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Brief Article

Immunogenicity of a Third Dose of the BNT162b2 mRNA Covid-19 Vaccine in Patients with Impaired B Cell Reconstitution After Cellular Therapy—A Single Center Prospective Cohort Study

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A B S T R A C T

Patients with delayed B-cell reconstitution/B-cell aplasia after cellular therapy show decreased immunogenicity to the BNT162b2 mRNA COVID-19 vaccine. We prospectively evaluated both humoral and cellular immune response to a third vaccine dose in patients after allogeneic HCT (n = 10) or CD19-based chimeric antigen receptor T cells (CAR-T) therapy (n = 6) with low absolute B cell numbers and who failed to mount a humeral response after 2 vaccine doses. Humoral response was documented in 40% and 17% after allogeneic HCT and CAR-T therapy, respectively. None of the patients with complete B-cell aplasia developed anti-vaccine antibodies. Cellular response was documented in all patients after allogeneic HCT and in 83% of the patients after CAR-T. T-cell subclasses levels were not predictive for response, while a longer duration from infusion of cells was associated with a better cellular response. We conclude that cellular response develops with repeated vaccine doses even in patients with B-cell aplasia or delayed B-cell reconstitution, and these patients should therefore be vaccinated. These results should be considered in future studies analyzing immunogenicity in this population. Larger and longer follow-up studies are required to confirm whether cellular immunogenicity translates into vaccine efficacy.

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The BNT162b2 mRNA COVID-19 (Pfizer/BioNTech) vaccine was approved by both the Food and Drug Administration and the European Medicines Agency for the prevention of COVID-19, based on a phase 3 study that showed 94.6% efficacy [1]. Patients after allogeneic hematopoietic cell transplantation (HCT) and chimeric antigen receptor T-cell therapy (CAR-T) targeting CD19^{+} cells often display a delayed B-cell reconstitution pattern [2−4]. The concern for delayed reconstitution has resulted in a general recommendation to start vaccinating against COVID-19 at least 3 to 6 months after HCT or CAR-T infusion [5,6]. We have previously shown that although post-vaccination positive serology was documented in 75% of patients after allogeneic HCT, only 36% of patients after CAR-T infusion had documented serum antibodies [7]. Based on

studies showing efficacy of a third vaccine dose in solid organ transplant recipients, we hypothesized that similarly, patients with poor B-cell reconstitution and no evidence for immunogenicity may benefit from a third dose of the anti-SARS-CoV2 vaccine [8].

METHODS
Patients

This prospective study was performed at the Bone Marrow Transplantation (BMT) Long-Term Follow-up Clinic at the Tel Aviv Sourasky Medical Center. Patients were eligible for this study if they had previously received 2 doses of the BNT162b2 mRNA COVID-19 (Pfizer, New York, NY/BioNTech, Mainz, Germany) vaccine, were tested negative for the presence of anti-spike antibodies after 2 vaccine doses, and had evidence of B-cell dysfunction (either complete B-cell aplasia or B-cell lymphopenia with an absolute CD19 count of <150 cell/μL). All patients received the BNT162b2 third dose 5 months after the second vaccine dose. The study was approved by the hospital ethics committee (no. 1067-20 amended) and was registered in ClinicalTrials.gov (NCT04724642). All patients signed informed consent before enrollment.

