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ORIGINAL RESEARCH

Analysis of the humoral and cellular immune response after a full course of BNT162b2 anti-SARS-CoV-2 vaccine in cancer patients treated with PD-1/PD-L1 inhibitors with or without chemotherapy: an update after 6 months of follow-up

A. Lasagna[1*†], **D. Lilleri**[2†], **F. Agustoni**[1], **E. Percivalle**[2], **S. Borgetto**[1], **N. Alessio**[1], **G. Comolli**[2], **A. Sarasini**[2], **F. Bergami**[2], **J. C. Sammartino**[2], **A. Ferrari**[2], **F. Zavaglio**[2], **F. Arena**[2], **S. Secondino**[1], **M. Falzoni**[1], **R. Schiavo**[3], **G. Lo Cascio**[3], **L. Cavanna**[4], **F. Baldanti**[2,5], **P. Pedrazzoli**[1,6†] & **I. Cassaniti**[2†]

[1]Medical Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Pavia; [2]Molecular Virology Unit, Department of Microbiology and Virology, Fondazione IRCCS Policlinico San Matteo, Pavia; [3]Microbiology Unit, Hospital Guglielmo da Saliceto, Piacenza; [4]Oncology Unit, Hospital Guglielmo da Saliceto, Piacenza; Departments of [5]Clinical, Surgical, Diagnostic and Pediatric Sciences; [6]Internal Medicine and Medical Therapy, University of Pavia, Pavia, Italy

Available online 11 December 2021

Background: The durability of immunogenicity of SARS-CoV-2 vaccination in cancer patients remains to be elucidated. We prospectively evaluated the immunogenicity of the vaccine in triggering both the humoral and the cell-mediated immune response in cancer patients treated with anti-programmed cell death protein 1/programmed death-ligand 1 with or without chemotherapy 6 months after BNT162b2 vaccine.

Patients and methods: In the previous study, 88 patients were enrolled, whereas the analyses below refer to the 60 patients still on immunotherapy at the time of the follow-up. According to previous SARS-CoV-2 exposure, patients were classified as SARS-CoV-2-naive (without previous SARS-CoV-2 exposure) and SARS-CoV-2-experienced (with previous SARS-CoV-2 infection). Neutralizing antibody (NT Ab) titer against the B.1.1 strain and total anti-spike immunoglobulin G concentration were quantified in serum samples. The enzyme-linked immunosorbent spot assay was used for quantification of anti-spike interferon-γ (IFN-γ)-producing cells/10^6 peripheral blood mononuclear cells. Fifty patients (83.0%) were on immunotherapy alone, whereas 10 patients (7%) were on chemo-immunotherapy. We analyzed separately patients on immunotherapy and patients on chemo-immunotherapy.

Results: The median T-cell response at 6 months was significantly lower than that measured at 3 weeks after vaccination [50 interquartile range (IQR) 20-118.8 versus 175 IQR 67.5-371.3 IFN-γ-producing cells/10^6 peripheral blood mononuclear cells; $P < 0.0001$]. The median reduction of immunoglobulin G concentration was 88% in SARS-CoV-2-naive subjects and 2.1% in SARS-CoV-2-experienced subjects. SARS-CoV-2 NT Ab titer was maintained in SARS-CoV-2-experienced subjects, whereas a significant decrease was observed in SARS-CoV-2-naive subjects (from median 1 : 160, IQR 1 : 40-1 : 640 to median 1 : 20, IQR 1 : 10-1 : 40; $P < 0.0001$). A weak correlation was observed between SARS-CoV-2 NT Ab titer and spike-specific IFN-γ-producing cells at both 6 months and 3 weeks after vaccination ($r = 0.467$; $P = 0.0002$ and $r = 0.428$; $P = 0.0006$, respectively).

Conclusions: Our work highlights a reduction in the immune response in cancer patients, particularly in SARS-CoV-2-naive subjects. Our data support administering a third dose of COVID-19 vaccine to cancer patients treated with programmed cell death protein 1/programmed death-ligand 1 inhibitors.

Key words: BNT162b2 anti-SARS-CoV-2 vaccine, cancer, PD-1/PD-L1 inhibitors, neutralizing antibody, spike-specific T-cell response, third dose

INTRODUCTION

Vaccines against SARS-CoV-2 have demonstrated high efficacy in reducing symptomatic infections and days of hospitalization.[1]

The first comprehensive meta-analysis about the immunogenicity and safety of anti-SARS-CoV-2 vaccines for patients with cancer showed that there is no reduced rate of

*Correspondence to: Dr Angioletta Lasagna, Medical Oncology Unit, Fondazione IRCCS Policlinico San Matteo, Piazzale C. Golgi 19, 27100 Pavia, Italy. Tel: +39-0382502287; Fax: +39-0382501601

E-mail: a.lasagna@smatteo.pv.it (A. Lasagna).

