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ORIGINAL RESEARCH

A snapshot of the immunogenicity, efficacy and safety of a full course of BNT162b2 anti-SARS-CoV-2 vaccine in cancer patients treated with PD-1/PD-L1 inhibitors: a longitudinal cohort study

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Background: Very few cancer patients were enrolled in coronavirus disease-2019 vaccine studies. In order to address this gap of knowledge, real-world studies are mandatory. The aim of this study was to assess both humoral and cellular response after a messenger RNA vaccination schedule.

Patients and methods: Eighty-eight consecutive cancer patients treated with programmed cell death protein 1/programmed death-ligand 1 inhibitors were enrolled from the beginning of the vaccination campaign for frail patients. Blood samples for humoral and cell-mediated immune response evaluation were obtained before vaccination (T0), before the second administration (T1) and 21 days after the second dose (T2). The primary endpoint was the evaluation of the percentage of participants showing a significant increase in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific T cells, measured by an enzyme-linked immunospot assay, after the second dose of BNT162b2 vaccine. The proportion of patients who reached the primary endpoint is computed together with its exact binomial 95% confidence interval.

Results: In SARS-CoV-2-naïve subjects, spike-specific T-cell response was almost undetectable at T0 [median 0.0 interferon-γ (IFN-γ) spot forming units (SFU)/million peripheral blood mononuclear cell (PBMC) interquartile range (IQR) 0-7.5] and significantly increased at T1 and T2 (median 15.0 IFN-γ SFU/million PBMC, 25th-75th 0-40 versus 90 IFN-γ SFU/million PBMC, 25th-75th 32.5-224, respectively) ($P < 0.001$). Focusing on naïve and experienced SARS-CoV-2 subjects, no differences were reported both in terms of CD4- and CD8-specific T-cell response, suggesting that BNT162b2 is able to elicit both adaptive responses after complete vaccination schedule, regardless of previous SARS-CoV-2 exposure. The level of SARS-CoV-2 neutralizing antibodies was low at T1 in SARS-CoV-2-naïve subjects [median 1 : 5 (IQR 1 : 5-1 : 20)] but reached a significantly higher median of 1 : 80 (25th-75th 1 : 20-1 : 160) at T2 ($P < 0.0001$). Moreover, no COVID-19 cases were documented throughout the period of study.

Conclusions: Our data have demonstrated that the administration of a full course of BNT162b2 vaccine elicited a sustained immune response against SARS-CoV-2 regardless of the type of cancer and/or the type of immune checkpoint inhibitors.

Key words: BNT162b2 anti-SARS-CoV-2 vaccine, cancer patients, PD-1/PD-L1 inhibitors, CD4+ T follicular response, CD8+ T-cell response

INTRODUCTION

After the emergence of the coronavirus disease-2019 (COVID-19) pandemic caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), many vaccines are being deployed, including messenger RNA (mRNA)-based vaccines. The BNT162b2 vaccine,[1] a lipid nanoparticle-formulated, nucleoside-modified RNA encoding the SARS-CoV-2 full-length spike, modified by

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two proline mutations to lock it in the prefusion conformation, was the first authorized for active immunization. This vaccine showed a 95% protection against SARS-CoV-2 infection in a phase II/III trial.[1] However, data on the immune response elicited by the vaccine are limited to a low number of subjects analyzed.[2,3] Previous reports related to SARS-CoV had suggested a protective role of both humoral and cell-mediated immunity, and that T-cell response could confer long-term protection while the antibody response in humans was found to be relatively short-lived in convalescent individuals.[4,5] Patients with cancer were not explicitly excluded, but the subjects receiving immunosuppressive therapy or immune-modifying drugs within 6 months of screening were not enrolled.

Generally, patients under chemotherapy or other immunosuppressive agents should not receive live vaccines and should preferably not receive inactivated vaccines, as recommended by the Infectious Diseases Society of America.[6] Expert consensus advocates that cancer patients should be vaccinated against SARS-CoV-2.[7] Immunoglobulin G (IgG) antibody response to SARS-CoV-2 infection does not seem to be different between healthy subjects and cancer patients,[8] but immune response against SARS-CoV-2 in cancer patients receiving vaccination against SARS-CoV-2 in terms of antibody titer (value and geometric mean) and specific T-cell response is yet unknown. People at higher risk, like patients with cancer, have been underrepresented in ongoing phase III clinical trials.[9]

Preliminary reports demonstrated that the response rates of initial immune response to the BNT162b2 vaccine among patients with myeloproliferative neoplasms were similar to those observed in the general population.[10] In a cohort prospective study of patients with cancer on systemic therapy, most of the patients were seropositive for SARS-CoV-2 anti-spike IgG antibodies after the full course of BNT162b2 vaccine, but their antibody titers were significantly lower than those of the control group, and in the multivariable analysis, the chemotherapy plus immune checkpoint inhibitors (ICIs) appears to be the only variable significantly associated with lower IgG titers.[11] ICIs promote antitumor response by interrupting co-inhibitory signaling pathways and immune-mediated elimination of tumor cells,[12] and based on preclinical data about their mechanism of action, ICIs are likely to enhance rather than diminish the immune response against vaccines.[13]

Many issues remain unanswered, including the time needed to develop immunity, the duration of immunity, the effects of different therapy on immunity and the optimal time points and schedule of vaccine administration in patients with cancer. The aim of the present study was to evaluate the characteristics and the magnitude of the T- and B-cell response in cancer patients treated with programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) inhibitors and receiving COVID-19 vaccine.

