Disease- and Therapy-Specific Impact on Humoral Immune Responses to COVID-19 Vaccination in Hematologic Malignancies

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ABSTRACT   Coronavirus disease-19 (COVID-19) vaccine response data for patients with hematologic malignancy, who carry high risk for severe COVID-19 illness, are incomplete. In a study of 551 hematologic malignancy patients with leukemia, lymphoma, and multiple myeloma, anti–SARS-CoV-2 spike IgG titers and neutralizing activity were measured at 1 and 3 months from initial vaccination. Compared with healthy controls, patients with hematologic malignancy had attenuated antibody titers at 1 and 3 months. Furthermore, patients with hematologic malignancy had markedly diminished neutralizing capacity of 26.3% at 1 month and 43.6% at 3 months, despite positive seroconversion rates of 51.5% and 68.9% at the respective time points. Healthy controls had 93.2% and 100% neutralizing capacity at 1 and 3 months, respectively. Patients with leukemia, lymphoma, and multiple myeloma on observation had uniformly blunted responses. Treatment with Bruton tyrosine kinase inhibitors, venetoclax, phosphoinositide 3-kinase inhibitors, anti-CD19/CD20–directed therapies, and anti-CD38/B-cell maturation antigen–directed therapies substantially hindered responses, but single-agent immunomodulatory agents did not.

SIGNIFICANCE: Patients with hematologic malignancy have compromised COVID-19 vaccine responses at baseline that are further suppressed by active therapy, with many patients having insufficient neutralizing capacity despite positive antibody titers. Refining vaccine response parameters is critical to guiding clinical care, including the indication for booster vaccines, for this vulnerable population.

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INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus responsible for coronavirus disease-19 (COVID-19), causes respiratory illness ranging from self-limited, mild respiratory tract symptoms to severe pneumonia with acute respiratory distress syndrome, multiorgan failure, and death. Due to their disease and/or associated treatments, patients with cancer are often immunocompromised and at increased risk of severe COVID-19 illness (1–3), with fatality rates far exceeding that of the general population (4). Within the cancer population, patients with hematologic malignancy are at greatest risk for COVID-19–related mortality (2, 3, 5–10).

The adverse outcomes of patients with hematologic malignancy to COVID-19 infection stem at least in part from intrinsic immune dysfunction. Patients with indolent B-cell malignancies, including chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and follicular lymphoma, have a high incidence of hypogammaglobulinemia, as well as T-cell dysfunction, which impairs long-term antiviral humoral immunity and response to vaccination (11). Patients with multiple myeloma have compromised humoral and cellular immunity from their plasma cell disorder, its associated hypogammaglobulinemia, and immunosuppression exacerbated by anti–multiple myeloma therapies (12). Patients with myeloid malignancies, including acute myeloid leukemia and high-risk myelodysplastic syndromes, have impaired innate immunity, antigen presentation, and quantitative defects in all immune cells, often at baseline before starting treatment and further exacerbated by therapies, including hematopoietic stem cell transplantation (13, 14).

Given their greater susceptibility to severe COVID-19 illness, patients with hematologic malignancy are a high-priority group for vaccination to mitigate COVID-19–related morbidity and mortality. Effective vaccination would enable receipt of disease-specific therapy and avoid delays in cancer care. There are limited comprehensive data on the efficacy of COVID-19 vaccines in patients with cancer, with many COVID-19 vaccine studies excluding patients with cancer and real-world outcomes for patients with cancer only recently starting to emerge. In this study, we aimed to define how vaccine humoral responses differ by hematologic malignancy subtype and disease-specific therapies across patient cohorts to provide an important metric for optimizing the clinical care of this vulnerable population.

RESULTS

Patient Characteristics

As of August 1, 2021, the study included 551 patients with leukemia ($n = 157$), lymphoma ($n = 173$), and multiple myeloma ($n = 221$), as well as a healthy volunteer control cohort ($n = 69$; Supplementary Fig. S1—study schema). Supplementary Table S1 lists patient demographics and disease subtypes. Median age was 65 (range, 22–97), with male predominance (56.3%). The most common disease subtypes were symptomatic multiple myeloma ($n = 211$), CLL/SLL ($n = 120$), diffuse large B-cell lymphoma ($n = 55$), follicular lymphoma ($n = 42$), marginal zone lymphoma ($n = 24$), Hodgkin lymphoma ($n = 14$), and mantle cell lymphoma ($n = 13$). Most

patients (75.5%) were on cancer therapy (i.e., treatment received within 6 months of initial COVID-19 vaccination). All patients received two doses of the mRNA-based vaccines, either BNT162b2 (75%) or mRNA-1273 (25%; Table 1). For the healthy controls, the median age was 31 (range, 22–67), with female predominance (78.3%; Supplementary Table S1). All healthy controls also received two doses of the mRNA-based vaccines, BNT162b2 (84.1%) or mRNA-1273 (15.9%; Table 1).

