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COVID-19 vaccination in cancer patients: Immune responses one year after the third dose

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ABSTRACT

Cancer patients (CPs), being immunosuppressed due to the treatment received or to the disease itself, are more susceptible to infections and their potential complications, showing therefore an increased risk of developing severe COVID-19 compared to the general population.

We evaluated the immune responses to anti-SARS-CoV-2 vaccination in patients with solid tumors one year after the administration of the third dose and the effect of cancer treatment on vaccine immunogenicity was assessed. Healthy donors (HDs) were enrolled. Binding and neutralizing antibody (Ab) titers were evaluated using chemiluminescence immunoassay (CLIA) and Plaque Reduction Neutralization Test (PRNT) respectively. T-cell response was analyzed using multiparametric flow cytometry.

CPs who were administered three vaccine doses showed lower Ab titers than CPs with four doses and HDs. Overall, a lower cell-mediated response was found in CPs, with a predominance of monofunctional T-cells producing TNFα. Lower Ab titers and a weaker T-cell response were observed in CPs without prior SARS-CoV-2 infection when compared to those with a previous infection. While no differences in the humoral response were found comparing immunotherapy and non-immunotherapy patients, a stronger T-cell response in CPs treated with immunotherapy was observed.

Our results emphasize the need of booster doses in cancer patients to achieve a level of protection similar to that observed in healthy donors and underlines the importance of considering the treatment received to reach a proper immune response.

1. Introduction

Since Coronavirus disease-19 (COVID-19) outbreak in 2020, several strategies to overcome the disease global health impact and its difficult management have been approved, including the introduction of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines [1,2]. Among SARS-CoV-2 vaccines, the mRNA vaccine BNT162b2 (Comirnaty®) has been widely employed showing protection from severe disease, hospitalization and death in immunocompetent and in frail populations as well [3–5]. However, vaccine immunogenicity in patients

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with solid tumors (cancer patients) is of particular interest due to the possible suppression and over-activation of immune system caused by the disease or the ongoing treatment [6]. Several studies have reported a greater risk of COVID-19 in cancer patients with a higher probability of severe or fatal complications due to the disease-related immune dysregulation or treatment induced immunosuppression that negatively affects this population vaccine immunogenicity [7–10].

The adaptive immune response plays an important role in COVID-19 severity, viral clearance, and disease resolution [11,12]. Variants of concern can partially escape the humoral response elicited by mRNA vaccines, but not T-cell mediated response [13]. Early induction of CD8+ T-cells could account for asymptomatic disease [14]. On the other hand, apoptosis-induced CD4+ and CD8+ T lymphopenia has been associated with severe COVID-19 [15]. Indeed, patients with severe COVID-19 present lymphopenia and low CD4+ and CD8+ T-cell counts, as well as high percentages of programmed cell death-1 (PD-1) expression on T-cells [16].

Upregulation of immune checkpoint receptors, such as PD-1, appears to be also associated with disease severity, and interpreted as T-cell exhaustion [17]. Nevertheless, conflicting evidence show that PD-1 positive cells are functionally active in the acute and early convalescent phases of COVID-19, raising the question of whether PD-1 could be considered a marker of activation rather than exhaustion in COVID-19 patients, or whether PD-1 may endow different functional subsets [18,19]. Cancer patients have been especially vulnerable to severe and life-threatening COVID-19, in addition to the disruption of their medical care during the worst periods of the pandemic [20–23]. Cancer patients and other immunocompromised populations were also excluded or underrepresented in the clinical trials for the SARS-CoV-2 mRNA vaccines [24,25].

In this framework, the efficacy of SARS-CoV-2 vaccines in immunocompromised populations is of paramount relevance for the design and implementation of vaccine strategies in these subjects. However, little is known regarding the long-term humoral and cellular responses triggered by SARS-CoV-2 mRNA vaccines in cancer patients after repeated booster doses and how much immunotherapies can affect it. Therefore, the question that remains unanswered is whether some frail subjects might benefit from the administration of repeated boosters of SARS-CoV-2 vaccine while others might not.

In this work, we studied the specific humoral and cellular immune responses one year after the administration of the third mRNA vaccine dose in cancer patients with solid tumors undergoing immunotherapy or not and with or without a previous infection.

2. Material and methods

2.1. Study design and participants

To evaluate humoral and specific T-cell response to mRNA BNT162b2 (Comirnaty®) vaccine, cancer patients (CPs) under immunotherapy, including anti-PD-L1 drugs, chemotherapy, a combination of immuno-chemotherapy or biological therapies, as well as age- and sex-matched healthy donors (HDs) were enrolled. Both CPs and HDs received at least three doses of mRNA BNT162b2 (Comirnaty®) vaccine according to schedule proposed by the current Italian national vaccination program [26].

Both CPs and HDs were also stratified according to SARS-CoV-2 infection onset after the third mRNA vaccine dose into two subgroups: experienced, including cancer patients or HDs that reported previous SARS-CoV-2 infection; naïve, including cancer patients or HDs that didn't referred SARS-CoV-2 infection. The differences in humoral and SARS-CoV-2 specific T-cell response among the subgroups were evaluated.

