufacturer's instructions. Positive results were defined as >8 interferon gamma-releasing cells/10^6 PBMCs; the assay was bound by a maximum value of 50 normalised counts.

141 patients provided three longitudinal samples ≥3 weeks following doses two (n=241), three (n=240) and four (n=229) (**Supp Table 1**). The median time between last vaccination and sample collection was longer after dose four at 105 days (vs. 66 days post-2nd and 70 days post-3rd doses) [p<0.0001]. Prior exposure to natural COVID-19 infection (Anti-N seropositivity) was more common after 4th dose (12.7%) compared to earlier doses (2.9-4.6%) [p<0.0001]. More patients received an adenoviral vector based vs. mRNA-based vaccine as their 2nd (48.1% vs. 35.3%) dose; however, mRNA-based vaccines comprised the majority of 3rd (93.3%) and 4th (95.6%) doses [p<0.0001]. At 4th dose, 41.9% of patients reported complete response (CR) or very good partial response (VGPR), and 17.5% were receiving anti-CD38/BCMA-targeting agents.

Patients with three serial samples were analysed for antibody titres (n=138) and T-cell IGRA counts (n=61) against COVID-19 spike (S) and nucleocapsid (N) antigens. Median Anti-S antibody titres increased between post-2nd (1,058 IU/mL; 93% seropositive) to post-3rd (5,954 IU/mL; 96% seropositive), and post-3rd to post-4th (10,995 IU/mL; 98% seropositive) doses [p<0.0001] (**Fig 1A**). Positive T-cell IGRA to S-antigen was observed in 62%, 56% and 70% of patients following doses two, three and four, respectively (**Fig 1B**). When examining the effect of booster doses, patients in the bottom quartile of Anti-S response after two doses had a robust increase after booster doses (median 98 vs. 4,218 IU/mL) [p=0.0013] albeit with lower titres than those in the top quartile [p<0.0001] (**Fig 1C**). Similarly, patients in the top 50% of T-IGRA response after two doses maintained stronger IGRA count values than the lower 50% after 3rd (mean 10 vs. 22) [p=0.0244] and 4th (mean 13 vs. 29) [p=0.0012] doses (**Fig 1D**). These findings support the benefit of booster doses in augmenting immunity but illustrate considerable variability within the MM patient cohort.

We then explored how response associated with factors related to vaccination. Firstly, patients with a concurrent humoral response to prior natural COVID-19 exposure (Anti-N seropositivity) had greater Anti-S titres (**Fig 2A**) [p<0.0001] after doses 2-4, respectively. Secondly, Anti-S titres were greater in those with a concurrently positive T-IGRA response after doses 2-4 [p<0.0001] (**Fig 2B**), suggesting a possible relationship between strength of humoral and cellular response. Thirdly, a greater proportion of patients achieved positive T-IGRA responses following the A-A-M-M (two adenoviral vector-based

followed by two mRNA-based vaccines) regimen compared with the M-M-M-M [four mRNA-based vaccines] regimen after doses 2-4 [$p<0.001$] (**Fig 2C**), suggesting a stronger T-cell response in patients who had received heterologous vaccine platforms.

Next, we examined clinical factors associated with response. IgG Anti-S titres, following dose 4, were positively correlated with total serum IgM [Spearman's $r=0.39$, $p<0.0001$] (**Fig 2D**), and serum IgA [Spearman's $r=0.36$, $p<0.0001$], but not with IgG [$p>0.05$]. Following 4th dose, T-cell IGRA counts were positively correlated with peripheral total lymphocyte count [Spearman's $r=0.35$, $p<0.0001$], CD4 [$r=0.33$, $p<0.0001$], CD8 [$r=0.32$, $p<0.0001$] and natural killer (NK) [$r=0.27$, $p=0.0006$] subsets (**Supp Table 2**). When assessing disease control and chemotherapy, patients achieving CR/VGPR at time of dose four had greater median Anti-S titres (24,278 IU/mL) than those with PR/stable (9,669 IU/mL) [$p<0.01$] or progressive/relapsed (3,530 IU/mL) disease [$p<0.0001$] (**Fig 2E**); all Anti-S seronegative patients had relapsed disease ($n=4$). Patients receiving anti-CD38 or BCMA-targeting agents at 4th dose had lower Anti-S titres (median 6,157 IU/mL) than those receiving other chemotherapy agents (median 16,102 IU/mL) [$p<0.05$] or no treatment (17,578 IU/mL) [$p<0.05$] (**Fig 2E**). Similarly, patients with progressive/relapsed disease or those receiving anti-CD38/BCMA-targeting agents at 4th dose had the lowest proportion achieving a positive T-cell IGRA (53.1% and 52.0%, respectively) (**Fig 2F**). Collectively, these analyses highlight immune and disease markers associated with variable vaccination-induced immunity after four doses.