Vaccination protocol and evaluation

Patients were vaccinated through the national Israeli vaccination program in mid-July 2021. All patients had a baseline quantification of absolute numbers of peripheral blood CD19+, CD4+, and CD8+ cells (cells/μL). All patients were reassessed for suitability to vaccination before administration of the third dose of BNT162b2 mRNA COVID-19 vaccine, including physical examination, assessment of graft-versus-host disease (GVHD) status, complete blood count and liver function tests. Patients were interviewed for post-vaccination adverse events 1 week after the administration of the third vaccine dose and underwent physical evaluation and repeated laboratory tests. Patients were then reassessed 21 days after the booster vaccine dose and had blood samples taken for SARS-CoV-2 serology and cellular response assays. The primary endpoint was evaluation of the humoral and cell-mediated response to the vaccine (measured by anti-spike IgG titers and intracellular cytokine staining, respectively). Adverse events were graded according to CTCAE v5.0 and chronic GVHD according to the NIH 2014 grading and response criteria [9]. Measurement of anti-spike antibody titers and evaluation of cellular response are described in the supplementary methods appendix. In brief, antibody titers were measured using a commercial automated SARS-CoV-2 IgG assay designed to detect IgG antibodies against the receptor binding domain of the S1 subunit of the SARS-CoV-2 wild-type spike protein (anti-spike) (Abbott Ireland, Sligo, Ireland). Cellular response was assessed by stimulating peripheral blood mononuclear cells (PBMCs) with a spike-protein peptide mix, followed by flow-cytometry based evaluation of stimulation induced CD154 upregulation, interferon-γ (IFN-γ) production or tumor necrosis factor-α (TNF-α) production (gating strategy is shown in Supplemental Figure S1). Complete cellular response was defined as production of both cytokines and CD154 upregulation, whereas partial cellular response was defined as either CD154 upregulation, IFN-γ production, TNF-α production or a combination of any two.

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 Chi-Square or t test, as appropriate. A 2-sided P value <.05 considered to be statistically significant. We compared both the humoral and the cellular responses of patients to healthy volunteers (n = 4). IBM SPSS Statistics, version 27 (IBM, Armonk, NY), was used to perform all analyses.

RESULTS

Patients and vaccination schedule

Twenty-five patients (32%), out of 77 patients who were included in the original vaccination program, did not have humoral response after 2 vaccine doses. Of these 25 patients, 16 patients had either complete B-cell aplasia (n = 7) or a low absolute CD19 count of (<150 cell/μL) (n = 9) and were eligible for enrollment according to this study protocol. Ten patients were post allogeneic HCT, and 6 were post CAR-T infusion. All 16 patients received the third dose of the vaccine in mid-July 2021, after a median time of 5.2 (range, 5.1-5.6) months from the second vaccine dose Table 1. depicts the characteristic of all on-protocol patients.

Tolerability and Safety

Nonhematologic vaccine-related adverse events were observed in 2 patients (13%) and included muscle cramps and generalized pain (both graded as grade 2). There were no grade 3-4 nonhematologic adverse events. One patient with moderate chronic GVHD developed a transient decrease in platelet count and exacerbation of chronic GVHD. Both adverse events resolved 2 weeks after the prednisone dose was increased.

Immunogenicity

None of the patients developed clinical COVID-19 infection during the study period. Positive serology was documented in 1 (17%) patient after CAR-T infusion and in 4 (40%) patients after allogeneic HCT. None of the patients with complete B-cell aplasia had positive serology, whereas 6 of 9 patients (67%) with low absolute CD19 count had a positive serology result. Mean anti-spike antibodies level was lower in the responding patients compared to controls (2746 $\pm$ 2326 versus 21067 $\pm$