Finally, all enrolled CPs were stratified according to cancer treatment into two subgroups: immunotherapy, including patients under immunotherapy and immuno-chemotherapy treatment, and non-immunotherapy, including cancer patients under chemotherapy and biological therapies. Patients in treatment with immunotherapy underwent anti-programmed cell death ligand 1 (PD-L1) therapy. The differences in humoral and SARS-CoV-2 specific T-cell response among the subgroups were evaluated.

2.2. SARS-CoV-2 anti-S antibodies

Whole blood samples were collected one year after the administration on the third dose of vaccine. Serum separation was performed through centrifugation at 1500× *g* for 15 min at room temperature and stored at −80 °C until analysis. The level of IgG antibodies against SARS-CoV-2 trimeric Spike protein was quantified using the LIAISON® SARS-CoV-2 TrimericS IgG kit (DiaSorin S.p.A., Saluggia, Italy), an indirect chemiluminescence immunoassay (CLIA) technology. Samples were considered positive when the test result was ≥33.8 Binding Antibody Units (BAU)/ml with the test quantification range going from 4.81 to 2080 BAU/ml. For results >2080 BAU/ml, a dilution of the sample was performed.

2.3. Neutralization assay

Sera samples from patients and healthy donors were tested to detect the presence of neutralizing antibodies (NAbs) against Wuhan strain, specimens of previously uninfected subjects were also tested against Omicron variant (24 patients and 6 healthy donors). NAbs titer was analyzed using the Plaque Reduction Neutralization Test (PRNT). The procedure was performed as follows: all specimens underwent serial dilutions using Minimum Essential Medium (MEM). For each sample, 3 dilutions were selected to be tested in duplicate. One hundred µl of each sample were mixed with 100 µl of previously diluted wild type (WT) or Omicron strain of SARS-CoV-2. After 1 h incubation at 37°, the suspension was added on a plate containing confluent Vero cells previously cultured and the mixture was kept at 37° for another hour. A semi-solid medium was then overlaid on the cell monolayer to restrict the spread of viral progeny and the plates were kept 5 days at 37°. The viral particles that were able to initiate a productive infection formed plaques that were counted after coloration with crystal violet and compared with the viral control to establish the percentage of reduction of viral infectivity. The PRNT50 titer was expressed as the reciprocal of the highest dilution able to reduce the number of plaque-forming units (PFU) by 50 % compared to the viral control [27].

2.4. SARS-CoV-2 specific T-cell stimulation

SARS-CoV-2 specific T-cell response was assessed using a multiparametric flow cytometry after overnight stimulation with SARS-CoV-2 peptide libraries on isolated peripheral blood mononuclear cells (PBMCs), as previously described [28–30]. Pools of lyophilized peptides, consisting mainly of 15-mer sequences with 11 amino acids overlap, covering the immunodominant sequence domains of the Wuhan wild-type (WT) Spike glycoprotein (S) (GenBank MN908947.3, Protein QHD43416.1) and the B.1.1.529 Omicron variant Spike Mutation Pool were purchased from Miltenyi Biotec. Specifically, PepTivator SARS-CoV-2 Prot_S1 covered the N-terminal S1 domain of the spike protein (amino acids [aa] 1–692). PepTivator SARS-CoV-2 Prot_S covered selected immunodominant sequence domains of the spike protein (aa 304–338, 421–475, 492–519, 683–707, 741–770, 785–802, and 885–1273). PepTivator SARS-CoV-2 Prot_S B.1.1529 selectively covered surface or spike mutated regions of BA.1, BA.2, BA.4/BA.5 variant of the SARS-CoV-2B.1.1.529 lineage. For each subject, an unstimulated and a positive phytohemagglutinin (PHA) 5 µg/ml control was also included. Brefeldin A at a final concentration of 10 µg/ml was added in the culture after 1 h of incubation.

PBMCs were then stained with an appropriate combination of fluorochrome-conjugated antibodies (PacificBlue-conjugated anti-

CD45, APC-Cy7-conjugated anti-CD4, APC-conjugated anti-CD8, BioLegend, San Diego). Subsequently, Fix/Perm solution (BioLegend, San Diego) was used prior intracellular staining (FITC-conjugated anti- IFNγ, PerCp-Cy 5.5-conjugated anti-TNFα and PE-Cy7-conjugated anti-IL2, BioLegend, San Diego), according to manufacturer's instructions. A fixable viability kit (Zombie Aqua™ BioLegend, San Diego) was used to exclude dead cells. Samples were acquired using MACSQuant (Miltenyi Biotec, Germany) and analyzed using FlowJo™ v10.8.1 software. Specifically, cytokine background obtained from the unstimulated condition was subtracted to the stimulated ones.

All possible combinations of intracellular expression of IFNγ, IL2 and TNFα in cytokine-producing T-cells were evaluated using the Boolean gate. "Responding T-cells" were defined as those cells producing any of IFNγ, IL2 and TNFα, while "triple-positive T-cells" were defined as those simultaneously producing all three cytokines, as previously described [28–30]. Display and analysis of the different cytokine combinations were performed with SPICE v6.1.