Finally, multivariate analysis identified independent predictors of persistently poor response after four doses (**Table 1**). Poor cellular response was defined by negative T-cell IGRA (below the manufacturer's recommended cut-off). As few patients had an Anti-S titre <50 IU/mL (assay positive cut-off), the World Health Organisation (WHO) threshold was used to define poor humoral response [7,352 IU/mL], as specified by the assay manufacturer. After 4th dose, patients with Anti-N seropositivity were less likely to have low Anti-S [$p=0.0011$]. Those with progressive/relapsed disease were more likely (vs. CR/VGPR) to have low Anti-S titres [adjusted OR 5.1, 95% CI=2.1-13.5, $p=0.0006$]. At borderline significance, patients taking anti-CD38 or BCMA-targeting agents at 4th dose were more likely to have negative T-cell IGRA [adjusted OR 3.2, 95% CI=1.0-10.7, $p=0.052$]. Patients who had received the A-A-M-M vaccine regimen were less likely to have negative T-cell IGRA in univariate [OR 0.42, 95% CI=0.19-0.93, $p=0.033$] but not multivariate [$p>0.05$] analysis. With every $1.0x10^9$/L increase in total lymphocyte count the odds of negative T-cell IGRA reduced [adjusted OR=0.26, 95% CI=0.11-0.54, $p=0.0007$], and for every 0.1g/L increase in serum IgM count the odds of low Anti-S titre also reduced [adjusted OR 0.65, 95% CI=0.53-0.79, $p<0.0001$]. These findings represent clinical predictors of ongoing poor vaccine response after four doses in MM patients.

In this study, we report a longitudinal analysis of immune response following COVID-19 vaccinations in MM patients and describe clinically available predictors of poor response after 4th dose. Relative to other cohorts[6] (**Supp Table 3**), our dataset has three main novelties. Firstly, we follow a large UK-wide cohort prospectively to understand how immunity evolves longitudinally. Secondly, our cohort received a mix of mRNA and adenoviral vector-based platforms (differing from most studies that have studied exclusively mRNA-based vaccine response)[6]. Thirdly, we report novel routinely available predictors of poor response after four doses.

We confirm reported clinical associations with poor response to earlier doses (lack of prior natural infection, poor disease control, anti-CD38/BCMA therapy) hold true after 4th dose. By univariate analyses vaccination with two adenoviral vector-based and two mRNA-based vaccines resulted in stronger T-cell IGRA responses compared to four mRNA-based vaccines. This is consistent with stronger immunogenicity shown with heterologous regimens in the general population[7–10] and other MM patient cohorts[11–13]. Multivariate analysis identified lower serum IgM as an independent predictor of low Anti-S titre after 4th dose, supporting an observation described after two doses[12]. Low total lymphocyte counts predicted lack of cellular response; a similar association is noted in patients with multiple sclerosis after COVID-19 vaccination[14].

There are some limitations to our analysis. Firstly, Anti-S and T-cell IGRA assays had maximum values (40,000 IU/mL and 50 normalised counts, respectively), limiting predictive power as stronger responses were not distinguished. Secondly, although Anti-S and T-IGRA values defining a positive antibody or T-cell response were based on historically established thresholds, the absolute values that correlate with clinical protection from COVID-19 remains unclear. Thirdly, current Omicron variants of concern (VOCs) have changed; however, a recent report has found that in heavily treated MM patients, multiple doses of vaccine-induced IgG Anti-S antibody cross-reacted well with a range of variants[15]. Therefore, our findings remain relevant to all MM patients in the present climate with current VOCs.

In conclusion, our study establishes the serial evolution of humoral and cellular immunity across doses 2-4 of COVID-19 vaccination in MM patients. Our data support the benefit of booster vaccination in augmenting robust COVID-19 immunity in MM. Additionally, we establish routinely available laboratory and clinical predictors of ongoing poor response after four doses, potentially enabling identification of vulnerable patients to target for booster doses or novel interventions to enhance immunity.