Table 1
Characteristics of Patients

Datum	Allogeneic HCT (n = 10)	CAR-T (n = 6)
Age (y), median (range)	66 (33-78)	68 (23-80)
Female sex	3 (33%)	2 (33%)
Time from HCT/CAR-T, median (range)	31 (11-65)	14 (8-17)
<12 months	3 (33%)	2 (33%)
Status of disease, no. of patients (%)		
Remission	10 (100%)	5 (83%)
Relapse	0	1 (17%)
Base line disease		
AML	8 (80%)	—
ALL	—	1 (17%)
DLBCL	—	5 (83%)
Other lymphoma	1 (10%)	—
Myeloproliferative neoplasm	1 (10%)	—
GVHD (allogeneic patients)		
Active chronic GVHD	7 (70%)	—
Previous (nonactive) chronic GVHD	1 (10%)	—
Patients on active IST	8 (80%)	—
Patients on active chemotherapy	1 (10%)	0 (0%)
Absolute lymphocyte count (cells/μL)		
Absolute CD19+ lymphocyte count, median (range)	98 (0-150)	0 (0-100)
N patients with complete B cell aplasia	2 (33%)	5 (83%)
Absolute CD4+ lymphocyte count, median (range)	304 (115-704)	146 (80-760)
Absolute CD8+ lymphocyte count, median (range)	583 (150-4224)	333 (105-1150)
CD4/CD8 ratio, median (range)	0.48 (0.2-1.1)	0.6 (0.2-1.1)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B cell lymphoma; IST, immunosuppressive therapy.
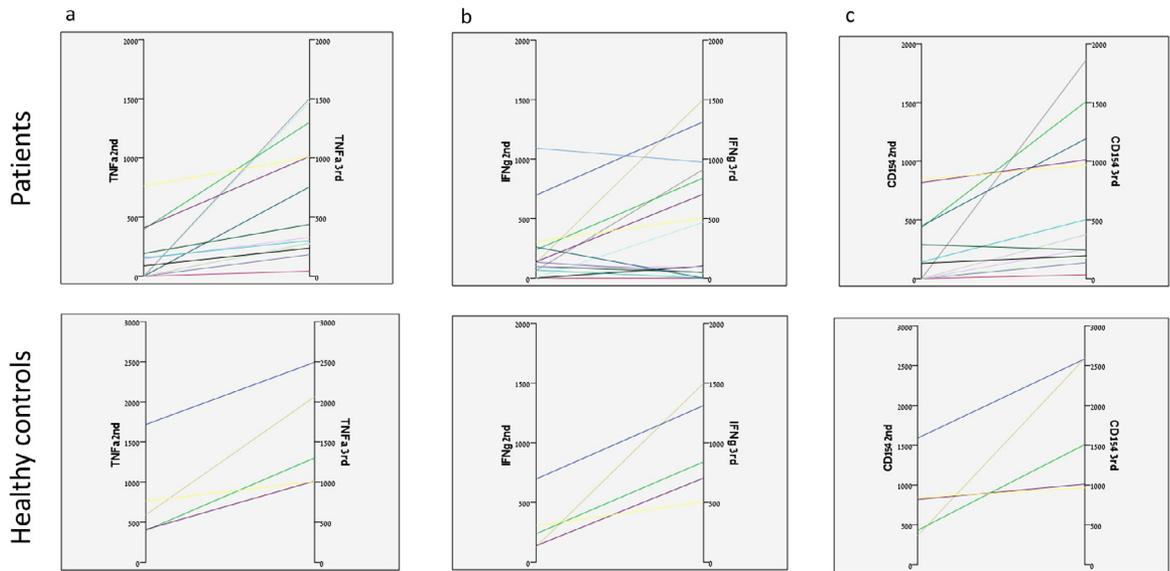
Figure 1. Parallel plots of absolute number of activated cells according to (a) TNF-α, (B) IFN-γ, and (c) CD154 (CD40L). Left side is before third vaccine results and right side is after third vaccine results.

6176 AU/mL, *P*= .002). Positive cellular response was documented in 5 (83%; 2 complete and 3 partial response) patients after CAR-T infusion and in all 10 (100%; 6 complete and 4 partial response) patients after allogeneic HCT (Figure 1).

There was no statistically significant difference in absolute CD4, CD8 or CD4/CD8 ratio values between patients with partial cellular response compared to patients with complete cellular response (*P*= .3, *P*= .4, and *P*= .7, respectively). Mean duration from infusion of cells was longer in patients with complete cellular response, compared to those with partial cellular response (34.1 ± 23.9 months versus 16.6 ± 7.4 months, *P*= .085); however, overall cellular response did not correlate with time from infusion (R^2 = .06 for CD154 and R^2 = .07 for both IFN-γ and TNF-α). In contrast, serology was modestly correlated with time from infusion (R^2 = .15; Figure 2). Mean B-cell blood levels were higher in patients who had complete

cellular response compared to patients who had partial cellular response; however, this was not statistically significant (47 ± S.D.67 versus 99 ± S.D. 60 cell/μL, respectively; *P*=.12).

