Adequate Immunogenicity And Low Rate of Severe Adverse Events After SARS-CoV-2 mRNA-Based Vaccination In Patients With Solid Malignancies On Active Treatment Starts To Decline 3 Months After Complete Primary Course of Vaccination

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Abstract

Background: SARS-CoV-2 vaccination in cancer patients is crucial since they are at increased risk of severe COVID-19 disease course, but data on efficacy and safety of vaccination are scarce.

Methods: We performed a prospective observational study of patients with solid cancers on active anticancer treatment (chemotherapy, immunotherapy with immune checkpoint inhibitors (ICI) or targeted therapy) that received mRNA-based SARS-CoV-2 vaccination at two institutions in Slovenia. The immunogenicity was assessed by the detection of anti-SARS-CoV-2 S1 IgG antibodies in serum; patients were sampled before, 2-3 weeks after the first dose, 2-3 weeks after the second dose, and 3 months after the complete primary course of vaccination. The results were also compared to controls, sampled at similar time points.

Results: Between March and July 2021 112 patients were included in the analysis. The seroconversion rate in patients without prior COVID-19 infection was 96% after the complete primary course of vaccination with 2 doses, compared to 100% for healthy controls. The seroconversion rate after vaccination for patients on chemotherapy, ICI, and targeted therapy was 100%, 91%, and 97%, respectively. All controls and the majority of patients on chemotherapy and targeted therapy, but only 83% for patients on ICI were adequate responders (anti-SARS-CoV-2 S1 IgG ≥ 880 ng/ml). Three months after the vaccination, a significant drop in antibody levels was observed in patients receiving ICI compared to controls ($P < 0.001$). Adverse events were mostly mild and predictable, none of the patients experienced serious adverse events after vaccination.

Conclusions: Immunogenicity after mRNA-based vaccination against SARS-CoV-2 in cancer patients is only slightly impaired, but influenced by the type of anticancer therapy received. Patients on ICI have the slightest and gradual antibody production. Since antibody levels decline after three months, a third vaccination dose is reasonable to provide adequate protection against severe COVID-19 disease course.

The study was approved by the National Ethics Committee (No. 0120-39/2021/6)

Background

COVID-19 pandemics had an enormous medical and socio-economic impact on the global population health, with over 250 million cases and over 5 million deaths reported so far. Moreover, the COVID-19 pandemics has left devastating consequences in the cancer community with a late cancer diagnosis, deferred treatment decisions, and immense mortality rates among cancer patients reaching as high as 30%. Furthermore, additional safety measures were incorporated such as telemedicine, and many patients’ treatment was delayed due to the circumstances created by the pandemics. Since COVID-19 disease adversely affects cancer patients, prophylactic strategies are crucial.

An unprecedented global effort has been made to develop effective and safe vaccines that could end the pandemics. Nevertheless, data on the safety and efficacy of current anti-SARS-CoV-2 (severe acute
respiratory syndrome coronavirus-2) vaccines is scarce for cancer patients that are receiving active
treatment. None of the more than 117,000 volunteers included in the phase III SARS-CoV-2 vaccination
trials were neither cancer patients recently diagnosed, on active cancer treatment, or any sort of
immunomodulatory treatment. Since vaccine efficacy is not always optimal in cancer patients and
is prone to many variables as seen by the influenza vaccine real-world evidence, more data on the
efficacy and safety of cancer patients being vaccinated against SARS-CoV-2 is urgently needed.

Many oncological organizations were quick to respond and suggested cancer patients be among the first
to be vaccinated and thus protected due to the nature of their disease and treatment, but also frequent
exposures at the healthcare system – all of that leaving them vulnerable, immunocompromised, and at
higher risk of contracting the virus. Initiatives were also taken to prospectively collect this data to provide
further safe options for cancer patients. Thus, we initiated a prospective observational study (PROONCO-COVID-19) of cancer patients with solid tumors treated in two oncology centers in Slovenia and
have also been voluntarily vaccinated with one of the available SARS-CoV-2 vaccines. Our study aimed to
assess the development of anti-SARS-CoV-2 S1 IgG antibodies (immunogenicity) and the safety of the
mRNA-based vaccines in cancer patients treated with chemotherapy, immunotherapy with immune
checkpoint inhibitors or targeted therapy.