PATIENTS AND METHODS

Study design

This study was an observational, longitudinal, multicenter study. Consecutive cancer patients treated with PD-1/PD-L1 inhibitors were enrolled from the beginning of the vaccination campaign for frail patients. The study was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement for reporting observational studies.[14]

Subjects were monitored during the overall period of vaccination at baseline (before vaccination; T0), before the second administration (T1) and 21 days after the second dose (T2).

Blood samples for humoral and cell-mediated immune response evaluation were obtained at each time point.

The primary endpoint was the evaluation of the percentage of participants showing a significant increase in SARS-CoV-2-specific T cells after the second dose of BNT162b2 vaccine.

The secondary endpoints were:

- Evaluation of the change in the rate of immunological response up to 21 days after the second dose of COVID-19 vaccine.
- Evaluation of the changes of neutralizing antibody and IgG antibody titer against SARS-CoV-2 (chemiluminescence immunoassay method) up to 21 days after the second dose of COVID-19 vaccine.
- Evaluation of the incidence of virologically confirmed COVID-19 cases after administration of at least one dose of COVID-19 vaccine.
- Evaluation of the incidence of adverse reactions to the COVID-19 vaccine, local and systemic, solicited and unsolicited, within the period of 4 weeks after each dose of vaccination.
- Evaluation of the incidence of 'new—onset' immune therapy-related adverse event (IRAE) in patients on immunotherapy 21 days after the second dose of vaccine.

The study (Co-Vax) was approved by the local ethics committee (Comitato Etico Area Pavia) and institutional review board (P-20210023530). All the subjects signed an informed written consent.

Patients' enrollment

Cancer patients who were programmed to receive a full course of vaccine during immunotherapy (anti-PD-1 or anti-PD-L1), in combination or not with chemotherapy were enrolled. The inclusion criteria were: (i) patients aged 18 years and older, regardless of sex; (ii) life expectancy (as estimated by the treating physician) $\geq$6 months; (iii) confirmed histological diagnosis of solid tumors; (iv) treatment with immunotherapy alone or in combination with chemotherapy; (v) signing of informed consent; (vi) patients with a history of a previous laboratory-confirmed diagnosis of SARS-CoV-2 infection will be also enrolled. Patients with

psychiatric illness/social situations that would limit compliance with study requirements were excluded from the study.

Patients were examined by PD-L1 status, TNM (tumor—node—metastasis), histology, type of treatment (anti-PD-1 or anti-PD-L1) with or without chemotherapy, treatment setting (first or second line, maintenance after chemo-radiotherapy), the time gap between the start of immunotherapy and vaccine administration and the history of a previous laboratory-confirmed diagnosis of SARS-CoV-2 infection. Patients' immunological profile was evaluated based on the following parameters: lymphocyte T-cell count (CD3CD4+, CD3CD8+, CD56 natural killer cells and CD19 B cells), neutrophils, neutrophil-to-lymphocyte ratio, lactate dehydrogenase, C-reactive protein.

Patients were enrolled in two oncology units of Northern Italy (Fondazione IRCCS Policlinico San Matteo, Pavia and AUSL Ospedale Guglielmo Da Saliceto, Piacenza).

Spike-specific T-cell response measured by ex vivo ELISpot assay

Immunological analysis has been limited to subjects vaccinated with mRNA BNT162b2 anti-SARS-CoV-2 vaccine to avoid confounding factors of different vaccines. Peripheral blood mononuclear cells (PBMCs) were isolated from heparin-treated blood by standard density gradient centrifugation. Briefly, PBMCs (2×10^5/100 μl culture medium per well) were stimulated in duplicate for 24 h in 96-well plates (coated with anti-interferon-γ (IFN-γ) monoclonal capture antibody) with peptide pools (15 mers, overlapping by 10 amino acids, Pepscan, Lelystad, The Netherlands) representative of the spike protein (S) at the final concentration of 0.25 μg/ml. Phytohemagglutinin (5 μg/ml) was used as positive control, and medium alone as negative control. Enzyme-linked immunospot (ELISpot) assay was carried out according to our previous protocol.[15]

Responses ≥10 net spots/million PBMCs were considered positive based on background results obtained with negative control [mean spot forming cell + 2 standard deviation (SD)].

Characterization of CD4+, CD4+ T follicular and CD8+ T-cell response

To evaluate T-cell subset proliferation, PBMCs (600 000/200 μl culture medium per well) collected from 20 vaccinated patients were stimulated in triplicate in 96-well round-bottom plates with peptide pools representative of the S protein, at the final concentration of 0.1 μg/ml for 7 days. Peptide pool from human actin was used as negative control antigen. Culture medium was RPMI 1640 supplemented with 2 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin, 5% of heat-inactivated human serum AB, 1 mM sodium pyruvate, 100 μM non-essential amino acids and 50 μM 2-mercaptoethanol. After culture, cells were washed with phosphate-buffered saline (PBS) 0.5 μM EDTA and stained in PBS with Live/Dead Fixable Violet Dye (Invitrogen, Waltham, Massachusetts, USA) at 4°C in our laboratory. After washing, cells were stained at room temperature in PBS 5% fetal calf serum with anti-CXCR5, followed by anti-IgG2b (biotinylated) and, subsequently, with Streptavidin BV421, CD3 PerCP 5.5, CD4 APC Cy7, CD8 FITC, CD25 PECy7 and CD278 (ICOS) APC antibodies. Finally, cells were washed and suspended in 1% paraformaldehyde. The frequency of CD25+ICOS+-expanded CD3+CD4+ and CD3+CD8+ T cells was determined by subtracting the frequency of PBMC incubated with actin peptides from the frequency of PBMC incubated with SARS-CoV-2 S and N peptides. Flow cytometry analyses were carried out with an FACS Canto II flow cytometer and DIVA software (BD Biosciences, Francklin Lakes, New Jersey, USA).

