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Immunogenicity and Safety of Booster SARS-CoV-2 mRNA Vaccine Dose in Allogeneic Hematopoietic Stem Cell Transplantation Recipients

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A B S T R A C T

Allogeneic hematopoietic stem cell transplantation (HSCT) recipients are susceptible to severe outcomes of Coronavirus disease 2019 (COVID-19). Most guidelines recommend a fourth dose (ie, booster) of COVID-19 vaccine to reduce the infection risk, and observational studies are needed to determine the immunogenicity and safety of the booster dose in this population. The primary outcome was to determine the quantitative anti-receptor-binding domain (RBD) antibody titers after the fourth dose of the COVID-19 vaccine. The secondary outcomes included adverse effects and all-cause mortality.

This single-group prospective cohort included allogeneic HSCT recipients age ≥18 years who received their fourth dose of COVID-19 mRNA vaccine between December 15, 2021, and August 2, 2022. We excluded patients with a history of COVID-19 diagnosis and those who received i.v. Ig within 21 days of antibody testing or rituximab within 6 months before study entry. We used regression models to determine the contributing factors significantly associated with post-fourth dose anti-RBD titer. Sixty-seven patients (median age, 59.5 years; IQR, 53.5 to 65.5 years; 33 males [61%]) received the fourth dose of vaccine, and 54 were included in the anti-RBD titer analysis. The median anti-RBD titers at 4 to 6 weeks after the third and fourth doses differed significantly (13,350 U/mL [IQR, 2618 to 34,740 U/mL] and 44,500 U/mL [IQR, 11,163 to 84,330 U/mL], respectively; $P < .0001$). In univariate analysis, the post-third dose anti-RBD titer (β = .70; 95% CI, .54 to .87; $P < .001$) and treatment with mycophenolate compounds (β = -1.05; 95% CI, -1.97 to -1.12; P = .03) significantly predicted the antibody response to the fourth dose. In multivariate analysis, the inverse association between treatment with mycophenolate compounds and the post-fourth dose anti-RBD antibody titer was not significant (β = -.57; 95% CI, -1.32 to .19; P = .14), whereas the significant association between the anti-RBD titers following the third and fourth doses did not change considerably (β = .66; 95% CI, .47 to .86; $P < .001$). The most frequent adverse event was vaccination site soreness (44%), followed by fatigue (16%), myalgia (4%), and headache (2%). No recipient experienced new or worsened preexisting graft-versus-host disease within 40 days of vaccination, and no patient died. Six patients (11%) developed breakthrough severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection not associated with hospitalization or severe outcomes. The fourth dose of the COVID-19 vaccine appears to be highly immunogenic and safe in allogeneic HSCT recipients. Further studies are needed to determine the neutralizing antibody titers against SARS-CoV-2 subvariants and the effectiveness and immunogenicity of bivalent vaccines in allogeneic HSCT recipients.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) vaccination is now widely recognized as the primary prevention strategy targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. The humoral immune response to the COVID-19 vaccine is critically important in reducing the risk of SARS-CoV-2 infection, hospitalization, and mortality; however, studies have shown that antibody levels decrease over time,

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potentially leading to reduced protection, particularly in immunocompromised patients, who are at greater risk of severe disease compared with the general population [1]. Booster vaccine doses are currently recommended for immunocompromised individuals [2,3].

Allogeneic hematopoietic stem cell transplantation (HSCT) recipients are at particular risk of severe COVID-19 outcomes, likely secondary to the suboptimal immune response to vaccination compared to immunocompetent individuals [4]. A fourth dose (ie, booster dose) has been incorporated into vaccination schedules for allogeneic HSCT recipients. Nonetheless, data defining the benefits of COVID-19 boosters in this population are limited. We conducted this prospective cohort study to assess the safety and humoral response to the booster dose of the COVID-19 vaccine in allogeneic HSCT recipients.

METHODS

Study Design

We conducted this prospective cohort study at the Hans Messner Allogeneic Transplant Program in Toronto. The University Health Network (UHN) Institutional Research Ethics Board approved the study, and all patients provided written informed consent.