Quantitative Antibody Response to Vaccination

Quantification of anti–SARS-CoV-2 antibody titers to assess humoral response after vaccination was determined using a chemiluminescent enzyme immunoassay at 1 and 3 months from initial vaccine (NB: "1 month" time point = 3 weeks from the first BNT162b2 vaccine and 4 weeks from the first mRNA-1273 vaccine). Individuals with a history of COVID-19 infection before vaccination were analyzed separately because of the likelihood of mounting extreme responses that would obscure the interpretation of the responses of those without prior infection. Based on a cutoff value of 50.0 AU/mL as the threshold for a positive result, postvaccination seroconversion rates and median antibody titers for patients with hematologic malignancy were 51.5% and 55.7 AU/mL [interquartile range (IQR) 4–465.7] at 1 month ($n = 167$) and 68.9% and 517.7 AU/mL (IQR 18.2–3,779) at 3 months ($n = 456$), with patients with multiple myeloma possessing the highest conversion rates of 61.1% and 86.5% at 1 and 3 months, respectively (Table 1). For most individuals, antibody titers peaked at 3 months (Supplementary Fig. S2), but when compared with healthy controls, patients with hematologic malignancy had markedly lower seroconversion rates and degree of response at both time points (Fig. 1A and Table 1). Patients and healthy controls with previous COVID-19 infection had robust responses, with notable exceptions of three patients on treatment with a Bruton tyrosine kinase (BTK) inhibitor ($n = 1$), venetoclax ($n = 1$), and belantamab mafodotin ($n = 1$; Fig. 1B and Table 1). Patients on active treatment had reduced responses at 3 months compared with patients on observation (Fig. 1C). Although seropositivity rates after two vaccine doses were similar for BNT162b2 (70.3%) and mRNA-1273 (67.7%), median antibody titer at 3 months was 389.2 AU/mL (IQR 24–2,896) for BNT162b2 ($n = 336$) and 2,042 AU/mL (IQR 14.3–10,632) for mRNA-1273 ($n = 122$; Fig. 1D). Female patients developed higher antibody titers than male patients, with median antibody titer at 3 months of 981.8 AU/mL (IQR 50–6,573) for women ($n = 211$) and 276.7 AU/mL (IQR 7.98–2,737) for men ($n = 246$; Fig. 1E). Younger patients generated higher antibody titers than older patients. Specifically, for patients age <50, median antibody titers were 147.2 AU/mL (IQR 10.3–787) at 1 month ($n = 24$) and 2,902 AU/mL (IQR 287–12,499) at 3 months ($n = 41$), compared with patients age 50 to 65, with median antibody titers 55.9 AU/mL (IQR 4.3–439.6) at 1 month ($n = 95$) and 496.1 AU/mL (IQR 20.9–3,553) at 3 months ($n = 179$) and patients age >65, with median antibody titers 12.6 AU/mL (IQR 3.2–186.7) at 1 month ($n = 48$) and 373.5 AU/mL (IQR 12.7–3,423) at 3 months ($n = 237$; Fig. 1F).

To account for the biological and treatment heterogeneity of the different hematologic malignancies, antibody

Table 1. Vaccine responses

		Healthy control	Leukemia	Lymphoma	Multiple myeloma	All patients
Total individuals		69	157	173	221	551
Vaccine subtype	BNT162b2	58 (84.1%)	105	136	172	413 (75%)
	mRNA-1273	11 (15.9%)	52	37	49	138 (25%)
SARS-CoV-2 spike IgG antibody response: Prior COVID-19 illness	1 mo seropositive rate	8/9 (88.9%)	1/1 (100%)	5/5 (100%)	6/6 (100%)	12/12 (100%)
	1 mo Ab titer (AU/mL): mean ± SD	16,881 ± 8,302	719.9 ± 0	13,748 ± 11,792	21,881 ± 4,984	16,729 ± 10,182
	1 mo Ab titer (AU/mL): median (IQR)	16,850 (12,763–24,672)	719.9 (719.9–719.9)	15,536 (1,602–25,000)	25,000 (16,607–25,000)	21,286 (5,787–25,000)
	3 mo seropositive rate	9/9 (100%)	5/7 (71.4%)	10/10 (100%)	10/11 (90.9%)	25/28 (89.3%)
	3 mo Ab titer (AU/mL): mean ± SD	17,737 ± 6,327	14,617 ± 12,973	19,003 ± 9,006	17,817 ± 8,492	17,441 ± 9,704
	3 mo Ab titer (AU/mL): median (IQR)	16,748 (11,566–25,000)	25,000 (4.9–25,000)	24,798 (12,669–25,000)	21,749 (11,244–25,000)	24,798 (10,604–25,000)
SARS-CoV-2 spike IgG antibody response: No prior COVID-19 illness	1 mo seropositive rate	59/59 (100%)	10/34 (29.4%)	18/38 (47.4%)	58/95 (61.1%)	86/167 (51.5%)
	1 mo Ab titer (AU/mL): mean ± SD	2,620 ± 5,104	1,137 ± 4,598	1,481 ± 4,015	1,716 ± 4,889	1,545 ± 4,624
	1 mo Ab titer (AU/mL): median (IQR)	886.2 (502.3–2,240)	4.5 (1.8–86.33)	46 (4.9–329.9)	96.6 (8.3–571.4)	55.7 (4–465.7)
	3 mo seropositive rate	54/54 (100%)	73/131 (55.7%)	74/132 (56.1%)	167/193 (86.5%)	314/456 (68.9%)
	3 mo Ab titer (AU/mL): mean ± SD	7,656 ± 4,701	3,827 ± 7,217	4,241 ± 7,752	4,176 ± 6,775	4,095 ± 7,182
	3 mo Ab titer (AU/mL): median (IQR)	7,720 (3,885–9,746)	187 (5–3,632)	127 (6.5–3,592)	1,218 (180.4–4,424)	517.7 (18.2–3,779)