Antibody response

Chemiluminescent assay (Liason SARS-CoV-2 S1/S2 IgG, Diasorin, Saluggia, Italy) for the quantitative characterization of SARS-CoV-2 anti-S1 and anti-S2 IgG antibodies, according to manufacturer's instructions, was carried out using serum samples. Results were given as AU/ml and a cut-off of 15 AU/ml was considered for the definition of positive samples. Results ranging from 12 to 15 AU/ml were considered borderline, while IgG titer <12 AU/ml was given as a negative result. Neutralizing antibody serum titer was determined as previously reported.[16] Results were considered positive if ≥1 : 10 serum titer.

Statistical analysis

The Stata software (release 17, StataCorp, College Station, TX) was used for computation. A two-sided *P* value <0.05 is considered statistically significant. Data are described with the median and 25th-75th percentiles if continuous and as counts and percentage if categorical. Log-transformation is applied to continuous variables for the purpose of the analysis. The proportion of patients who reached the primary endpoint is computed together with its exact binomial 95% confidence interval (95% CI). Potential correlates of the primary endpoint are evaluated using logistic models; odds ratios (ORs) and 95% CI are presented. In case of null cells, exact logistic regression is used. The modifying effect of age (≤/>65 years) is assessed by including an interaction term in the model. In no case, we observed heterogeneity in the effect of the potential correlates based on age class (data not shown). The rate of immunological response at the several time points is computed together with its exact binomial 95% CI. Changes over time are evaluated with a generalized linear model for repeated measures for binomial or continuous data. Huber—White robust standard errors are computed to account for intra-patient correlation over time. Differences with respect to baseline and 95% CI are computed. The rate of adverse events is presented together with their 95% CI.

RESULTS

Patients' characteristics

Eighty-eight subjects (23 females and 65 males; median age 68 years, 25th-75th 61.5-73 years) were enrolled between 24 March and 23 April 2021. Sixty-seven (76.1%) had lung cancer, eight (9.1%) had melanoma, seven (7.9%) had kidney cancer and the remaining six patients (6.9%) had head and neck cancer (three patients), bladder cancer (one patient), breast cancer (one patient) and squamous cell skin cancer (one patient). The most common treatment was ICI alone (66 patients, 75.0%) and the most common ICI was pembrolizumab (54 patients, 61.4%). Eleven patients (12.5% of the study population) were receiving durvalumab for maintenance in chemo-radiotherapy-treated unresectable stage III non-small-cell lung cancer and two patients (2%) were receiving nivolumab for adjuvant melanoma.

Forty-one (46.6%) of 88 patients had no comorbidities; in those with comorbidities, the most common were cardiovascular diseases and diabetes mellitus. Sixty-three (71.6%) patients had undergone influenza vaccination (Supplementary Table 1, available at https://doi.org/10.1016/j.esmoop.2021.100272).

Based on positive serology results at baseline and/or documented positive SARS-CoV-2 RNA PCR in nasal swabs, 13 patients (14.8%) were considered as positive for previous SARS-CoV-2 infection (SARS-CoV-2-experienced patients) while 75 (85.2%) were considered naïve for SARS-CoV-2 infection. The flow chart with the patients' disposition has been represented in Supplementary Figure 1, available at https://doi.org/10.1016/j.esmoop.2021.100272.

Vaccination schedule

Seventy-eight patients (88.6%) received a full course of BNT162b2 vaccine and four patients (5.7%) received only the first dose: in particular, three patients had progression of disease leading to a rapid decline in clinical conditions, hence the second dose was missed. The latter patient presented two immune-related side-effects (hepatitis G3 and colitis G3) 10 days after the first dose of vaccine: she required hospitalization and she received high-dose steroid therapy, obtaining clinical remission. The median time between the first administration of immunotherapy and the first dose of vaccine was 8.32 months (25th-75th 2.47-15.69 months).

Sustained spike-specific T-cell response elicited by BNT162b2 vaccine in SARS-CoV-2-naïve patients after complete vaccination schedule. Cell-mediated response elicited by BNT162b2 vaccine was assessed in SARS-CoV-2-experienced patients and SARS-CoV-2-naïve patients at T1 and T2. Complete analysis was carried out in 73 of 78 (93.6%) subjects receiving full vaccination schedule.

Overall, S-specific T-cell response measured at T0 was 5.0 IFN-γ spot forming units (SFU)/million PBMC (25th-75th 0.0-15.0) and it reached 125.0 IFN-γ SFU/million PBMC (25th-75th 52.5-345) at T2 ($P < 0.001$) with a proportion of responder subjects at T2 of 0.92 (95% CI 0.83-0.97) (Figure 1). The proportion was 0.72 (95% CI 0.60-0.82) at T1.