The study cohort comprised allogeneic HSCT recipients age ≥18 years who received the fourth dose (ie, booster dose) of COVID-19 mRNA vaccine between December 15, 2021, and August 2, 2022. The COVID-19 mRNA vaccines were monovalent and provided through the provincial vaccination campaign. Similar to our previous study investigating the immunogenicity and safety of COVID-19 vaccination following the 3-dose schedule in this population [5], we collected participants' blood samples at 4 to 6 weeks after the fourth dose to assess anti-RBD antibody titers. We excluded patients with a prior diagnosis of COVID-19, patients who did not return for anti-RBD antibody titer testing, and those who received i.v. immunoglobulin (IVIG) within 21 days of antibody testing or received at least 1 dose of rituximab within 6 months of study entry.

The primary outcome was to assess the quantitative anti-RBD antibody titers after the fourth dose of the COVID-19 mRNA vaccines. Secondary outcomes included SARS-CoV-2 breakthrough infection and adverse events following COVID-19 mRNA vaccination, including graft-versus-host disease (GVHD) within 40 days of vaccination [6] and all-cause mortality. For all outcomes except death, the observation period ended on September 30, 2022. The closing date for the outcome of death was October 30, 2022.

Laboratory Methods

The Roche Elecsys anti-SARS-CoV-2 S enzyme immunoassay (Roche Diagnostics, Rotkreuz, Switzerland) was used to measure anti-RBD antibodies. The assay was performed according to the manufacturer's instructions in a certified biochemistry laboratory. A positive response was defined as ≥.8 U/mL, and the assay has a lower limit of quantitation of .4 U/mL.

Safety Assessment

The Food and Drug Administration toxicity grading scale for volunteers in vaccine trials was used to categorize adverse events as follows: grade 1, no interference in daily activities;, grade 2, some interference in daily activities; grade 3, participants unable to perform daily activities; and grade 4, potentially life-threatening [7]. All patients were under follow-up after vaccination during the observation period. Furthermore, we continued follow-up until September 30, 2022 (October 30 for death) to determine the occurrence of study outcomes (ie, new onset or worsening of preexisting GVHD, COVID-19 diagnosis, hospitalization, adverse events, or death). Acute GVHD was graded according to the Keystone criteria, and chronic GVHD was defined according to the National Institutes of Health Chronic Graft-versus-Host Disease Consensus criteria [8]. GVHD outcome (ie, new onset or worsening of preexisting GVHD) had to have occurred within 40 days of the latest vaccine dose for inclusion in our analysis [6]. Reverse-transcription PCR or rapid antigen testing of upper respiratory tract specimens confirmed SARS-CoV-2 infection.

Statistical Analysis

The primary outcome of the study was to measure anti-RBD antibody titer at 4 to 6 weeks after receiving the fourth dose of COVID-19 mRNA vaccine. The antibody titer was expressed in U/mL and converted into a base-10 logarithmic scale (ie, $\log_{10}$ scale). We provided descriptive statistics to summarize the baseline characteristics of the study population. Median and interquartile range (IQR) were used for quantitative variables. Differences in the median values of paired observations were analyzed using the Wilcoxon matched-pairs signed-rank test. The Spearman rank correlation test was used to determine the degree of correlation between the anti-RBD antibody titer after the third and fourth doses. A P value <.05 was considered statistically significant.

Univariate and multivariate linear regression analyses were performed to identify variables that might impact anti-RBD titer as the primary outcome. Although we considered all variables associated (ie, $P < .2$) with anti-RBD antibody titer in the univariate analysis as potential covariates, we restricted the number of covariates to a maximum of 6, considering the number of patients in the multivariate model. To prevent collinearity, we used a correlation matrix and found no variables with strong correlation (ie, absolute correlation coefficient >.7). We also tested for interactions between covariates using the F test. We used a systematic approach to select the most parsimonious model based on the Akaike information criterion and the Bayesian information criterion [9]. We assessed the goodness of fit of the linear regression model using R^2 statistics.

Statistical analyses were performed with Stata version 15.1 (StataCorp, College Station, TX), and figures were created using Prism version 9.4.1 (GraphPad Software, San Diego, CA).

