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Transplantation and Cellular Therapy 000 (2022) 1–9

Transplantation and Cellular Therapy

journal homepage: www.tctjournal.org

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Infectious Disease

Humoral and Cellular Immune Response to Covid-19 Vaccination in Patients with Chronic Graft-versus-Host Disease on Immunosuppression

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Article history:
Received 9 June 2022
Accepted 25 August 2022

Key Words:
COVID-19
SARS-CoV-2
Vaccine
HCT
Immune response

A B S T R A C T

Chronic graft-versus-host disease (cGVHD) and its management with immunosuppressive therapies increase the susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, as well as progression to severe Coronavirus 19 disease (COVID-19). Vaccination against COVID-19 is strongly recommended, but efficacy data are limited in this patient population. In this study, responses to COVID-19 vaccination were measured at 3 time points—after the initial vaccine series, before the third dose, and after the third dose—in adults with cGVHD receiving immunosuppressive therapy. Humoral response was measured by quantitative anti-spike antibody and neutralizing antibody levels. Anti-nucleocapsid antibody levels were measured to detect natural infection. T cell response was evaluated by a novel immunosequencing technique combined with immune repertoire profiling from cryopreserved peripheral blood mononuclear cell samples. Present or absent T cell responses were determined by the relative proportion of unique SARS-CoV-2-associated T cell receptor sequences ("breadth") plus clonal expansion of the response ("depth") compared with those in a reference population. Based on both neutralizing antibody and T cell responses, patients were categorized as vaccine responders (both detected), nonresponders (neither detected), or mixed (one but not both detected). Thirty-two patients were enrolled for the initial series, including 17 (53%) positive responders, 7 (22%) mixed responders, and 8 (25%) nonresponders. All but one patient categorized as mixed responders had humoral responses while lacking T cell responses. No statistical differences were observed in patient characteristics among the 3 groups of patients categorized by immune response, although sample sizes were limited. Significant positive correlations were observed between the robustness of cellular and humoral responses after the initial series. Among the 20 patients with paired samples (pre- and post-third dose), a third vaccination resulted in increased neutralizing antibody titers. cGVHD worsened in 10 patients (26%; 6 after the initial series and 4 after the third dose), necessitating escalation of immunosuppressive doses in 5 patients, although 4 had been tapering immunosuppression and 5 had already worsening cGVHD at the time of vaccination, and a clear association between COVID-19 vaccination and cGVHD could not be drawn. Among the patients with cGVHD on immunosuppressive therapy, 72% demonstrated a neutralizing antibody response after a 2-dose primary COVID-19 vaccination, two-thirds of whom also developed a T cell response; 25% had neither a humoral nor a T cell response. A third dose further amplified the antibody response.

INTRODUCTION

The rapid development of vaccines has had a major impact on the trajectory of the Coronavirus disease 2019 (COVID-19) pandemic. Current vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) confer significant protection against infection, severe illness, and hospitalization in healthy adults [1,2]. Immunocompromised patients are

Financial disclosure: See Acknowledgments on page XXX.
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https://doi.org/10.1016/j.jtct.2022.08.026

especially vulnerable to SARS-CoV-2 infection, yet they were excluded from most of the vaccine trials; consequently, whether these vaccines and the current vaccination schedules are effective in these patients remains unclear. Several studies have reported impaired vaccine responses in solid organ transplant recipients, those with rheumatologic diseases who are on immunosuppressive medications, and those with certain hematologic malignancies, such as multiple myeloma and chronic lymphocytic leukemia [3–8].

Recipients of allogeneic hematopoietic stem cell transplantation (HCT) who are experiencing chronic-graft-versus-host disease (cGVHD) receive immunosuppressive medications for treatment. Both the immune dysfunction associated with cGVHD and the pharmacologic immunosuppression can impair humoral and cellular immune responses. One study reported a high incidence of severe infection and mortality with SARS-CoV-2 infection among allogeneic HCT recipients [9]. Currently, for adult allogeneic HCT patients, the Centers for Disease Control and Prevention (CDC) recommends a 3-dose primary mRNA vaccine, followed by a first booster dose at least 3 months later, in contrast to a 2-dose primary series for healthy adults with 1 booster after at least 5 months. Individuals who are immunocompromised or age >50 years are encouraged to get a second booster dose at least 4 months after the first booster, for a total of 5 vaccinations. Alternatively, the CDC endorses 2-dose primary series of the Ad26.COV2.S vaccine, followed by mRNA vaccine booster doses at 2 months and 7 months after the primary series. However, data are lacking as to the effectiveness of SARS-CoV-2 vaccines in individuals with cGVHD or the appropriate vaccine regimen; for example, whether these patients should receive heterologous vaccine formulations. There are also emerging data showing that both humoral and T cell responses are vital to fully and durably protect against severe infection [10]. Here we report humoral and cellular responses to the COVID-19 initial vaccine series (a 2-dose primary series, as was recommended at the time) and a third dose (given as a booster) in patients with cGVHD on immunosuppressive medications.

METHODS

Study Protocol

This was a prospective study designed to evaluate immune response over time in allogeneic HCT recipients who were experiencing cGVHD, were still on systemic immunosuppression, and were scheduled to receive any of the COVID-19 vaccines. Patients were eligible to enroll before or after initiating the COVID-19 vaccine series, including the mRNA vaccine (BNT162b2 [Pfizer] or mRNA-1273 [Moderna]) or the Ad.26.COV2.S (J&J/Janssen) vaccine. All patients enrolled in the study had undergone allogeneic HCT as treatment for hematologic malignancy and were in remission when enrolling in the study.