In SARS-CoV-2-experienced subjects, the level of S-specific T-cell response increased significantly at T1 than respect to baseline (median 352.5 IFN-γ SFU/million PBMC, 25th-75th 96.3-522.5 versus median 77.5 IFN-γ SFU/million PBMC, 25th-75th 36.3-155; $P = 0.001$), while the level did not further increase at T2 (median 362.5 IFN-γ SFU/million PBMC, 25th-75th 236.3-2059; $P = 0.156$). Focusing on SARS-CoV-2-naïve subjects, spike-specific T-cell response was almost undetectable at T0 (median 0.0 IFN-γ SFU/million PBMC, IQR 0-7.5) and significantly increased at T1 and T2 (median 15.0 IFN-γ SFU/million PBMC, 25th-75th 0-40 versus 90 IFN-γ SFU/million PBMC, 25th-75th 32.5-224, respectively) ($P < 0.001$) (Figure 2).

Overall, the proportion of the responders in the group of SARS-CoV-2-naïve subjects was 0.69 (95% CI 0.56-0.80) at T1 and 0.91 (95% CI 0.81-0.96) at T2. When experienced subjects were considered, the proportions were 0.72 (95% CI 0.60-0.82) and 0.92 (95% CI 0.83-0.97), respectively.

None of the analyzed clinical variables (PD-L1 status, TNM staging, histology, type of treatment, with or without chemotherapy, treatment setting, time gap between the start of immunotherapy and vaccine administration, history of a previous laboratory-confirmed diagnosis of SARS-CoV-2 infection) showed a statistically significant correlation with the increase in SARS-CoV-2-specific T cells. In particular, in all patients with stage III disease (13 patients, 100%) spike-specific T-cell response significantly increased at T2, whereas of the 53 stage IV patients, only in 47 (47.9%) the level of S-specific T-cell response increased significantly at T2. These data unfortunately do not show a statistically significant association, despite an odd of significant response at T2 twice as high in stage III patients (exact logistic regression OR 2.15, 95% CI 0.28 to $+\infty$, $P = 0.529$). Only in patients treated with chemo-immunotherapy T-cell response seems to be lower with borderline statistical significance (OR 0.20 95% CI 0.04-1.14; $P = 0.0778$).

Phenotypical characterization of spike-specific T cells revealed that BNT162b2 elicits both CD4 and CD8 T-cell response. Twenty vaccinated ICI patients (13 SARS-CoV-2-naïve and 7 SARS-CoV-2-experienced subjects) were tested for phenotypical analysis of spike-specific T-cell proliferative response. Overall, if the median CD4+ T-cell response was higher than respect to CD8+ T-cell response (median 3.67, 25th-75th 0.23-12.92 versus 2.25, 25th-75th 0.57-9.06), the difference was not statistically significant ($P = 0.573$). Focusing on naïve and experienced SARS-CoV-2 subjects, no differences were reported both in terms of CD4+- and CD8+-specific T-cell response, suggesting that BNT162b2 is able to elicit both adaptive responses after complete vaccination schedule, regardless of previous SARS-CoV-2 exposure (Figure 3).

Humoral response elicited by BNT162b2 vaccine

Overall, a significant increase of S1/S2 IgG response in SARS-CoV-2-naïve subjects at T1 (median 7.6, IQR 3.5-27