†Equal contributions.

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seroconversion compared with the control [Relative risk 0.95, 95% confidence interval (CI) 0.90-1.01, $P = 0.09$, $I^2 = 73.5\%$, $P = 0.01$] in solid cancer patients, even if a significant decrease was observed in hematological patients (Relative risk 0.62, 95% CI 0.41-0.92, $P = 0.02$, $I^2 = 96.2\%$, $P < 0.001$).[2] Additionally, patients with solid tumors vaccinated during chemotherapy courses showed both reduced anti-receptor-binding domain antibody concentrations and neutralizing antibody responses at 28 days after the booster administration.[3]

To date, only one study reported a 6-month follow-up of SARS-CoV-2 vaccine immunogenicity, efficacy, and safety in cancer patients with respect to the control group, revealing no differences between the two cohorts. Additionally, the decline of antibody concentration was similar 6 months after the second dose in both groups, though the majority of patients were still seropositive.[4]

In our previous paper,[5] we highlighted the immunogenicity of the vaccine in triggering both the humoral and the cell-mediated immune response in cancer patients treated with anti-programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) with or without chemotherapy after a full course of COVID-19 vaccine.

This study prospectively evaluated these outcomes 6 months after BNT162b2 anti-SARS-CoV-2 vaccine.

PATIENTS AND METHODS

Patients and study design

Patients with cancer receiving a full course of vaccine during anti-PD-1/anti-PD-L1 therapy with or without chemotherapy were enrolled. As detailed in our previous report, the inclusion criteria were: (i) patients aged 18 and older; (ii) life expectancy $\geq$6 months; (iii) confirmed histological diagnosis of solid tumors; (iv) vaccination with the BNT162b2 messenger RNA (mRNA) vaccine; and (v) signing of informed consent. A previous infection with SARS-CoV-2 was not an exclusion criterion.

Patients were defined as 'SARS-CoV-2-experienced' if they had a documented past positive SARS-CoV-2 RNA in a nasopharyngeal swab and/or positive anti-spike immunoglobulin G (IgG) at the time of enrollment (before vaccination). Otherwise, they were classified as 'SARS-CoV-2-naive'.

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

The study (Co-Vax) was conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for reporting observational studies[6] and was approved by the local ethics committee (Comitato Etico Area Pavia) and institutional review board (P-20210023530). All subjects signed an informed written consent before the enrollment.

This is a prospective follow-up report of the primary study.

For these conclusive analyses 26-27 weeks after the second dose of BNT162b2 anti-SARS-CoV-2 vaccine we have considered only the patients who remained on immunotherapy at this time point (T3).

Assessments

The patients were monitored 26-27 weeks after the second dose with blood samples for humoral and cell-mediated immune response evaluation. Throughout the study, all patients underwent a nasopharyngeal swab before each cycle of immunotherapy.

Study endpoints

In the first publication of this study,[5] the primary endpoint was the percentage of patients with a significant increase in spike-specific interferon-γ (IFN-γ)-producing T cells between baseline and 3 weeks after the second vaccination dose. In the present study, we provided an update on the duration of immune response after BNT162b2 mRNA vaccination at 26-27 weeks (6 months), analyzing both spike-specific IFN-γ-producing T cells and humoral response (total IgG concentration and SARS-CoV-2 NT Ab titer). Subjects were defined as 'full responders' if there was a positive anti-spike IgG concentration, a SARS-CoV-2 NT Ab titer, and spike-specific IFN-γ-producing T cells. Additionally, we evaluated the incidence of virologically confirmed COVID-19 cases during the entire period of the study.

Spike-specific T-cell response measured by ex vivo enzyme-linked immunosorbent spot assay

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-IFN-γ monoclonal capture antibody) with peptide pools (15mers, overlapping by 10 amino acids, Pepscan, Lelystad, the Netherlands) representative of the spike protein (S) at a final concentration of 0.25 µg/ml. Phytohemagglutinin (5 µg/ml) was used as a positive control, and medium alone as a negative control. Enzyme linked immunospot assay was carried out according to our previous protocol.[5] Responses $\geq$10 spike-specific IFN-γ-producing T cells/million PBMCs were considered positive based on background results obtained with the negative control (mean spot forming units + two standard deviations).