Peripheral blood samples were collected before receiving the vaccine and after each vaccination at timed intervals up to 12 months. Clinical data collection included baseline patient characteristics, transplantation information, cGVHD characteristics, and immunosuppressive treatment. Medical records were reviewed to determine whether confirmed SARS-CoV-2 infections occurred while actively enrolled in the study or if cGVHD symptoms worsened in the 6 weeks after each vaccination. The study was approved by the Fred Hutchinson Cancer Center Institutional Review Board. All participants provided signed informed consent.

Sample Collection

Whole blood samples were collected in standard sodium heparin venipuncture tubes and separated into plasma and peripheral blood mononuclear cells (PBMCs). Samples were frozen at -80 °C and tested in batches.

Assessment of Humoral Response

Humoral immune response was determined by quantitative assessment of plasma for neutralizing antibody, anti-spike (anti-S) antibody, and anti-nucleocapsid (anti-N) antibody levels. Neutralizing antibody levels were assessed using a D614G SARS-CoV-02 spike pseudotyped lentivirus neutralization assay (HTS-LV-pseudo/293T) [11,12]. A 50% neutralization dilution (ND50) ≥20 (≥.004 IU/mL) was considered a positive response and converted to IU/mL. Anti-S and anti-N antibody levels were assessed using an electrochemiluminescence-based immunoassay (Elecsys anti-SARS-CoV-2 S assay; Roche Diagnostics, Basel, Switzerland). The manufacturer classifies samples with ≥.8 AU/mL as positive for anti-S antibodies and those with a cutoff index ≥1.0 as positive for anti-N antibodies, with the latter used to identify patients with prior natural infection with SARS-CoV-2.

Assessment of Cellular Response

The patients' T cell response to vaccination was assessed by Adaptive Biotechnologies (Seattle, WA) using immunoSEQ, a novel immunosequencing platform targeting CDR3 regions of human TCR-β chains. In brief, extracted genomic DNA from whole blood was amplified in a bias-controlled multiplex PCR assay, followed by high-throughput sequencing [13]. The resulting repertoire of TCR-β sequences in each sample were queried for 4469 specific public sequences previously identified as associated with SARS-CoV-2 (ie, SARS-CoV-2-associated enhanced sequences) [14]. The relative absolute abundance of SARS-CoV-2-associated enhanced sequences in the T cell fraction was characterized. The clonal breadth and depth of SARS-CoV-2-associated enhanced sequences were calculated, and a categorical assessment of the relative enrichment of SARS-CoV-2-associated enhanced sequences in each sample was determined through the application of a classifier developed for assessment of the T cell response to natural infection, as described previously [14]. Breadth is calculated as the number of unique SARS-CoV-2-associated rearrangements out of the total number of unique productive rearrangements, whereas depth accounts for the frequency of those rearrangements in the repertoire. The SARS-CoV-2-specific T cell response in each sample was characterized as present or absent based on the relative abundance of SARS-CoV-2-associated enhanced sequences using a classifier that applies a logistic regression with two dependent variables: the number of unique sequences associated with SARS-CoV-2-associated TCR-β DNA sequences and the total number of unique TCR-β DNA sequences in the sample, as described previously [14].

This T cell response classifier is the same as that leveraged in the T-Detect COVID assay Adaptive Biotechnologies (Seattle, WA), which received Emergency Use Authorization from the US Food and Drug Administration for the detection of recent or prior infection with SARS-CoV-2 [15].

Statistical Analysis

Based on neutralizing antibody and T cell responses, patient vaccine responses were categorized as positive responders (both detected), nonresponders (neither detected), or mixed responders (one but not both detected). Patient and transplant characteristics were compared between the groups, using the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables. The T cell response was correlated with the humoral response using Spearman correlation. All statistical tests were 2-sided using an α = .05 level of significance. SAS statistical software (SAS Institute, Cary, NC) was used to perform all statistical analyses of clinical data.

RESULTS

Demographics

A total of 38 patients participated in the study, 32 of whom had enrolled for the 2-dose primary vaccination (initial series) of COVID-19 vaccines. After the initial series, 13 patients from the initial cohort did not provide subsequent samples, for reasons detailed in Figure 1. The remaining 19 patients from the initial cohort were assessed for immune response after the third vaccine dose. An additional 6 patients enrolled at the time of the third dose. All patients had completed the initial 2-dose series at least 3 months before the CDC changed the recommendation to a 3-dose initial series for the mRNA vaccines. One patient from the initial cohort who received the J&J vaccine remained in the study and received a dose of the Moderna vaccine as a booster, which was considered equivalent to a third dose for those who received only the mRNA vaccine. Further references to the third dose of vaccine in this article include this patient.