		Healthy control	Patients with hematologic malignancies
Neutralizing antibody	1 mo positive inhibition	55/59 (93.2%)	21/80 (26.3%)
	1 mo: mean % inhibition ± SD	62.8 ± 20.3	22.8 ± 26.3
	1 mo: median % inhibition (IQR)	63.6 (51.3–78.4)	11.3 (4.9–30.6)
	3 mo positive inhibition	21/21 (100%)	17/39 (43.6%)
	3 mo: mean % inhibition ± SD	94 ± 4.2	36 ± 40.4
	3 mo: median % inhibition (IQR)	95.6 (93.8–95.9)	9.3 (1–86.7)

Abbreviations: Ab, antibody; AU/mL, artificial units/milliliter; IQR, interquartile range; mo, month; SD, standard deviation.

responses were assessed by disease subcategory (Fig. 2A). Figure 2B and Table 1 summarize leukemia patient responses, including the following categories: (i) observation: median antibody titer 5.3 AU/mL (IQR 2–465.7) at 1 month (n = 11) and 1,945 AU/mL (IQR 176.2–11,556) at 3 months (n = 53); (ii) BTK inhibitors: median antibody titer 3.5 AU/mL (IQR 0.7–12.9) at 1 month (n = 10) and 6.2 AU/mL (IQR 3–189) at 3 months (n = 32); and (iii) venetoclax: median antibody titer 2.9 AU/mL (IQR 2.6–51.5) at 1 month (n = 5) and 4.3 AU/mL (IQR 1.4–29.5) at 3 months (n = 19). As expected
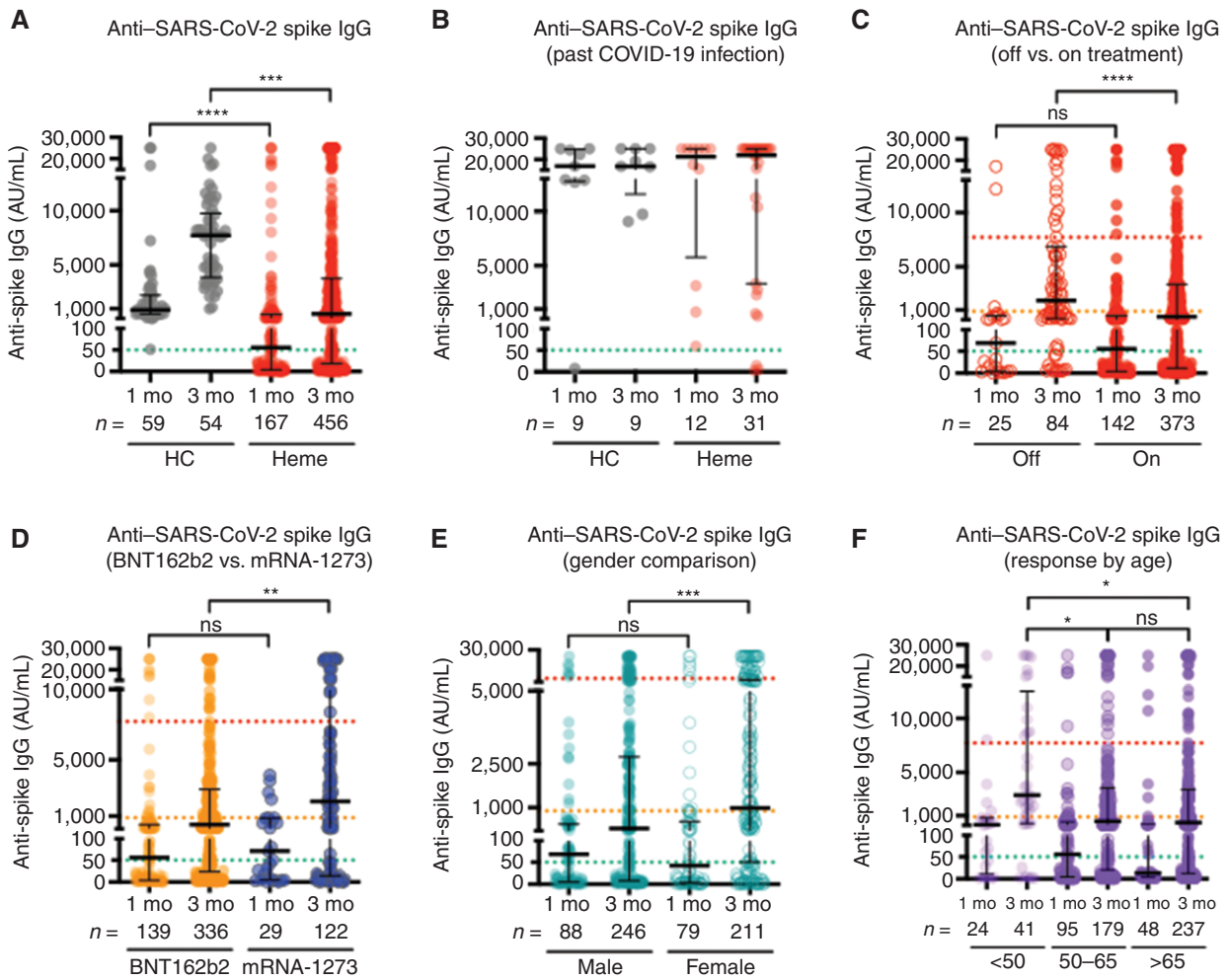
Figure 1. Quantitative antibody responses to COVID-19 vaccines. Anti–SARS-CoV-2 spike IgG antibody titers were measured at 1 and 3 months after initial COVID-19 vaccination and summarized using scatter plots with median and interquartile range. Note: "1 month" time point = 3 weeks from the first BNT162b2 vaccine and 4 weeks from the first mRNA-1273 vaccine. mo, month. **A,** Patients with hematologic malignancy (Heme; red dots) compared with healthy controls (HC; gray dots), excluding those with previous COVID infection. **B,** Heme and HC with history of COVID-19 infection. **C,** Heme off (open circles) and on (filled red circles) cancer treatment. **D,** Heme responses after receiving BNT162b2 (orange circles) and mRNA-1273 (blue circles). **E,** Heme responses by gender (male = filled teal circles; female = open teal circles). **F,** Heme responses by age. For all plots, green dashed line denotes the threshold for a positive result (50.0 AU/mL), orange dashed line denotes the median value at 1 month for HC (886 AU/mL), and red dashed line denotes the median value at 3 months for HC (7,720 AU/mL). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; ns = not significant. n = number of individuals per category.