Phenotypical characterization of spike-specific IFN-γ-producing T cells

In a subset of patients, phenotypical characterization of the spike-specific IFN-γ-producing T-cell response was carried out, according to our previous protocol.[5] In detail, 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 a final concentration of 0.1 µg/ml for 7 days. A peptide pool from human actin was used as a 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, MA) 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 PBMCs incubated with actin peptides from the frequency of PBMCs 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, Franklin Lakes, NJ).

Antibody response

The quantitative characterization of spike-specific IgG antibodies was carried out by Trimeric assay (Liaison, Diasorin, Saluggia, Italy) and results were given as BAU/ml (positive results >33.8 BAU/ml). Additionally, the neutralizing antibody (NT Ab) titer against the B.1.1 strain was measured as previously reported[5] and results were given as positive when NT Ab was ≥1 : 10.[7,8]

Statistical analysis

GraphPad Prism 8.3.0 (GraphPad Software, La Jolla, CA) was used for statistical analyses. A two-sided P value <0.05 is considered statistically significant. Data were described as median and interquartile range (IQR) if continuous and as counts and percentage if categorical. Comparison between two groups was carried out using the Mann−Whitney test (unpaired samples) or Wilcoxon test (paired samples), whereas the Spearman's test was used for correlation analysis. The Fisher exact test was used for comparison of categorical variables.

RESULTS

Patients' characteristics

The original study cohort[5] consisted of 88 patients with solid tumor (23 females and 65 males; (median age 68 years, IQR 61.5-73). The current study included 60 patients with solid tumors (17 females and 43 males; median age 66 years, IQR 60-71) who were receiving anti-PD-1/anti-PD-L1 therapy at the time of T3 (6 months after COVID-19 vaccination). Forty-six patients (76.7%) had lung cancer, six (10%) had melanoma, and four (6.7%) had kidney cancer; the remaining four patients (6.7%) had head and neck cancer (two patients), bladder cancer (one patient), and squamous cell skin cancer (one patient).

In the first paper, complete analyses were carried out in 73/78 subjects who received the complete vaccination schedule. In the follow-up paper, we were able to collect samples at 6 months in only 60 out of 73 subjects. In particular, we excluded 13 patients: 1 patient refused the blood sample, 6 patients died, and 6 patients were no longer on immunotherapy.

A total of 50 patients (50/60, 83.0%) were on immunotherapy alone, whereas 10 patients (10/60, 7%) were on chemo-immunotherapy (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2021.100359).

Spike-specific T-cell response elicited by BNT162b2 anti-SARS-CoV-2 vaccine

Spike-specific IFN-γ-producing cells/10^6 PBMCs measured 6 months post-vaccination were compared with the response elicited by BNT162b2 vaccine 3 weeks after a complete vaccination schedule administration. From the analysis of 60 paired samples, we observed that the rate of subjects with a detectable spike-specific T-cell response was 0.93 (95% CI 0.84-0.97) at 3 weeks and 0.83 (95% CI 0.72-0.90) at 6 months. Moreover, spike-specific IFN-γ-producing cells/10^6 PBMCs at 6 months were significantly lower than those measured at 3 weeks after vaccination (50 IQR 20-118.8 versus 175 IQR 67.5-371.3 IFN-γ-producing cells/10^6 PBMCs; $P < 0.0001$).

Spike-specific IFN-producing cells/10^6 PBMCs 6 months and 3 weeks after vaccination were 42.5 (IQR 16.2-111.3) and 125 (IQR 52.5-357.5), respectively ($P < 0.0001$) (Figure 1A). A reduction in terms of spike-specific IFN-γ-producing cells/10^6 PBMCs was observed in SARS-CoV-2-experienced subjects ($n = 8$), since median responses were 72.5 (IQR 55-940) and 245 (IQR 212.5-1399) spike-specific IFN-γ-producing cells/10^6 PBMCs at 6 months and 3 weeks, respectively ($P = 0.035$; Figure 1B). Of note, none of the SARS-CoV-2-experienced subjects showed negative spike-specific T-cell responses at 6 months post-vaccination.