Methods

Study design and participants

We performed a prospective observational study of patients with solid cancers that are currently receiving
or have received systemic cancer therapy in the past year and have also been vaccinated for COVID-19.
Between March 1st and July 21st, 2021, patients with known solid malignancy treated at two academic
institutions in Slovenia (University Clinic Golnik and University Medical Centre Maribor) that were also
fully vaccinated, were included. All patients gave written Informed consent before study inclusion. The
study was approved by the National Ethics Committee (No. 0120-39/2021/6).

Procedures

Cancer patients included in this study were receiving systemic oncological treatment without interruption
by the vaccination procedures. Data collected included patient age, sex, time of cancer diagnosis, stage
and histological type of cancer, concomitant immunomodulatory therapy and systemic cancer therapy
that they were receiving - chemotherapy, immunotherapy with immune checkpoint inhibitors, a
combination of the latter two or targeted therapy (either tyrosine kinase inhibitors or endocrine therapy).

Blood samples from patients were obtained at four prespecified timepoints: Timepoint 1: Before the
vaccination (-7 days), Timepoint 2: 2-3 weeks after the first vaccination dose, Timepoint 3: 2-3 weeks
after the second vaccination dose, Timepoint 4: 3 months after the complete primary course of
vaccination (+/- 14 days). Blood was then centrifuged and stored if not analyzed shortly for the presence
of anti-SARS-CoV-2 S1 IgG antibodies. The antibody levels produced by cancer patients were then
compared to healthy controls, which were previously tested at similar time points after vaccination. All laboratory analyses were conducted at Laboratory for Clinical Immunology and Molecular Genetics at University Clinic Golnik.

Vaccination was carried out according to the manufacturing instructions, BNT162b2 (Pfizer/BioNTech) and mRNA-1273 (Moderna) being received 3 and 4 weeks apart, respectively. Since the adenoviral AZD1222 (AstraZeneca) vaccine boost dose is recommended 4 – 12 weeks apart and since soon only mRNA-based vaccines were recommended for cancer patients, we only included patients vaccinated with mRNA-based vaccines in this analysis. Vaccination of patients conferred to the National Vaccination Strategy, which was regularly updated by the Slovenian COVID-19 Vaccination Advisory Group.

Telephone consultations were carried out 24 – 48 hours after each vaccination dose to assess possible safety concerns and adverse events (AE), which were then noted on pre-prepared forms. AEs were graded with grades (G) 0 – 4 (G0 – no AE; G1 – mild AE; G2 – moderate AE which does not interfere with daily activities; G3 – severe AE which interferes with daily activities; G4 – life-threatening AE, hospitalization needed). The AEs being evaluated were either local (pain, redness, or swelling on injection site) or systemic (fever, chills, fatigue, myalgia, arthralgia, headache, vomiting, diarrhea) in line with trials of mRNA vaccines.\textsuperscript{10,11} All of the AEs were also reported by the national pharmacovigilance system to the National Institute for Public Health, as appropriate.

Detection of anti-SARS-CoV-2 S1 IgG Antibodies in serum

The immunogenicity of mRNA-based vaccines was assessed by S1-protein-based commercial ELISA assay IDK® anti-SARS-CoV-2 IgG by Immundiagnostik AG (Bensheim, Germany) according to the manufacturer’s instructions. The assay is designed to detect IgG antibodies directed against the receptor-binding domain of the S1 subunit of the spike protein of SARS-CoV-2. The test has no cross-reactivity to plasma probes for Adenovirus, Epstein-Barr Virus, Influenza A/B, HCoV-229E, HCoV-HKU1, HCoV-NL63, and HCoV-OC4. The results of quantitative anti-SARS-CoV-2 IgG ELISA show excellent correlation with the WHO International Standard for anti-SARS-CoV-2 immunoglobulin measurement (R2=0.9909; NIBSC code: 20/136) and strong correlation with SARS-CoV-2 neutralization.\textsuperscript{18} Results were interpreted according to the manufacturer’s recommendation: samples with \( \geq 175 \) ng/ml were considered positive; measurements > 2020 ng/ml (upper limit of the assay) were truncated at 2020 ng/ml. To obtain the concentration in binding antibody units (BAU)/ml, the results in ng/ml were divided by a factor of 20 (20 ng/ml \( \equiv 1 \) BAU/ml). Subjects with values \( \geq 880 \) ng/ml (\( \geq 44 \) BAU/mL) were considered to have a high probability of immune protection against SARS-CoV-2 and were classified as adequate responders.