from underlying disease pathology and B-cell–directed therapies, patients with CLL/SLL, who constituted 76.4% of patients in the leukemia cohort, had extremely blunted responses at 3 months with median antibody titer 20.5 AU/mL (IQR 3.6–1,863; $n = 105$) compared with myeloid leukemia patient responses with median antibody titer 2,555 AU/mL (IQR 214.3–5,843; $n = 23$; Supplementary Fig. S3). Figure 2C and Table 1 summarize lymphoma patient responses, including the following categories: (i) observation: median antibody titer 219 AU/mL (IQR 15.9–5,763) at 1 month ($n = 9$) and 4,743 AU/mL (IQR 496.1–20,251) at 3 months ($n = 43$); (ii) BTK inhibitors: median antibody titer 105.3 AU/mL (IQR 1.1–2,997) at 1 month ($n = 4$) and 28 AU/mL (IQR 1.5–172.1) at 3 months ($n = 15$); (iii) phosphoinositide 3-kinase (PI3K) inhibitors: median antibody titer 11.7 AU/mL (IQR 3.6–19.8) at 1 month ($n = 2$) and 5 AU/mL (IQR 3.5–286.9) at 3 months

($n = 5$); and (iv) anti-CD19/anti-CD20–directed therapies: median antibody titer 13.6 AU/mL (IQR 1.4–87.6) at 1 month ($n = 14$) and 8.4 AU/mL (IQR 3.7–66.8) at 3 months ($n = 38$). Figure 2D and Table 1 summarize multiple myeloma patient responses, including the following categories: (i) observation: median antibody titer 282.2 AU/mL (IQR 36.8–9,305) at 1 month ($n = 8$) and 1,298 AU/mL (IQR 664.3–5,459) at 3 months ($n = 11$); (ii) immunomodulatory agents: median antibody titer 198.8 AU/mL (IQR 36.85–624.5) at 1 month ($n = 53$) and 2,397 AU/mL (IQR 798.1–8,816) at 3 months ($n = 85$); (iii) anti-CD38–directed therapies: median antibody titer 12.3 AU/mL (IQR 1.7–56.7) at 1 month ($n = 18$) and 318 AU/mL (IQR 50.1–1,594) at 3 months ($n = 53$); and (iv) anti–B-cell maturation antigen (BCMA)–directed therapies: median antibody titer 9.6 AU/mL (IQR 9.6–9.6) at 1 month ($n = 1$) and 36.4 AU/mL (IQR 9.4–78.7) at 3 months ($n = 7$).
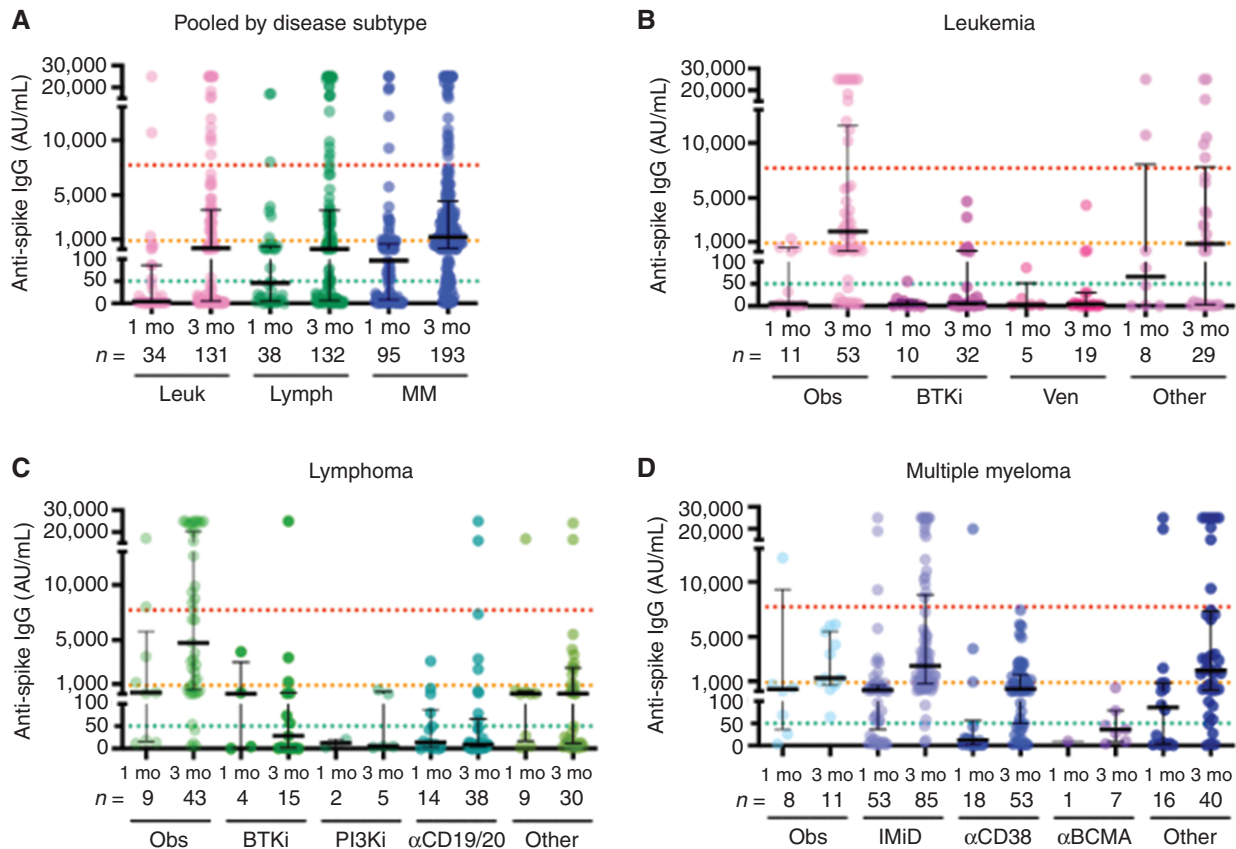
Figure 2. Disease-specific antibody responses to COVID-19 vaccines. Anti–SARS-CoV-2 spike IgG antibody titers measured at 1 and 3 months after initial COVID-19 vaccination grouped by disease subtype and summarized using scatter plots with median and interquartile range. Note: "1 month" time point = 3 weeks from the first BNT162b2 vaccine and 4 weeks from the first mRNA-1273 vaccine. **A,** Patients with leukemia (Leuk), lymphoma (Lymph), and multiple myeloma (MM). mo, month. **B,** Patients with leukemia on observation (Obs) and patients receiving BTK inhibitors (BTKi), venetoclax (Ven), and other