2.5. Statistical analyses

All data are reported as median and interquartile range [IQR]. Differences between CPs and HDs as well as among subgroups were assessed using a two-tailed Mann-Whitney test for quantitative variables. Differences among CPs subgroups and HDs were assessed using a non-parametric Kruskal-Wallis test with Dunn's multiple comparison post-test for quantitative variables. Results were considered statistically significant if the p-value was <0.05. To evaluate the relationship between the antibody level detected through CLIA and the PRNT50 titer against both Wuhan strain and Omicron variant, Spearman's correlation and a linear regression model were estimated.

Statistical analyses were performed using GraphPad Prism 9. Finally, distribution differences of the different cytokine combinations between groups and subgroups were performed using the nonparametric Permutation test using SPICE, distributed by the National Institute of Allergy and Infectious Diseases, NIH.

The study was granted ethical approval by the local ethical committee, protocol number 0897/2022.

3. Results

3.1. Study population

From November 2022 to January 2023, 66 CPs reffered to the Oncology Unit of Policlinico Umberto I Hospital, Sapienza University of Rome, were enrolled. Among them, 47 CPs were vaccinated with three doses of mRNA vaccine between December 2020 and December 2021 (CPs-3) while 19 received an additional 4th dose of vaccine on June 2022 (CPs-4) (Table 1).

As control group, 27 age- and sex-matched HDs (consisted predominantly of healthcare workers and employees) vaccinated with three doses of mRNA vaccine between December 2020 and December 2021 were enrolled.

In the CPs-3 group, 21 (45 %) were in treatment with immunotherapy, specifically: 11 (23 %) underwent immunotherapy, 20 (43 %) chemotherapy, 10 (21.2 %) with a combination of chemo- and immunotherapy and 6 (12.8 %) underwent biological therapies. Meanwhile, among CPs-4 group, 3 (16 %) were in treatment with immunotherapy, 9 (47 %) chemotherapy, 3 (16 %) with both immune- and chemotherapy and 4 (21 %) underwent biological therapies.

Finally, previous SARS-CoV-2 infection after the third dose of mRNA vaccine was reported in 64 % and 58 % of the CPs-3 and CPs-4 groups, respectively. Similarly, 58 % of HDs reffered previous SARS-CoV-2 infection after the third dose of mRNA vaccine. Demographic and clinical features of the study population are reported in Table 1.

Table 1
Demographic and clinical features of the study population.

	CPs-3 (n=47)	CPs-4 (n=19)	HDs (n=27)
Age, median (IQR), years	60 (51–69)	58 (55–65)	57 (48–62)
Male/Female, n	25/22	5/14	9/18
SARS-CoV-2 infection, n	30	11	15
Months from treatment start (IQR)	7 (2–22)	18 (4–36)	
Immunotherapy, n	21	7	
Primary tumor, n			
Lung	18	3	
Breast	9	10	
Colon	4	3	
Bladder	3	0	
Head-neck	2	0	
Prostate gland	2	0	
Rectum	2	0	
Stomach	2	0	
Esophagus	1	0	
Kidney	1	0	
Ovary	1	0	
Pancreas	1	2	
Skin	0	1	
Surrenal gland	1	0	
Comorbidities yes/no, n	28/19	13/6	
Cardiovascular	19	12	
Endocrinological	8	4	
Gastrointestinal	5	2	
Pneumological	4	1	
Autoimmune diseases	2	1	
Neurological	1	2	
Infectious	1	1	
Psychological/psychiatric	1	0	
Urological	1	0	

Data are shown as median (IQR) or number of subjects. CPs-3: cancer patients who received 3 vaccine doses; CPs-4: cancer patients who received 4 vaccine doses; HDs: healthy donors; n: number; IQR: interquartile range.

3.2. Humoral response

One year after receiving the third dose of anti-SARS-CoV-2 vaccine, a lower anti-Spike antibody titer in CPs-3 group compared to CPs-4 one was observed ($p = 0.0209$). Compared to HDs, only CPs-3 group showed lower anti-Spike antibody titer ($p = 0.0079$). No differences between CPs-4 and HDs were observed (Fig. 1A).

Stratifing all groups according to SARS-CoV-2 infection after the third vaccine dose, a lower anti-Spike antibody titer among naïve compared to experienced patients in CPs-3 group was observed ($p = 0.0004$) (Fig. 1B). Conversely, no differences among naïve and experienced subjects in both CPs-4 and HDs were observed (Fig. 1B). Additional comparisons showed a lower anti-Spike antibody titer among naïve in CPs-3 group compared to naïve from CPs-4 group ($p = 0.0060$) and naïve from HDs ($p = 0.0380$) (Fig. 1B). No differences in anti-Spike antibody titer were found when experienced subjects of every group were compared (Fig. 1B).