Characteristics of these patients assessed in conjunction with their initial series are detailed in Table 1. The median patient age was 61 years (interquartile range [IQR], 39.5 to 65.5 years), and the median time from transplantation was 4.0 years (IQR, 2.1 to 5.6 years). The majority of patients received the Pfizer-BioNTech vaccine (BNT162b2) (n = 24; 75.0%), 7 patients (21.9%) received the Moderna vaccine

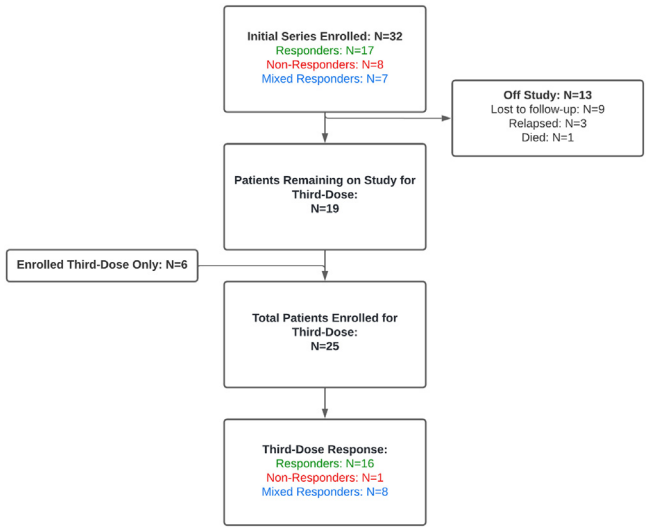

Figure 1. Population distribution.

(mRNA-1273), and 1 patient received the J&J vaccine (Ad.26.COV2.S) for their initial series.

Based on National Institutes of Health criteria for cGVHD, at the time of enrollment in the study, 18 patients (56.3%) had severe cGVHD, 8 (25.0%) had moderate cGVHD, and 6 (18.8%) had mild cGVHD. Twenty-three patients were on systemic corticosteroids for treating cGVHD at the time of the initial vaccine series. There were no differences in clinical characteristics among the 3 groups (Table 1). When patients were dichotomized into humoral responders and nonresponders or T cell responders and nonresponders, no differences in clinical characteristics were observed (data not shown).

Immune Response after the Initial Vaccine Series

Among the 32 patients who received the initial vaccine series, 17 (53%) showed positive B and T cell immune responses, 8 (25%) had no B or T cell response, and 7 (22%) had a mixed response. Samples were drawn at a median of 25 days (IQR, 21 to 35 days) after completing the initial series. Among the 23 patients with a B cell response, the median neutralizing antibody titer was .19 IU/mL (IQR, .08 to .99 IU/L) and the median anti-S antibody level was 999.5 AU/mL (IQR, 227 to 6720 AU/mL). Among all patients, a significant positive correlation was observed between T cell breadth and depth and serology titers (breadth versus anti-S, ρ = .615 [P < .001]; depth versus anti-S, ρ = .823 [P < .001]; breadth versus ND50, ρ = .444 [P < .05]; depth versus ND50, ρ = .657 [P < .001]) (Figure 2). Six of the 7 patients classified as mixed responders had a B cell response only. A modest increase in T cell clonal depth was seen in those with a mixed response (Figure 2), indicating some degree of T cell clonal expansion although insufficient for classification as a "positive" T cell response. One patient in the cohort of mixed responders had a T cell response detected without a B cell response after the initial series; however, a short interval of only 4 days between the second vaccine dose and the sample drawn for the immune response assessment is the likely reason for the negative result. This is further supported by the fact that the patient was positive for neutralizing antibody (.30 IU/mL) when B cell response was assessed before a subsequent vaccine dose.

No patient tested positive for SARS-CoV-2 by PCR or antigen testing or had anti-N antibodies before or after the initial vaccination series. No significant differences in baseline characteristics, transplant type, GVHD prophylaxis, or current receipt of systemic steroids were noted across the 3 groups of patients categorized by response to the vaccine, although the sample size was small.

Immune Response after the Third Dose

Twenty-five participants received their third vaccine dose at a median of 136 days (IQR, 114 to 175 days) after the second vaccine dose. Among the 20 patients with paired pre- and post-third dose samples, the pre-third dose median neutralizing antibody titer and anti-S antibody titer were .08 IU/mL (IQR, .01 to .21 IU/mL) and 546.5 AU/mL (IQR, 178.1 to 2141 AU/mL), respectively. Samples drawn at a median of 32 days (IQR, 21 to 41 days) after the third dose showed a median 9.5-fold increase in neutralizing antibody titers compared to the pre-third dose levels, with a median neutralizing antibody titer of 1.11 IU/mL (IQR, .29 to 1.83 IU/mL). Similarly, the anti-S antibody titer increased by a median 14.5-fold to a median titer of 18,636 AU/mL (IQR, 2284 to 25,000 AU/mL) (Figure 3). In contrast, the post-third dose T cell response assessment demonstrated only modest increases in the clonal depth, breadth, and T cell model score (Supplementary Table S2).

Fifteen patients had samples from all 3 relevant assessment time points (post-initial series, pre-third dose, post-third dose) allowing comparisons over time. In this subcohort, both B cell and T cell responses persisted even at 4 months after the initial series, and a third dose of vaccine further amplified the humoral response (Figure 4). Improvement in T cell clonal breadth after the third dose was observed mainly in patients who previously had a T cell response to the initial vaccine series (Figure 4). Among the 8 patients who failed to respond to the initial vaccine series, only 3 remained in the study to receive the third dose. One of these 3 patients tested positive for anti-N antibody before the third dose, another had an antibody response after the third dose, and the third patient remained a nonresponder even after the third dose (Supplementary Figure S1).

cGVHD

Patients were monitored for cGVHD flares for up to 6 weeks after vaccine doses. cGVHD worsened in 6 patients (19%) after the initial vaccination. Of these 6 patients, 3 already had worsening cGVHD before vaccination, and 3 had been tapering immunosuppression. Immunosuppressive treatment was