Outcomes

The primary outcome was defined as the development of anti-SARS-Cov-2 S1 IgG antibodies after vaccination of cancer patients with mRNA-based vaccines against SARS-CoV-2 measured 2-3 weeks after the first and second vaccine dose. Antibodies were also monitored later at three months to assess the longevity of antibody protection.
Secondary endpoints included safety analysis of adverse events and reactogenicity following the first and second vaccination doses. Another secondary endpoint was the proportion of patients with SARS-CoV-2 infection after the complete primary course of vaccination and with worse COVID-19 disease course after vaccination.

**Statistical analysis**

Data distribution was evaluated using the D’ Agostino and Pearson test. Wilcoxon signed-rank test and Mann Whitney test were used as appropriate to calculate differences of anti-SARS-Cov-2 S1 IgG antibody levels between different time points and groups. The frequency distribution of patients with anti-SARS-Cov-2 S1 IgG antibodies between different time points and groups was compared with Fisher's exact test based on contingency tables. Statistical analysis was performed using GraphPad PRISM software (version 9.2 for Windows; GraphPad Software, San Diego, CA, USA). A P value of less than 0.05 was considered statistically significant.

**Results**

During the enrollment phase, a total of 125 patients were recruited in the study and signed the informed consent form. The primary analysis included 112 patients; 2 died due to disease progression, 3 were vaccinated only once and refused second vaccination dose, 4 were either lost to follow up or did not adhere to study protocol, and 4 were vaccinated with adenoviral vaccine and were excluded due to different dosing schedule. Consort diagram of patient flow is shown in Figure 1.

The median age was 62 years (range 24 – 81 years), 58% were females and 88% were currently receiving anticancer therapy. Most of the patients were metastatic (70%) and treated for non-small cell lung cancer (NSCLC) – 71%. Types of cancer therapy being applied currently or within the last year are shown in Table 1. Four patients (4%) were on continuous steroid therapy that was not used as part of routine oncological schemes and one patient was on immunomodulatory therapy with the anti-IL-23 drug due to psoriasis. Detailed demographic and clinical data are presented in Table 1.