Finally, stratifing CPs according to the treatment received, lower anti-Spike antibody titers in immunotherapy CPs-3 subgroup compared to immunotherapy CPs-4 were found ($p = 0.0043$). Moreover, comparing both immunotherapy CPs-3 and CPs-4 subgroups to HDs, only immunotherapy CPs-3 subgroup showed lower antibody titer ($p = 0.0085$). Conversely, no differences in immunotherapy CPs-4 subgroup compared to HDs were found as well as in both non-immunotherapy CPs-3 and CPs-4 subgroups (Fig. 1C).

3.3. Neutralization assay

Comparing the PRNT50 titer against Wuhan strain, a lower titer in CPs-3 group compared to HDs was observed, although statistical significance was not reached ($p = 0.0707$) (Fig. S1 A). A lower PRNT50 titer against Wuhan strain among naïve compared to experienced

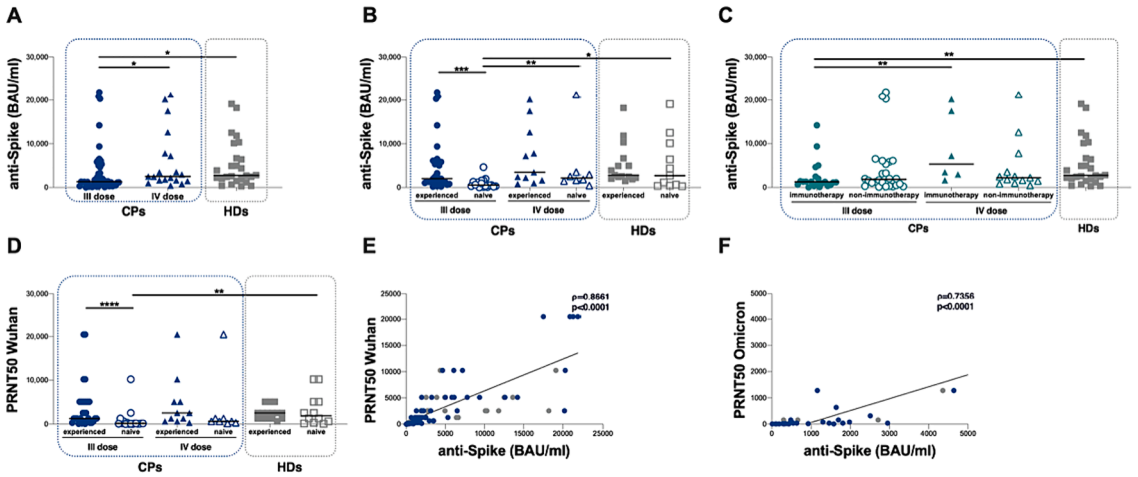

Fig. 1. Evaluation of humoral response in the study population. Anti-Spike antibody titer in CPs stratified according to (A) number of mRNA vaccine doses (B) previous SARS-CoV-2 infection and (C) immunotherapy. (D) PRNT50 titer against Wuhan strain in CPs stratified according to previous SARS-CoV-2 infection. (E) Positive correlation between anti-Spike antibody titer and PRNT50 titer against Wuhan strain. Linear correlation was evaluated using the regression test ($R^2 = 0.5848$, $p < 0.0001$). (F) Positive correlation between anti-Spike antibody titer and PRNT50 against Omicron strain. Linear correlation was evaluated using the regression test ($R^2 = 0.9075$, $p < 0.0001$).

patients in CPs-3 group was observed ($p < 0.0001$) (Fig. 1D). Conversely, no differences among naïve and experienced subjects in both CPs-4 and HDs were observed (Fig. 1D). A lower PRNT50 titer against Wuhan strain was observed in naïve CPs-3 subgroup compared to naïve HDs one ($p = 0.0045$) (Fig. 1D). Conversely, no differences in PRNT50 titer against Wuhan strain among naïve CPs-4 and naïve HDs subgroups neither among both experienced CPs-3 and CP-4 subgroups compared to experienced HDs subgroup were found (Fig. 1D). Finally, no differences in PRNT50 titer against Wuhan strain were found when CPs were stratified according to the treatment received (Fig. S1 B). However, in immunotherapy CPs-3 a lower PRNT50 titer against Wuhan strain compared to HDs was observed, although statistical significance was not reached ($p = 0.0731$) (Fig. S1B). The PRNT50 titer against Omicron strain was measured for all subjects naïve to the infection. Overall, the PRNT50 titer against Omicron was lower than PRNT50 titer against Wuhan ($p < 0.0001$). Lower PRNT50 titer against Omicron in CPs-3 group compared to HDs was observed, although statistical significance was not reached ($p = 0.0817$). Conversely, no difference between CPs-4 and HDs was observed (Fig. S1 C).