Of the 10 patients who did not have evidence for humoral response, cellular response was documented in 90%. The only patient who did not have either cellular nor humoral response was a patient with acute lymphoblastic leukemia in complete disease remission. This patient is currently 17 months after anti-CD19 CAR-T therapy with complete B-cell aplasia.

DISCUSSION

In this study we evaluated the response to a third dose of the BNT162b2 mRNA COVID-19 vaccine in patients with a low B-cell count or complete B-cell aplasia who did not develop humoral response after 2 vaccine doses. To our knowledge, this is the first study that focused on this subgroup of patients
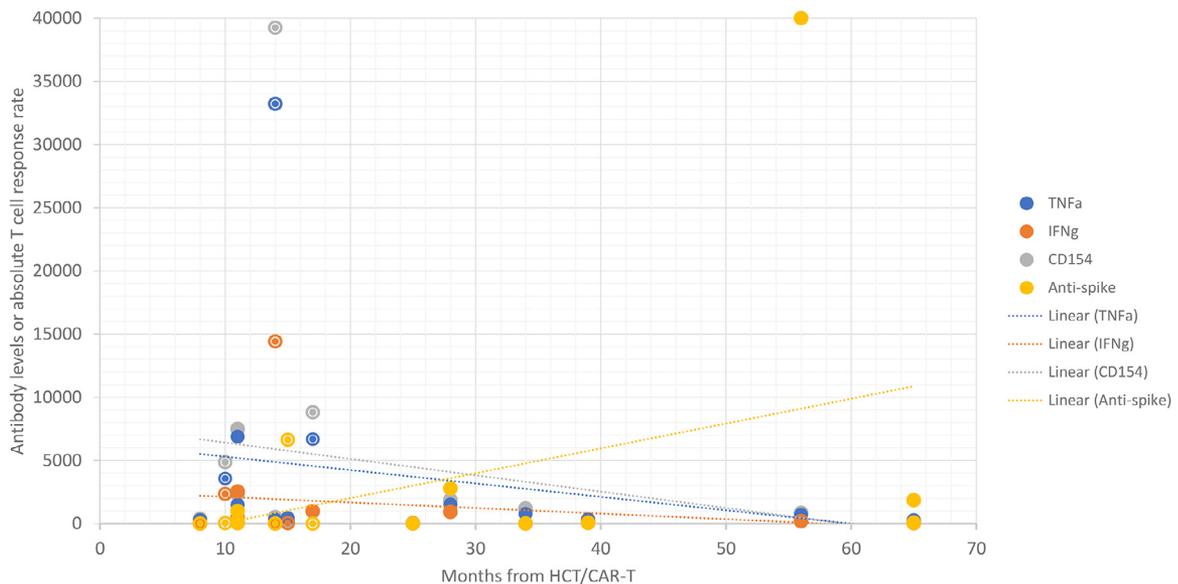
Figure 2. Anti-spike level and absolute T cell response rate to TNF-α, IFN-γ, and CD154 (CD40L) according to the number of months from transplantation or CAR-T infusion. *Filled dots* represent samples from patients post HCT, and *target-shape dots* represent samples from patients after CAR-T infusion.

who are post cellular therapy. We show that in this specific population, the vaccine was relatively safe, and although humoral response was documented in a minority of the patients (17% after CAR-T and 40% after allogeneic HCT), cellular response (whether documented in all 3 domains or only in 1 or 2) was more prevalent.