Table 1: Demographic and clinical characteristics of included patients.
|                                | n   |
|--------------------------------|-----|
| **Age in years, median (range)** | 62  (24 – 81) |
| **Sex, n [%]**                                                                 |
| - Male                         | 47  (42%)        |
| - Female                       | 65  (58%)        |
| **Cancer type, n [%]**                                                   |
| - NSCLC\(^*\)                  | 80  (73%)        |
| - Breast cancer                | 13  (12%)        |
| - Gastrointestinal cancer      | 9   (8%)         |
| - Malignant mesothelioma       | 5   (4%)         |
| - SCLC\(^**\)                  | 4   (4%)         |
| - Ovarian cancer               | 3   (3%)         |
| - Gastrointestinal cancer      | 2   (2%)         |
| **Stage, n [%]**                                                            |
| - Limited                      | 21  (20%)        |
| - Locoregionally advanced      | 11  (10%)        |
| - Metastatic                   | 79  (79%)        |
| **Currently receiving anticancer therapy, n [%]**                           |
| - Yes                          | 90  (88%)        |
| - No                           | 14  (12%)        |
| **Anticancer therapy, n [%]**                                              |
| - Chemotherapy alone           | 26  (23%)        |
| - Age in yrs, median (range)   | 61  (40 – 76)    |
| - Male                         | 11  (44%)        |
| - Currently receiving therapy  | 15  (58%)        |
| - Immune checkpoint inhibitors | 44  (39%)        |
| - Age in yrs, median (range)   | 65  (24 – 73)    |
| - Male                         | 26  (58%)        |
| - Currently receiving therapy  | 42  (90%)        |
| - Targeted therapy (tyrosine kinase inhibitors or endocrine therapy) | 41  (38%)        |
| - Age in yrs, median (range)   | 61  (35 – 81)    |
| - Male                         | 9   (21%)        |
| - Currently receiving therapy  | 41  (98%)        |
| **Currently receiving steroids, n [%]\(^\text{III}\)**                      |
| - No                           | 108 (96%)        |
| - Yes                          | 4   (4%)         |
| **Other immunomodulatory therapy, n [%]\(^\text{IV}\)**                     |
| - No                           | 111 (99%)        |
| - Yes                          | 1   (1%)         |
| **Type of vaccination received, n [%]\(^\text{V}\)**                        |
| - mRNA-based BNT.1621 (Pfizer/BioNtech)                                    | 109 (97%)        |
| - mRNA-based mRNA-1273 (Moderna)                                          | 9  (3%)          |
| **History of SARS-CoV-2 infection, n [%]\(^*\)**                           |
| - No                           | 99  (88%)        |
| - Yes                          | 11  (12%)        |
| **Positive SARS-CoV-2 IgG antibodies prior to vaccination, n [%]\(^\text{I}\)** |
| - No                           | 90  (81%)        |
| - Yes                          | 22  (20%)        |

Legend: *NSCLC – non-small cell lung cancer; **SCLC – small cell lung cancer; *** - all patients received 2 doses; ¥ - receiving either methylprednisolone, dexamethasone or prednisolone not received within cancer treatment regimens; ∑ - one patient receiving anti-IL-23 therapy for psoriasis; μ - SARS-CoV-2 status was positive at baseline if the patient had clinical or virological evidence of COVID-19 illness either by positive patient history and positive RT-PCR test (reverse transcriptase-polymerase chain reaction) or positive RT-PCR test alone; δ - Patients were considered to have positive SARS-CoV-2 IgG antibodies if the level of anti-SARS-CoV-2 S1 IgG was above the threshold of 175 ng/mL.
Most patients (97%) received the mRNA-based BNT162b2 (Pfizer/BioNTech) vaccine. There were 13 patients (12%) with a history of COVID-19 infection before the first vaccination. Additional 9 patients (8%) tested positive for anti-SARS-CoV-2 S1 IgG before vaccination but had an asymptomatic disease course. Hence, 22 patients were classified as patients with prior COVID-19 infection. For comparison analysis we included 64 healthy controls with a median age of 58 years (range 22 – 77 years), 75% were females. All controls received 2 doses of the mRNA-based BNT162b2 vaccine. Twenty-two controls were classified as individuals with prior COVID-19 infection.

**Primary outcomes**

All but four patients in our cohort developed anti-SARS-CoV-2 S1 IgG antibody concentration of more than 175 ng/ml (≥ 8.8 BAU/ml) resulting in 96% of seropositive samples 2-3 weeks after the complete primary course of vaccination mRNA-based vaccines. Subjects with values ≥ 880 ng/ml (≥ 44 BAU/mL) are considered to have a high probability of immune protection against SARS-CoV-2, and in our cohort, all but seven cancer patients achieved that level and were classified as adequate responders.

