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Safety and Immunogenicity of the BNT162b2 mRNA COVID-19 Vaccine in Patients after Allogeneic HCT or CD19-based CART therapy—A Single-Center Prospective Cohort Study

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A B S T R A C T

Data are scarce regarding both the safety and immunogenicity of the BNT162b2 mRNA COVID-19 vaccine in patients undergoing immune cell therapy; thus, we prospectively evaluated these two domains in patients receiving this vaccine after allogeneic hematopoietic cell transplantation (HCT; $n = 66$) or after CD19-based chimeric antigen receptor T cell (CART) therapy ($n = 14$). Overall, the vaccine was well tolerated, with mild non-hematologic vaccine-reported adverse events in a minority of the patients. Twelve percent of the patients after the first dose and 10% of the patients after the second dose developed cytopenia, and there were three cases of graft-versus-host disease exacerbation after each dose. A single case of impending graft rejection was summarized as possibly related. Evaluation of immunogenicity showed that 57% of patients after CART infusion and 75% patients after allogeneic HCT had evidence of humoral and/or cellular response to the vaccine. The Cox regression model indicated that longer time from infusion of cells, female sex, and higher CD19$^+$ cells were associated with a positive humoral response, whereas a higher CD4$^+$/CD8$^+$ ratio was correlated with a positive cellular response, as confirmed by the ELISpot test. We conclude that the BNT162b2 mRNA COVID-19 vaccine has impressive immunogenicity in patients after allogeneic HCT or CART. Adverse events were mostly mild and transient, but some significant hematologic events were observed; hence, patients should be closely monitored.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has variable presentations. An increased risk for severe disease and death has been noted among patients after allogeneic hematopoietic cell transplantation (HCT), with a case fatality rate of 9% to 30% [1, 2]. The BNT162b2 mRNA COVID-19 (Pfizer-BioNTech; Pfizer, New York, NY) vaccine was recently approved by both the US Food and Drug Administration (FDA) and European Medicines Agency for the prevention of COVID-19, based on a phase III study that showed 94.6% efficacy [3]. Although this vaccine is recommended by the FDA, European Society for Blood and Marrow Transplantation (EBMT), and National Marrow Donor Program for immunosuppressed patients, data are scarce regarding vaccine effectiveness and safety in patients undergoing immune cell therapy. It is reasonable to assume that, like the response to other vaccines, patients after cell therapy would demonstrate a lower response rate compared with the general population [4, 5]. In addition, immunologic alterations that may result in exacerbation of graft-versus-host disease (GVHD) and other immune phenomena are also potential concerns. In this study, we

aimed to evaluate the safety and immunogenicity of the BNT162b2 mRNA COVID-19 vaccine in patients who underwent either allogeneic HCT or CD19-based chimeric antigen receptor T cell (CART) therapy.

METHODS
Patients

This was a prospective study performed at the BMT Long-Term Follow-up (LTFU) clinic, Tel Aviv Sourasky Medical Center. All sequential patients who have undergone allogeneic HCT and CART therapy at the center are followed in the LTFU clinic. Patients were eligible for this study if they fulfilled the EBMT criteria for COVID-19 vaccination (Version 2.0, December 21, 2020), including age >18 years and at least a 3-month interval between cell infusion and referral to vaccination. In patients after CART infusion, if the blood level of CD19$^+$ cells was 0 after 3 months, vaccination was deferred to 6 months after CART infusion. After 6 months, patients were vaccinated irrespective of the CD19$^+$ cell count. In addition, our protocol exclusion criteria included grade 3 or 4 acute GVHD, treatment for acute or chronic GVHD with ≥0.5 mg/kg of prednisone (or an equivalent steroid formula), treatment with rituximab within the previous 6 months, treatment with mesenchymal cells within 1 month, hematologic relapse, treatment with maintenance therapy (excluding tyrosine kinase inhibitors), previous infection with SARS-CoV-2 or recent exposure to a SARS-CoV-2−infected person, or known allergy to vaccine components. In case of GVHD exacerbation, patients were not eligible for the second dose of vaccine until they returned to the baseline status of GVHD. The study was approved by the hospital ethics committee (#1067-20) and was registered in ClinicalTrials.gov (NCT04724642). All patients signed informed consent prior to enrolment.

Protocol Vaccination and Evaluation

Patients were vaccinated through the national Israeli vaccination program that started in mid-December 2020. All patients had a baseline serology test to detect anti-nucleocapsid antibodies to ensure a SARS-CoV-2−negative status and a baseline quantification of peripheral blood CD19$^+$, CD4$^+$, and CD8$^+$ cells. Prior to the first dose of the BNT162b2 mRNA COVID-19 vaccine, patients were reassessed for suitability for vaccination, including physical examination, assessment of GVHD status, complete blood count, and liver function tests. One week after administration of the first vaccine dose, patients were interviewed for post-vaccination adverse events, underwent physical evaluation and repeated laboratory tests, and were then scheduled for their second vaccine dose. Patients were reassessed 7 to 14 days after the second vaccine dose and had a blood test for SARS-CoV-2 serology and enzyme-linked immunosorbent spot (ELISpot) assay. Patient demographics, disease characteristics, and GVHD parameters were collected prospectively. Concomitant immunosuppressive therapy (IST) was also documented, and we defined high-intensity IST (as opposed to low-intensity IST) as either a prednisone dose of ≥.25 mg/kg/day or another IST medication.

