Patients with Multiple Myeloma (MM) have an impaired immunity due to both the underlying disease and the myeloma treatment-inducing immunosuppression [1]. We report here a single-institution retrospective analysis of 127 consecutive patients with symptomatic MM (71 males, 56 females), medians 69.5 years (range 45–85); 63 patients had newly diagnosed MM and 64 patients had MM refractory to one or more previous treatment lines. Myeloma therapies included PI-I/MID combos, IMiD-based regimens, anti-CD38 mAb-based therapies, antibody-drug conjugates (Belantamab Mafodotin monotherapy), dexamethasone and high dose melphalan. The treatments received are summarized in Table 1. Anti-spike IgG antibodies were detected also in 50 healthy volunteers of similar age and sex, who served as controls. Patients with symptomatic MM and healthy controls received two doses of COVID-19 mRNA vaccine (Pfizer BioNTech) on days 1 and 21 between 29 April and 15 May 2021. Patients with a prior history of SARS-CoV-2 were excluded from the analysis. All MM patients promised strict compliance with the general recommendations for infection risk reduction.

Time of seroconversion is a key factor in establishing the time window for serology test usage. Zhao et al. and Okba et al. [2, 3] indicate that the median time for IgG seroconversion is between 9 and 14 days after the disease onset. Therefore, quantitative determination of anti-spike S1/S2 IgG antibody was performed at 4 weeks from vaccination completion (LIAISON® SARS-COV-2 S1/S2 IgG, LIAISON®) [4]. A threshold >15 AU/ml of anti-Spike IgG, related to the neutralizing activity of anti-SARS-COV-2 antibodies (cut off <12.0 AU/ml = negative; cut off from 12 to 15 AU/ml = non diagnostic; cut off ≥15 AU/ml = positive), had previously been established.

Primary endpoints of this analysis were the percentage of patients responding to COVID-19 mRNA vaccine (by quantifying the anti-spike antibodies titre 4 weeks after vaccination completion), the identification of factors associated with antibody response, as well as the long-term protection of the COVID-19 mRNA vaccine [5]. Chi-square and Wilcoxon’s rank-sum tests were used to compare categorical and continuous variables, respectively. P-values were considered significant if less than 0.05.

Of the 127 patients, 65 were evaluable for response. Patients with >50% of missing data were excluded from the analysis. Anti-spike IgG antibodies were detected in 50/65 (76.9%) MM patients, defined as responders [177 AU/mL (range 26.4 – 1430)], MM patients (23.1%, 15/65) who failed to respond to two doses of COVID-19 mRNA vaccine [3.8 AU/mL (range 0.65 – 9.33)] were defined as non-responders. All healthy controls (100%) responded to the vaccine [249 AU/mL (range 104 – 2430)] (Table 2). No clinically relevant side-effects were noted.

We examined the differences between the two subgroups of patients (responders vs. non-responders), stratified by their “myeloma disease status” (relapsed/refractory MM vs. newly diagnosed MM), “plasmacytosis” (bone marrow plasma cells <60% vs. bone marrow plasma cells >60%) [6, 7], serum levels of “lactate dehydrogenase” (LDH) [6, 7], serum levels of “beta2microglobulin” (B2M) [6, 7], “gamma globulin levels”, “white blood cell count” (WBC), “absolute neutrophil count” (ANC), “lymphocytes”, “haemoglobin concentration”, “age” and “sex”. Many of these parameters, predictive of myeloma outcomes, are inter-dependent. No statistically significant differences were found between the two subgroups of patients in terms of myeloma disease status, LDH, residual gammaglobulin levels, WBC, ANC, lymphocytic response, age and sex (Table 3). Conversely, plasmacytosis, B2M and haemoglobin concentration were associated with a different response to the vaccine. Twenty-nine patients (44.6%) with extreme plasmacytosis (60.0 ± 20.3 mean ± SD) (Table 3) had a mean titre of less than 15 AU/ml of anti-Spike IgG [3.8 AU/mL (range 0.65 – 9.33)], compared with 36 patients (55.4%) with low plasmacytosis (28.2 ± 18.8 mean ± SD) (Table 3); by contrast, the latter showed significantly higher antibody mean titers [177 AU/mL (range 26.4 – 1430)]. B2M was significantly higher in non-responders compared to responders (4.6 ± 4.1 vs. 3.2 ± 3.6 mean ± SD; p = 0.006) (Table 3). The haemoglobin value was significantly lower in non-responders compared to responders (10.8 ± 1.8 vs. 12.1 ± 1.8 mean ± SD; p = 0.008) (Table 3). Multivariate analysis confirmed the bone marrow infiltration pattern and haemoglobin value as statistically significant variables (Table 4). In the present
most of our patients achieved at least a partial response to MM therapy. Spike IgG amongst patients with chronic lymphocytic leukaemia [8]. with MM, has been associated with an inferior development of anti-

KRd: carfilzomib, lenalidomide, dexamethasone; Kd: carfilzomib, dexametha
thalidomide, dexamethasone; DVd: daratumumab, bortezomib, dexametha
sone; R Maintenance: lenalidomide; VMP: bortezomib, melphalan, prednisone;
EloRd: elotuzumab, lenalidomide, dexamethasone.