Twenty-two patients were considered previously infected, thus leaving 90 patients that were COVID-19 naïve. Results are shown in Figure 2. At time point 2, 52/90 (58%) of previously non-infected patients achieved seroconversion with anti-SARS-CoV-2 S1 IgG levels ≥ 175 ng/ml and one-third of patients [31/90 (35%)] achieved anti-SARS-CoV-2 S1 IgG levels ≥ 880 ng/ml (≥ 44 BAU/ml), being adequate responders. On the other hand, all but one patient in the previously infected group were adequate responders after the first vaccination. Differences were significant comparing the non-infected group before and after the first vaccination, \( P < 0.001 \). Median anti-SARS-CoV-2 S1 IgG levels after first vaccination for non-infected and infected group were 255 ng/ml (0 – 2020) and 2020 ng/ml (196 – 2020 ng/ml), \( P < 0.001 \), respectively. At time point 3 (after the second vaccination), 86/90 (96%) of previously non-infected patients achieved seroconversion with median SARS-CoV-2 S1 IgG titres of 2020 ng/ml (0 – 2020) which was statistically significant compared to the levels before and after the first vaccination – both \( P < 0.001 \). At time point 4 (3 months after the complete primary course of vaccination) anti-SARS-CoV-2 S1 IgG levels were still significantly higher compared to before and after the first vaccination dose for previously non-infected patients \( P < 0.001 \), and comparable to the antibody levels after the second dose of vaccine. In the non-infected group, 92% and 87% of patients were adequate responders after the complete primary course of vaccination and three months after vaccination, respectively. The difference between the non-infected and previously infected group was statistically significant 3 months after the completing primary course of vaccination with \( P = 0.033 \).

**Seroconversion according to anticancer therapy**

Different antibody response was observed when comparing previously non-infected patients according to different anticancer therapy, especially after the first vaccine course as shown in Figure 3. Compared to healthy controls, who had seroconversion rates of 100% after the first vaccination dose and median anti-SARS-CoV-2 S1 IgG levels of 2020 ng/ml [678 – 2020], patients on chemotherapy, ICI, or targeted therapy had seroconversion rates of 55%, 49%, and 70%, respectively. Similarly, 88% of controls were adequate
responders, while this rate was much lower in patients on chemotherapy, ICI or targeted therapy, reaching 41% ($P = 0.040$), 23% ($P = 0.001$) and 42% ($P = 0.045$), respectively. Compared to healthy controls, median anti-SARS-CoV-2 S1 IgG levels after only one vaccine dose for patients receiving chemotherapy were significantly lower: 519 ng/ml [0 – 2020], $P = 0.005$, as they were for patients on ICI: median levels 169 ng/ml [0 – 2020], $P < 0.001$ and targeted therapy: median levels 541 ng/ml [0 – 2020], $P = 0.001$. Patients on ICI had the lowest anti-SARS-CoV-2 S1 IgG levels compared to patients on targeted therapy ($P = 0.033$) and combined patients on chemotherapy and targeted therapy ($P = 0.045$).

After the complete primary course of vaccination, similar seroconversion rates were observed, with 100%, 100%, and 97% for healthy controls, patients receiving chemotherapy, and targeted therapy, respectively. Conversely, patients receiving ICI had significantly lower seroconversion (91%) and anti-SARS-CoV-2 S1 IgG compared to healthy controls ($P = 0.001$). Equally, rates of adequate responders, were similar in healthy controls (100%), in patients on chemotherapy (100%) and targeted therapy (97%), but only 83% of patients treated with ICI.

Patients and healthy controls previously infected with COVID-19 both achieved high levels of anti-SARS-CoV-2 S1 IgG even after the first dose of vaccination and with no significant differences in antibody levels after the first and second vaccination as shown in Figure 4.

**Long term outcomes (after 3 months)**

Three months after the complete primary course of vaccination data for 102 patients are available, of those 84 were previously non-infected patients. Results show consistent positive anti-SARS-CoV-2 S1 IgG levels in cancer patients (95%), although a significant drop is observed. Patients receiving ICI have significantly lower levels of anti-SARS-CoV-2 S1 IgG compared to healthy controls 3 months after vaccination ($P < 0.0001$). At this time point one patient in the chemotherapy group, four in the ICI group, and three in the targeted therapy group exhibit anti-SARS-CoV-2 S1 IgG levels below 880 ng/ml (< 44 BAU/ml) – the level, which is considered to offer protection against SARS-CoV-2 with high probability. Results are shown in Figures 3 and 4.

