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Induction of robust humoral immunity against SARS-CoV-2 after vaccine administration in previously infected haematological cancer patients

The coronavirus disease 2019 (COVID-19) is an ongoing, global pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Impaired seroconversion has been repeatedly reported in patients with haematological malignancies (HM) and an important question remains as to whether the current anti-SARS-CoV-2 vaccine-induced immune response offers adequate protection from severe COVID-19 in 'frail' individuals, such as HM patients.[1–5] Using a disease-overarching cohort of HM patients ($n = 120$) already tested for their humoral response at post-infection,[6] we launched this study to assess the immune reactions after vaccine administration and compared the results at post-infection with those at post-vaccination. Patients' baseline demographic and disease characteristics are summarised in Table 1 (see also Data S1). The overall percentage of seroconversion in the study cohort at the end of the vaccination programme (post-vaccination) was 94.2% (113/120) for anti-s IgG, as judged by COVID-SeroIndex ELISA (Data S1). The fact that our study cohort displayed a rate of seroconversion at post-infection of 84.2% (101/120), with a total of 19 seronegative patients, meant that only 12 of the seronegative patients had seroconverted upon vaccination (Table S1). The seroconversion rate and characteristics of the 26 patients who had undergone haematopoietic stem cell transplantation are shown in Table S2. When patients were stratified according to single- or double-dose vaccine administration, no significant variation was found between these two groups ($p = 0.85$). In contrast, when patients were stratified according to cancer diagnosis, the anti-s IgG antibody titre was significantly higher in patients with myeloid neoplasms (median: 1896.7, interquartile range [IQR]: 818.8–4712.7) than in patients with lymphoid malignancies or plasma cell disorders (median: 715.0; IQR: 42.6–1755.9 and median: 685.3; IQR: 186.0–1602.5 respectively; $p = 0.0031$). In addition, the cancer status at the time of vaccine administration influenced the levels of anti-s IgG titres as these were generally higher in patients belonging to the 'watch and wait' group (median: 1040.4, IQR: 431.9–4712.7) and 'complete/partial response' group (median: 913.0, IQR: 233.8–2542.5) compared to 'stable/progressive disease' patients (median: 500, IQR: 155.0–1436.9), albeit the difference was not statistically

significant ($p = 0.38$) (Figure 1A). Accordingly, univariable and multivariable logistic regression analyses showed that patients displaying anti-s antibody titres higher than the median level were less likely to be found in the 'lymphoid disorder' or 'plasma cell disorder' groups (Table S3).

Next, quantification of neutralising antibody (NAb) levels by testing the sera against rVSV-SARS-CoV-2-S$_{\Delta 21}$ infection of Vero E6-TMPRSS2 cells[7] (see Data S1) revealed that the median effective dose (ID50) neutralisation titres poorly correlated with the anti-s titres (Spearman's correlation $r = 0.29$, $p = 0.001$). As observed with anti-s IgG titres, no significant variation was found between single or double vaccine administrations, geometric mean (GeoMean) ID50 neutralisation titres of 2071.0 (95% confidence interval [CI]: 1444.4–2969.4) and 2034.0 (95% CI: 1215.3–3404.3), respectively. The GeoMean ID50 neutralisation titre was 1693.4 (95% CI: 1042.7–2750.3) in patients with lymphoid malignancies, while it was higher in patients with plasma cell disorders or myeloid neoplasms [2357.6 (95% CI: 1276.9–4352.9) and 2555.9 (95% CI: 1589.4–4110.3), respectively]. When patients were categorised by their cancer disease status, the NAb titres were higher in the 'watch and wait' group (2446.9) in comparison with the 'complete/partial response' and 'stable/progressive disease' groups (2068.3 and 1424.3, respectively) (Figure 1B). As shown in Figure 1C, the reverse cumulative distribution curves for each variant according to the proportion of participants were quite similar in patients stratified by cancer diagnosis, whereas they showed a reduced response in the 'stable/progressive disease' group (Figure 1D). The correlation between the ID50 neutralisation titres and anti-s titres was stronger at post-infection than at post-vaccination as the Spearman's rank correlation coefficient dropped from 0.47 to 0.29 ($p = 0.11$). The median value of the anti-s IgG titres was 66.9 (IQR 24.7–154.3) at post-infection and increased to 892.1 (95% CI: = 222.9–2520.7) at post-vaccination ($p < 0.0001$). The median value ID50 neutralisation titre at post-infection was 518.6 (IQR: 103.5–1191.0), while at post-vaccination it rose to 1925.7 (IQR: 828.5–9279.8) ($p < 0.0001$). In addition, and consistent with other reports, we also found that patients treated with chemo-free therapies, such as those based on antibodies