The primary endpoint was the incidence of grade 3 or 4 adverse events and GVHD exacerbation.

Secondary endpoints included overall adverse effects and humoral and cell-mediated response to vaccine (measured by anti-S immunoglobulin G and ELISpot tests, respectively).

We graded adverse events according to Common Terminology Criteria for Adverse Events 5.0, acute GVHD according to the Mount Sinai Acute GVHD International Consortium criteria, and chronic GVHD according to the National Institutes of Health 2014 grading and response criteria [6]. Causality of adverse events was defined according to the World Health Organization−Uppsala Monitoring Centre categories (certain, probable, possible, and unlikely; http://who-umc.org/Graphics/24734.pdf).

Serology Detection of Antibodies to the Spike Protein

Seroprotective antibodies were analyzed and measured 7 to 14 days after the second vaccine dose using the Elecsys Anti-SARS-CoV-2 S assay on the Cobas e411 (Roche Diagnostics, Basel, Switzerland), an electrochemiluminescence immunoassay intended for qualitative and semiquantitative detection of antibodies (including immunoglobulin G) to the SARS-CoV-2 spike protein receptor binding domain (RBD) in human serum. Based on the manufacturer's instructions, an antibody concentration of ≥0.80 U/mL is considered positive (upper limit, 250 U/mL). Units are specific for this assay only, and 20 U/mL correspond to a 1-nM mixture of two anti-RBD monoclonal antibodies.

Evaluation of Cellular Response by ELISpot Assay

Following initial evaluation of cytokine production by flow cytometry and understanding that stimulation with a pooled M-peptide mix induces the strongest interferon gamma (IFN-γ) and interleukin 2 (IL-2) production (Supplementary Figure S1A,B), further evaluation of the anti-S (spike glycoprotein) cellular response was evaluated using the ELISpot assay for the detection of peptide-induced IFN-γ and IL-2 secretion (Human IFN-γ/IL-2 Dual ELISpot, 874.040.005S; Diaclone, Besançon, France) according to the

manufacturer's instructions. For this purpose, donor cells were plated at 100,000 cells/100 mL and were stimulated with the relevant peptides for 19 hours at 37°C. Cells were stimulated with spike glycoprotein peptides for evaluation of anti-vaccine responses (peptide concentration of 0.9 nmol/mL), membrane glycoprotein peptides for evaluation of previous exposure to SARS-CoV-2 and detection of convalescent samples (peptide concentration of 0.9 nmol/mL), or phorbol 12-myristate 13-acetate (PMA)/ionomycin as control to confirm cell viability and responsiveness (PMA concentration, 5 ng/mL; ionomycin concentration, 500 mg/mL), in addition to pre-stimulation trypan blue staining. Cytokine detection was evaluated by manual spot counting. Peptides used for stimulation included a pool of lyophilized peptides of the viral spike glycoprotein (S) or membrane glycoprotein (M) (PepTivator SARS-CoV-2 Prot_S and Prot_M; Miltenyi Biotec, Bergisch Gladbach, Germany). Per the manufacturer's information, these peptide pools consist of 15-mer sequences with an overlap of 11 amino acids, covering the immunodominant sequence domains of the S glycoprotein (amino acids 304 to 338, 421 to 475, 492 to 519, 683 to 707, 741 to 770, and 785 to 802 and the sequence end 885 to 1273) and the complete sequence of the M glycoprotein. Based on control groups of pre-vaccinated individuals ($n = 8$) and convalescent mildly affected individuals ($n = 4$), we set the threshold for positive cellular response at four spots per well.

Intracellular Cytokine Staining

In order to evaluate the ability of convalescent donor T cells to recognize viral peptides, intracellular cytokine staining was performed (Figure 1). For this purpose, peripheral blood mononuclear cells were stimulated in 96-well plates (150,000 cells/150mL) using a pool of lyophilized peptides of the viral S glycoprotein, M glycoprotein, or nucleocapsid (N) protein (PepTivator SARS-CoV-2 Prot_S, Prot_M, and Prot_N; Miltenyi Biotec). Per the manufacturer's information, these peptide pools consist of 15-mer sequences with an overlap of 11 amino acids, covering the immunodominant sequence domains of the S glycoprotein (amino acids 304 to 338, 421 to 475, 492 to 519, 683 to 707, 741 to 770 and 785 to 802, and the sequence end 885 to 1273), as well as the complete sequence of the M glycoprotein and N protein.