RESULTS

Sixty-seven allogeneic HSCT recipients were assessed for eligibility after receiving the fourth dose of a COVID-19 mRNA

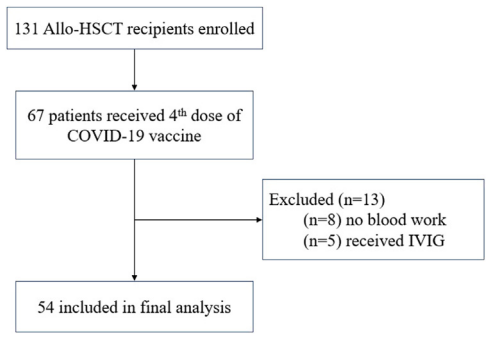

Figure 1. Study flow diagram.

vaccine. Thirteen patients (19.4%) were excluded from the analysis. Among these, 8 patients did not return for bloodwork, and 5 were treated with IVIG (Figure 1). The final analysis included 54 patients (33 males; 61%), with a median age of 59.5 years (IQR, 53.5 to 65.5 years). The participants underwent hematopoietic HSCT from a matched unrelated donor (n = 28; 52%), matched related donor (n = 14; 26%), a haploidentical donor (n = 8; 15%), or a mismatched unrelated donor (n = 4; 17%). The most frequent underlying hematologic malignancy was acute myeloid leukemia (n = 21 of 54; 39%), followed by chronic myelomonocytic leukemia (n = 9; 17%), and myelodysplastic syndrome (n = 7; 9%). The median time from transplantation to the fourth vaccine dose was 553 days (IQR, 430 to 1098 days), and 6 patients (11.1%) were in their first-year post-transplantation. Four patients were treated with calcineurin inhibitors (2 with cyclosporine and 2 with tacrolimus), and the cyclosporine median trough level was 100.5 ng/mL (IQR, 43.5 to 184.5 ng/mL). The median lymphocyte count at the time of vaccination was 1350 cells/μL (IQR, 1000 to 2500 cells/μL), and 13 patients (24.1%) had a count $<$1000 cells/μL. The most frequent comorbidity was hypertension (44%), followed by chronic kidney disease (17%). Twenty-four patients (44%) had chronic graft-versus-host disease (GVHD) at administration of the fourth dose. The baseline characteristics and demographic data of the cohort are summarized in Table 1.

Antibody Responsiveness

The median anti-RBD antibody titer after the fourth dose of vaccine was 4.6 $\log_{10}$ U/mL (IQR, 4.0 to 4.9 $\log_{10}$ U/mL), which was significantly higher than the median anti-RBD antibody titer after the third dose (4.1 $\log_{10}$ U/mL; IQR, 3.4 to 4.5 $\log_{10}$ U/mL) (Figure 2). The median change in antibody titers between the third and fourth doses was .4 $\log_{10}$ U/mL (IQR, .1 to .7 $\log_{10}$ U/mL).

Six patients in this cohort had a suboptimal response (ie, anti-RBD $<$100 U/mL) to the primary series, which included the BNT162b2 vaccine. One of these patients had laboratory evidence of hematology disease relapse. Three patients had chronic GVHD involving the gut (n = 2), skin (n = 3), mouth (n = 1), eye (n = 1), lung (n = 1), and liver (n = 2) and received ruxolitinib (n = 1), mycophenolate compounds (n = 3), and systemic corticosteroids (n = 2). Three patients received mRNA-1273 monovalent vaccine and 3 received monovalent BNT162b2 vaccine as booster doses. Only one patient subsequently developed COVID-19, which was mild, not requiring hospitalization. Of these 6 patients, 3 (50%) did not achieve anti-RBD antibody levels $>$2 $\log_{10}$ U/mL after the booster dose. These 3 patients had underlying diseases of acute myeloid leukemia, acute lymphoblastic leukemia, and chronic myelomonocytic leukemia. Two patients received transplants from haploidentical donors, and 1 patient had a matched unrelated donor. All 3 patients received a myeloablative conditioning regimen, and 2 had chronic GVHD.

We observed a significant positive correlation in anti-RBD titers after the third and fourth COVID-19 vaccine doses (Spearman ρ = .6578; $P < .001$) (Figure 3). Table 2 summarizes the results of the univariate and multivariate linear regression analyses for predictors of anti-RBD antibody titer after the fourth dose of the COVID-19 mRNA vaccine. In the univariate analysis, anti-RBD titer following the third dose was directly associated with the titer after the fourth dose. On the other hand, haploidentical donor status, myeloablative conditioning regimen, and treatment with mycophenolate compounds had

Table 1
Baseline Characteristics (N = 54)