therapies. **C,** Patients with lymphoma on Obs and patients receiving BTKi, phosphoinositide 3-kinase (PI3K) inhibitors, anti-CD19/CD20–directed therapies (αCD19/20), and other therapies. **D,** Patients with multiple myeloma on Obs and patients receiving immunomodulatory agents (IMiD), anti-CD38–directed therapy (αCD38), anti–B-cell maturation antigen-directed therapies (αBCMA), and other therapies. For all plots, green dashed line denotes the threshold for a positive result (50.0 AU/mL), orange dashed line denotes the median value at 1 month for healthy controls (886 AU/mL), and red dashed line denotes the median value at 3 months for healthy controls (7,720 AU/mL). n = number of individuals per category.

Overall, patients with lymphoma on observation, the group with the largest fraction of patients in remission, had the highest median antibody response at 3 months. Patients on BTK inhibitors, venetoclax, PI3K inhibitors, anti-CD19/CD20–directed therapies, and anti-CD38/BCMA–directed therapies had markedly attenuated seroconversion rates and absolute antibody titers. In contrast, patients with multiple myeloma on maintenance therapy with immunomodulatory agents (lenalidomide or pomalidomide) had relatively intact responses, with median antibody titers at 3 months approaching that of patients with lymphoma on observation.

Qualitative Antibody Response to Vaccination

Because neutralizing antibody levels against SARS-CoV-2 are predictive of protection from symptomatic COVID-19 (15), we analyzed available samples for neutralizing activity at 1 and 3 months from initial vaccine, with at least 30% inhibition required as a positive result per assay standardization against a plaque-reduction neutralization test. For patients with hematologic malignancy, the percentage of individuals with positive neutralizing activity and the median percentage inhibition were 26.3% and 11.3% (IQR 4.9–30.6) at 1 month (n = 80) and 43.6% and 9.3% (IQR 1–86.7) at 3 months (n = 39; Fig. 3A; Table 1). Furthermore, median percentage inhibition at both 1 and 3 months was lowest for patients with leukemia, all of whom had CLL/SLL, and highest for patients with multiple myeloma, the majority of whom were on lenalidomide maintenance therapy (Supplementary Fig. S4). In contrast, the percentage of healthy controls with positive neutralizing activity and the median percentage inhibition were 93.2% and 63.6% (IQR 51.3–78.4) at 1 month (n = 59) and 100% and 95.6% (IQR 93.8–95.9) at 3 months (n = 21; Fig. 3A and Table 1).