References

1. Chari A, Kemal Samur M, Martinez-Lopez J, et al. Clinical features associated with COVID-19 outcome in multiple myeloma: first results from the International Myeloma Society data set. Blood. 2020;136(26):3033-3040.
2. Ramasamy K, Sadler R, Jeans S, et al. Immune response to COVID-19 vaccination is attenuated by poor disease control and antimyeloma therapy with vaccine driven divergent T-cell response. Br J Haematol. 2022;197(3):293-301.
3. Aleman A, Upadhyaya B, Tuballes K, et al. Variable cellular responses to SARS-CoV-2 in fully vaccinated patients with multiple myeloma. Cancer Cell. 2021;39(11):1442-1444.
4. Van Oekelen O, Gleason CR, Agte S, et al. Highly variable SARS-CoV-2 spike antibody responses to two doses of COVID-19 RNA vaccination in patients with multiple myeloma. Cancer Cell. 2021;39(8):1028-1030.
5. Ramasamy K, Sadler R, Jeans S, et al. COVID symptoms, testing, shielding impact on patient-reported outcomes and early vaccine responses in individuals with multiple myeloma. Br J Haematol. 2022;196(1):95-98.
6. Chuleerarux N, Manothummetha K, Moonla C, et al. Immunogenicity of SARS-CoV-2 vaccines in patients with multiple myeloma: a systematic review and meta-analysis. Blood Adv. 2022;6(24):6198-6207.
7. Schmidt T, Klemis V, Schub D, et al. Immunogenicity and reactogenicity of heterologous ChAdOx1 nCoV-19/mRNA vaccination. Nat Med. 2021;27(9):1530-1535.
8. Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. Lancet. 2021;398(10295):121-130.
9. Andrews N, Stowe J, Kirsebom F, et al. Covid-19 Vaccine Effectiveness against the Omicron (B.1.1.529) Variant. N Engl J Med. 2022;386(16):1532-1546.
10. Naranbhai V, Garcia-Beltran WF, Chang CC, et al. Comparative Immunogenicity and Effectiveness of mRNA-1273, BNT162b2, and Ad26.COV2.S COVID-19 Vaccines. J Infect Dis. 2022;225(7):1141-1150.
11. Goldwater MS, Stampfer SD, Sean Regidor B, et al. Third dose of an mRNA COVID-19 vaccine for patients with multiple myeloma. Clin Infect Pract. 2023;17:100214.
12. Stampfer SD, Goldwater MS, Jew S, et al. Response to mRNA vaccination for COVID-19 among patients with multiple myeloma. Leukemia. 2021;35(12):3534-3541.
13. Storti P, Marchica V, Vescovini R, et al. Immune response to SARS-CoV-2 mRNA vaccination and booster dose in patients with multiple myeloma and monoclonal gammopathies: impact of Omicron variant on the humoral response. Oncoimmunology. 2022;11(1):2120275.
14. Tortorella C, Aiello A, Gasperini C, et al. Humoral- and T-Cell-Specific Immune Responses to SARS-CoV-2 mRNA Vaccination in Patients With MS Using Different Disease-Modifying Therapies. Neurology. 2022;98(5):e541-e554.
15. Faustini SE, Hall A, Brown S, et al. Immune responses to COVID-19 booster vaccinations in intensively anti-CD38 antibody treated patients with ultra-high-risk multiple myeloma: results from the Myeloma UK (MUK) nine OPTIMUM trial. Br J Haematol. 2023;201(5):845-850.

Table 1: Independent predictors of persistently poor COVID-19 vaccination-induced immunity in MM patients.
Two separate binary logistic regression models were developed. Low titre is defined as COVID-19 anti-spike (Anti-S) antibody titre below World Health Organisation (WHO) cut-off threshold of 7,352 IU/mL, as per kit assay manufacturer. *N*=85 low Anti-S vs. *N*=140 high Anti-S. *N*=49 negative T-cell interferon gamma releasing assay (IGRA) vs. *N*=117 positive T-cell IGRA to COVID-19 spike antigen.

