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

Multiple myeloma (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 4 doses. Here, we report results of a longitudinal prospective observational study that measured COVID-19 vaccination responses after doses 2, 3 and 4 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. The study was 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 2, 3 and 4. Collected serum samples were analyzed 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, respectively, were considered a positive result; the assay was bound by a maximum value of 40,000 IU/mL. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized samples; lymphocyte subsets were determined by immunophenotyping, and an interferon γ-release assay (Oxford Immunotec T IGRA) was used to quantify COVID-19 specific effector T cells (separately against S and N antigens), as per the manufacturer's instructions. Positive results were defined as >8 interferon γ-releasing cells/10^6 PBMC; the assay was bound by a maximum value of 50 normalized counts.

A total of 141 patients provided three longitudinal samples ≥3 weeks following doses 2 (N=241), 3 (N=240), and 4 (N=229) (*Online Supplementary Table S1*). The median time between last vaccination and sample collection was longer after dose 4 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 the 4th dose (12.7%) compared to earlier doses (2.9-4.6%) (*P*<0.0001). More patients received an adenoviral vector-based *versus* mRNA-based vaccine as their 2nd dose (48.1% *vs*. 35.3%); however, mRNA-based vaccines comprised the majority of 3rd (93.3%) and 4th (95.6%) doses (*P*<0.0001). At the 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 3 serial samples were analyzed for antibody titers (N=138) and T-cell IGRA counts (N=61) against COVID-19 spike (S) and nucleocapsid (N) antigens. Median anti-S antibody titers 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) (Figure 1A). Positive T-cell IGRA to S-antigen was observed in 62%, 56%, and 70% of patients following doses 2, 3 and 4, respectively (Figure 1B). When examining the effect of booster doses, patients in the bottom quartile of anti-S response after 2 doses had a robust increase after booster doses (median 98 *vs*. 4,218 IU/mL; *P*=0.0013), albeit with lower titers than those in the top quartile (*P*<0.0001) (Figure 1C). Similarly, patients in the top 50% of T-IGRA response after 2 doses maintained stronger IGRA count values than the lower 50% after the 3rd (mean 10 *vs*. 22; *P*=0.0244) and 4th (mean 13 *vs*. 29; *P*=0.0012) doses (Figure 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 was associated with factors related to vaccination. Firstly, patients with a concurrent humoral response to prior natural COVID-19 exposure (anti-N sero-positivity) had greater anti-S titers (*P*<0.0001) after doses 2-4, respectively (Figure 2A). Secondly, anti-S titers were greater in those with a concurrently positive T-IGRA response after doses 2-4 (*P*<0.0001) (Figure 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 (2 adenoviral vector-based followed by 2 mRNA-based vaccines) regimen compared with the M-M-M-M (4 mRNA-based vaccines) regimen after doses 2-4 (*P*<0.001) ((Figure 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 titers, following dose 4, were positively correlated with total serum IgM (Spearman's *r*=0.39, *P*<0.0001) (Figure 2D), and serum IgA (Spearman's *r*=0.36, *P*<0.0001), but