Although in patients after solid organ transplantation a third vaccine dose resulted in reappearance of humoral immunogenicity, patients after allogeneic HCT may have both quantitative and qualitative defects of the B-cell compartment, and thus revaccination may not necessarily result in augmentation of antibody production [8,10]. In addition, B-cell aplasia after additional cellular therapies is further associated with decreased response to vaccination. Conversely, both patients with B-cell aplasia and with B-cell dysfunction, exhibited a substantial cellular response, which may be sufficient for protective immunogenicity. Similar to other publications, we found that humoral and a more robust cellular immunity were positively associated with a longer time since HCT [7,11,12]. Interestingly, our findings that a greater cellular response in patients after CAR-T infusion also depends on the time elapsed from the CAR-T infusion suggests that recovery of the B-cell number and function also impacts T-cell responsiveness. In addition, it is possible that longer duration of disease control allows better reconstitution of the T-cell compartment.

Our study is limited by the small number of patients. Despite this, it is reassuring to see that patients in this study developed significant cellular reactivity even in the absence of humoral immunity. The significance of this reactivity and its relevance to vaccine efficacy is unknown. Evidence for persistence of post-vaccine anti-SARS-CoV-2 specific T-cell clones is scarce. In fact, there are only limited data that show effective cellular immunogenicity in the absence of humoral function [13]. In a broader view, no clinical or immunologic variables, as well as tests to monitor immune function, have been established to predict vaccine responsiveness in patients after cellular therapy. In the absence of such data, we recommend that all patients continue following general preventive precautions. Longitudinal studies in this vulnerable population are essential to gain more experience and to confirm whether cellular immunogenicity translates into vaccine efficacy. In addition, exploring novel ways of vaccine combinations and prophylactic, or early administration of monoclonal antibodies or the use of novel anti-viral drugs, are warranted.

SUPPLEMENTARY METHODS
Serology

The presence of anti-SARS-CoV-2 IgG antibodies was evaluated by using a commercial automated SARS-CoV-2 IgG assay (Abbott Ireland). The chemiluminescent microparticle immunoassay provided qualitative and quantitative determination of anti-SARS-CoV-2 receptor binding domain IgG antibody levels (SARS-CoV-2 IgG II Quant, Cat no. 6S60; Abbott Ireland). Results were provided in arbitrary units (AU/mL) as defined by the manufacturer, ranging between 0 to 40,000AU/mL (level > 150 AU/mL was considered positive).

Cellular Immune Response

T cells response was assessed by stimulating donor PBMCs with pooled complete-spike peptide mix in the presence of protein transport inhibitor, followed by staining for the activation marker CD154 (CD40L) and intracellular cytokines (TNF-α and IFN-γ). For this purpose, we used a SARS-CoV-2 T-Cell Analysis Kit for human PBMCs (Cat no. 130-128-156; Miltenyi Biotec, Bergisch Gladbach, Germany), and assay was performed according to manufacturer instructions. Briefly, donor PBMCs were plated in a 96-well plate at a concentration of 0.5 to 1×10^6 PBMCs/100 μL and incubated at 37°C and 5% CO_2 with 2 μL of either complete pooled S-peptide mix, CytoStim for positive control or 10% DMSO in sterile water for negative control. After 2 hours, Brefeldin A was added to each well, and cells were incubated for an additional 4 hours. Cells were then stained with viability dye, followed by fixation, permeabilization, and staining for surface markers (CD3, CD20, CD14, CD4, CD8, CD154) and intracellular cytokines (TNF-α and IFN-γ). After staining, samples

were acquired using BD FACSCanto II, and 20,000 CD4$^+$ events were collected for each sample.

Analysis was performed on gated CD4$^+$ T cells and the absolute number of activated CD154$^+$, INFγ^+ or TNF-α^+ cells was recorded and normalized for 1×10^6 CD4$^+$ T cells. To calculate the actual response rate, the absolute number of positive events in the unstimulated negative control was deducted from the absolute number of events in the S-stimulated samples, as shown in the following formula:

$$\left(\frac{1 \times 10^6 (\#S - stimulated\ Cytokine + CD4s)}{\#Total\ recorded\ CD4s} - \frac{1 \times 10^6 (\#\ Unstimulated\ cytokine + CD4s)}{\#Total\ recorded\ CD4s} \right)$$

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jtct.2022.02.012.

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