For the purpose of intracellular cytokine staining, plated cells were stimulated with the relevant peptides and controls as described in the main text. Brefeldin A was added 1 hour after stimulation, and cells were incubated overnight for an additional 14 hours. Cells were then fixed, permeabilized, and stained intracellularly for IFN-γ, IL-2, and IL-17. The following Invitrogen (Waltham, MA) antibodies were used: CD4 PB (clone RPA-T4), CD8a PE (clone RPA-T8), IL-17a FITC (clone BL168), IFN-γ APC (clone 4S.B3), and IL-2 PE-Cy7 (clone MQ1-17H12). Cells were acquired using a BD FACSCanto II flow cytometer (BD Biosciences, Franklin Lakes, NJ) and analyzed using FlowJo 10.0 software.

Statistical Analysis

Continuous variables were described as the mean, median, standard deviation, and range of values, as applicable. Categorical data were described with contingency tables including frequency and percent. Antibody titers were compared between patient groups using either Pearson's chi-square or t-test, as appropriate. The associations between various parameters and the serology/ELISpot test results were determined using bilinear logistic regression, with two-sided $P < .05$ considered to be statistically significant. SPSS Statistics 27 (IBM, Armonk NY) was used to perform all analyses.

RESULTS
Patients and Vaccination Schedule

Beginning December 23, 2020, all patients who were part of the BMT LTFU clinic at the Tel Aviv Sourasky Medical Center ($n = 155$), were assessed for eligibility based on the EBMT recommendations (Figure 2). Of those, 28 medical-tourism patients were given recommendations for vaccination in their countries of origin and were vaccinated off-protocol. Patients were excluded because of an unwillingness to come to the clinic for post-vaccination surveillance ($n = 24$), uncontrolled GVHD ($n = 4$), short period after HCT or CART (<3 months; $n = 5$), and lack of complete remission after CART therapy ($n = 6$). Eight patients (7.1% out of 112 eligible patients) refused the vaccination program (six due to general opposition to vaccinations and two because of specific worries about receiving the new COVID-19 vaccine). Three patients were diagnosed with SARS-CoV-2 infection after receiving the first vaccination and thus were not evaluated for the immunogenicity endpoints.
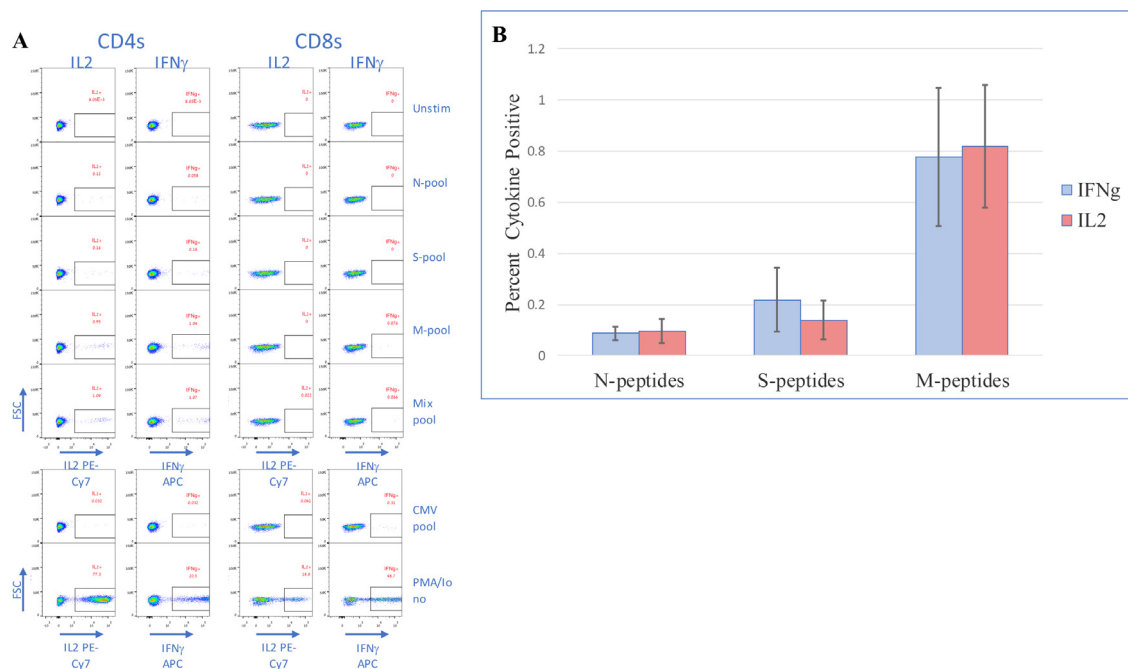
Figure 1. Intracellular cytokine staining. (A) Representative flow cytometry plots of convalescent stimulated peripheral blood mononuclear cells (PBMCs). Cells were stimulated overnight with the indicated peptide pools in the presence of brefeldin A. The following day, cells were fixed, permeabilized, and intracellularly stained for IL-2 and IFN-γ. Left columns show results of gated CD4s and right columns of gated CD8s. As can be seen, stimulation with pooled M peptides resulted in the highest IL-2 and IFN-γ staining in CD4$^+$ cells, but the S peptide pool resulted in lower levels of secretion, which were even lower for the N peptide pool. Of note, CD8$^+$ cells were negative for the stained cytokines, which could theoretically suggest that the peptide pools used preferentially bind to major histocompatibility complex II. (B) Bar graph showing the average percent of cytokine-positive cells within CD4$^+$ T cells after stimulation of PBMCs with different mixed peptide pools (N, S, or M). The average of five convalescent donors is shown. Stimulation with the M peptide pool led to a significantly higher percent of IL-2$^+$ and IFN-γ^+ cells.
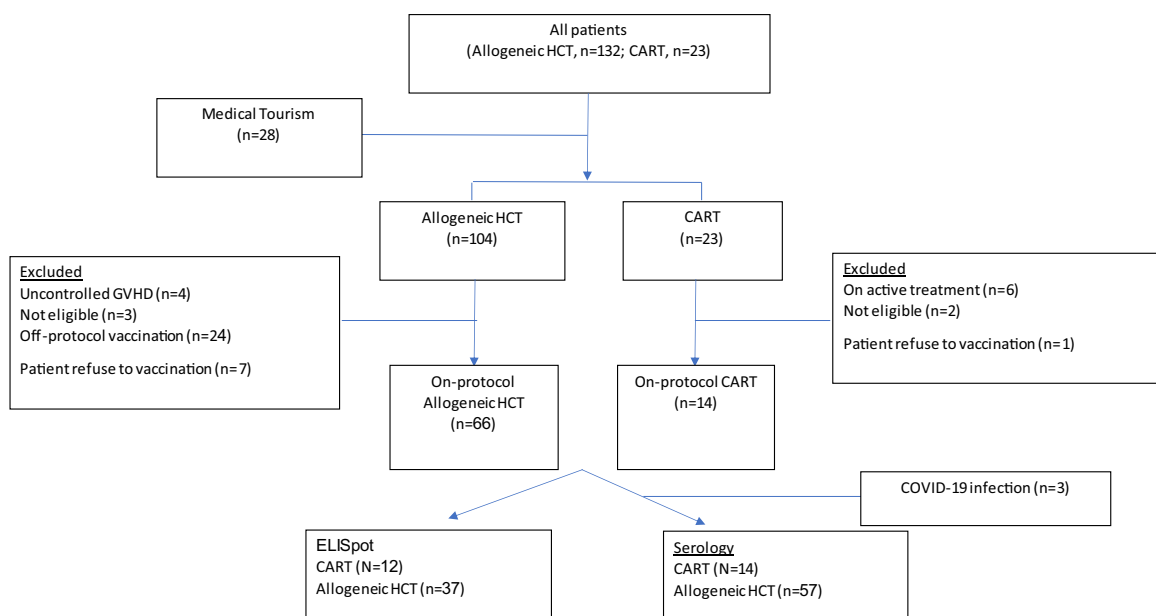
Figure 2. Disposition of patients.