A total of 20 vaccinated patients on immunotherapy alone (13 SARS-CoV-2-naive and 7 SARS-CoV-2-experienced subjects) were tested for phenotypical analysis of spike-specific T-cell proliferative response at 6 months after vaccination and the data were compared with those obtained at 3 weeks after vaccination. Overall, median CD4+ T-cell response was maintained at 6 months (median 3.67, IQR 0.22-12.92) compared with that observed at 3 weeks after vaccination (median 3.79, IQR 0.17-38.18; $P = 0.2024$). Similarly, no differences were reported in terms of median CD8+ T-cell response between 6 months (median 2.25, IQR 0.57-9.06) and 3 weeks (median 0.63, IQR 0.04-5.4) after vaccination ($P = 0.5768$).

Humoral response elicited by BNT162b2 anti-SARS-CoV-2 vaccine

The rate of subjects with positive IgG concentration decreased 6 months post-vaccination compared with 3 weeks after vaccine [1.0 (95% CI 0.94-1.0) versus 0.87 (95% CI 95 0.76-0.93), respectively]. Overall, the median reduction of IgG concentration was 88% (IQR 77.5%-92.55%) in SARS-CoV-2-naive subjects and median IgG concentration at 6 months was 205.2 (IQR 73.2-654.6) BAU/ml whereas it was 1938 (IQR 834-2080) BAU/ml after 3 weeks ($P < 0.0001$; Figure 2A).

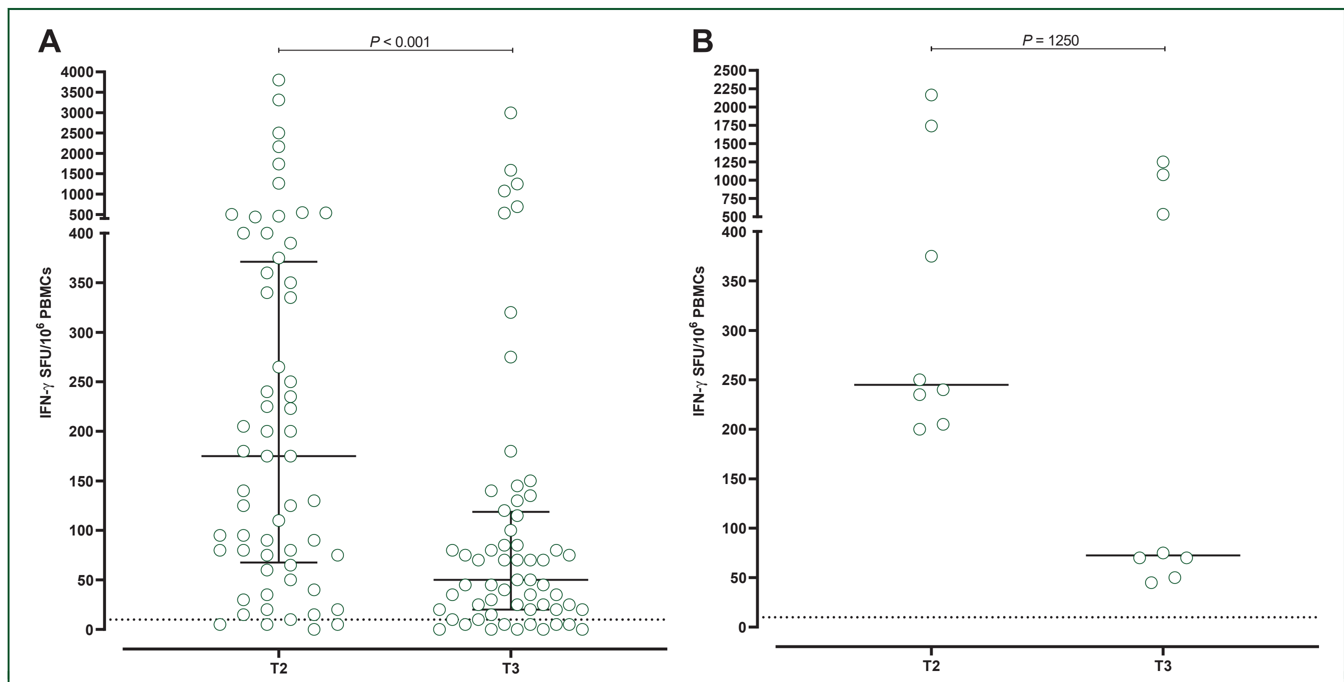

Figure 1. Spike-specific T-cell response was measured at 3 weeks (T2) and 6 months after vaccination (T3) in SARS-CoV-2-naive (n = 52; A) and SARS-CoV-2-experienced subjects (n = 8; B).

Median T-cell responses and P values are shown.