3.4. Correlation analysis

Overall, positive correlations between the antibody level and both the PRNT50 titer against Wuhan ($\rho = 0.8661$, $p < 0.0001$) (Fig. 1E) and

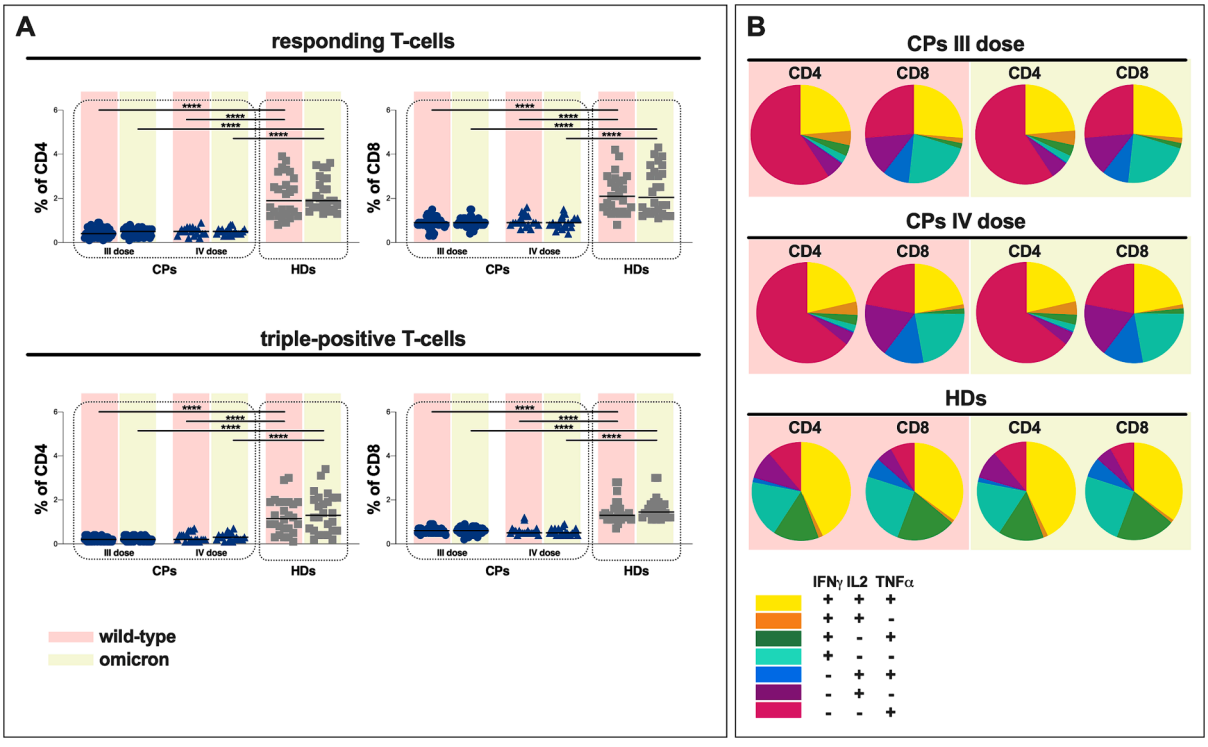

Fig. 2. Evaluation of S-specific T-cell response in study population. (A) Percentage of responding and triple-positive T-cells in CPs and HDs. Data are shown as median (lines). (B) Pie charts representing multifunctional cytokine analysis of specific T-cells in CPs and HDs.

Omicron strains ($\rho = 0.7356$, $p < 0.0001$) were found (Fig. 1F). Further analysis evaluating separately CPs and HDs groups showed positive correlation between the antibody level and both the PRNT50 titer against Wuhan and Omicron strains for both groups (CPs: $\rho = 0.8847$, $p < 0.0001$; HDs: $\rho = 0.7242$, $p < 0.0001$) (Fig. 1E and 1F).

3.5. Cellular response

Regarding the specific T-cell response, we first compared data from wild-type and omicron stimulations for each study population group. As shown in Fig. 2A, in each of the three enrolled groups (CPs-3, CPs-4 and HDs) no statistically significant difference in the percentages of responding and triple-positive T-cells stimulated with wild-type or omicron peptides was observed (Table S1). A lower percentage of CD4+ responding T-cells was found in both CPs-3 and CPs-4 subgroups compared to HDs for both wild-type ($p < 0.0001$ and $p < 0.0001$, respectively) and omicron stimulation ($p < 0.0001$ and $p < 0.0001$, respectively) (Fig. 2A) as well as a lower percentage of CD8+ responding T-cells for both wild-type ($p < 0.0001$ and $p < 0.0001$, respectively) and omicron stimulation ($p < 0.0001$ and $p < 0.0001$, respectively) (Fig. 2A). Moreover, CPs-3 and CPs-4 subgroups showed a statistically lower percentage of CD4+ triple-positive T-cells compared to HDs for both wild-type ($p < 0.0001$ and $p < 0.0001$, respectively) and omicron stimulation ($p < 0.0001$ and $p < 0.0001$, respectively) as well as a lower percentage of CD8+ triple-positive T-cells for both wild-type ($p < 0.0001$ and $p < 0.0001$, respectively) and omicron stimulation ($p < 0.0001$ and $p < 0.0001$, respectively) (Fig. 2A). All median values and IQR are reported in the Table S1.