TABLE 1 Patients' characteristics at the time of vaccine administration

Characteristics	N (%) Patients (n = 120)
Age, years median (range)	63.1 (21–86)
Sex	
Male	79 (65.8)
Female	41 (34.2)
Presence of comorbidities (≥1)	54 (45.0)
Haematopoietic stem cell transplantation	26 (21.7)
Autologous	23 (88.5)
Allogeneic	3 (11.5)
Cancer diagnosis	
Lymphoid malignancies	58 (48.3)
Myeloid neoplasms	33 (27.5)
Plasma cell disorders	29 (24.2)
Cancer status	
Watch and wait	26 (22.0)
Stable/progressive disease	13 (11.0)
Complete/partial response	81 (67.0)
Past severe COVID-19	36 (30.0)
Vaccine regimen	
Single dose	66 (55.0)
Pfizer/BNT162b2	51 (77.3)
Moderna/mRNA1273	5 (7.6)
AstraZeneca/ChAOx1-S/AZD1222	7 (10.6)
Janssen/Ad26COV51	3 (4.5)
Second dose	54 (45.0)
Pfizer/BNT162b2	51 (94.4)
Moderna/mRNA1273	2 (3.7)
AstraZeneca/ ChAOx1-S/AZD1222	1 (1.9)
Anti-cancer treatment in the last 6 months	34 (28.3)
Chemotherapy-based treatment in the last 6 months	14 (11.7)
Chemotherapy-free treatment in the last 6 months	20 (16.7)
Time from first vaccine dose to antibody testing, days, median (range)	91 (12–245)
Time from last dose of vaccine to antibody testing, days median (range)	74 (12–245)
Time from first SARS-CoV-2-positive test to first dose of vaccine, days median (range)	173 (66–564)
Time from SARS-CoV-2-positive test to antibody testing, days median (range)	277 (149–644)

against CD38 or CD20, or Bruton's tyrosine kinase (BTK) inhibitors during vaccination, tend to exhibit lower anti-s and NAb titres than those measured in patients under chemo-based regimens.[8–10]

Another important finding from this study comes from the analysis performed using a mathematical modelling which provides a quantitative prediction of the link between NAb levels and clinical protection against severe COVID-19.[6,11] Using this predictive model, we found that patients with a diagnosis of myeloid neoplasm and in 'watch and wait' status displayed the highest percentage of subjects above the cut-off level (945.5) required for 50% protection (81.8% and 77% respectively). When these values were compared between post-infection and post-vaccination, the percentage of patients above the 50% protective neutralisation level was consistently increased in all categories, especially in patients with lymphoid malignancies (Figure S1).

One limitation of our study is that we did not stratify the patients according to the type of vaccine administered. However, the fact that only 9.0% of HM patients were vaccinated with adenovirus-based vaccines led us to conclude that this could not have biased our statistical analysis.