Table 1 provides the characteristic of all 80 on-protocol patients. Median age was 65 years (range, 23 to 83). The majority of the patients after allograft (n = 40, 62%) had active chronic GVHD, and 58% were on active IST. Complete B cell aplasia was documented in nine patients (11.3%): in eight of the patients after CART infusion and in one patient after allogeneic HCT with ongoing severe chronic GVHD.

Assessment of Eligible Patients for Suitability for Vaccination Program

All patients underwent baseline physical examination, general laboratory testing, and lymphocyte subpopulation analysis to assess suitability to the first vaccine dose. Among the patients after CART infusion (n = 14), all were recommended to continue with the vaccination program. Three patients after

Table 1
Characteristics of Patients

Characteristic	Entire Cohort (N = 80)
Age (yr), median (range)	65 (23-83)
Female, n (%)	36 (45)
Months from infusion, median (range)	
Allogeneic HCT	32 (3-263)
CART	9 (3-17)
Status of disease, n (%)	
Remission	77 (96)
Relapse[a]	3 (4)
Baseline disease, n (%)	
AML	37 (46)
MDS	7 (9)
ALL	8 (10)
DLBCL	13 (15)
Other lymphoma	7 (10)
Myeloproliferative neoplasm	7 (9)
Other	1 (1)
Preparative regimen, n (%)	
Myeloablative	40 (61)
Reduced intensity/non-myeloablative	26 (39)
Donor, n (%)	
Matched sibling	17 (26)
Mismatched family donor	3 (5)
Matched unrelated donor	44 (68)
Mismatched unrelated donor	2 (2)
GVHD, n (%)	
Active acute	0 (0)
Active chronic	40 (62)
Previous (non-active) chronic	3 (5)
Patients on active IST, n (%)	
Low-intensity[†]	6 (16)
High-intensity[†]	32 (84)
Patients on active chemotherapy, n (%)	6 (8)
Patients with complete B cell aplasia, n (%)	9 (11.3)
Total lymphocyte count, median (range)	
Total CD19$^+$ lymphocyte	89 (0-1078)
Total CD4$^+$ lymphocyte	325 (10-1575)
Total CD8$^+$ lymphocyte	756 (48-4158)
CD4$^+$/CD8$^+$ ratio, median (range)	0.48 (0.14-2.9)