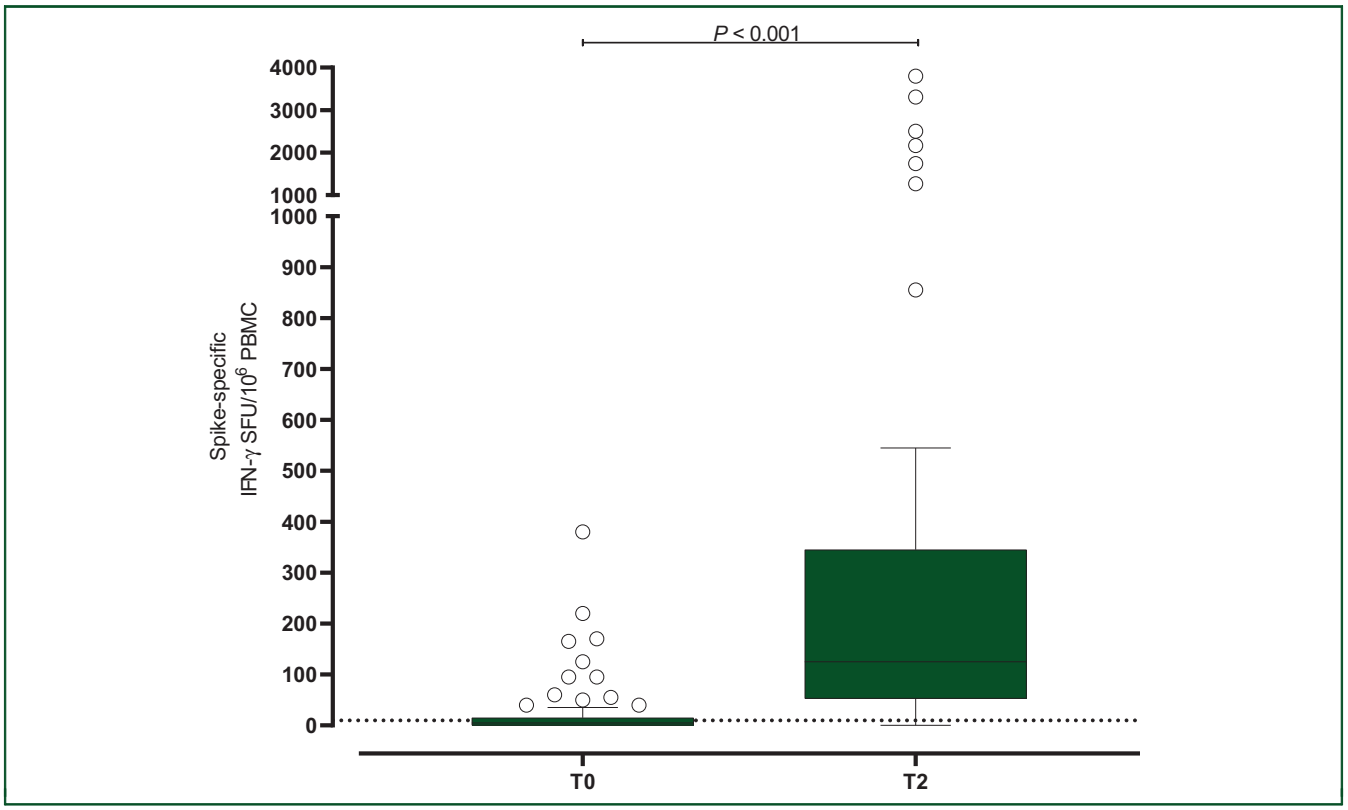

Figure 1. Spike-specific T-cell response at baseline (T0) and T2 (42 days) in 73 ICI patients.
Responses are given as spike-specific IFN-γ SFU/106 PBMC and *P* value of the comparison between the two time points is given in the graph. Horizontal dotted line indicates the cut-off level. ICI, immune checkpoint inhibitor; IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; SFU, spot forming units.

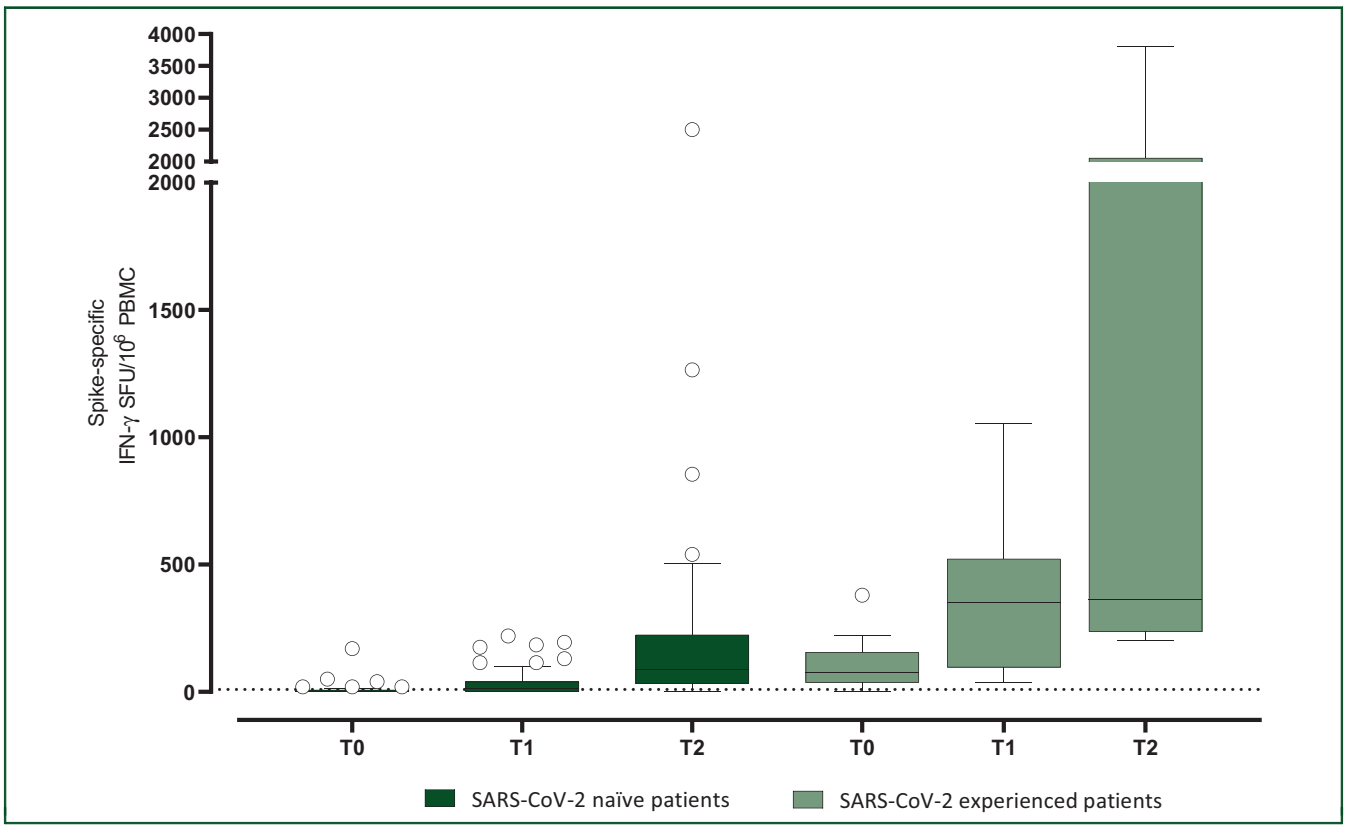

Figure 2. Spike-specific T-cell response at baseline (T0), day 21 (T1) and day 42 (T2) measured in SARS-CoV-2-naïve patients and SARS-CoV-2-experienced subjects.
Responses are given as spike-specific IFN-γ SFU/106 PBMC. Horizontal dotted line indicates the cut-off level. IFN-γ, interferon-γ; PBMC, peripheral blood mononuclear cell; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SFU, spot forming units.