IFN-γ, interferon- γ; PBMCs, peripheral blood mononuclear cells.

IgG concentration in SARS-CoV-2-experienced subjects after two doses of BNT162b2 anti-SARS-CoV-2 vaccine was 2035 (IQR 1411-2080) BAU/ml at 6 months and 2077 (IQR 2074-2080) BAU/ml at 3 weeks after vaccination with an overall median reduction of 2.1% (IQR 0.0%-32.2%) (Figure 2B).

Looking at the SARS-CoV-2 NT Ab titer, a significant decrease was observed in SARS-CoV-2-naive subjects [median 1 : 20 (IQR 1 : 10-1 : 40) at 6 months versus median 1 : 160 (IQR 1 : 40-1 : 640) at 3 weeks; $P < 0.0001$]. Two subjects (3.8%) were negative at 3 weeks for SARS-CoV-2 NT Ab titer, whereas the number of negative subjects reached 11 (21.2%) at 6 months ($P = 0.015$; Figure 3A).

The SARS-CoV-2 NT Ab titer was maintained in SARS-CoV-2-experienced subjects since 5/8 (63% patients) showed a stable NT Ab titer of 1 : 640, whereas in 3/8 (37%) we observed a sixfold decrease at most (Figure 3B). A weak correlation was observed between SARS-CoV-2 NT Ab titer and IFN-γ-producing cells specific for the spike protein both at 6 months and 3 weeks after vaccination ($r = 0.467$; $P = 0.0002$ and $r = 0.428$; $P = 0.0006$, respectively).

Type of therapy and long-term BNT162b2 immune response

Immune responses at 6 months after vaccination were compared in 9 subjects treated with chemo-immunotherapy and 43 subjects treated with only immunotherapy. In order to avoid confounding factors, only SARS-CoV-2-naive subjects vaccinated with two BNT162b2 doses were analyzed. Overall, the rate of 'full responders' was 0.78 (95% CI 0.45-0.96) and 0.98 (95% CI 0.88-0.99) in the two groups, respectively; the difference was not statistically significant ($P = 0.0738$).

As shown in Figure 4A, the median number of spike-specific IFN-γ-producing cells/10^6 PBMCs in patients treated with chemo-immunotherapy was slightly lower (median 20, IQR 5-60 IFN-γ-producing cells/10^6 PBMCs) compared with that observed in only immunotherapy patients (median 45, IQR 20-120 IFN-γ-producing cells/10^6 PBMCs; $P = 0.0569$).

Similarly, even if the differences were not statistically significant, the humoral response against the spike antigen at 6 months was lower in patients receiving chemo-immunotherapy in terms of both IgG concentration [median 80.6 (IQR 31.2-198.8) BAU/ml versus median 196.3 (IQR 72.3-432.0) BAU/ml; $P = 0.1303$; Figure 4B) and SARS-CoV-2 NT Ab titer [median 1 : 10 (IQR 1 : 5-1 : 20) versus median 1 :20 (IQR 1 : 10-1 : 80) $P = 0.1852$] (Figure 4C).

In our cohort, age is not correlated with humoral response at 6 months, even if a negative weak correlation was observed between age and spike-specific IFN-γ-producing cells/10^6 PBMCs recorded at 6 months after vaccination ($r = -0.3593$; $P = 0.0065$) but not at 3 weeks after vaccine ($r = -0.1541$; $P = 0.2567$). No differences were reported when gender, tumor stage (III versus IV), and type of tumor (lung versus other) were compared.

DISCUSSION

A full course of mRNA anti-SARS-CoV-2 vaccines is highly effective in preventing a symptomatic SARS-CoV-2 infection,[9] but the durability of this protection is not known yet. The first real-world data on the duration of the humoral response 6 months after the full course of BNT162b2 COVID-19 vaccine indicate a considerable decrease of both IgG concentration