Finally, an uneven T-cell subset distribution in both CPs-3 and CPs-4 subgroups was observed, with a predominance of monofunctional T-cells producing TNFα (IFNγ-IL2-TNFα +) for both wild-type and omicron stimulations. On the other hand, in HDs, a heterogeneous distribution of T-cell cytokines producers was found (Fig. 2B). Indeed, comparing T-cell CD4 subset distribution of both CPs-3 and CPs-4 subgroups with HDs, a statistically significant difference was observed ($p < 0.0001$) as well as in T-cell CD8 subset distribution ($p < 0.0001$) (Fig. 2B).

According to previous SARS-CoV-2 infection, CPs and HDs were stratified into two subgroups: experienced and naïve. Among CPs-3 and CPs-4 subgroups, higher percentages of responding T-cells to wild-type and omicron peptide stimulation were observed in experienced CPs compared to naïve CPs (for CPs-3 group CD4: $p = 0.0373$ and $p = 0.0482$, respectively; CD8: $p = 0.0169$ and $p = 0.0402$, respectively. For CPs-4 group CD4: $p = 0.0559$ and $p = 0.0485$, respectively; CD8: $p = 0.0422$ and $p = 0.0371$, respectively) (Fig. 3). Similarly, a higher percentage of triple-positive T-cells to wild-type and omicron peptide stimulation were observed in experienced CPs compared to naïve CPs (for CPs-3 group CD4: $p = 0.0373$ and $p = 0.0131$, respectively; CD8: $p = 0.0402$ and $p = 0.0003$, respectively. For CPs-4 group CD4: $p = 0.0061$ and $p = 0.0379$, respectively; CD8: $p = 0.0127$ and $p = 0.0306$, respectively) (Fig. 3). No significant diffeences among experienced and naïve HDs were observed. Compared to HDs, CPs showed a lower percentages of responding and triple-positive T-cells in both experienced and naïve subgroups (Fig. 3, Table S2).

Finally, stratifing CPs according to treatment, higher percentages of responding and triple-positive T-cells were observed in CPs treated with immunoterapy compared to non-immunotherapy CPs (Fig. 4, Table S3). Compared to HDs, a lower percentages of responding and triple-positive T-cells in non-immunotherapy CPs of both CPs-3 and CPs-4 subgroups was found (Fig. 4, Table S3). Similarly, a lower a lower percentages of responding and triple-positive T-cells in immunotherapy CPs-3 subgroup was observed (Fig. 4, Table S3). Otherwise, no differences in the percetanges of responding and triple-positive T-cells in immunotherapy CPs-4 subgroup compared to HDs was found (Fig. 4, Table S3).

4. Discussion

The extent of COVID-19 pandemic resulted in the need of a rapid development of vaccines and to the implementation of vaccination campaigns. To date, more than 5 billion people have completed the primary vaccination series [31]. Beside healthcare workers, the first categories of individuals to receive the vaccination have been frail persons. Among these, cancer patients are known to be at increased risk of developing severe COVID-19 compared to the general population