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphocytic leukemia; DLBCL, diffuse large B cell lymphoma.
*All patients with stable mixed chimerism and myeloproliferative neoplasms.
[†] Of the total 38 patients given IST.

allogeneic HCT were recommended to postpone the first dose of the vaccine because of suboptimal control of GVHD. All three of these patients completed the vaccination program after stabilization of their GVHD. Median time from onset of the national vaccination program to the first vaccine dose was 15 days (range, 1 to 50). Six patients did not receive the second dose of vaccine (development of SARS-CoV-2 infection, n = 3; unwillingness to receive the second dose because of presumably vaccine-related adverse events, n = 3). Median time between the first and second dose of the vaccine was 21 days (range, 17 to 36).

Tolerability and Safety

Overall, non-hematologic vaccine-reported adverse events were observed in 11 patients (14%) after the first vaccine dose and in 18 patients (24%) after the second dose (Figure 3). There were no grade 3 or 4 non-hematologic adverse events, and the majority

of adverse events were graded as grade 1. A patient after allogeneic HCT developed a grade 2 vasculitic rash on the leg. Further investigation of this case revealed that there was no evidence of infection. Blood tests were negative for antinuclear antibodies and antineutrophil cytoplasmatic antibodies. Pathologic examination revealed lymphohistiocytic infiltrate. The event was graded as possibly related to the vaccine. The patient was treated with steroid cream and the rash resolved within 2 weeks (Figure 4a).

Several hematologic adverse events were considered by the investigators to be possibly vaccine related.

Cytopenia development/exacerbation. Twelve percent of the patients developed cytopenia after the first dose, and 10% developed cytopenia after the second dose. The majority of the cases were grade 1 or 2 (thrombocytopenia, n = 7 [8.8%]; neutropenia, n = 3 [3.8%]) and anemia (n = 1 [1.3%]); however, four patients (5%) developed grade 3 or 4 thrombocytopenia (n = 3) or neutropenia (n = 1). In most cases, cytopenia resolved within 2 weeks and patients subsequently received the second vaccine dose. None of these resulted in significant morbidity or required hospitalization. Hemoglobin continued to decrease for 2 weeks in a 69-year-old female with a history of myelodysplastic syndrome and refractory anemia; 6 months earlier, this patient had undergone allogenic HCT from a matched unrelated donor (Figure 4b). Of note, there was no evidence of GVHD, and she was still receiving low-dose cyclosporine. To investigate this, we performed bone marrow aspiration that showed trilineage hematopoiesis with no evidence for increased blast percentage and hypolobulated megakaryocytes. The latter finding was not noted in a previous bone marrow evaluation. Cytogenetic analysis showed normal karyotype; however, the percentage of the donor's chimerism gradually dropped from 97% to 59%. Cyclosporine was stopped, and she received two separate doses of donor lymphocyte infusion (1×10^6/kg and 5×10^6/kg) (Figure 4b). This impending secondary rejection was considered to be a possible vaccine-associated adverse event.

GVHD exacerbation. After the first vaccine dose, there were three cases (4.5%) of exacerbation of GVHD, all of which developed within the first week after injection. The grade 2 oral GVHD resolved 18 days after intervention with steroid mouthwash (n = 1); the grade 2 liver and grade 1 oral GVHD resolved within a week with no intervention (n = 1); and the grade 2 lower gut GVHD resolved within 3 weeks after a short steroid course of prednisone 0.25 mg/kg per day (n = 1). All three patients subsequently received the second dose. After the second dose of vaccine there were also three cases of exacerbation of GVHD, all of which occurred within 1 week after injection. The grade 2 liver GVHD and arthralgia resolved within 2 weeks with a short course of low-dose steroids (n = 1); the grade 3 fasciitis and skin rash returned to baseline after the prednisone dose was increased from 0.15 mg/kg to 0.25 mg/kg for 2 weeks; and the grade 2 oral GVHD resolved within 2 weeks with the use of steroid mouthwash (n = 1). Because all patients had a stable chronic GVHD for at least a month prior to vaccination, we considered all cases of GVHD exacerbation to be possibly related to vaccination.