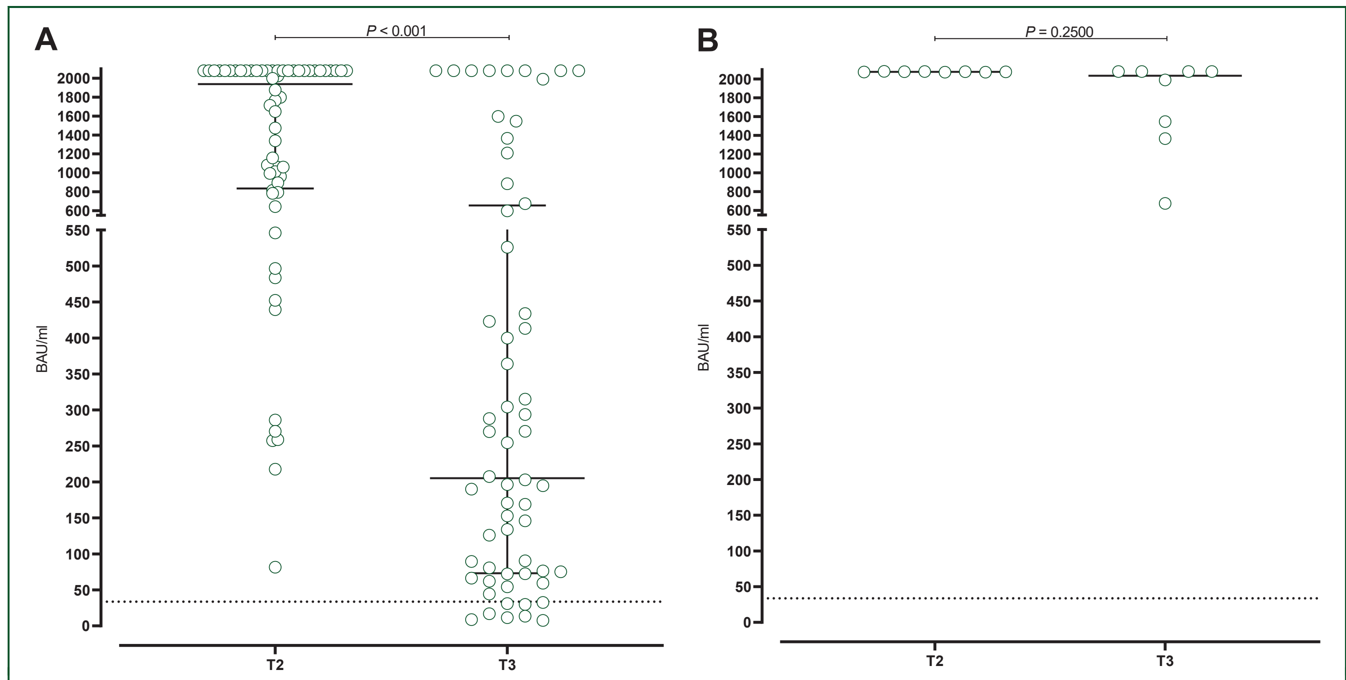

Figure 2. Total anti-spike immunoglobulin G (IgG) concentration was measured at 3 weeks (T2) and 6 months after vaccination (T3) in SARS-CoV-2-naive ($n = 52$; A) and SARS-CoV-2-experienced subjects ($n = 8$; B).
Median IgG concentrations and *P* values are shown.

and NT Ab titer, especially among men, people 65 years of age or older, and immunocompromised subjects.[10]

Our current study is a longitudinal follow-up of patients with cancer who had been on immunotherapy at the time of vaccination and had remained on treatment throughout the 6-month study period.

Our data confirm the reduction of both humoral and cell-mediated responses after 6 months of a full course of a COVID-19 vaccine. In particular, the median number of spike-specific IFN-γ-producing cells was significantly lower than that measured at 3 weeks after vaccination in SARS-CoV-2-naive patients, whereas in SARS-CoV-2-experienced