Table 1
Baseline Characteristics of Participants Receiving the Initial Vaccine Series According to Vaccine Response

Characteristic	Total Cohort (N = 32)	Vaccine Response Group			
		Responders (N = 17)	Nonresponders (N = 8)	Mixed (N = 7)	*P* Value
Age, yr, median (IQR)	61.0 (39.5-65.5)	56.0 (37.0-62.0)	62.5 (49.0-70.5)	64.0 (49.0-67.0)	.26
Female sex, n (%)	13 (40.6)	8 (47.1)	3 (37.5)	2 (28.6)	.80
Years since HCT, median (IQR)	4.0 (2.1-5.6)	4.3 (2.3-5.8)	3.2 (1.2-4.6)	4.1 (2.0-6.2)	.57
Preparative regimen intensity, n (%)					.42
Myeloablative	18 (56.3)	11 (64.7)	3 (37.5)	4 (57.1)	
Reduced intensity	5 (15.6)	1 (5.9)	2 (25.0)	2 (28.6)	
Nonmyeloablative	9 (28.1)	5 (29.4)	3 (37.5)	1 (14.3)	
Donor type, n (%)					.11
HLA-identical sibling	10 (31.3)	8 (47.1)	2 (25.0)	0 (0)	
Matched unrelated	19 (59.4)	7 (41.2)	6 (75.0)	6 (85.7)	
Mismatched unrelated	3 (9.4)	2 (11.8)	0 (0)	1 (14.3)	
Months from cGVHD to sample, median (IQR)	36.3 (14.7-56.2)	37.6 (19.9-58.5)	25.0 (11.1-46.3)	33.9 (7.2-52.9)	.46
NIH cGVHD severity at the time of vaccination, n (%)					.83
Mild or less	6 (18.8)	4 (23.5)	1 (12.5)	1 (14.3)	
Moderate	8 (25.0)	5 (29.4)	1 (12.5)	2 (28.6)	
Severe	18 (56.3)	8 (47.1)	6 (75.0)	4 (57.1)	
On prednisone, n (%)	23 (71.9)	11 (64.7)	7 (87.5)	5 (71.4)	.51
Prednisone dose, mg/kg/d, median (IQR), n	.1 (.1-.2); n = 23	.1 (.1-.3); n = 11	.1 (.1-.3); n = 7	.1 (.1-.1); n = 5	.32
Other immunosuppressive medications, n (%)					
Ruxolitinib	6 (18.8)	3 (17.6)	1 (14.3)	2 (25)	
Tacrolimus	3 (9.4)	3 (17.6)			
Mycophenolate mofetil	5 (15.6)	2 (11.8)		3 (37.5)	
Sirolimus	3 (9.4)	2 (11.8)	1 (14.3)		
Axatilimab	2 (6.3)	1 (5.9)	1 (14.3)		
Extracorporeal photopheresis	2 (6.3)	1 (5.9)		1 (12.5)	
Glasdegib	1 (3.1)	1 (5.9)			
Belumosudil	1 (3.1)		1 (14.3)		
Itacitinib	1 (3.1)		1 (14.3)		
None	4 (12.5)	3 (17.6)	1 (14.3)		
WBC, median (IQR)	6.8 (5.4-8.1)	6.9 (5.9-7.8)	7.7 (5.3-10.4)	6.2 (4.7-7.4)	.42
ALC, median (IQR)	131.5 (73.8-233.6)	145.0 (109.4-224.3)	67.2 (45.7-148.1)	193.5 (109.0-299.2)	.11
ANC, median (IQR)	383.0 (230.8-580.3)	385.5 (221.8-549.1)	615.9 (366.1-736.6)	245.8 (230.6-395.5)	.12
Vaccine type					.57
Pfizer	24 (75.0)	12 (70.6)	6 (75.0)	6 (85.7)	
Moderna	7 (21.9)	5 (29.4)	1 (12.5)	1 (14.3)	
J&J	1 (3.1)	0 (0)	1 (12.5)	0 (0)	
Chronic GVHD flare after initial series	6 (18.8)	3 (17.6)	3 (37.5)	0 (0)	.20

P values are based on the chi-square test for categorical variables and the Kruskal-Wallis test for continuous variables.
ALC indicates absolute lymphocyte count; ANC, absolute neutrophil count.

escalated in 5 of them. cGVHD flares were seen in 3 patients who had no response to initial vaccination.

Four patients experienced cGVHD flares after the third dose requiring escalation of immunosuppression. Two of these patients were on an immunosuppression taper, and 1 patient was experiencing progression of cGVHD even before the third dose.