In summary, our findings indicate that most patients with HM who have recovered from SARS-CoV-2 infection and received vaccine administration are able to mount a robust humoral response, with a percentage of patients displaying protection against severe COVID-19 falling in the range of 69%–82%. Specifically, we found that one dose of vaccine in HM patients with prior infection is sufficient to reach anti-s IgG titres and neutralising activities as high as those elicited by two doses, and that the robustness of the humoral response very much resembles that observed in healthy subjects with prior infection.[12] Therefore, additional vaccine administration might be avoided in these patients, at least in the short-term (see comparison of the overall titres in Figure 1E,F). In good agreement, three studies[13–15] have recently claimed that smaller subsets of HM patients with prior SARS-CoV-2 infection in their study cohorts displayed enhanced immune response compared to infection-naïve patients. In addition, and consistent with other reports, we also show that patients treated with chemo-free therapies during vaccination tend to exhibit lower anti-s and NAb titres than those measured in patients under chemo-based regimens, suggesting that the humoral response to vaccination in HM patients may be affected by continuous treatment with antineoplastic agents, chiefly BTK inhibitors, that impair innate immunity.[8–10]
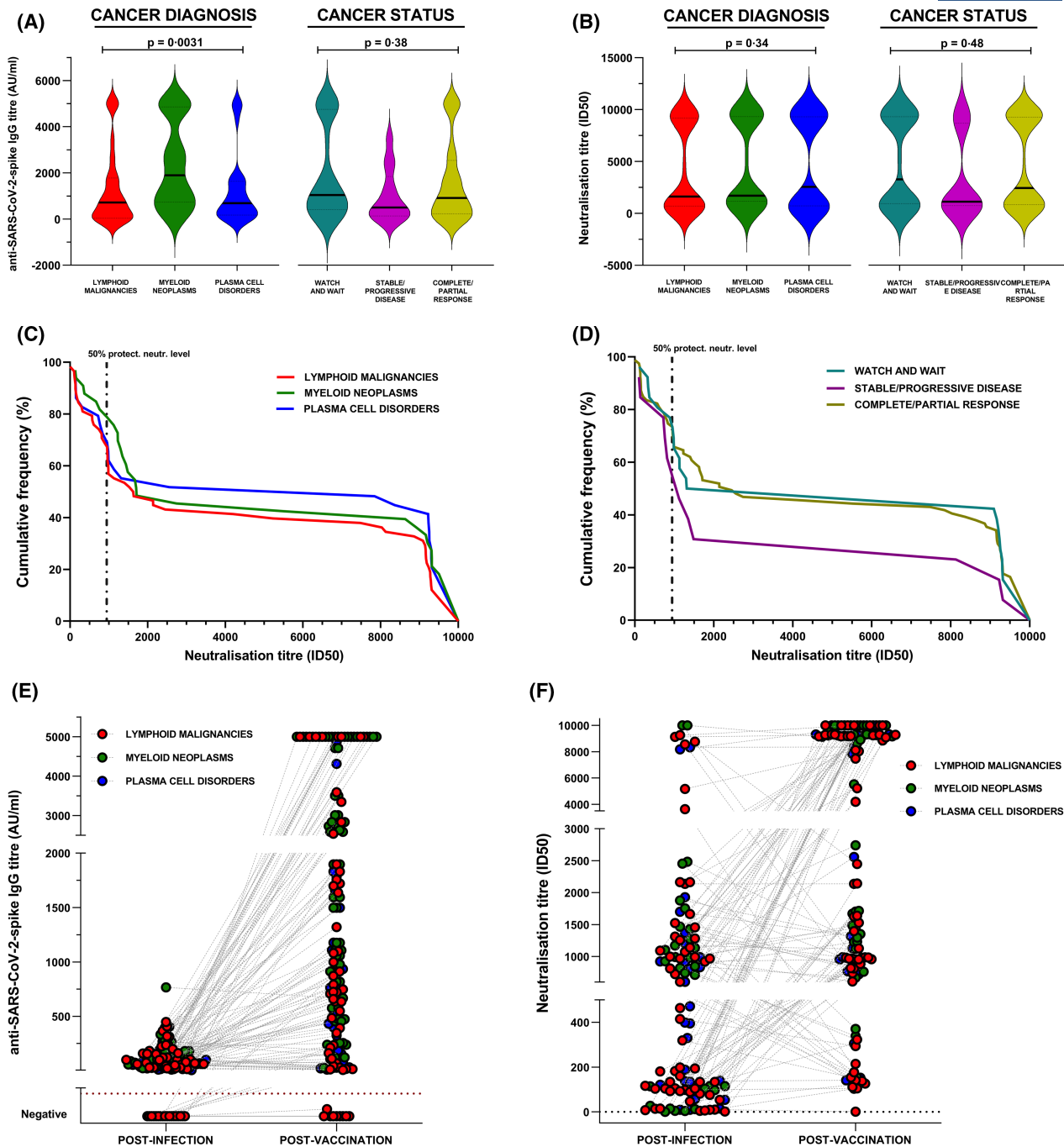
FIGURE 1 Humoral response in haematologic malignancy (HM) patients. Violin plots depicting (A) anti-s IgG titres and (B) anti-SARS-CoV-2 neutralising activity in HM patients with prior SARS-CoV-2 infection analysed after completing the SARS-CoV-2 vaccination. HM patients are grouped according to cancer diagnosis (left handed panel) or cancer status (right handed panel). Bars represent median (thick line) and interquartile range (dotted line). Statistical analysis was performed using the Kruskal–Wallis test. (C, D) Reverse cumulative distribution of the anti-SARS-CoV-2 ID50 neutralisation titre at post-vaccination analysis in HM patients with prior SARS-CoV-2 infection grouped according to (C) cancer diagnosis or (D) cancer status Reverse cumulative distribution curves denote the percentage of subjects that have reached each titre threshold. The vertical black dotted line indicates the 50% protective neutralisation level against SARS-CoV-2 infection, which was estimated to be 945.5 in our cohort calculated as 20.2% of the ID50 mean level. (E) Longitudinal analysis of the anti-SARS-CoV-2-spike IgG or (F) ID50 neutralisation titres in HM patients at post-infection versus post-vaccination. Coloured dots indicate cancer diagnosis. The horizontal red dotted line in (E) indicates the lower limit of quantification (3.2 AU/ml). The horizontal black dotted line in (F) indicates the zero value. The anti-s titres at post-vaccination were only diminished in 8% of patients when compared to those detected at post-infection. The neutralising activity decreased in 25% of the patients and those who were above the median level of the neutralisation titre at post-infection displayed lower titres in 43% of cases at post-vaccination testing.