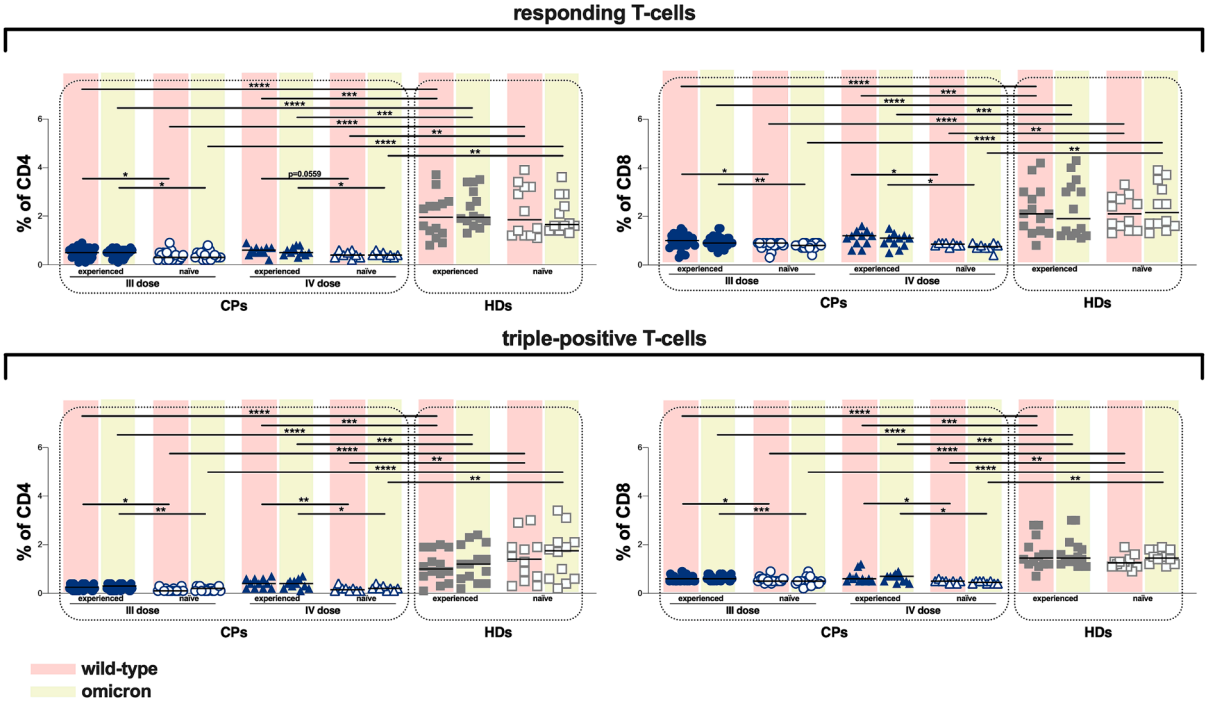

Fig. 3. Evaluation of S-specific T-cell response in CPs and HDs stratified according to previous SARS-CoV-2 infection.

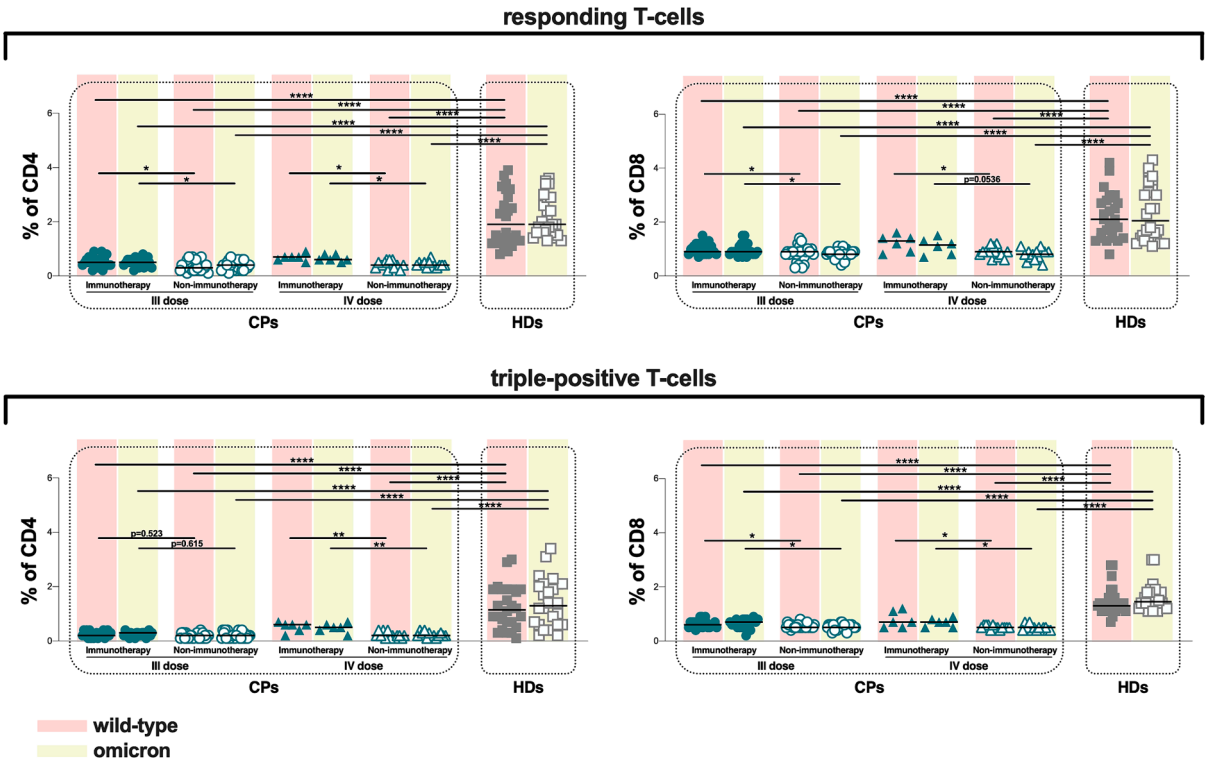

Fig. 4. Evaluation of S-specific T-cell response in CPs and HDs stratified according to immunotherapy.

[32–35]. In addition, most of these patients have comorbidities and are immunosuppressed due to the treatment received or to the disease itself, making them more susceptible to infections and their potential complications.

Our study was designed to evaluate the serological status and immunogenicity of the third dose of mRNA vaccine in a cohort of patients with solid malignancies under different cancer treatments investigating immunotherapy effects on humoral and/or cellular responses.

The scarce existing evidence points towards an enhanced humoral and cell mediated response after the second dose [36] as well as an enhanced humoral response after an additional booster dose [37], although the latter seems of lower intensity compared to healthy subjects [38]. Moreover, limited data exist concerning cell-mediated immunity and the potential exhaustion of T-lymphocytes in the event of repeated booster doses of SARS-CoV-2 vaccine in this population.