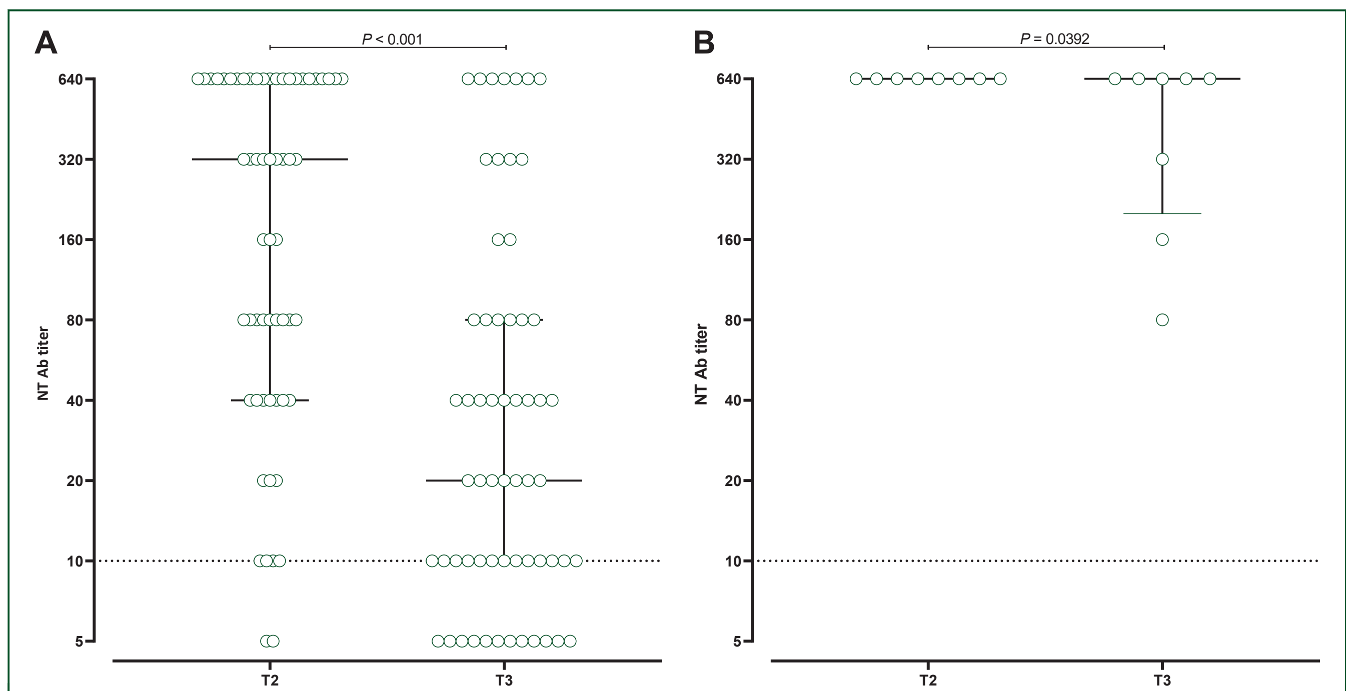

Figure 3. Neutralizing antibody titer (NT Ab) was measured at 3 weeks (T2) and 6 months after vaccination (T3) in SARS-CoV-2-naive ($n = 52$; A) and SARS-CoV-2-experienced subjects ($n = 8$; B).
Median NT Abs and *P* values are shown.

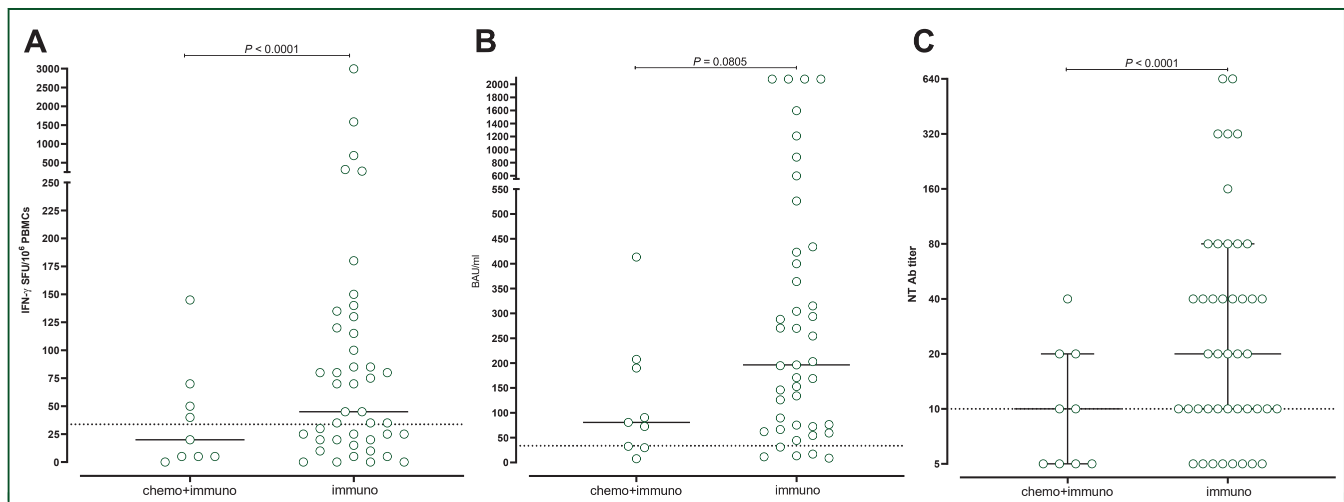

Figure 4. Total spike-specific T-cell response (A), anti-spike immunoglobulin G (B), and NT Ab titer (C) were measured in cancer patients treated with a combination of chemotherapy and immunotherapy (chemo+immuno) and immunotherapy only (immuno).
Median response and *P* value are shown in each graph.
IFN-γ, interferon- γ; NT Ab, neutralizing antibody titer; PBMCs, peripheral blood mononuclear cells.