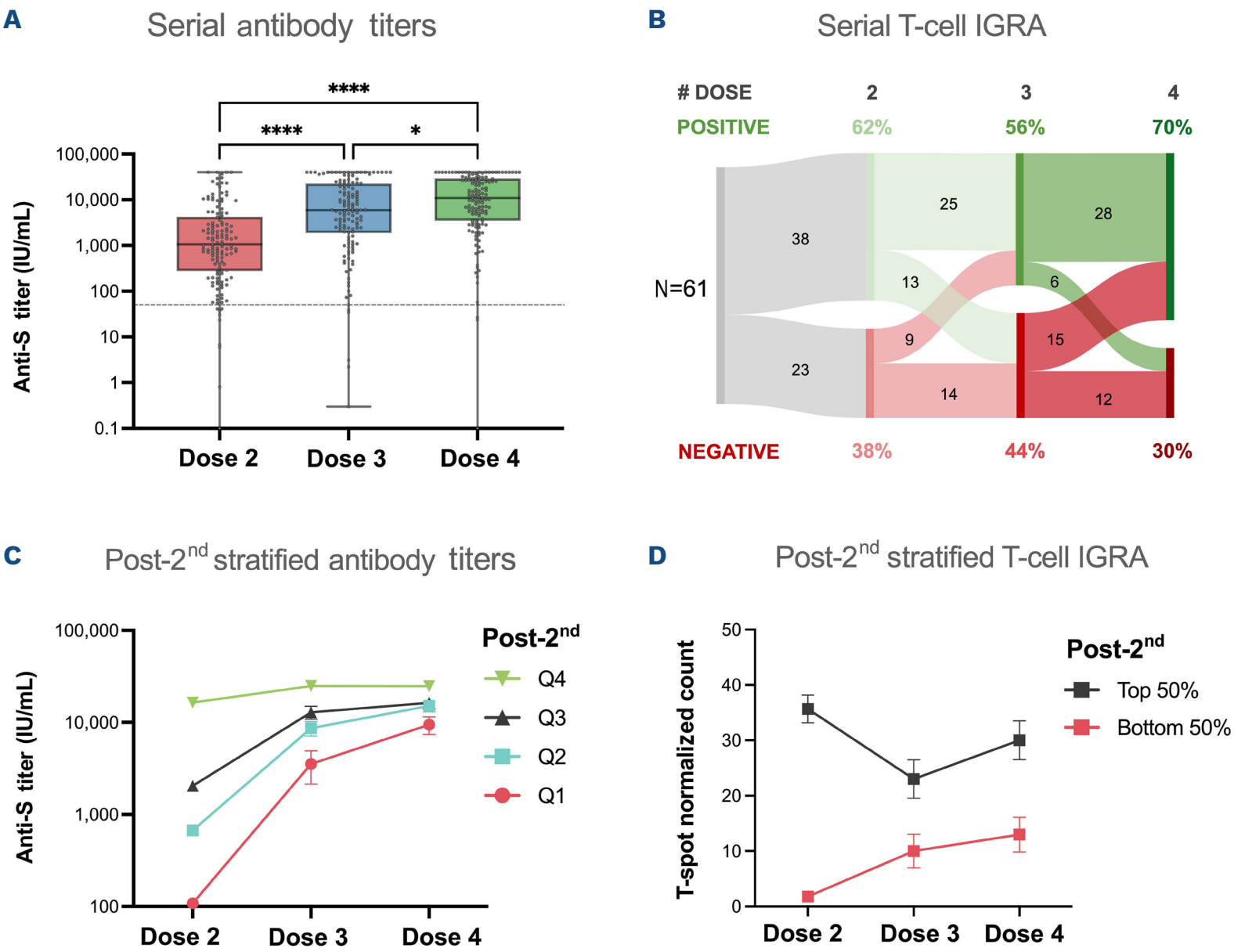

Figure 1. Longitudinal immune responses to 4 COVID-19 vaccinations in multiple myeloma patients. (A) Longitudinal change in anti-S antibody titers in uniform cohort of 138 patients providing 3 serial samples ≥3 weeks following doses 2-4. Kruskal-Wallis with Dunn's multiple comparison test, *P<0.05, ****P<0.0001. (B) Sankey diagram showing longitudinal change in T-cell interferon γ-release assay (IGRA) positivity (normalized T-cell IGRA count ≥8) in uniform cohort of 61 patients with 3 serial T-cell assays following doses 2-4. (C) Longitudinal anti-S titers in patients stratified into 4 anti-S quartiles following 2nd dose (Q1 = bottom 25%; Q4 = top 25%) and prospectively followed after doses 3 and 4. Mean ± Standard Error of Mean (SEM). N=138 total. (D) Longitudinal normalized 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 2nd dose and prospectively followed after doses 3 and 4. Mean ± SEM.

not with IgG (P>0.05). Following the 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 (*Online Supplementary Table S2*). When assessing disease control and chemotherapy, patients achieving CR/VGPR at the time of dose 4 had greater median anti-S titers (24,278 IU/mL) than those with PR/stable disease (9,669 IU/mL) (P<0.01) or progressive/relapsed (3,530 IU/mL) disease (P<0.0001) (Figure 2E); all anti-S seronegative patients had relapsed disease (N=4). Patients receiving anti-CD38 or BCMA-targeting agents at the 4th dose had lower anti-S titers (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) (Figure 2E). Similarly, patients with progressive/relapsed disease or those receiving anti-CD38/BCMA-targeting agents at the 4th dose had the lowest proportion achieving a positive T-cell IGRA (53.1% and 52.0%, respectively) (Figure 2F). Collectively, these analyses highlight immune and disease markers associated with variable vaccination-induced immunity after 4 doses.