- experience, no differences were found when comparing type and num-
ber of myeloma specific treatments.

In conclusion, we identified extreme plasmacytosis (p < 0.001), B2M (p = 0.006), and haemoglobin concentration (p = 0.008) as the main predictive factors for non-response. The reduced serological response might confirm the possible primary role of extensive plasma cell bone marrow infiltration. In our analysis, there was no statistically significant difference in terms of response to vaccine between patients with relapsed/refractory MM compared to patients with newly diagnosed MM, confirming the extreme importance of the clonal plasma cells rate in the bone marrow, regardless of other prognostic factors. Immunopa-
resis during the disease course, which may also be major in patients with MM, has been associated with an inferior development of anti-

Spike IgG amongst patients with chronic lymphocytic leukaemia [8]. Terpos [9] and Stamper [5] indicate that also advanced age is responsible for a reduced immune response and suggest that the administration of a second timely vaccine dose is essential to develop an adequate anti-Spike IgG titre. Unexpectedly, our data indicated that the reduced levels of uninvolved immunoglobulins and age, that are negative prog-
nostic indicators of outcomes in MM, did not affect the response to the COVID-19 vaccine. Anti-CD-38 and anti-SLAMF7 monoclonal antibo-
dies, as well as BCMA-targeting agents, have a B-cell-depleting ac
tivity and may impede seroconversion [10]. In this cohort, in line with

Table 1

| Characteristic | All patients (n = 64) | Non-Responders (n = 15) | Responders (n = 49) | p-value for comparison |
|---------------|----------------------|------------------------|---------------------|-----------------------|
| Sex           |                      |                        |                     |                       |
| Female        | 32 (50.0)            | 8 (53.3)               | 24 (48.9)           | 0.77                  |
| Male          | 32 (50.0)            | 7 (46.7)               | 25 (51.0)           |                       |
| Age           |                      |                        |                     |                       |
| <60           | 12 (18.7)            | 2 (13.3)               | 10 (20.4)           |                       |
| 60–69         | 14 (21.9)            | 2 (13.3)               | 12 (24.5)           |                       |
| 70–79         | 29 (45.3)            | 7 (46.7)               | 22 (44.9)           |                       |
| ≥80           | 9 (14.1)             | 4 (26.7)               | 5 (10.2)            | 0.36                  |
| Mean age ± SD | 69.5 ± 9.5           | 72.8 ± 9.4             | 68.5 ± 9.4          | 0.07                  |
| Disease status |                     |                        |                     |                       |
| At onset      | 22 (34.4)            | 5 (33.3)               | 17 (34.7)           |                       |
| Relapse/     | 42 (65.6)            | 10 (66.7)              | 32 (65.3)           | 0.92                  |
| refractory    |                      |                        |                     |                       |
| B2M (mcg/mL), mean ± SD | 3.6 ± 3.8       | 4.6 ± 4.1              | 3.2 ± 3.6           | 0.006                 |
| LDH (U/L), mean ± SD | 192.1 ± 62.0    | 191.4 ± 70.4           | 192.4 ± 60.1        | 0.64                  |
| Bone marrow infiltration pattern (%) | 35.6 ± 23.3 | 60.0 ± 20.3 | 28.2 ± 18.8 | <0.001 |
| WBC (x 1000/ul), mean ± SD | 5.4 ± 1.9       | 5.3 ± 2.7              | 5.5 ± 1.7           | 0.37                  |
| Lymphocytes (x 1000/ul), mean ± SD | 1.4 ± 0.7 | 1.2 ± 1.0              | 1.5 ± 0.7           | 0.02                  |
| Neutrophils (x 1000/ul), mean ± SD | 3.3 ± 1.5 | 3.4 ± 1.9              | 3.3 ± 1.3           | 0.68                  |
| Haemoglobin (g/dL), mean ± SD | 11.8 ± 1.9 | 10.8 ± 1.8             | 12.1 ± 1.8          | 0.008                 |
| IgG (mg/dL), mean ± SD | 928.5 ± 739.4 | 942.3 ± 479.3         | 924.3 ± 806.5      | 0.36                  |

1 The p-values for comparison between non-responders vs. responders were computed using chi-square test (for categorical variables) or Wilcoxon’s rank-
sum test (for continuous variables). All the p-values <0.01 are highlighted in bold.