Figure 3B shows the correlation between anti–SARS-CoV-2 antibody titers and neutralizing activity, demarcating responses into quadrants based on test results. For individuals (patients and healthy controls) with negative antibody titers and negative neutralizing activity (n = 60; group Q1 in Fig. 3B), the median antibody titer was 5.4 AU/mL (IQR 1.8–12) with median percentage inhibition 2.2% (IQR 0–6.5). For individuals with positive antibody titers but negative
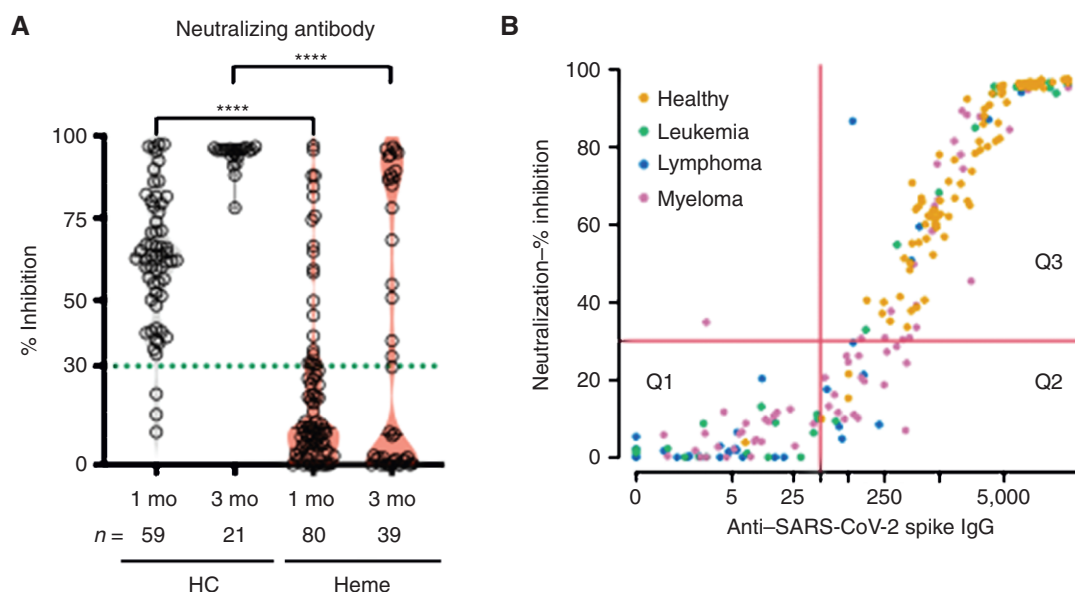
Figure 3. Neutralizing antibody levels after COVID-19 vaccines. Circulating neutralizing antibodies against SARS-CoV-2 were assessed at 1 and 3 months after initial COVID-19 vaccination. Note: "1 month" time point = 3 weeks from the first BNT162b2 vaccine and 4 weeks from the first mRNA-1273 vaccine. **A,** Violin plots comparing patients with hematologic malignancy (Heme; red fill) with healthy controls (HC; gray fill). Green dashed line denotes the threshold for a positive result (30% inhibition). ****, $P < 0.0001$. mo, month; n = number of individuals per category. **B,** Scatter plot of anti–SARS-CoV-2 spike IgG antibody titers and neutralizing antibodies assessed at 1 and 3 months after initial COVID-19 vaccination. Red horizontal line denotes threshold for the positive neutralizing result (30% inhibition). Red vertical line denotes threshold for the positive antibody result (50.0 AU/mL). Q1 = negative antibody titer and negative neutralizing activity. Q2 = positive antibody titer and negative neutralizing activity. Q3 = positive antibody titer and positive neutralizing activity.

neutralizing activity (n = 27; group Q2 in Fig. 3B), the median antibody titer was 101.7 AU/mL (IQR 78–150.4) with median percentage inhibition 17.5% (IQR 9.9–24.3). For individuals with positive antibody titers and positive neutralizing activity (n = 44; group Q3 in Fig. 3B), the median antibody titer was 1,891 AU/mL (IQR 557.2–8,733) with median percentage inhibition 84.9% (IQR 51.8–95.2). Overall, every individual with an antibody titer of at least 500 AU/mL (n = 113) had positive neutralizing activity with median percentage inhibition 87.9% (IQR 65.4–95.9), whereas only 1 of 61 individuals with an antibody titer <50 AU/mL had positive neutralizing activity. For individuals with positive antibody titers but negative neutralizing activity after one vaccine (n = 23; group Q2 in Fig. 3B), the median antibody titer after receiving a second vaccine increased to 2,121 AU/mL (IQR 991.4–4,680), an antibody level with a high likelihood of adequate neutralizing capacity (i.e., antibody titer ≥500). In contrast, for individuals with negative antibody titers and negative neutralizing activity after one vaccine (n = 45; group Q1 in Fig. 3B), the median antibody titer after receiving a second vaccine increased incrementally to 15.7 AU/mL (IQR 2.1–345.6), an antibody level with low likelihood of adequate neutralizing capacity (i.e., antibody titer <50). Of the patients in the Q1 subgroup who failed to mount positive antibody titers after receiving a second vaccine (n = 29; Fig. 3B), perivaccine treatments included anti-CD19/CD20–directed therapies (n = 11), PI3K inhibitors (n = 5), anti-CD38–directed therapy (n = 4), BTK inhibitors (n = 3), anti-BCMA–directed therapies (n = 2), venetoclax (n = 2), lenalidomide (n = 1), and prednisone (n = 1).