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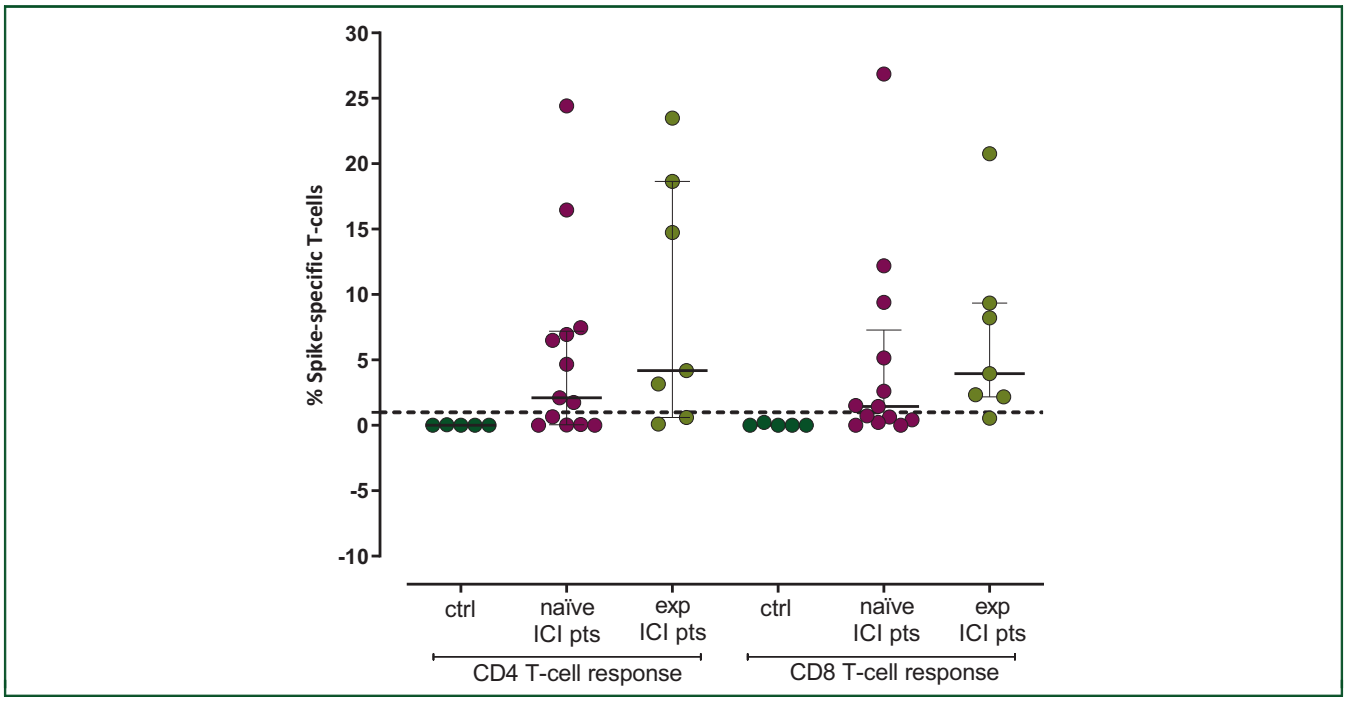

Figure 3. Spike-specific T-cell proliferative response in 13 SARS-CoV-2-naïve patients (naïve ICI pts; green dots), 7 SARS-CoV-2-experienced subjects (exp ICI pts; dark purple dots) and 5 healthy controls (ctrl, green dots) analysis at T2 (day 42).
Horizontal lines indicate median and interquartile range. Responses are given as percentage (%) of spike-specific T cells. Horizontal dotted line indicates the cut-off level. ICI, immune checkpoint inhibitor; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

AU/ml) than respect to baseline (median 3.5, 25th-75th 3.5-3.5 AU/ml; $P < 0.0001$). Similarly, S1/S2 IgG response in SARS-CoV-2-experienced subjects was significantly higher than that detected at T1 (median 152, 25th-75th 92.7-259.5; $P < 0.001$) (Figure 4A). The proportion of SARS-CoV-2-naïve subjects with positive S1/S2 IgG level was 0.95 (95% CI 0.90-1.01) at T2.

Neutralizing antibodies (NT Abs) against SARS-CoV-2 B.1 strain were measured at T1 and T2. The level of SARS-CoV-2 NT Abs was low at T1 in SARS-CoV-2-naïve subjects [median 1 : 5 (IQR 1 : 5-1 : 20)] but reached a significantly higher median of 1 : 80 (25th-75th 1 : 20-1 : 160) at T2 ($P < 0.0001$) (Figure 4B). The proportion of SARS-CoV-2 NT Abs-positive subjects at T2 was 0.77 (95% CI 0.68-0.87).