In line with other studies [39,40], our data showed that the humoral response was weaker in cancer patients who received 3 vaccine doses compared to both healthy donors and cancer patients who received an additional dose, whereas similar antibody titers were observed comparing these last two groups. Frail subjects can be less responsive or show a more variable response to the anti-SARS-CoV-2 vaccination [30,41]. Moreover, previous studies demonstrated an improved immune response after one or more additional doses of vaccine in frail subjects. For instance, in cancer patients, a second dose of anti-influenza vaccine was recommended after/during the 2009 H1N1 pandemic [42,43] as well as additional doses of the varicella-zoster virus vaccine are suggested to supply a stronger protection [44,45].

Conversely, among the three groups, no significant difference in the neutralizing capacity of the antibodies was found, althought lower antibody titers against both Wuhan and Omicron variant in cancer patients who underwent 3 vaccine doses compared to the healthy donors were observed. The different results between binding and neutralizing antibodies could be due to the type of test performed, automated and manual, respectively. However, as already reported by several authors [46–49], a positive correlation was found comparing the binding and neutralizing antibody titer against both Wuhan and Omicron strains underlining the utility of antibody titers as a suitable tool to estimate B-cell response.

In addition, we found that cancer patients had an overall impaired T-cell response compared to healthy donors with a lower cytokines production by both CD4+ and CD8+ T-cells, regardless of the vaccine dose received and the viral variant used for T-cell stimulation. Our results are in agreement with other studies that analyzed the T-cell response after vaccination in patients bearing solid and hematological malignancies, in which the percentages of viral specific CD4+ and CD8+ T-cells were lower than in healthy individuals [38,50]. When compared to healthy donors, cancer patients showed a different T-cell subset distribution, in particular cancer patients showed a higher percentage of TNF-α producing T-cells. This result is in accordance with what observed in other immunocompromised population, like people living with HIV [29] and solid organ transplant recipients [30], which exhibit a less heterogeneous T-cell response, with a higher proportion of CD4+ and CD8+ T-lymphocytes producing only one cytokine, a circumstance generally associated to a poorer protection against infections [51].

More than half of the subjects in our cohort reported a previous SARS-CoV-2 infection. Cancer patients who had been administered three doses of the vaccine, naïve to the infection, showed a lower humoral response than those with a previous infection as well as lower than healthy donors lacking previous infection [52,53]. From our observations experiencing the infection as well as receiving an additional vaccine dose, brings the antibody titer of cancer patients to a comparable level of that observed in healthy subjects, stressing the need of an extra dose of vaccine in this population. Regarding the T-cell response, experienced cancer patients showed a more vigorous response when compared to naïve, unrelatedly of the received vaccine dose, suggesting the possibility of a boosting effect of the natural infection on T-cell response [54–56]. However, when healthy donors were included in the comparison, a lower T-cell response was detected in all patients.

Finally, investigating the influence of therapy on the immune response to vaccination, no differences in humoral response comparing immunotherapy and non-immunotherapy patients were found, in contrast with previous studies [57–59]. However, immunotherapy

patients developed a stronger T-cell response compared to non-immunotherapy patients, with no differences regarding the number of doses. These results are consistent with the purpose of anti-PD-L1 therapy, of stimulating the immune response as already proved for influenza vaccination [60]. Our observations emphasize that a possible effect of different therapies should be considered when vaccinating cancer patients. While chemotherapy and radiotherapy aim to directly kill cancer cells [61,62], the purpose of immunotherapy is to restore the patient's immune system [63]. Treatment involving programmed cell death protein 1 (PD-1)/(PD-L1) blockade that reverse T-cell exhaustion might also improve the immune response after vaccination against infectious diseases [58,60].

The main limitations of this study include the absence of information on immune response immediately after the third dose; in addition, a small number of patients were enrolled in our study, which resulted in an even smaller group size when the population was stratified according to previous infection and treatment received.

Despite WHO declared the end of the emergency state [64], COVID-19 keeps representing a threat in terms of clinical outcome for cancer patients. Our study underlines the usefulness of binding antibody levels as a tool to evaluate the immunogenicty provided after anti-SARS-CoV-2 vaccination, as well as the involvement of T-cells in immunigenicity. Moreover, it highlights the importance of adjusting the vaccination schedule and considering the therapy received in this population to reach a proper immune response.

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CRediT authorship contribution statement

Roberta Campagna: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Federica Dominelli:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Maria Antonella Zingaropoli:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Fabio Ciurluini:** Visualization, Investigation, Data curation. **Giorgia Grilli:** Visualization, Methodology. **Alessandra Amoroso:** Visualization, Methodology. **Angelo De Domenico:** Visualization, Methodology. **Donatella Amatore:** Visualization, Methodology. **Maria Stella Lia:** Visualization, Methodology. **Enrico Cortesi:** Visualization, Funding acquisition. **Vincenzo Picone:** Visualization, Funding acquisition. **Claudio Maria Mastroianni:** Visualization, Funding acquisition. **Maria Rosa Ciardi:** Visualization, Funding acquisition. **Riccardo De Santis:** Visualization, Funding acquisition. **Florigio Lista:** Visualization, Funding acquisition. **Guido Antonelli:** Visualization, Funding acquisition. **Ombretta Turriziani:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.03.017.

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