Variable	Value
Male sex, n (%)	33 (61)
Age, yr, median (IQR)	59.5 (53.5-65.5)
Lymphocyte count, cells/μL, median (IQR)	1350 (1000-2500)
Creatinine, μmol/L, median (IQR)	89 (69-106)
Interval between transplantation and fourth dose, d, median (IQR)	553 (430-1098)
Interval between third and fourth doses, d, median (IQR)	135.5 (121.7-161.5)
Duration of follow-up, d, median (IQR)	253 (200.2-270)
Neutrophil count at receipt of fourth dose ($\times 10^9$/L), median (IQR)	2.95 (2.4-3.9)
Lymphocyte count at receipt of fourth dose ($\times 10^9$/L), median (IQR)	1.3 (1-2.5)
Donors, n (%)	
Matched related donor	14 (26)
Unrelated matched donor	28 (52)
Haploidentical donor	8 (15)
Mismatched unrelated donor	4 (7)
Indication for transplantation, n (%)	
Acute myeloid leukemia	22 (41)
Acute lymphoblastic leukemia	5 (9)
Myelodysplastic syndrome	7 (13)
Non-Hodgkin lymphoma	1 (2)
Hodgkin lymphoma	1 (2)
Chronic lymphoblastic leukemia	1 (2)
Severe aplastic anemia	1 (2)
Chronic myelomonocytic leukemia	9 (16)
Mixed phenotype acute leukemia	2 (4)
Thalassemia	1 (2)
Myelofibrosis	4 (7)
Comorbidities, n (%)	
Chronic kidney disease	9 (16)
Diabetes	4 (7)
Hypertension	22 (41)
Chronic liver disease	4 (7)
Conditioning regimen, n (%)	
Myeloablative	22 (41)
Reduced intensity	32 (59)
Total body irradiation	35 (65)
Disease status	
Relapse	2 (4)
GVHD prophylaxis/treatment, n (%)	
Antithymocyte globulin	38 (70)
Methotrexate	7 (13)
Corticosteroids	12 (23)
Tacrolimus (FK506)	2 (4)
Cyclosporine	2 (4)
Mycophenolic acid	9 (17)
Post-transplantation cyclophosphamide	40 (74)
Chronic GVHD, n (%)	24 (44.5)
Grade	
Mild	8 (15)
Moderate	15 (28)
Severe	1 (1.8)
Patients receiving active treatment	16 (30)
Vaccine, fourth dose, n (%)	
BNT162b2 mRNA (Pfizer-BioNTech)	40 (74)
mRNA-1273 (Moderna COVID-19 vaccine)	14 (26)

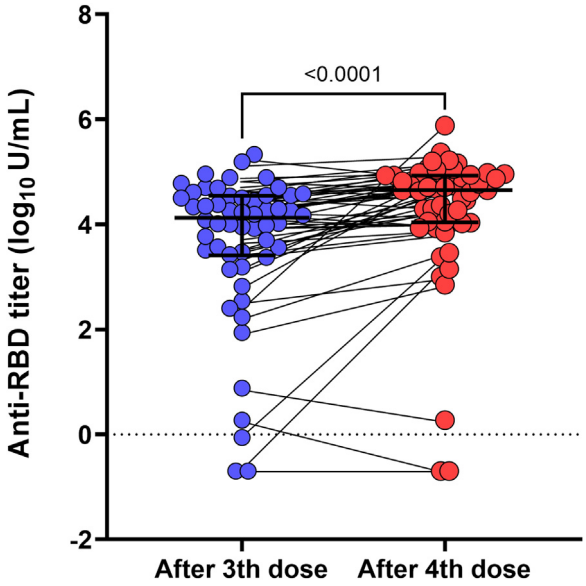

Figure 2. Anti-RBD titers after third and fourth doses of COVID-19 mRNA vaccine. The dot plots with connectors show anti-RBD antibody titers at 4 to 6 weeks after the third vaccine dose and 4 to 6 weeks after the fourth vaccine dose. Each dot represents 1 patient at each time point, and the connectors join each patient's antibody titer after each dose of the vaccine. Horizontal lines represent, from top to bottom, the 75th percentile, median (50th percentile), and 25th percentile. The vertical line represents the IQR. *P* values were estimated using the Wilcoxon matched-pair signed-rank test.

significant inverse associations with the primary outcome. No other significant associations were observed.

In multivariate analysis, the post-third dose anti-RBD antibody titer significantly predicted the post-fourth dose anti-RBD titer. No other variable significantly predicted the post-fourth dose anti-RBD titer in the multivariate regression model (Table 2).