Despite receiving a complete primary course of vaccination, four patients did not develop detectable antibodies against the spike (S1) protein of SARS-CoV-2. One patient was treated for metastatic non-small cell lung cancer (NSCLC) with ICI but was also receiving glucocorticoids due to a severe adverse event of these treatments, the second was treated for metastatic NSCLC with a combination of chemoinmunotherapy but also had an underlying chronic lymphocytic leukemia in remission that did not require treatment at the moment. The third patient was treated for metastatic renal cell carcinoma with mTOR inhibitor and the fourth patient was treated for metastatic NSCLC with a combination of chemoinmunotherapy – the latter two patients had no other significant history. Additional three previously non-infected patients had serum anti-SARS-CoV-2 S1 IgG values < 880 ng/ml after the complete primary course of vaccination which is considered suboptimal. Eleven patients altogether had serum anti-SARS-CoV-2 S1 IgG values < 880 ng/ml after 3 months of the complete primary course of vaccination, owing to the rapid drop of antibody levels.
Safety

There were no new safety concerns after vaccine administration and none of the patients had life-threatening adverse events (AE). Patients with local, systemic, or both types of AE were recorded in 38%, 7%, and 31% after the first vaccination and 39%, 5%, and 43% after the second vaccination, respectively. Most of the AE were local, either pain, redness, or swelling on the injection site, with 69% of patients experiencing them after the first and 82% of patients after the second vaccination course. The proportion of AE and their severity are shown in Figure 5. No major differences were noted in terms of adverse event frequency when comparing previously non-infected and infected cancer patients, with 78% of previously non-infected reporting AEs after first, 91% after the second, and 73% after both vaccination courses. Similarly, 73% of previously infected patients reported AEs after first, 73% after second and 64% after both vaccinations, respectively.

COVID-19 infection after vaccination

After reviewing all of the patient electronic health records on December 15th 2021, there were three cases of PCR confirmed COVID-19 infection among our group of vaccinated patients. The first patient got infected 3 months after the second vaccine dose, his anti-SARS-CoV-2 S1 IgG value was on the upper limit (2020 ng/ml), but he also had disease progression during the infection and died, probably due to cancer progression and not due to severe COVID-19 course. The second patient got infected just before the 6-month timepoint, her anti-SARS-CoV-2 S1 IgG value after 3 months was 1648 ng/ml, but then tested negative at the time of infection, thus receiving antibody cocktail with indevimab and casivirimab. She had a moderate disease course with cough and fatigue. The third patient got infected 6 months after the vaccination and only had a sore throat with no other symptoms – her anti-SARS-CoV-2 S1 IgG level was on the upper limit (2020 ng/ml) at the time of infection. Both patients survived the COVID-19 disease without sequelae and are now receiving anticancer treatment normally.

Discussion

To our knowledge, this is the first report of cancer patients on active treatment for solid malignancies, which provides not only immediate data about seroconversion after the complete primary course of vaccination with mRNA-based SARS-CoV-2 vaccines but also extended data three months after. Compared to healthy controls, cancer patients on anticancer treatment achieved adequate seroconversion rates after completing the primary course of vaccination regardless of the type of anticancer therapy received. However, anti-SARS-CoV-2 S1 IgG antibody production was built more gradually than in healthy controls, which was especially prominent after the first vaccination dose. However, anti-SARS-CoV-2 S1 IgG production was moderate in patients receiving ICI compared to patients receiving chemotherapy or targeted therapy. Similarly, after three months following vaccination, there was a decline in anti-SARS-CoV-2 S1 IgG levels of patients receiving chemotherapy and ICI, but not targeted therapy, compared to healthy controls.
Since COVID-19 pandemics have had devastating consequences in the frail population of cancer patients, it is vital to gather as much reliable data as to demonstrate whether vaccination benefit this population.\(^2\)\(^-\)\(^7\)\(^,\)\(^9\) Due to the paucity of data available, patients are often reluctant to be vaccinated since the efficacy and safety profile of vaccines in this population is unknown, leaving them at low vaccination levels – from 40–60\%.\(^{19\text{-}21}\)

From reports available so far, it is evident that patients with hematological malignancies do poorly in terms of seroconversion rates compared to patients treated for solid tumors. That is both due to the impaired immune system caused by the disease itself and due to highly immune-suppressive therapy.\(^{15,22\text{-}25}\)