subjects, the reduction of the median number of spike-specific IFN-γ-producing cells was less marked, thus suggesting that a triple exposure to SARS-CoV-2 antigens may be able to induce a higher and more persistent cell-mediated immune response. This difference was also confirmed by the analysis of humoral response kinetics, since positive SARS-CoV-2 NT Ab titer and IgG concentration were maintained in SARS-CoV-2-experienced subjects, but not in SARS-CoV-2-naive patients.

With respect to the analyzed clinical variables [age, sex, PD-L1 status, TNM (tumour—node—metastasis) staging, type of treatment, with or without chemotherapy], chemo-immunotherapy seemed to determine a reduction in humoral and spike-specific IFN-γ-producing cells, even if the difference was not statistically significant. The lack of significance might be due to the small sample size; thus, larger prospective studies are mandatory. In our cohort, age is not correlated with humoral response at 6 months, whereas a negative weak correlation was observed between age and spike-specific IFN-γ-producing cells/10^6 PBMCs recorded at 6 months after vaccination.

In our cohort of cancer patients, the administration of a full course of the mRNA vaccine provides a durable immune response in SARS-CoV-2-experienced subjects, while in SARS-CoV-2-naive patients the durability seems less marked. Prior natural COVID-19 history leads to the formation of a strong immune response and our data highlight that the vaccine could act as a booster for SARS-CoV-2-experienced subjects.

In this setting, a ‘real life’ study reported vaccine protection against acquisition of SARS-CoV-2 infection of 83% in the overall population and of 93% in SARS-CoV-2-experienced subjects.[11] Importantly, in our cohort, including seronegative patients, no cases of COVID-19 were documented. Since no control group was included in this study, real data on vaccine efficacy in our cohort are difficult to be extrapolated. So far, the absence of SARS-CoV-2-positive cases after vaccination might be related to vaccination itself, but also to the strict adherence of patients to social distancing, facial mask use, and hand sanification.

To date, the third dose administration programs are ongoing worldwide, but data about the need of its administration are scarce, above all in frail and immune-compromised patients. Preliminary data confirmed that a third BNT162b2 dose leads to an increase NT Ab titer in cancer patients.[12] Our paper confirms the immunogenicity of the BNT162b2 anti-SARS-CoV-2 vaccine even at 6 months, but highlights a reduction in the immune response in cancer patients, particularly in SARS-CoV-2-naive subjects. These data strongly suggest a priority to administer the third dose of COVID-19 vaccine and add a significant contribution to the management of patients with cancer.

Strengths and limitations

The strengths of our paper are the simultaneous tracking of humoral and cellular immune responses with the detection of both anti-spike IgG concentration and NT Ab titers, and enzyme-linked immunosorbent spot (ELISpot) assay in a well-defined and homogeneous population over time. 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 vaccine eliminating other confounding factors. The use of the *ex vivo* ELISpot assay, however, limited the analysis of T-cell response to only IFN-γ-producing cells in response to the spike antigen. Thus, other cells producing different cytokines have not been studied. Furthermore, the absence of COVID-19 cases in our cohort limits speculations in terms of a protective T-cell response. Of note, we carried out the assessment at the 6-month follow-up before the patients received their third dose of vaccine; in this way, we were able to eliminate an important confounding factor. The limitations of our paper are the lack of a control group and the small sample size that enable us to extend our conclusions in a definitive way.

Conclusion

Our data confirm the durability of the vaccine both in the humoral and in the cell-mediated immune response in patients with cancer treated with anti-PD-1/PD-L1, but demonstrate also a significant reduction, particularly in SARS-CoV-2-naive patients. Our data support administering a third dose of COVID-19 vaccine to patients with cancer treated with PD1/PD-L1 inhibitors.

FUNDING

This work was supported by Ricerca Corrente [grant number 08067620], Fondazione Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Policlinico San Matteo, Italy, Ricerca Finalizzata [grant BIAS number 2020-12371760], and from European Commission—Horizon 2020, European Union [EU project 101003650—ATAC].

DISCLOSURE

The authors have declared no conflicts of interest.

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