Breakthrough SARS-CoV-2 Infection

Six patients (11.1%) presented with a breakthrough SARS-CoV-2 infection after the fourth vaccine dose. The median time between vaccination and infection was 91.5 days (IQR, 43 to 151 days). All infections were mild, and no patient required hospitalization. There was no significant difference in the median anti-RBD antibody titers between the participants who developed breakthrough SARS-CoV-2 infection and those who did not (4.32 $\log_{10}$ U/mL [IQR: 3.92 to 4.71] versus 4.68 $\log_{10}$ U/mL [IQR, 4.05 to 4.94]; $P = .24$).

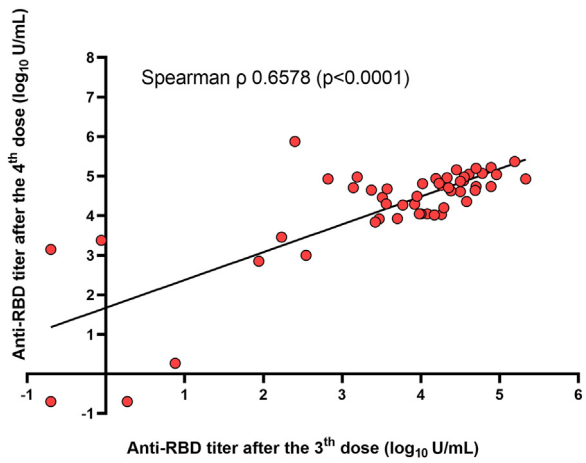

Figure 3. Correlation between anti-RBD antibody titer after the third and fourth doses of COVID-19 mRNA vaccine. Correlation is seen between the anti-RBD titer after the third vaccine dose (*x*-axis, expressed in $\log_{10}$) and the anti-RBD titer after the third dose (*y*-axis, expressed as well in $\log_{10}$). Each dot represents the anti-RBD titer of 1 individual. The diagonal line denotes the line of best fit by regression.

Adverse Events

The most frequent adverse event was tenderness over the vaccination site, reported by 44% of recipients, mostly at grade 1 intensity. Other reported adverse effects included fatigue (16%), myalgia (4%), and headache (2%). We observed no severe adverse events, and no recipient experienced new-onset GVHD within 40 days of vaccination. There were no relapses or mortality during the follow-up period. Reported adverse effects are summarized in Supplementary Table S1.

DISCUSSION

The declining antibody titer after the primary 3-dose COVID-19 vaccination series in allogeneic HSCT recipients is associated with breakthrough SARS-CoV-2 infection risk, and a booster dose is required [10]. It is of utmost importance to determine whether mounting an antibody response following booster doses of COVID-19 vaccines is expected in this increased-risk population. In this cohort, the fourth dose of mRNA COVID-19 vaccine significantly increased the anti-RBD antibody titer in allogeneic HSCT recipients. Moreover, the booster dose considerably improved the anti-RBD antibody titer in one-half of the patients with a poor immune response (ie, anti-RBD antibody titers <2 $\log_{10}$ U/mL) following the primary 3-dose vaccination series. In multivariable analysis, the anti-RBD antibody titer after the booster dose was positively correlated with the post-third dose antibody titer. Similar to the study reported by Kimura et al. [5], we found inverse associations between antibody response to the fourth dose of vaccine and haploidentical donor HSCT in univariate regression analyses. We did not observe any significant adverse effects associated with the fourth dose of the COVID-19 mRNA vaccines. Overall, the findings in this cohort demonstrate that the fourth dose is safe, effectively increases the humoral response, and supports the COVID-19 booster dose strategy in allogeneic HSCT recipients.

In our previous study, we observed new onset or exacerbation of preexisting GVHD in 9% of patients after the first dose, in 7.6% after the second dose, and in 5.4% after the third dose [5]. However, in current study, we observed no incidence of new or worsening GVHD following the administration of the booster dose. Breakthrough SARS-CoV-2 infections occurred in our study despite high antibody levels, but we did not observe any severe COVID-19 outcomes. These findings support the booster dose strategy in allogeneic HSCT recipients.