SARS-CoV-2-experienced subjects reached a higher level of S1/S2 IgG and SARS-CoV-2 NT Abs even after one dose of vaccine (2425 AU/ml, 25th-75th 1540-3768 AU/ml and 1 : 640, 25th-75th 1 : 640-1 : 640, respectively) than respect to SARS-CoV-2-naïve subjects tested at T2 ($P < 0.0001$), suggesting that one dose of vaccine may act as a booster in subjects with previous SARS-CoV-2 exposure, regardless of time of previous infection.

COVID-19 cases after vaccine administration

No COVID-19 cases were documented throughout the period of study.

Side-effects and IRAEs

The most common side-effects observed after the first dose of vaccine were pain at the injection site (28.57%, 22/88) and fever (2.6%, 2/88). Fever (oral body temperature $\geq$38°C) occurred after the second dose of BNT162b2 in 6.94% (5/78). In general, systemic events were reported more often after the second dose. Of note, headache and myalgia were the most frequently reported systemic events. Local effects were less commonly reported after dose 2 than after dose 1 of vaccine. No thrombosis, hypersensitivity adverse events or vaccine-related anaphylaxis were signaled. Side-effects were typically reported within the first 24 h after vaccination. (Supplementary Table 2, available at https://doi.org/10.1016/j.esmoop.2021.100272). Only one patient has reported two immune-related side-effects (hepatitis G3 and colitis G3) 10 days after the first dose of vaccine.

DISCUSSION

It is well known that cancer patients are at increased risk of morbidity and mortality from SARS-CoV-2 infection.[17] Despite this evidence, very few patients with cancer were enrolled in COVID-19 vaccine studies and so many unanswered questions remain about the risk—benefit ratio of these vaccines in this frail population. As there is an urgent need to protect cancer patients from COVID-19, the main professional societies and organizations, e.g. the American Association of Clinical Oncology, the European Society of Medical Oncology and the Associazione Italiana di Oncologia Medica, strongly endorsed prioritization of such patients for SARS-CoV-2 vaccination,[7,18,19] although there are still many unclear issues about their efficacy and safety.