Similar to our study, others also showed more gradual anti-SARS-CoV-2 S1 IgG antibody build-up after the first vaccination.\(^{15,22\text{-}26}\) A French group clearly showed that both vaccination doses are necessary to achieve adequate protection, since only 55\% of cancer patients developed specific anti-SARS-CoV-2 antibodies after a single dose, compared to 100\% of healthy individuals. Also, levels of anti-SARS-CoV-2 antibodies are lower in cancer patients compared to healthy individuals and irrespective of the timing of anticancer therapy received.\(^{26,27}\) This is further confirmed in our group of patients, where the seroconversion rate after the first dose was significantly lower than after the second (64\% versus 96\%; \(P < 0.0001\)). In the largest prospective trial reported so far, cancer patients performed exceptionally well in terms of seroconversion after vaccination. Moreover, they defined a threshold of anti-SARS-CoV-2 S1 IgG levels based on their neutralizing capacity, that categorized patients to adequate and suboptimal responders – the latter probably needing the third vaccine dose to achieve sufficient SARS-CoV-2 S1 IgG levels to offer reliable protection against severe disease.\(^{28}\) A recent systematic review and meta-analysis provided data on promising antibody response after COVID-19 vaccination in cancer patients with seroconversion rates > 90\%.\(^{29}\)

We have shown that patients receiving ICI have the lowest anti-SARS-CoV-2 antibody production, especially after the first dose of vaccine. After vaccination with two doses, antibody levels seem to be comparable regardless of anticancer therapy received. Similarly, an Israeli group provides data on 102 solid cancer patients being vaccinated and the lowest anti-SARS-CoV-2 IgG titres achieved in patients receiving chemo-immunotherapy or immunotherapy plus targeted therapy.\(^{30}\) This data is further supported by the Greek group that found significantly lower antibody production after only one dose of vaccine in patients receiving ICI.\(^{31}\) Since ICI are showing an immunomodulatory mode of action in patients infected with SARS-CoV-2, thus reducing cytotoxic effects and cytokine release, there could also be a potential effect on delaying anti-SARS-CoV-2 S1 IgG antibody production after vaccination.\(^{32}\)

The present study shows that antibody levels against SARS-CoV-2 are high for most cancer patients on active anticancer treatment after vaccination with both doses, offering enough protection to escape a severe disease development. This is further supported by the fact that out of our cohort of 112 patients, only three were infected with COVID-19 and symptomatic after a complete vaccination course. Most of
the patients were adequate responders after vaccination with both doses and were considered to have a high probability of protection against severe COVID-19 infection, having anti-SARS-CoV-2 S1 IgG Ab above 880 ng/ml (44 BAU/ml).

Nevertheless, there was a marked decline in anti-SARS-CoV-2 S1 IgG following three months after vaccination with both doses, especially in the group of patients receiving chemotherapy or ICI. That further supports the idea of a third vaccination dose in this patient population, which is also safe and feasible as shown by recent publications.  

In our study, there were no new safety concerns raised in terms of reactogenicity or adverse events (AE) following vaccination. Other reports also show less frequent emergence of AE after vaccination in cancer patients than healthy subjects as seen in the trials.\textsuperscript{10,11,15,28,34} Much fewer local or systemic AEs are reported, especially after the second vaccination, with 70% of cancer patients reporting no AE whatsoever.\textsuperscript{15,28} Similar to our results, a large US study showed 70% of patients experiencing local and up to 50% of patients systemic AEs after either vaccination.\textsuperscript{34} It should be noted that cancer patients are already affected by their disease, symptom,s and possible adverse effects of anticancer treatment, thus leaving them less perceivable for possible vaccination AEs.