Overall, the immune response to COVID-19 vaccines may be inadequate in allogeneic HSCT recipients. Canti et al. [11] reported detectable antibodies in only 49% of allogeneic HSCT recipients at 7 weeks after a 2-dose BNT162b2 mRNA (Pfizer-BioNTech) vaccination. Considering the inadequate immune response to the 2-dose vaccination series, a third dose was included in the primary COVID-19 vaccination series for moderately or severely immunocompromised patients, such as allogeneic HSCT recipients [12,13]. Recently reported data in allogeneic HSCT recipients showed that 18% of participants had poor anti-RBD antibody titers (ie, <2 $\log_{10}$ U/mL) at 4 to 6 weeks after the third dose [5]. Therefore, a considerable proportion of allogeneic HSCT recipients may have an inadequate antibody response following the complete primary 3-dose

Table 2
Predictors of anti-RBD Antibody Titer after the Fourth Dose of COVID-19 mRNA Vaccine, Univariate and Multivariate Stepwise Linear Regression

Variable	Univariate Analysis			Multivariate Analysis		
	β	95% CI	*P* Value	β	95% CI	*P* Value
Anti-RBD antibody titer, post-third dose*	.70	.54 to .87	<.001	.66	.47 to .86	<.001
Age	.007	−.021 to .03	.50	-	-	-
Sex	.26	-.47 to 1.00	.48	-	-	-
Days after stem cell transplantation	−.0004	−.0009 to .0001	.18	-	-	-
Type of donor						
Matched related	Reference					
Unrelated matched	−.14	−.96 to .68	.74	-	-	-
Haploidentical	−1.42	−2.53 to −.32	.012	-	-	-
Mismatched unrelated	−.23	−1.64 to 1.18	.74		-	-
Number of comorbidities*	−.26	−.63 to .11	.16	.03	−.31 to .24	.81
Myeloablative conditioning	−.78	−1.48 to −.09	.028	-	-	-
Total body irradiation	.13	−.63 to .89	.74	-	-	-
Relapse of hematologic disease	.39	−1.51 to 2.29	.68			
GVHD prophylaxis/treatment						
Antithymocyte globulin	.39	−.39 to 1.16	.32	-	-	-
Methotrexate	.43	−.63 to 1.49	.42	-	-	-
Corticosteroids*	−.56	−1.42 to .29	.19	.01	−.5 to .72	.71
Calcineurin inhibitors*	−.97	−2.32 to .38	.15	−.4	−1.39 to .59	.42
Mycophenolic acid*	−1.05	−1.97 to −.12	.03	−.57	−1.32 to .19	.14
Post-transplantation cyclophosphamide	.47	−1.42 to 2.36	.62	-	-	-
Chronic GVHD	−.21	−.93 to .51	.56	-	-	-
Mild	.27	−.77 to1.32	.60	-	-	-
Moderate	−.42	−1.26 to .41	.32	-	-	-
Severe	−.95	−3.62 to 1.72	.48	-	-	-
Receiving active treatment	−.42	−1.20 to .36	.28	-	-	-
Neutrophil count at receipt of the fourth dose	−.069	−.33 to .20	.60	-	-	-
Lymphocyte count <1000 cells/μL at the fourth dose*	−.68	−1.49 to .14	.10	.11	−.50 to .73	.71

* Variables with $P < .2$ in univariate regression analysis were included in the multivariate linear regression model (ie, post-third dose anti-RBD titer, treatment with mycophenolate compounds, steroids, calcineurin inhibitors and lymphocyte count <1000 cells/μL at the time of the fourth dose). The adjusted coefficient β and *P* values for these variables are displayed. The donor status and the conditioning regimen were not included in the multivariate analysis due to significant association and interaction with other variables (eg, rabbit antithymocyte globulin) and lack of biological plausibility considering the interval between transplantation and the fourth dose administration (see text).

series. Additionally, a recent multicenter registry showed a significant decline in antibody titers at 3 to 6 months after the complete primary vaccination series in allogeneic HSCT recipients associated with a 15% risk of breakthrough infection [10]. These data, along with our current results, highlight the importance of booster vaccinations in COVID-19 prevention strategies.