SARS-CoV-2 Infections

Ninety-two percent of all patients enrolled in the study have remained COVID-free to date. One patient had no response after the initial series but detectable anti-N antibodies at the time of their third vaccine dose, although they did not have a history of symptoms or a positive PCR test. Another patient with a mixed response after their third dose developed symptoms and later tested positive. A third patient who was classified as a mixed responder (detectable neutralizing antibodies but lack of T cell response after the third dose) had a positive COVID-19 PCR test approximately 6 months after receiving their third vaccine dose. This patient had not received monoclonal antibody prophylaxis (which was not yet available), and a fourth dose of COVID-19 vaccine was not yet

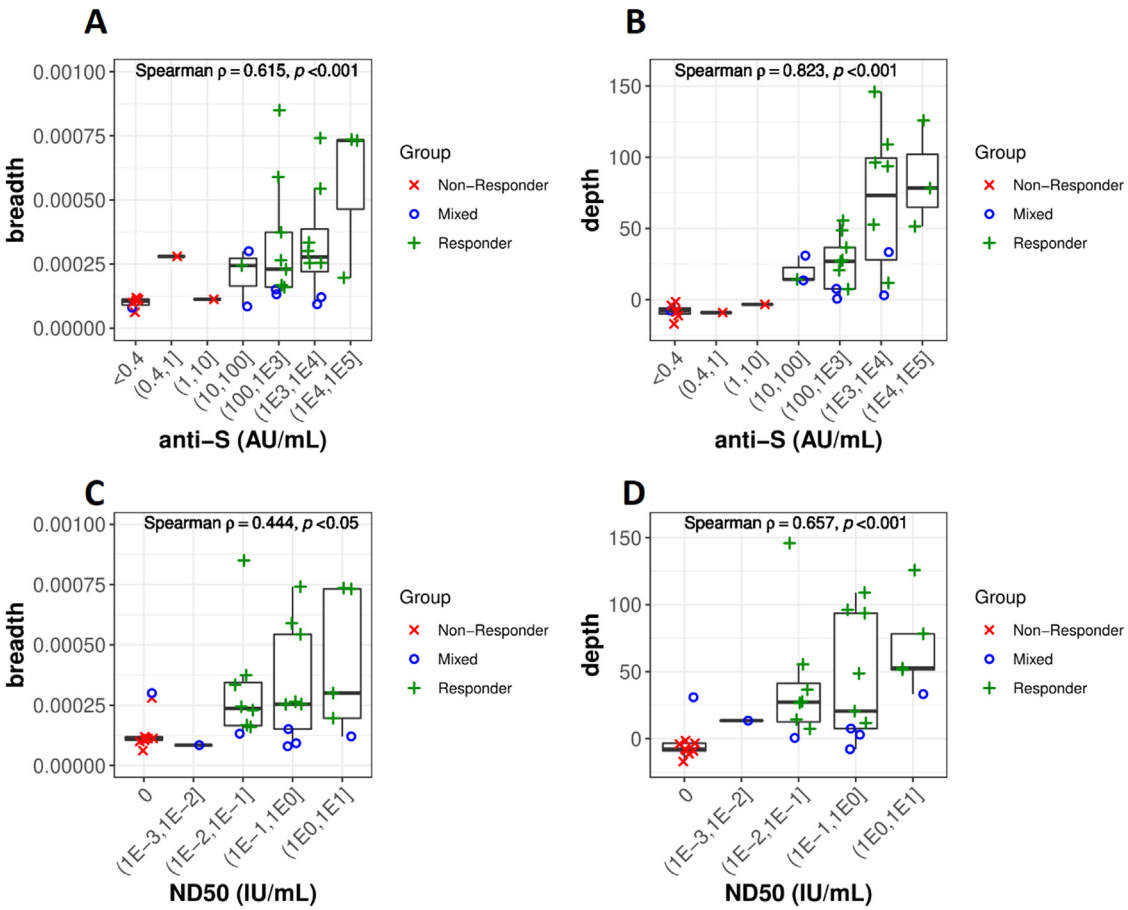

Figure 2. T cell clonal response stratified by humoral response after initial vaccine series. Shown are box-and-whisker plots of cellular response stratified by humoral response after initial vaccine series. (A and B) T cell clonal breadth (A) and depth (B) on the *y*-axis, and anti-S antibody titer on the *x*-axis. (C and D) Comparisons of T cell clonal breadth (A) and depth (B) (*y*-axis) with neutralizing antibody titer (*x*-axis).

recommended by the CDC, and death subsequently occurred from complications related to severe COVID-19.

DISCUSSION

SARS-CoV-2 prevention strategies have been evolving through the COVID-19 pandemic, with the availability of monoclonal antibodies to provide passive immunity. Nonetheless, vaccination remains the cornerstone of prevention. Most vaccine studies in HCT recipients have relied on antibody testing as a surrogate to assess vaccine efficacy; however, with accumulating data, it is becoming clear that B cell and T cell responses are both vital for effectively combating SARS-CoV-2 infection and preventing severe disease [16]. This is one of the first studies reporting findings from an evaluation of both B cell and T cell responses following the initial vaccine series as well as after the third vaccine dose in patients with cGVHD.

After receiving the initial series, only 53% of our study patients had both B cell and T cell responses. An additional 19% of the patients had a B cell response without a T cell response (mixed responders). Thus, 72% of patients had neutralizing antibodies after the initial series, similar to the proportion found in another study that included 57 patients with cGVHD [17]. The seroconversion rate was higher after the initial series in our study compared with that in patients with hematologic malignancies as reported in other studies [5,6], although the seroconversion rate and titers were lower compared with those in healthy adults [1,2,18]. Emerging data suggest that roughly 70% to 80% of allogeneic survivors are able to mount B cell responses [19–21] unless they have B cell aplasia due to disease or treatment [22,23].

T cell response was assessed using T-Detect, a novel method developed by Adaptive Biotechnologies that combines immunosequencing of the CDR3 region of the TCR-β locus with immune repertoire profiling to determine the SARS-CoV-2-specific clonal depth and breadth as described previously. Compared with functional assays, this method can assess the breadth and depth of T cell expansion at a higher resolution to provide a greater understanding of the cellular immune response, as well as its dynamics over time [14,24].