Finally, multivariate analysis identified independent predictors of persistently poor response after 4 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 titer <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 the 4th dose, patients with anti-N seropositivity were less likely to have low anti-S (P=0.0011). Those with progressive/relapsed dis-

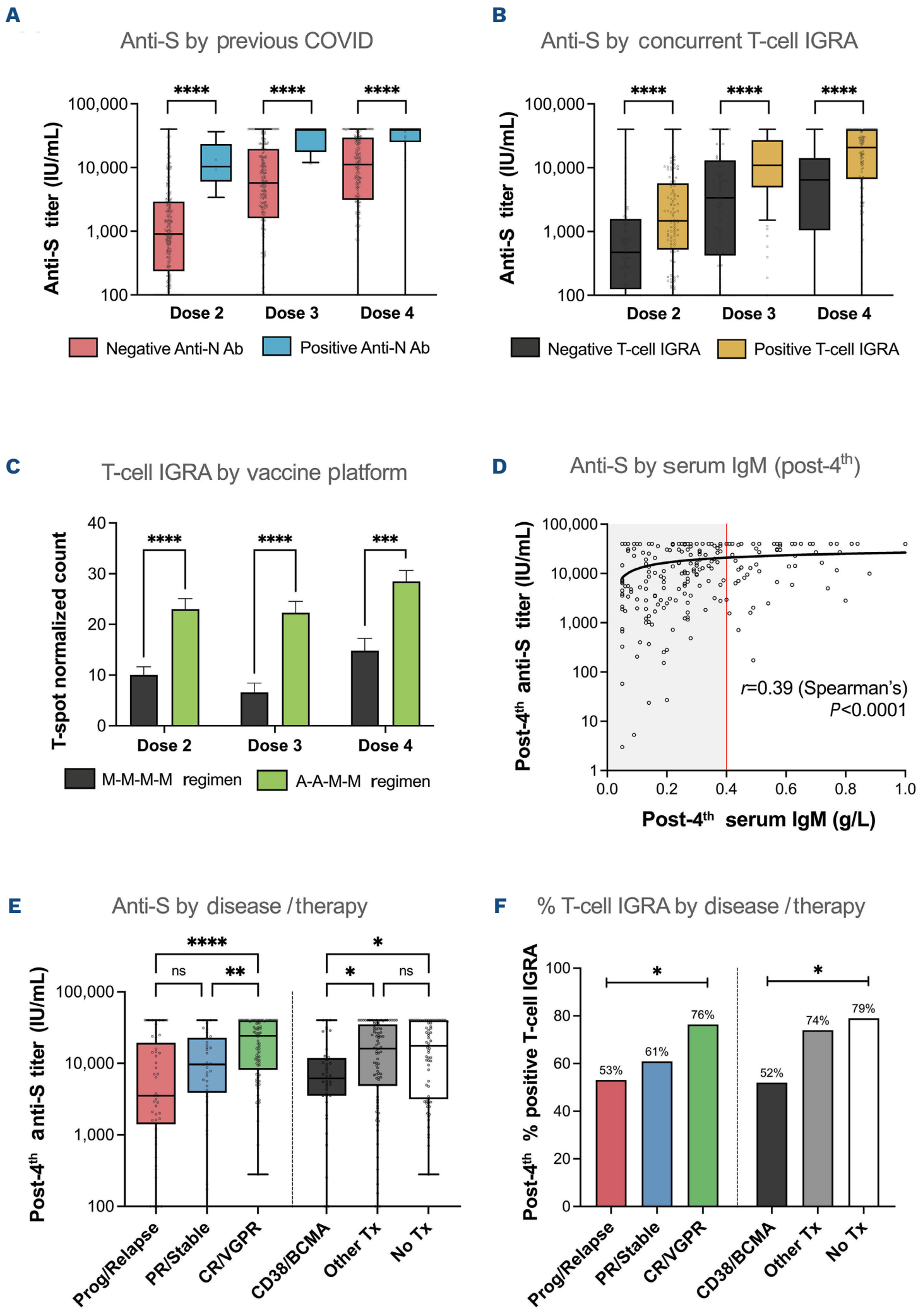

Figure 2. Vaccine and patient factors associated with variable immune response. (A) Anti-S titer in patients with *versus* without serological evidence of previous COVID-19 infection (defined by anti-N antibody titer ≥1.4 IU/mL), longitudinally after doses 2 (N=232 *vs*. 7), 3 (N=227 *vs*. 11) or 4 (N=196 *vs*. 29). Mann-Whitney test, ****P<0.0001. (B) Anti-S titer in patients with concurrently negative *versus* positive T-cell interferon γ-release assay (IGRA), longitudinally after doses 2 (N=77 *vs*. 112), 3 (N=64 *vs*. 79) or 4

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(N=48 *vs*. 115). Mann-Whitney test, ****P<0.0001. (C) T-cell IGRA (normalized counts) to S antigen between cohorts of patients receiving the M-M-M-M (4 mRNA-based vaccines) *versus* A-A-M-M (2 adenoviral vector-based followed by 2 mRNA-based vaccines) regimens, longitudinally after doses 2 (N=65 *vs*. 94), 3 (N=51 *vs*. 72) or 4 (N=49 *vs*. 88). Mean ± Standard Error of Mean (SEM), Mann-Whitney test, ***P<0.001, ****P<0.0001. (D) Relationship between IgG anti-S titer and total serum IgM after 4th dose. N=225. Spearman's Rank correlation coefficient displayed. (E and F) Anti-S titer (E) or % positive T-cell IGRA (F) following 4th dose, by concurrent multiple myeloma disease control (International Myeloma Working Group classification of therapy response) or concurrent anti-myeloma therapy. CR: complete response; Prog: progressive; PR: partial response; TX: treatment; VGPR: very good partial response. *P<0.05, **P<0.01, ****P<0.0001, ns: not significant.

Table 1. Independent predictors of persistently poor COVID-19 vaccination-induced immunity in MM patients.

Factor	Predictors of low anti-S titer						Predictors of negative T-cell IGRA					
	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	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

Two separate binary logistic regression models were developed. Low titer is defined as COVID-19 anti-spike (Anti-S) antibody titer 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 γ-release assay (IGRA) *vs*. N=117 positive T-cell IGRA to COVID-19 spike antigen. [a]A-A-M-M (2 adenoviral vector-based followed by 2 mRNA-based vaccines) regimen, compared to M-M-M-M (4 mRNA-based vaccines) regimen. [b]Myeloma disease control at time of 4th dose, defined by International Myeloma Working Group (IMWG) classification of therapy response. [c]Concurrent anti-myeloma 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. CI: Confidence Intervals; CR: complete response; OR: Odds Ratio; PR: partial response; VGPR: very good partial response.