It was also necessary to examine whether clinical factors observed in heuristic approaches determining the risk factors for suboptimal response to the primary series (eg, haploidentical donor status, lymphopenia) affect the antibody response to the booster vaccine doses. Chaekal et al. [14] found that low lymphocyte count (<1000 cells/μL), chronic GVHD, and vaccination within the first year after transplantation were independent predictors of poor response to primary vaccination series, but donor source was not. On the other hand, Kimura et al. [5] showed that haploidentical donor status is significantly associated with a suboptimal immune response to a 3-dose COVID-19 mRNA vaccination series. In our cohort, this variable had a significant association with study outcome in univariate analysis. We did not include the conditioning regimen and donor type in the multivariate analysis. These variables had a significant association (eg, the association between rabbit antithymocyte globulin and myeloablative conditioning: 29/32 versus 9/22; $P < .001$) or interaction (eg, the interaction between the conditioning regimen and post-third dose anti-RBD titer: $\beta = .56$; 95% CI, .23 to .88) with other covariates. The conditioning regimens and donor status likely were surrogates for other variables. In addition, the effect of these 2 variables on the antibody response to the booster dose lacks biological plausibility, considering the interval between transplantation and the booster dose administration (median, 553 days; IQR, 430 to 1098 days).

We did not observe any significant association between anti-RBD antibody titer after the fourth dose and vaccination timing, chronic GVHD, or lymphocyte count. On the other hand, there was a significant association between treatment with mycophenolate compounds and post-fourth dose anti-RBD titer in the univariate analysis. The effect of this variable did not remain significant in multivariate analysis. In the study conducted by Kimura et al. [5], treatment with mycophenolate compounds had no significant association with the post-third dose anti-RBD titer. In a recent multicenter European study, treatment with mycophenolate compounds did not considerably change the COVID-19-associated outcomes in allogeneic HSCT patients receiving vaccines [4]. Similar to our data, Hill et al. [15] did not observe any significant relationships between antibody response to COVID-19 mRNA vaccines and such clinical factors as vaccination timing, presence of GVHD, immunosuppressive therapy at the time of vaccination, and absolute lymphocyte counts. Of note, in that study, only 10 patients received the fourth dose of COVID-19 mRNA vaccines, and the

details of antibody response in those 10 individuals were not provided. Overall, the immune response to the booster dose of the COVID-19 mRNA vaccine in allogeneic HSCT recipients appears reassuring.

The differences between our cohort and the populations in previous studies investigating the immune response to the primary COVID-19 vaccine series should be considered when interpreting our current findings. First, the patients in our cohort had already completed a 3-dose primary series of COVID-19 vaccination and increasing the number of doses is associated with a greater likelihood of optimal response to vaccines [16]. Second, the COVID-19 vaccination campaign for allogeneic HSCT recipients typically begins at least 3 to 6 months post-transplantation [17]. Considering the interval between vaccine doses, individuals who are eligible to receive the booster dose are typically beyond the first-year post-transplantation (88.9% of our cohort). Third, most patients receiving the fourth dose achieved lymphocyte reconstitution, and we observed a poor association between lymphocyte count and post-fourth dose anti-RBD titer. The predictors of suboptimal immune response to the second or third doses of COVID-19 mRNA vaccines that are time-dependent are not typically present when investigating the immune response to COVID-19 mRNA vaccine booster doses.

Our study was associated with some limitations. Since all patients were recruited from the outpatient clinic and patients with chronic GvHD are frequently seen in the outpatient setting, we observed a relatively high frequency of patients with chronic GvHD in our cohort. We did not measure variant-specific neutralizing antibodies, which might have provided additional information for the circulating variants; however, anti-RBD titer measurement is a standard approach to determining overall humoral immunity. Health Canada approved the bivalent vaccines as boosters in December 2022, after our study period; therefore, we could not evaluate the efficacy of bivalent COVID-19 vaccines, which is the subject of future studies. Some patients might have developed asymptomatic SARS-CoV-2 infection between the third and fourth doses. Eventually, we did not determine the effect of anti-CD20 monoclonal antibodies on anti-RBD titers, because this variable was an exclusion criterion.

In conclusion, we have shown that the booster dose of the COVID-19 vaccine in allogeneic HSCT recipients is safe and induces a robust humoral immune response. Most adverse events were mild and transient, and we observed no severe adverse events, new-onset or worsening existing GVHD, relapse, or mortality during the follow-up period. These findings provide important insight into the optimal vaccination strategy for this vulnerable population and support the use of booster vaccine doses to enhance the protective immune response against SARS-CoV-2 infection. Further studies are needed to determine the neutralizing antibody titers against SARS-CoV-2 subvariants and the effectiveness and immunogenicity of bivalent vaccines in allogeneic HSCT recipients.

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Authorship statement: A.M. and J.T.S. contributed equally as co-first authors.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2023.08.008.