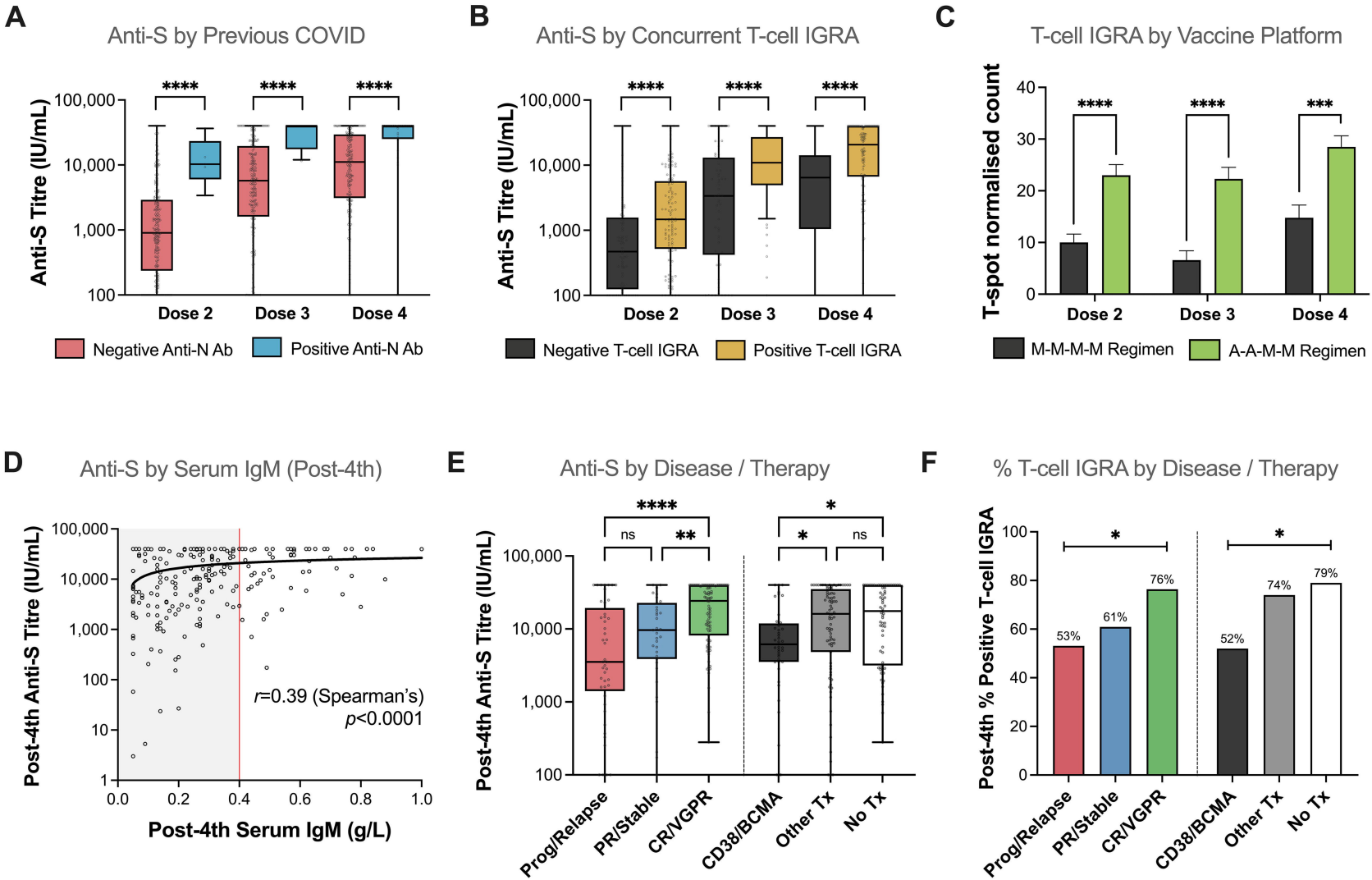
	Predictors of Low Anti- Titre						**Predictors of Negative T-cell IGRA**					
Factor	**Unadjusted Model**			**Adjusted Model**			**Unadjusted Model**			**Adjusted Model**		
	OR	**CI**	**p**	**OR**	**CI**	**p**	**OR**	**CI**	**p**	**OR**	**CI**	**p**
Age [per year increase]	0.99	0.97-1.02	0.687	0.99	0.95-1.02	0.455	1.03	0.99-1.08	0.112	1.02	0.98-1.08	0.337
Male sex [vs. female sex]	0.75	0.43-1.28	0.288	0.70	0.35-1.39	0.308	**2.15**	**1.08-4.46**	**0.034**	1.83	0.82-4.18	0.145
A-A-M-M vaccines [vs. M-M-M-M] [a]	1.08	0.58-2.03	0.802	1.27	0.60-2.70	0.531	**0.42**	**0.19-0.93**	**0.033**	0.50	0.20-1.26	0.142
PR/stable disease [vs. CR/VGPR] [b]	2.09	0.91-4.76	0.080	1.78	0.69-4.63	0.232	2.08	0.75-5.64	0.151	2.05	0.62-6.70	0.234
Progressive/relapse [vs. CR/VGPR] [b]	**4.70**	**2.22-10.21**	**0.00007**	**5.11**	**2.06-13.46**	**0.0006**	**2.85**	**1.18-6.98**	**0.020**	**2.52**	**0.91-7.12**	**0.076**
Anti-CD38/BCMA Tx [vs. No Tx] [c]	**2.96**	**1.33-6.78**	**0.008**	0.88	0.32-2.43	0.808	**3.44**	**1.24-9.85**	**0.019**	**3.19**	**1.00-10.65**	**0.052**
Other treatment [vs. No Tx] [c]	1.18	0.59-2.35	0.642	0.52	0.21-1.23	0.141	1.30	0.54-3.22	0.562	0.60	0.19-1.82	0.365
Anti-N seropositivity [d]	**0.10**	**0.02-0.35**	**0.002**	**0.07**	**0.01-0.27**	**0.0011**						
Serum IgM	**0.66**	**0.55-0.77**	**0.00002**	**0.65**	**0.53-0.79**	**0.00005**						
Positive T-cell IGRA [e]	0.78	0.39-1.56	0.470	0.51	0.21-1.23	0.137						
Total lymphocyte count							**0.28**	**0.13-0.54**	**0.0004**	**0.26**	**0.11-0.54**	**0.0007**