A positive T cell response via the binary classifier was observed in only 56% of the patients. This classifier, which applies the same analytics as used in the T-Detect COVID test, is designed to identify the TCR sequences that are commonly and reliably associated with natural infection with the SARS-CoV-2 virus and performs well in detecting vaccine response as well [25]. The sensitivity of this classifier may be impacted by (1) the restricted antigen pool (ie, spike only) of vaccination compared with natural infection, which could limit the breadth of the overall T cell response; (2) the highly individualized nature of the T cell response, which can generate TCRs unique to that individual, so- called “private” TCR sequences and therefore not identified as an enhanced sequence via this approach, which relies on public sequences, (3) kinetics of the T cell compartment, which is known to contract between 2 and 8 weeks postvaccination [25,26] and thus may be impacted by variability in time from vaccine to sample

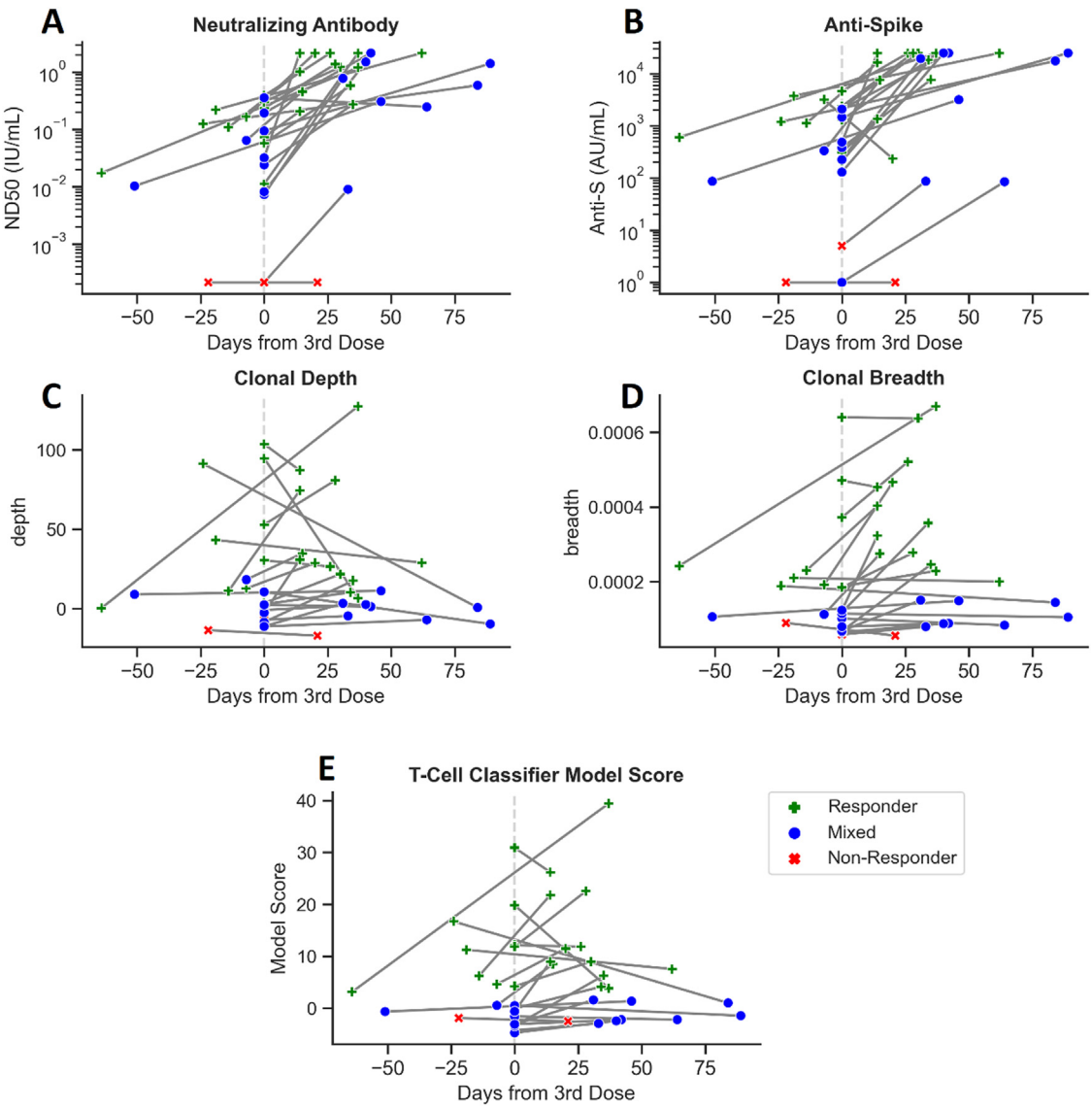

Figure 3. Individual pre- and post-third-dose B cell and T cell responses. Among 20 patients with pre- and post-third-dose samples, responses for neutralizing antibodies in IU/mL (A), anti-S antibodies in AU/mL (B), T cell clonal depth (C), T cell clonal breadth (D), and T cell model score (E) are plotted.

collection; and (4) underlying immunodeficiency with a more restricted TCR spectrum owing to transplantation or treatment with medications that inhibit T cells. These factors together could explain the lower rate of T cell response compared with serologic responses observed in our study. No significant differences in baseline characteristics were observed between those with a T cell response and those with no response, however. The sample size is too small to allow assessment of the differences or identification of correlations with specific immunosuppressive medications.