Immunogenicity

Three patients (all after allogeneic HCT) who developed COVID-19 infection after the first vaccine dose were excluded from this analysis. All 14 patients after CART infusion were evaluated by serology and 12 patients (86%) by ELISpot assay. Among the patients after allogenic HCT (n = 63), 57 patients (90%) and 37 patients (59%), respectively, were evaluated.
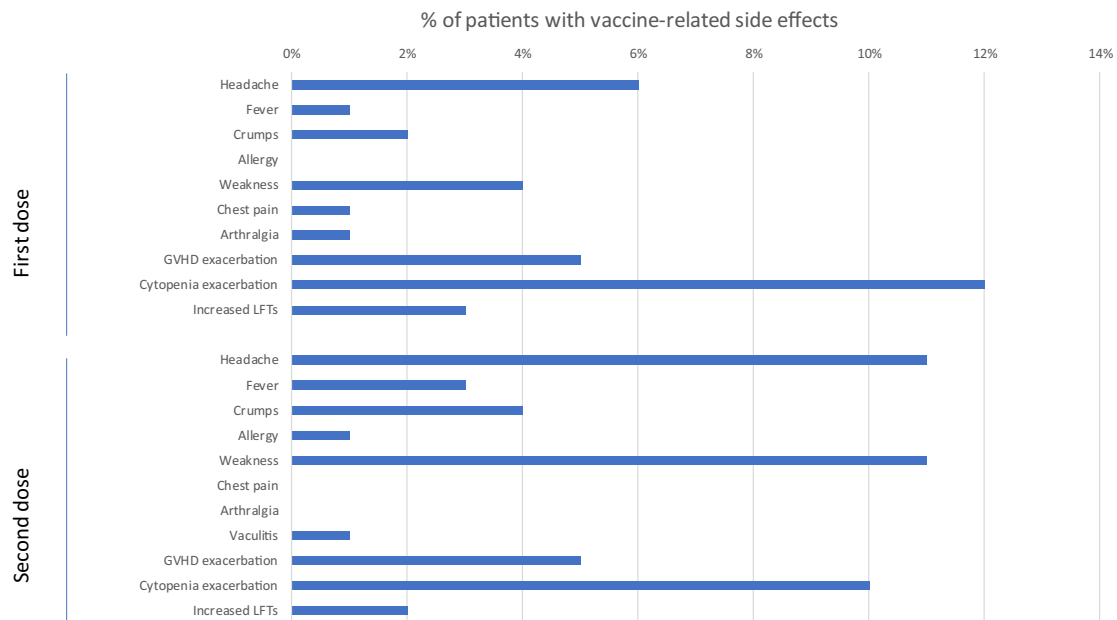
% of patients with vaccine-related side effects

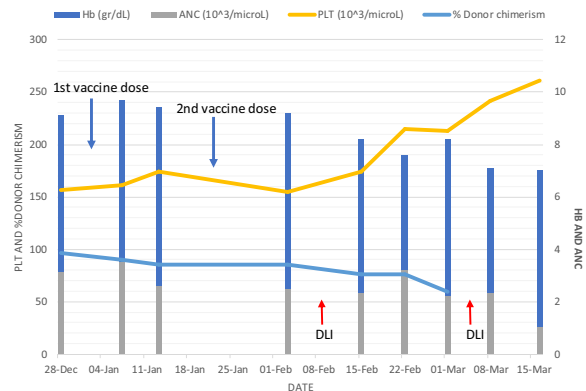**Figure 3.** Adverse events of vaccine.

A

B

Figure 4. (A) Skin rash consistent with dermal vasculitis after the first vaccine dose. (B) Hb, ANC, PLT, and percentage of donor chimerism in a patient with impending secondary graft rejection. Hb indicates hemoglobin; ANC, absolute neutrophil count; PLT, platelets; DLI indicates donor lymphocyte infusion.

Positive serology was documented in five patients (36%) after CART infusion and in 47 patients (75%) after allogeneic HCT. Median titer levels were 0.4 units/mL (range, 0.4 to 250) and 178 units/mL (range, 0.4 to 250), respectively (Figure 5). Positive ELISpot was documented in six patients (50%) after CART infusion and in seven patients (19%) after allogeneic HCT. Of the 13 patients with negative serology and available ELISpot, three patients (23%) showed a cellular response with positive ELISpot results. All three patients had complete B cell aplasia and all were after CART infusion. Overall, taking into consideration both the serology and the ELISpot tests, eight (57%) after CART infusion and 47 patients (75%) after allogeneic HCT had evidence of either humoral or cellular response to the vaccine.

On multivariate analysis, a positive humoral response to the vaccine was associated with increased time from infusion of cells (P= .032), female sex (P = .028), and higher number of CD19^{+} cells (P = .047), whereas age, active GVHD, and intensity of concomitant IST did not predict response (Table 2). Focusing on the subgroup of patients after CART infusion revealed that patients with B cell reconstitution had a higher incidence of positive serology compared with those with B cell aplasia (66% vs. 11%; P = .025). In the allogeneic HCT group, only one patient had complete B cell aplasia, and this patient had a negative serology test.