* Prior cellular response against natural COVID-19 infection (suggested by positive Anti-N IGRA) showed perfect prediction for T-cell IGRA negativity [*p*=0.002] and so were not included in the multivariate analyses due to the model not converging.
[a] 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.
[b] Myeloma disease control at time of 4th dose, defined by International Myeloma Working Group (IMWG) classification of therapy response; CR = complete response; VGPR = very good partial response; PR = partial response.
[c] Concurrent antimyeloma therapy at time of 4th dose; BCMA = B-cell maturation antigen targeting agents; No Tx = no treatment.
[d] Anti-N seropositivity indicative of prior natural COVID-19 exposure; effect compared to those who are Anti-N seronegative.

Figure 1: Longitudinal immune responses to four COVID-19 vaccinations in MM patients.
(A) Longitudinal change in Anti-S antibody titres in uniform cohort of *N*=138 patients providing three serial samples ≥3 weeks following doses 2-4. Kruskal-Wallis with Dunn's multiple comparison test, * $P < 0.05$, **** $P < 0.0001$.
(B) Sankey diagram showing longitudinal change in T-cell IGRA positivity (normalised T-cell IGRA count ≥8) in uniform cohort of *N*=61 patients with three serial T-cell assays following doses 2-4.
(C) Longitudinal Anti-S titres in patients stratified into four Anti-S quartiles following second dose (Q1 = bottom 25%; Q4 = top 25%) and prospectively followed after doses three and four. Mean ± SEM, *N*=138 total.
(D) Longitudinal normalised T-cell IGRA count to S antigen in patients stratified as top 50% (*N*=31) or bottom 50% (*N*=30) of T-cell IGRA following second dose and prospectively followed after doses three and four. Mean ± SEM.