ease were more likely (*vs*. CR/VGPR) to have low anti-S titers (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 the 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 were 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 titer were 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 4 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 the 4th dose. Relative to other cohorts[6] (*Online Supplementary Table S3*), our dataset has 3 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 4 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 the 4th dose. By univariate analyses, vaccination with 2 adenoviral vector-based and 2 mRNA-based vaccines resulted in stronger T-cell IGRA responses compared to 4 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 titer after the 4th dose, supporting an observation described after 2 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 normalized 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 remain unclear. Thirdly, current Omicron variants of concern (VOC) 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 VOC.

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 4 doses, potentially enabling identification of vulnerable patients to target for booster doses or novel interventions to enhance immunity.

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Contributions

MJK is responsible for study concept and design, drafting the manuscript, statistical analysis, obtained funding, and supervised the study. KR is responsible for study concept and design, drafting the manuscript, obtained funding, supervised the study, 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. RS is responsible for study concept and design, and drafting the manuscript. SM is responsible for study concept and design, and drafting the manuscript. SG is responsible for study concept and design and drafting the manuscript. SMcD and SmcK are responsible for study concept and design. GA is responsible for drafting the manuscript and statistical analysis. CoK is responsible for statistical analysis. LYC, SV, StB, ChK, MD and SuB are responsible for drafting the manuscript. VG, JB, OC, SV, AT and NG are responsible for administrative, technical, or material support. All authors are responsible for the acquisition, analysis, or interpretation of data, and critical revision of the manuscript for important intellectual content.

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Data-sharing statement

Data are available on request to the corresponding author.

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