Because of the low number of positive ELISpot results, we did not perform an analysis to identify potential predictors; however, the ELISpot results were correlated with the CD4^{+}/CD8^{+} ratio (Pearson correlation = .54; 95% confidence interval, .29 to .72; P< .001). The number of CD19^{+}, CD4^{+}, and CD8^{+} cells did not significantly correlate with the probability of positive test results.

DISCUSSION

In this study, we evaluated the safety profile and response to the BNT162b2 mRNA COVID-19 vaccine. To our knowledge, this is the first study that has focused on patients after cell therapy, including allogeneic HCT and CART therapy. We showed that in
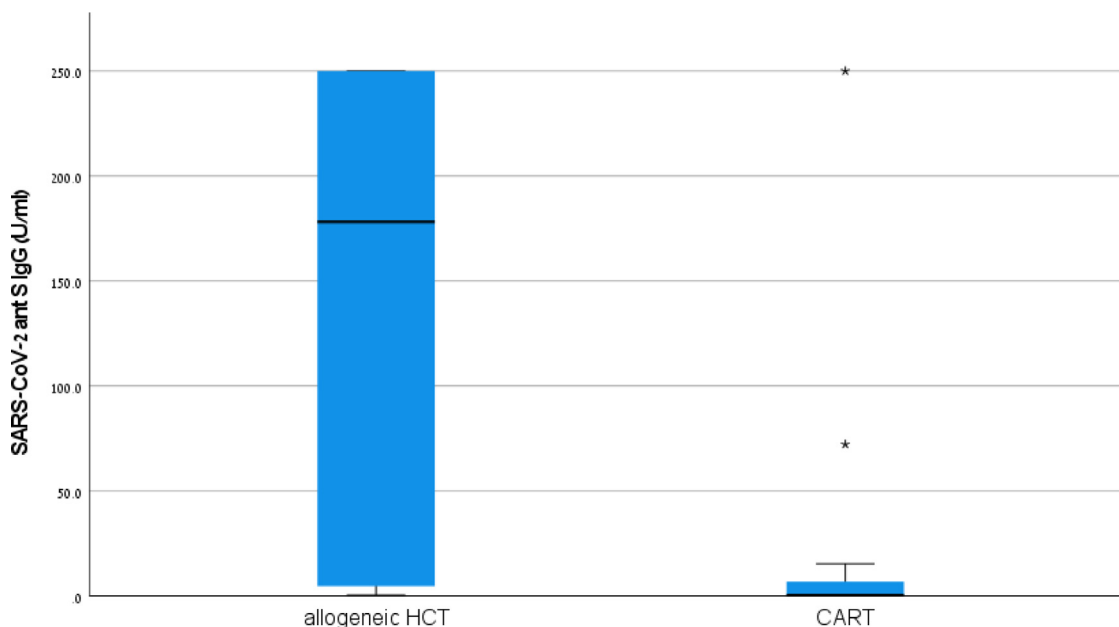
Figure 5. Box plot of serology blood levels in patients after allogeneic HCT and after CART infusion.

Table 2
Analyses of Predictors Using Logistic Binary Regression for Positive Serology

Positive Serology Test	Univariate		Multivariate		
	β	P	β	P	95% CI
Age	.987	.493	—	—	—
Months from infusion of cells	1.04	.012	1.05	.032	1.01-1.38
Sex (female vs. male)	2.16	.041	2.26	.028	1.6-3.06
Active GVHD	0.73	.467	—	—	—
Intensity of IST (low)	1.374	.563	—	—	—
CD19+ cells	1.32	.012	1.88	.047	1.0-2.12

CI indicates confidence interval.

this population the vaccine was relatively safe and there were no grade 3 or 4 non-hematologic adverse events; however, ~5% of the patients developed transient grade 3 or 4 cytopenia. GVHD exacerbation was noted in ~5% of the patients and was easily controlled. Remarkably, a humoral response was documented in 82% of the patients after allogeneic HCT. Perhaps even more surprising, a humoral response was observed in 36% of patients after B cell−depleting CART therapy, and 50% of those had evidence of a cellular response. After measuring overall (both humoral and cellular) response, an in vitro immune response was documented in 75% and 57% of allogeneic HCT and CART recipients, respectively.

Patients after allogeneic HCT or CART therapy are often immunosuppressed for months and years due to conditioning regimens, maintenance therapy, immunosuppressive medications, persistent hypogammaglobulinemia, and GVHD, all of which blunt immune response and reduce vaccine efficacy [7, 8]. Although these patients are at a high risk for complications from viral infections, the efficacy of both specific antiviral therapy and vaccination is inferior compared with healthy subjects. The mRNA SARS-CoV-2 vaccines were administered in the pivotal trials, and safety data have not raised any significant concerns [3, 9]. Similarly to the reported data, non-hematologic adverse events were mild in our cohort and resolved within 2 days, and we did not observe any grade 3 or 4 non-hematologic toxicity [9].