Figure 2: Vaccine and patient factors associated with variable immune response.
(A) Anti-S titre in patients with vs. without serological evidence of previous COVID-19 infection (defined by Anti-N antibody titre ≥1.4 IU/mL), longitudinally after doses two (*N*=232 vs. 7), three (*N*=227 vs. 11) or four (*N*=196 vs. 29). Mann-Whitney test, **** $P < 0.0001$.
(B) Anti-S titre in patients with concurrently negative vs. positive T-cell IGRA, longitudinally after doses two (*N*=77 vs. 112), three (*N*=64 vs. 79) or four (*N*=48 vs. 115). Mann-Whitney test, **** $P < 0.0001$.
(C) T-cell IGRA (normalised counts) to S antigen between cohorts of patients receiving the M-M-M-M [four mRNA-based vaccines] vs. A-A-M-M [two adenoviral vector-based followed by two mRNA-based vaccines] regimens, longitudinally after doses two (*N*=65 vs. 94), three (*N*=51 vs. 72) or four (*N*=49 vs. 88). Mean ± SEM, Mann-Whitney test, *** $P < 0.001$, **** $P < 0.0001$.
(D) Relationship between IgG Anti-S titre and total serum IgM after fourth dose. *N*=225, Spearman's Rank correlation coefficient displayed.
(E+F) Anti-S titre (E) or % positive T-cell IGRA (F) following fourth dose, by concurrent MM disease control (IMWG classification of therapy response) or concurrent anti-myeloma therapy. Prog = progressive; PR = partial response; VGPR = very good partial response; CR = complete response; Tx = treatment. Ns = not significant, * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.


A
Serial Antibody Titres
****
****
*
Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
10
1
0.1
Dose 2
Dose 3
Dose 4
B
Serial T-cell IGRA
DOSE
2
3
4
POSITIVE
62%
56%
70%
n=61
38
23
25
13
9
14
28
6
15
12
NEGATIVE
38%
44%
30%
C
Post-2nd Stratified Antibody Titres
Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
Dose 2
Dose 3
Dose 4
Post-2nd
Q4
Q3
Q2
Q1
D
Post-2nd Stratified T-cell IGRA
T-spot normalised count
50
40
30
20
10
0
Dose 2
Dose 3
Dose 4
Post-2nd
Top 50%
Bottom 50%


A
Anti-S by Previous COVID
Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
****
****
****
Dose 2
Dose 3
Dose 4
Negative Anti-N Ab
Positive Anti-N Ab
B
Anti-S by Concurrent T-cell IGRA
Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
****
****
****
Dose 2
Dose 3
Dose 4
Negative T-cell IGRA
Positive T-cell IGRA
C
T-cell IGRA by Vaccine Platform
T-spot normalised count
40
30
20
10
0
****
****
***
Dose 2
Dose 3
Dose 4
M-M-M-M Regimen
A-A-M-M Regimen
D
Anti-S by Serum IgM (Post-4th)
Post-4th Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
10
1
0.0
0.2
0.4
0.6
0.8
1.0
r=0.39 (Spearman's)
p<0.0001
Post-4th Serum IgM (g/L)
E
Anti-S by Disease / Therapy
Post-4th Anti-S Titre (IU/mL)
100,000
10,000
1,000
100
****
ns
**
*
*
ns
Prog/Relapse
PR/Stable
CR/VGPR
CD38/BCMA
Other Tx
No Tx
F
% T-cell IGRA by Disease / Therapy
Post-4th % Positive T-cell IGRA
100
80
60
40
20
0
*
*
53%
61%
76%
52%
74%
79%
Prog/Relapse
PR/Stable
CR/VGPR
CD38/BCMA
Other Tx
No Tx

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**

1. Aleman A, Van Oekelen O, Upadhyaya B, et al. Augmentation of humoral and cellular immune responses after third-dose SARS-CoV-2 vaccination and viral neutralization in myeloma patients. Cancer Cell. 2022;40(5):441-443.
2. Azeem MI, Nooka AK, Shanmugasundaram U, et al. Impaired SARS-CoV-2 Variant Neutralization and CD8+ T-cell Responses following 3 Doses of mRNA Vaccines in Myeloma: Correlation with Breakthrough Infections. Blood Cancer Discov. 2023;4(2):106-117.
3. Ntanasis-Stathopoulos I, Karalis V, Gavriatopoulou M, et al. Second Booster BNT162b2 Restores SARS-CoV-2 Humoral Response in Patients With Multiple Myeloma, Excluding Those Under Anti-BCMA Therapy. Hemasphere. 2022;6(8):e764.
4. Keppler-Hafkemeyer A, Greil C, Wratil PR, et al. Potent high-avidity neutralizing antibodies and T cell responses after COVID-19 vaccination in individuals with B cell lymphoma and multiple myeloma. Nat Cancer. 2023;4(1):81-95.
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 on Excel 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