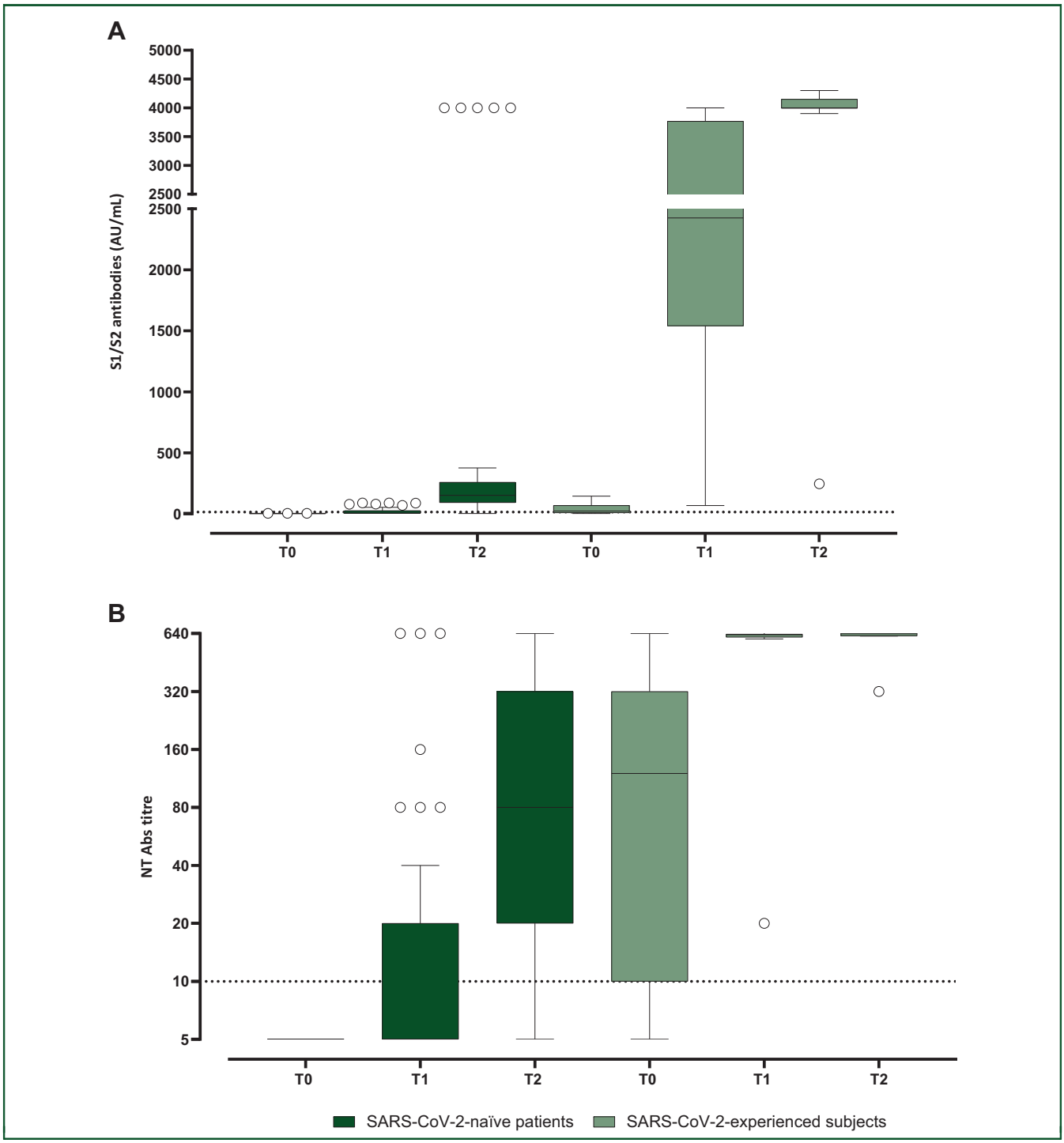

Figure 4. Serological response (A) and neutralizing antibodies (NT Abs) (B) in SARS-CoV-2-naïve patients and SARS-CoV-2-experienced subjects at T0 (baseline), T1 (day 21) and T2 (day 42).

Horizontal lines indicate median and interquartile range. Responses are given as AU/ml and NT Abs titer, respectively. SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Recently, some studies have begun to describe the spectrum of early vaccine response among larger subsets of patients with cancer on active therapy.

Thakkar et al. evaluated anti-spike IgG titers in 200 cancer patients (67% with solid tumors and 33% with hematologic tumors): a significantly lower seroconversion rate was observed in patients with hematologic malignancies (85%) versus solid tumors (98%). Instead, patients receiving ICI therapy had high seroconversion rates.[20]

In our cohort study, we aimed to evaluate the humoral and cell-mediated immune response in cancer patients treated with PD-1/PD-L1 inhibitors and receiving BNT162b2 anti-SARS-CoV-2 vaccine. We chose to specifically evaluate only cancer patients treated with ICIs for

their mechanism of action on the immune system. Our data confirmed that the rate of SARS-CoV-2-naïve subjects developing positive antibody level measured by S1/S2 assay is high as expected following two vaccine doses (95%), even if only one-third of patients developed a positive antibody response after the first dose, confirming previous results obtained in cancer patients[21] and in health care workers.[22,23]

Furthermore, we also investigated the development of spike-specific cell-mediated immune response using a home-made *ex vivo* ELISpot assay. Interestingly, >90% of patients developed a sustained spike-specific T-cell response at T2, suggesting that adaptive immune response is not compromised in this cohort of subjects. Furthermore, a sustained CD4 and CD8 T-cell response was elicited by vaccination. Thus, in our cohort of subjects with solid cancer, the administration of a full course of an mRNA vaccine provides good protection against COVID-19 and these results do not depend upon the type of cancer and/or the type of ICIs. Our data offer another interesting observation: SARS-CoV-2-experienced patients mounted a robust neutralizing immune response even after a first dose suggesting that the past infection may be an immune enhancer condition and may not be a reason for vaccine hesitancy.

Goshen-Lago et al. reported that the adverse events after the two doses of BNT162b2 vaccine in cancer patients were similar to data in the studies comprising healthy population.[24] In addition, Waissengrin and colleagues described the safety of the BNT162b2 mRNA vaccine in a cohort of patients treated with ICIs.[25] They compared side-effects in the patients treated with ICIs with a healthy control group matched by sex and year of birth. In this paper, they observed no new immune-related side-effects or exacerbation of existing immune-related side-effects and a side-effect profile similar in the healthy controls and patients with cancer.[25]

Since both ICI treatment and COVID-19 vaccines stimulate the immune response, it has been hypothesized that these vaccines may increase the incidence of IRAEs with ICI treatment. To date, there are no data demonstrating the direct answer.

In our study, only one patient reported two immune-related side-effects (hepatitis G3 and colitis G3) 10 days after the first dose of vaccine. Unfortunately, such small numbers do not allow any kind of conclusion, but the description of further cases may be interesting.

Strengths and limitations

The strength of our data consists in the simultaneous detection of both anti-spike and neutralizing antibody titers and IFN-γ release assay giving us a comprehensive tracking of humoral and cellular immune response. In the same time, we have collected data at baseline and after a full course of vaccination instead of only a single dose and so far. The availability of baseline data for each patient of both cellular and immunological status allows us to demonstrate that the response depends only on the vaccination eliminating other confounding factors. Moreover, all patients are receiving PD-1/PD-L1 inhibitors, making our results homogeneous about the type of treatment. Our paper has several limitations. To begin with, the lack of a control group that is not possible to study for ethical reasons and the small sample size of patients evaluated. Secondly, the study cohort represents an older population, with a median age of 68 years. The intriguing phenomenon of the immunosenescence is well known[26] and also that the immune response to many other vaccines is reciprocally associated with age.[27] Thirdly, the IFN-γ release assay results are difficult to interpret because the comparison data are lacking.

CONCLUSION

Mounting a robust immune response against SARS-CoV-2 requires two phases: neutralization and effector T-cell functions. Our data confirm the efficacy of the vaccine in triggering both the humoral and the cell-mediated immune response in patients with cancer treated with anti-PD-1/PD-L1. The SARS-CoV-2 BNT162b2 vaccine appears to be safe and achieves satisfying serologic status in cancer patients during immunotherapy. Many future real-world data are warranted to confirm these results and to determine the long-term efficacy of the vaccine.

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DISCLOSURE

The authors have declared no conflicts of interest.

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