Einarsdottir et al. [27] recently demonstrated that after 2 doses, humoral and functional cellular immunity were significantly correlated in long-term HCT survivors. However, their cohort comprised only a small number of patients with cGVHD (n = 18) or on ongoing immunosuppressive treatment (n = 9), and the ongoing prednisone dose in these patients was low (median, 5 mg/day), such that an effect of these clinical factors on humoral or functional cellular responses after 2 vaccine doses was not demonstrable [27]. Intriguingly, the lack of early humoral response predicted a lack of eventual functional T cell responsiveness, and the lack of adequate functional T cell response at 3 to 4 weeks after the second vaccine dose predicted a suboptimal humoral response 4 to 5 months later [27]. These observations shift the focus to the utility of subsequent doses, which were explored in our study. The third dose of vaccine induced a robust increase in antibody titers among those who previously had mounted a B cell response and is consistent with other studies showing enhanced antibody titers [28]. Interestingly, the third dose resulted in only modest increases in the depth and breadth of T cell response. A similar observation was reported by Shroff et al. [29] among solid organ transplant patients when tested by ELISspot and by Mohammed et al. [30] among healthy adults by TCR sequencing. It is possible there are only a limited number of epitopes within the spike protein that elicit a maximal response after the initial series, and that with subsequent doses, fewer epitopes remain that have already not elicited a T cell response and thus there is no increase in clonal breadth [30]. Antibody response has been shown to improve in both affinity and efficacy in clearing the spike protein over time [31], and similarly,

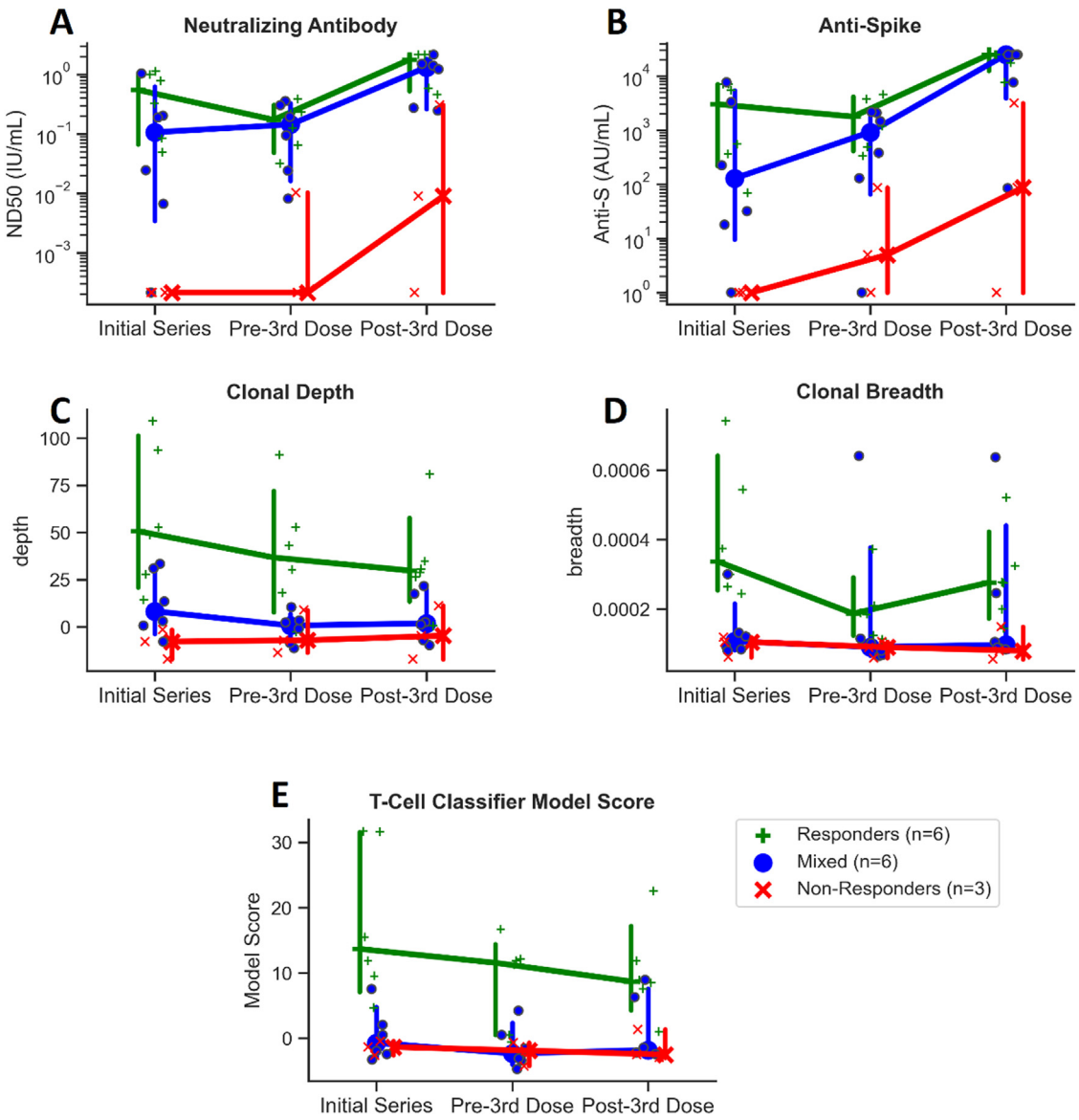

Figure 4. Group post-initial series and pre- and post-third-dose B cell and T cell responses according to response group after the initial series. For 15 patients with samples available for immune response assessment from all 3 time points—post-initial series, pre-third dose and post-third dose—median responses for neutralizing antibodies in IU/mL (A), anti-S antibodies in AU/mL (B), T cell clonal depth (C), T cell clonal breadth (D), and T cell model score (E) are plotted.

memory T cells generated from the initial series might mount an anamnestic response that is more efficient in clearing the spike protein after the third dose and could explain the lack of increase in depth [32].