Table 4

Univariate and multivariate analyses of factors associated to vaccine response.

**Table 2**

Serological response to BNT162b2 vaccine.

| Characteristic | All patients (n = 64) | Non-Responders (n = 15) | Responders (n = 49) | p-value for comparison |
|---------------|----------------------|------------------------|---------------------|-----------------------|
| Sex           |                      |                        |                     |                       |
| Female        | 32 (50.0)            | 8 (53.3)               | 24 (48.9)           | 0.77                  |
| Male          | 32 (50.0)            | 7 (46.7)               | 25 (51.0)           |                       |
| Age           |                      |                        |                     |                       |
| <60           | 12 (18.7)            | 2 (13.3)               | 10 (20.4)           |                       |
| 60–69         | 14 (21.9)            | 2 (13.3)               | 12 (24.5)           |                       |
| 70–79         | 29 (45.3)            | 7 (46.7)               | 22 (44.9)           |                       |
| ≥80           | 9 (14.1)             | 4 (26.7)               | 5 (10.2)            | 0.36                  |
| Mean age ± SD | 69.5 ± 9.5           | 72.8 ± 9.4             | 68.5 ± 9.4          | 0.07                  |
| Disease status |                     |                        |                     |                       |
| At onset      | 22 (34.4)            | 5 (33.3)               | 17 (34.7)           |                       |
| Relapse/     | 42 (65.6)            | 10 (66.7)              | 32 (65.3)           | 0.92                  |
| refractory    |                      |                        |                     |                       |
| B2M (mcg/mL), mean ± SD | 3.6 ± 3.8       | 4.6 ± 4.1              | 3.2 ± 3.6           | 0.006                 |
| LDH (U/L), mean ± SD | 192.1 ± 62.0    | 191.4 ± 70.4           | 192.4 ± 60.1        | 0.64                  |
| Bone marrow infiltration pattern (%) | 35.6 ± 23.3 | 60.0 ± 20.3 | 28.2 ± 18.8 | <0.001 |
| WBC (x 1000/ul), mean ± SD | 5.4 ± 1.9       | 5.3 ± 2.7              | 5.5 ± 1.7           | 0.37                  |
| Lymphocytes (x 1000/ul), mean ± SD | 1.4 ± 0.7 | 1.2 ± 1.0              | 1.5 ± 0.7           | 0.02                  |
| Neutrophils (x 1000/ul), mean ± SD | 3.3 ± 1.5 | 3.4 ± 1.9              | 3.3 ± 1.3           | 0.68                  |
| Haemoglobin (g/dL), mean ± SD | 11.8 ± 1.9 | 10.8 ± 1.8             | 12.1 ± 1.8          | 0.008                 |
| IgG (mg/dL), mean ± SD | 928.5 ± 739.4 | 942.3 ± 479.3         | 924.3 ± 806.5      | 0.36                  |

1 The p-values for comparison between non-responders vs. responders were computed using chi-square test (for categorical variables) or Wilcoxon’s rank-
sum test (for continuous variables). All the p-values <0.01 are highlighted in bold.

Table 3

Frequency distribution and comparison of characteristics of patients with negative vs. positive vaccine response.
These data require confirmation and larger studies are particularly important to elucidate the mechanisms of immune response to this novel pathogen.

In the subgroup of patients with a good response to the vaccine, after a median follow-up of 7 months from the second dose of COVID-19 mRNA vaccine, there were no cases of COVID-19. Only in two (13.3%) of the 15 patients who failed to respond to two doses of COVID-19 mRNA vaccine did COVID-19 infection occur.

A limit of our analysis is the fact that the new LIAISON® SARS-CoV-2 S1/S2 IgG test, used for our analysis, enables the detection only of IgG neutralizing antibodies against S1/S2 antigens of SARS-CoV-2. Low baseline IgM levels are predictive of an incomplete response to the vaccine [5], while post-vaccine spike IgG levels may help to guide decisions regarding future re-vaccination strategies for this vulnerable patient population. In any case, it is not yet clear whether IgG neutralizing antibodies against S1/S2 antigens of SARS-CoV-2 provide long term immunity to the virus, or protect patients against re-infection [9].

The duration of protective immunity following the COVID-19 mRNA vaccine, the number of doses and the optimal dosing intervals warrant evaluation in clinical trials.

Credit Authors statement

All authors have contributed to the preparation of the manuscript. In detail, Mele G. has contributed to the acquisition, analysis and interpretation of data, contributed to drafts of the manuscript. Romano C. was involved in the collection of clinical and laboratory data. Pastore D., Miccoli A. and Santoro A. were involved in the critical revision of the article.

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