In contrast, hematologic/immunologic adverse events may be of concern in the context of cell therapy. Studies performed in healthy individuals showed that, concurrent with the production of neutralizing antibodies and the stimulation of virus-specific CD4+ and CD8+ T cells, there was a robust release of immunomodulatory cytokines, such as IFN-γ, TNF, IL-1, IL-2, and IL-12 [10,11]. These proinflammatory cytokines may alter T cell function and induce, or exacerbate, GVHD [12]. In our cohort, ~5% of allogeneic HCT recipients had exacerbation of GVHD. Half of the patients required systemic steroids to control the exacerbation, and all exacerbations resolved within several weeks. In line with that, we observed grade 3 or 4 cytopenia in ~5% of the patients, mainly thrombocytopenia. Although rare, this phenomenon was also reported in healthy individuals, and it was postulated that these individuals may have had preformed antibodies against components of the vaccine nanoparticles or impaired platelet production as part of the post-vaccination systemic inflammatory response [13, 14]. We favor the latter explanation in our cohort, as all patients showed spontaneously recovered counts and were closely monitored for months prior to vaccination, suggesting that the possibility of preformed antibodies was unlikely.

The single case of impending late graft rejection is worrisome. Although vaccination-associated rejection has been extensively reported in recipients of solid organ transplants, it has been rarely reported after HCT [15]. Possible mechanisms that facilitate rejection after vaccination are indirect activation by cytokines or natural killer cells and alternating endothelial progenitor cells that impair the bone marrow microenvironment and subsequently accelerate the rejection process [16, 17]. In the single case in our cohort, the patient received repeated doses of donor lymphocyte infusion and is being monitored weekly for both toxicity and marrow recovery.

In the current study, we used two methods to test the immunogenicity of BNT162b2. Although humoral immunity evaluation by serology testing is simple and relatively inexpensive, evaluation of cellular immunity by ELISpot is more complicated. Furthermore, because of low B cell numbers, which may prevent antibody production, serology testing was of limited use in our cohort of patients. Indeed, 57% of the patients after CART infusion in our

cohort had a complete B cell aplasia prior to vaccination. In the allogeneic HCT group, B cell aplasia was less common and could have resulted from either delayed immune reconstitution or concomitant administration of rituximab for the treatment of GVHD. In our cohort, by using both methods, we identified an immune response to the vaccine in 57% of patients after CART infusion and 75% of the patients after allogeneic HCT. This is an important finding that suggests that B cell aplasia should not by itself preclude patients from starting a vaccination program. Of note, the observed response rate was significantly higher than that reported for other vaccines in the same population and was similar to the responses of patients with chronic inflammatory diseases or immune dysfunction to BNT162b2 [4, 18, 19]. The high response rate may partially reflect an inherent selection bias, as our inclusion criteria were based on the EBMT recommendations. Although these results support the EBMT recommendations, we postulate that the vaccine response rate in an unselected transplanted population may be inferior. In line with the published data, we found that both a longer period from the infusion of cells (a composite outcome to encompass immune reconstitution) and absolute B cell counts were associated with better humoral response and, in addition, that the CD4+/CD8+ ratio was associated with cellular response [18, 20]. Interestingly, intensive IST and active GVHD did not predict responses to the vaccine; thus, patients may be referred to vaccination even if they are undergoing intensive IST therapy.

Our study is limited by several factors. First, the cohort of patients was small, and patients with relapse of malignancy or who were on high-dose steroids were excluded. Thus, although the majority of post-transplant patients were eligible, it is difficult to generalize the overall response results to other transplant or CART subgroups. Second, we were able to test cellular responses in only half of the cohort; therefore, any predictors of response should be interpreted with caution, as they were only tested in a small number of patients. Third, currently there is no clear definition of what level of humoral response correlates with clinical protection, and different laboratory tests do not correlate well. Finally, there is an open question of persistence of immunogenicity and the relationship between the in vitro assays and the overall vaccine efficacy in patients with substantial immunodeficiency state.

To conclude, this study, to our knowledge, is the first to show the immunogenicity of the BNT162b2 mRNA COVID-19 vaccine in patients after allogeneic HCT or CART. Adverse events were mostly mild and transient, but some significant hematologic events were observed, and patients should be closely monitored for hematologic and immunologic toxicity. Larger surveillance studies are needed to verify our findings, and a prospective longitudinal monitoring of antibody levels is warranted to test persistence of the humoral response and to verify false-positive results in patients with persistent B cell aplasia. In the meantime, general preventive precautions should be continued until more data are available.

ACKNOWLEDGMENTS

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