The correlation between our assays and functional immunity is unknown; nevertheless, in other immunocompromised populations, experts have suggested testing for antibodies after vaccination since their absence could identify a highly vulnerable population that should be prioritized for monoclonal antibody prophylaxis. Others argue that even among responders, whether the B cell and T cell responses against the vaccine will translate into protection against severe COVID-19 disease is unclear, and so monoclonal prophylaxis should be indicated irrespective of surrogate markers of immunity.

Several studies have also attempted to identify factors associated with vaccine response among immunosuppressed patients. In their study of allogeneic HCT recipients, Cuffel et al. [33] noted a trend toward lower anti-S titers in patients with active acute GVHD or cGVHD and on immunosuppressive therapy, suggesting that apart from time to transplantation, immunosuppressive therapy and active GVHD may impact postvaccination seroconversion rates. Tamari et al. [34] evaluated humoral response to COVID-19 vaccine and aimed to identify predictors of vaccine response in 217 patients who received cellular therapy at their institution, including allogeneic HCT (n = 149), autologous HCT, and chimeric antigen receptor T cell therapy. In a multivariate analysis of the allogeneic HCT recipients, CD19 cell count and IgG level were associated with mounting a spike Ab response [34]. However, the authors did not report the number of patients who had cGVHD or the number of patients on immunosuppressive medications at the time of vaccination in their multivariate analysis, and thus whether the aforementioned predictive factors are valid in patients with active cGVHD and receiving immunosuppressive therapy is unclear. In contrast, our study focused exclusively on patients with active cGVHD who were on immunosuppressive therapy

and evaluated both humoral and cellular responses to COVID-19 vaccines. However, our small sample size precluded us from evaluating factors that might be able to predict response to the COVID-19 vaccines. Larger studies are needed to evaluate predictive factors of immune response to COVID-19 vaccines and may help identify patients less likely to respond to vaccine and direct future treatment strategies.

Ten patients in our study experienced a cGVHD flare after receiving their initial vaccine series or third dose. Five of these patients were on an immunosuppressive therapy taper, and 4 had been experiencing cGVHD progression even before receiving the vaccine doses. With limited numbers, a clear correlation could not be drawn between the COVID vaccine and exacerbation of cGVHD, but it remains a theoretical concern, given the 4% to 25% rate of cGVHD flares reported in other vaccination studies [28,35-37]. Nevertheless, the cGVHD flares appeared clinically mild and did not result in any change in organ scoring according to the National Institutes of Health consensus criteria [38] or in new organ involvement. Symptoms reverted to baseline within a couple of months with increased doses of immunosuppressive medication, except in 1 patient who had experienced cGVHD progression before vaccination and remained refractory.

Our study has several limitations, including its small sample size and some missing longitudinal immune response data. Once a third dose was recommended, many participants received it in the community, resulting in missed research samples. Another limitation is the lack of a healthy control cohort that received a third dose to put our longitudinal T cell results in context.

In summary, our study shows that 53% of patients with cGVHD on immune suppression therapy mounted both B cell and T cell responses to their initial vaccine series. Although the immune response to the vaccine was lower in our cohort compared with healthy subjects, 72% mounted a neutralizing antibody response. These data support vaccination in patients with cGVHD. Although assessment of the response to vaccination is not recommended at present, some have suggested that testing could be helpful in immunocompromised populations to help guide other preventive interventions [39]. Larger studies with long-term follow-up will help us better understand the kinetics, effectiveness, and durability of the immune response to COVID vaccination in patients with cGVHD.

ACKNOWLEDGMENTS

Financial disclosure: Funding for this study was provided by the David and Patricia Giuliani/Oliver Press Endowed Chair in Cancer Research. Adaptive Biotechnologies provided immunosequencing and computational biology services.

Conflict of interest statement: L.W.L. and D.B.G. are employees of Adaptive Biotechnologies; S.J.L. has received consulting fees from Mallinckrodt, Equillium, and Kadmon; research funding from Amgen, AstraZeneca, Incyte, Kadmon, Novartis, Pfizer, Syndax, and Takeda; and drug supply from Janssen and serves on an Incyte clinical trial steering committee. J.A.H. has received consulting fees from Allovir, Covance/CSL, CRISPR, Karius, Octapharma, OptumHealth, Pfizer, Symbio, and Takeda and research funding from Allovir, Deverra, Gilead, Karius, Merck, and Oxford Immunotec. The other authors have no conflicts of interest to report.

Authorship statement: Study conception and design: S.M., H.Q.P., L.W.L., D.B.G., J.A.H., and S.J.L.; data collection: S.M. and H.Q.P.; data analysis: L.W.L. and L.O.; drafting of manuscript: S.M., H.Q.P., and S.J.L.; critical review of manuscript: all authors. S.M. and H.Q.P. contributed equally to this work and should be considered co-first authors.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.08.026.

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