APPENDIX

PREVenT COVID Group Investigators

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REFERENCES

1. Chemaitelly H, Tang P, Hasan MR, et al. Waning of BNT162b2 vaccine protection against SARS-CoV-2 infection in Qatar. *N Engl J Med*. 2021;385:e83.
2. Duly K, Farraye FA, Bhat S. COVID-19 vaccine use in immunocompromised patients: a commentary on evidence and recommendations. *Am J Health Syst Pharm*. 2022;79:63–71.
3. City of Toronto. COVID-19: vaccine eligibility & doses. 2021. Available at: https://www.toronto.ca/community-people/health-wellness-care/health-programs-advice/respiratory-viruses/covid-19/covid-19-vaccines/covid-19-vaccine-eligibility-doses/ Accessed May 15, 2023.
4. Busca A, Salmanton-García J, Marchesi F, et al. Outcome of COVID-19 in allogeneic stem cell transplant recipients: results from the EPICOVIDEHA registry. *Front Immunol*. 2023;14: 1125030.
5. Kimura M, Ferreira VH, Kothari S, et al. Safety and immunogenicity after a three-dose SARS-CoV-2 vaccine schedule in allogeneic stem cell transplant recipients. *Transplant Cell Ther*. 2022;28. 706.e1-706.e10.
6. Ali H, Ngo D, Aribi A, et al. Safety and tolerability of SARS-CoV2 emergency-use authorized vaccines for allogeneic hematopoietic stem cell transplant recipients. *Transplant Cell Ther*. 2021;27. 938.e1-938.e6.
7. Federal Register. Guidance for industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials; availability. September 27, 2007. Available at: https://www.federalregister.gov/documents/2007/09/27/E7-19155/guidance-for-industry-toxicity-grading-scale-for-healthy-adult-and-adolescent-volunteers-enrolled-in. Accessed February 18, 2023.
8. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. The 2014 Diagnosis and Staging Working Group report. *Biol Blood Marrow Transplant*. 2015;21. 389-401.e1.
9. Portet S. A primer on model selection using the Akaike Information Criterion. *Infect Dis Model*. 2020;5:111–128.
10. Piñana JL, Martino R, Vazquez L, et al. SARS-CoV-2-reactive antibody waning, booster effect and breakthrough SARS-CoV-2 infection in hematopoietic stem cell transplant and cell therapy recipients at one year after vaccination. *Bone Marrow Transplant*. 2023;58:567–580.
11. Canti L, Humblet-Baron S, Desombere I, et al. Predictors of neutralizing antibody response to BNT162b2 vaccination in allogeneic hematopoietic stem cell transplant recipients. *J Hematol Oncol*. 2021;14:174.
12. Britton A, Embi PJ, Levy ME, et al. Effectiveness of COVID-19 mRNA vaccines against COVID-19-associated hospitalizations among

immunocompromised adults during SARS-CoV-2 Omicron predominance - VISION Network, 10 states, December 2021-August 2022. *MMWR Morb Mortal Wkly Rep*. 2022;71:1335–1342.
13. Bar-On YM, Goldberg Y, Mandel M, et al. Protection of BNT162b2 vaccine booster against Covid-19 in Israel. *N Engl J Med*. 2021;385:1393–1400.
14. Chaekal OK, Gomez-Arteaga A, Chen Z, et al. Predictors of Covid-19 vaccination response after in-vivo T-cell-depleted stem cell transplantation. *Transplant Cell Ther*. 2022;28. 618.e1-618.e10.
15. Hill JA, Martens MJ, Young JH, et al. SARS-CoV-2 vaccination in the first year after allogeneic hematopoietic cell transplant: a prospective, multicentre, observational study. *eClinicalMedicine*. 2023;59: 101983. [Internet]Available at: https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(23)00160-8/fulltext. Accessed May 15, 2023.
16. Clémenceau B, Guillaume T, Coste-Burel M, et al. SARS-CoV-2 T-cell responses in allogeneic hematopoietic stem cell recipients following two doses of BNT162b2 mRNA vaccine. *Vaccines (Basel)*. 2022;10:448.
17. Government of Ontario Ministry of Health and Long-Term Care. MERS-CoV (novel coronavirus) - Ministry Programs - Health Care Professionals. Available at: https://www.health.gov.on.ca/en/pro/programs/publichealth/coronavirus/covid19_vaccine.aspx. Accessed March 14, 2023.