DISCUSSION

Hematologic malignancies are biologically heterogeneous with a spectrum of inherent immune impairment that is further exacerbated by disease-directed therapies. Understanding the impact of hematologic malignancy subtype and treatments on immune responses to COVID-19 vaccines is essential for optimizing vaccination strategies for this vulnerable population. To address this vital issue, we show that patients with hematologic malignancy have compromised COVID-19 vaccine humoral responses at baseline that are further suppressed in the setting of active therapy and, importantly, that antibody titers alone are an imperfect measure of immunity, as many patients with positive anti–SARS-CoV-2 antibody titers have insufficient neutralizing capacity.

A growing series of studies from multiple groups using various testing methods have reported blunted COVID-19 vaccine responses in patients with cancer, including those with hematologic malignancies (16–22). In addition to confirming impaired humoral capacity of patients receiving B-cell–directed treatments targeting CD20, CD38, and BCMA, as well as BTK inhibitors, our results show impaired responses in the setting of PI3K inhibitors and venetoclax. Further illustrating the profound dampening effect of therapy on vaccine responses was a subset of patients in our study with past COVID-19 infection who failed to mount robust responses after two vaccine doses, contrasting with the amplified antibody titers expected after a single vaccine dose in healthy individuals with prior COVID-19 infection (23). Our findings also highlight preserved humoral responses for patients with

multiple myeloma on maintenance therapy with immunomodulatory agents. Insights into the underlying mechanisms of therapies that diminish or enhance responses to COVID-19 vaccines will aid the future development and refinement of vaccination and/or therapeutic strategies to control the current and future pandemics.

Consistent with other reports, our findings emphasize the importance of a two-dose approach when administering mRNA-based vaccines for patients with hematologic malignancy due to the extremely low response rates and absolute antibody titers after a single vaccine. Interestingly, at 3 months after receiving two vaccine doses, hematologic malignancy recipients of mRNA-1273 generated higher median antibody titers (2,042 AU/mL) than recipients of BNT162b2 (389.2 AU/mL), which was also noted in another study (20). However, our sample size and cross-sectional observation preclude determination of clinical significance. It has been posited that the higher amount of mRNA encoding the SARS-CoV-2 spike protein in mRNA-1273 (100 μg for mRNA-1273 vs. 30 μg for BNT162b2) may account for the difference in antibody titers between the two vaccines (20). Thus, similar to influenza vaccine efficacy in older individuals, administration of a higher mRNA total dose to increase antigen delivery may be required to optimize responses of individuals immunocompromised by disease and/or therapy. In addition, because germinal center reactions in response to mRNA vaccination last up to 12 weeks (24), the delayed timing of the second dose of mRNA-1273 (28 days for mRNA-1273 vs. 21 days for BNT162b2) may induce higher spike-specific antibody levels from the positive effects of delayed boosting following the priming dose, a strategy used early in the COVID-19 pandemic in the United Kingdom.

In this study, we evaluated antibody neutralization capacity using a surrogate ACE-2/receptor binding domain (RBD) inhibition assay, which provides results comparable to conventional live virus or pseudovirus neutralization assays (25). Although the various antibody neutralization assays have not been validated specifically in patients with cancer, significant variation in assay accuracy for patients with cancer, as compared with noncancer populations, is unlikely. Importantly, regardless of the neutralization assay platform, there are known deficiencies of each regarding their true representation of activity *in vivo*, as none of the assays measure actual effector function mediated by the Fc fragment of the antibody. In addition, viral clearance is only partially related to neutralization, as has been demonstrated for many viruses including SARS-CoV-2 (26). Our results also demonstrate a threshold effect between absolute antibody titers and neutralizing activity, thus supporting strategies to augment responses including consideration of booster vaccines. Although our current study evaluated neutralization capacity against the SARS-CoV-2 wild-type variant, further investigation into neutralization capacity against the B.1.617.2 (delta) variant, as well as other new variants of concern, in the setting of booster vaccination is warranted.

Vaccine efficacy requires the induction of vigorous and durable antibody and cellular responses (27). Although humoral immunity is a key measure of vaccine response, inadequate antiviral antibody titers and/or neutralizing activity do not necessarily signify absence of vaccine benefit. In one study, patients with cancer with impaired B-cell responses but preserved CD8 T-cell responses during COVID-19 infection had lower viral loads and mortality than patients without T-cell responses (28), underscoring the contribution of the adaptive arm of immunity in conferring immune protection. Although B-cell targeting therapies like anti-CD20 antibodies or BTK inhibitors may allow for adequate T-cell immunity, others like anti-CD38–directed therapies have broader effects, including targeting activated T-cell subsets. Studies evaluating the qualitative and quantitative aspects of cellular immune responses to COVID-19 vaccines for our study population are ongoing.

An important caveat to our study is that response assessments were specific for the original SARS-CoV-2 strain. The breadth and depth of antibody responses, as well as other aspects of vaccine efficacy, in the context of cancer therapy require additional investigation, especially as SARS-CoV-2 variants arise in the community. A strength of this study is the inclusion of a vaccinated healthy volunteer cohort, with the provision that the healthy volunteer pool skewed younger with female predominance. However, the overall vaccine responses of this cohort were consistent with those seen in adults for other studies (29, 30). More diverse, age-matched control groups will be important moving forward as data emerge on waning immunity in those age >65 and the role for booster vaccination. Another limitation of this study is the relative underrepresentation of some of the hematologic malignancy subsets, which merits follow-up studies of additional patients to confirm response outcomes. Finally, because virtually all patients in the study received mRNA-based vaccines, the applicability of our findings to alternative vaccine platforms is unknown.