CONFLICT OF INTEREST
Marco Ladetto declares the following relationships in the last 5 years in terms of consultancy, participation to advisory boards, invitation to scientific meetings, institutional research support and contracts with: AbbVie, Acerta, Amgen, ADC Therapeutics, Astra Zeneca, BeiGene Celgene, GSKI, Gentili, Gilead/Kite, Novartis, Incyte J&J, Jazz, Regeneron, Roche, Sandoz, Takeda. He has been principal or strategic investigator in studies supported by: Celgene, J&J, BeiGene, ADC Therapeutics. Gianluca Gaidano declares in the last 5 years the following relationships in terms of consultancy, participation to advisory boards, invitation to scientific meetings, institutional research support and contracts with: AbbVie, Astra Zeneca,

BeiGene, Incyte, Janssen, Roche. All other authors declare no competing interests.

DATA AVAILABILITY STATEMENT
Data sharing requests for access to data should be addressed to the corresponding author (M.G.). The individual participant data collected (including the data dictionary) will be available, after pseudonymisation. All proposals requesting data access will need to specify how the data will be used, and all proposals will need the approval of the investigator team before data release. Data will be shared through the online platform REDCap (https://www.project-redcap.org/).

PATIENT CONSENT
All participants provided written informed consent; samples and associated data were pseudonymised and recorded on the REDCap (https://www.project-redcap.org/) web application in compliance with current GDPR and Italian legislation on the protection of sensitive data and privacy.

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SUPPORTING INFORMATION
Additional supporting information can be found online in the Supporting Information section at the end of this article.