In conclusion, intrinsic deficits in humoral immunity, further compounded by cancer-directed therapies, hinder antibody responses of patients with hematologic malignancy to COVID-19 vaccines. Because antibody titers alone are an imperfect surrogate of immunity, comprehensive evaluation of other parameters of response are warranted to define the role of booster vaccines and other adjunctive approaches to optimize vaccination strategies and clinical care for these patients.

METHODS

Patients

Patients at Memorial Sloan Kettering Cancer Center (MSKCC) with leukemia, lymphoma, and multiple myeloma participated in this observational study and received their initial mRNA-based vaccines between December 2020 and April 2021, with vaccine responses measured at 1 and 3 months from initial vaccination (NB: "1 month" time point = 3 weeks from the first BNT162b2 vaccine and 4 weeks from the first mRNA-1273 vaccine). Any patient with a response assessment from at least one of the time points was included in the data analysis. Parallel assessments from a healthy volunteer cohort provided controls. The study was conducted through the Division of Hematologic Malignancies at MSKCC in accordance with the Declaration of Helsinki guidelines. Informed consent was waived under a retrospective research protocol (protocol 20-390) approved by the Institutional Review and Privacy Board of Memorial Hospital/MSKCC.

Anti–SARS-CoV-2 Spike IgG Assay

A chemiluminescent microparticle immunoassay (AdviseDx SARS-CoV-2 IgG II assay; Abbott) detected anti–SARS-CoV-2 spike IgG

antibody titers. Briefly, serum samples were combined with paramagnetic particles coated with recombinant SARS-CoV-2 protein specific for the RBD of the S1 protein, followed by incubation, washing, and addition of a conjugate and chemiluminescent substrate. The resulting chemiluminescent reaction was measured as a relative light unit (RLU), with a direct relationship between the amount of IgG antibodies to SARS-CoV-2 in the sample and the RLU detected by the system optics (Architect i2000 analyzer). The assay uses a 4-Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration. The cutoff is 50.0 AU/mL.

Surrogate Virus Neutralization Assay

The SARS-CoV-2 surrogate virus neutralization test kit (Genescript) measured circulating neutralizing antibodies against SARS-CoV-2 that block the interaction between the RBD of the viral spike glycoprotein with the ACE2 cell-surface receptor. Briefly, serum samples were preincubated with the horseradish peroxidase (HRP)–conjugated recombinant SARS-CoV-2 RBD fragment (HRP-RBD, wild-type variant) to allow binding of circulating neutralization antibodies to HRP-RBD, then added to a capture plate precoated with the human ACE2 receptor (hACE2) protein, followed by additional incubation and washing steps before addition of a stop solution for endpoint reaction reading on a microplate reader at 450 nm. The absorbance of the sample is inversely dependent on the titer of the anti–SARS-CoV-2 neutralizing antibodies. Percentage inhibition was calculated per manufacturer's instructions with a positive cutoff value of 30% and validated with a panel of confirmed COVID-19 patient and healthy control sera. This value was determined from a comparator plaque-reduction neutralization test (PRNT) assay performed per World Health Organization guidelines, showing 100% agreement with $PRNT_{50}$ and $PRNT_{90}$ levels.

Statistical Analyses

Descriptive analyses included mean±standard deviation (SD) and median with the IQR. The Wilcoxon signed rank test and the Wilcoxon rank-sum test compared anti–SARS-CoV-2 neutralizing antibody titers and neutralizing activity across groups. Statistical analyses were calculated using Prism 8 software (GraphPad) and R 4.1.0 (R Core Team), with statistical significance requiring $P < 0.05$.

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Authors' Contributions

D.J. Chung: Conceptualization, resources, data curation, formal analysis, supervision, funding acquisition, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **G.L. Shah:** Conceptualization, visualization, project administration, writing–review and editing. **S.M. Devlin:** Formal analysis, visualization, writing–review and editing. **L.V. Ramanathan:** Investigation, writing–review and editing. **S. Doddi:** Investigation, writing–review and editing. **M.S. Pessin:** Writing–review and editing. **E. Hoover:** Project administration, writing–review and editing. **L.T. Marcello:** Investigation, writing–review and editing. **J.C. Young:** Investigation, writing–review and editing. **S.R. Boutemine:** Investigation, writing–review and editing. **E. Serrano:** Investigation, writing–review and editing. **S. Sharan:** Investigation, writing–review and editing. **S. Momotaj:** Investigation, writing–review and editing. **L. Margetich:** Investigation, writing–review and editing. **C.D. Bravo:** Project administration, writing–review and editing. **G.A. Papanicolaou:** Writing–review and editing. **M. Kamboj:** Writing–review and editing. **A.R. Mato:** Writing–review and editing. **L.E. Roeker:** Writing–review and editing. **M. Hultcrantz:** Writing–review and editing. **S. Mailankody:** Writing–review and editing. **A.M. Lesokhin:** Writing–review and editing. **S.A. Vardhana:** Conceptualization, supervision, funding acquisition, visualization, project administration, writing–review and editing. **D.A. Knorr:** Conceptualization, supervision, funding acquisition, visualization, project administration, writing–review and editing.

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