Since anti-SARS-CoV-2 S1 IgG assay has high sensitivity and specificity, it is easily reproducible, quick, and non-costly, and correlates well with immunogenicity achieved after infection, it is thought reasonable to expect that a high level of anti-SARS-CoV-2 S1 IgG antibodies after vaccination correlates well with the protection against worse disease outcome as shown in the early phases of vaccine development.\textsuperscript{35,36} However, antibody levels only represent humoral immunity, whereas cellular immunity protecting from severe courses may be preserved despite lower antibody levels in cancer patients compared to controls. The upper limit of antibody measurements was at 2020 ng/ml, so the differences between different groups in our cohort could be even greater than observed here. Another shortcoming might be the underrepresentation of certain groups of patients or malignancies, mirroring the frequency of certain patient groups being treated at certain centers. The strength of our study is a centralized laboratory for analysis of antibody levels, which reduces the chances of differences in methodology and execution of assay. In addition, the study was prospectively planned with every time point for blood sampling being pre-arranged, thus reducing the inconsistency in study execution. Also, only patients with solid cancers were included, which further harmonizes the group being investigated and pronounces potential other variables that play a role in the antibody production.

Conclusion

In conclusion, our study exhibits high levels of anti-SARS-CoV-2 S1 IgG antibodies produced by patients with solid malignancies on active cancer treatment after a complete primary course of vaccination. However, the dynamic of antibody production after vaccination is highly influenced by anticancer therapy, with patients receiving ICI having the slightest and gradual antibody production. This supports the incentive to further protect cancer patients with vaccination, not only to protect them from worse disease
outcomes in case of infection but also to safely apply cancer therapy even in the ongoing COVID-19 pandemics, considering all other safety measures as well. Furthermore, since anti-SARS-CoV-2 S1 IgG levels start to decline after three months, initiatives for a third vaccination dose to provide adequate protection against severe COVID-19 disease in case of infection are sensible.

**Abbreviations**

anti-SARS-CoV-2 S1 IgG – Immunoglobulin G antibodies against spike protein of severe acute respiratory syndrome coronavirus-2

Ab – antibody

BAU - binding antibody units

ICI – immune checkpoint inhibitors

ChT - chemotherapy

AE – adverse event

G - grade

**Declarations**

**Ethics approval and consent to participate:** All methods and procedures were carried out in accordance with Declaration of Helsinki. The study was approved by the Slovenian National Ethics Committee (No. 0120-39/2021/6). All patients gave written Informed consent before study inclusion.

**Consent for publication:** Not applicable

**Availability of data and materials:** Data generated or analysed during this study are included in this published article. Any needed clarifications and/or additional supporting data are available from the authors upon request.

**Competing interests:** The authors declare no competing interests.

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**Author’s contributions:** All authors were involved in study design and execution. MR and UBS supervised antibody detection and data collection. UJ, MR, JD, and UBS were involved in data interpretation and graphical imaging processing. UJ and MR were the major contributors in writing the manuscript. All authors have read and approved the final manuscript.
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**Figures**
Figure 1

CONSORT diagram showing the included and analysis population

125 patients included in study

13 patients excluded from analysis:
- 2 patients died due to cancer progression
- 3 patients vaccinated only once
- 4 patients lost to FU / did not adhere to study protocol
- 4 patients vaccinated with AZD1222

112 patients included in analysis
(vaccinated and provided blood samples per protocol)
Figure 2

Anti-SARS-CoV-2 S1 IgG antibody levels in previously infected and non-infected cancer patients

Legend: (A) Differences in antibody (Ab) titers against spike (S1) protein of SARS-CoV-2 in cancer patients at different time-points in patients without previous COVID-19 infection (blue colour) and in patients with prior COVID-19 infection (red colour). Wilcoxon signed rank test. (B) Proportion of cancer patients with antibodies against spike (S1) protein of SARS-CoV-2 at different time-points in patients without previous COVID-19 infection (blue borders) and in patients with prior COVID-19 infection (red borders). Chi-square test. Patients with IgG values $\geq 880$ ng/ml ($\geq 44$ BAU/ml) were considered to have a high probability of immune protection against SARS-CoV-2.
Anti-SARS-CoV-2 S1 IgG antibody levels in previously non-infected patients according to anticancer therapies

Legend: (A) Differences in antibody (Ab) titres against spike (S1) protein of SARS-CoV-2 in cancer patients at different time-points according to anticancer therapy received and in control subjects. All without previous COVID-19 infection. Wilcoxon signed rank test & Mann Whitney test. (B) Proportion of cancer patients with antibodies against spike (S1) protein of SARS-CoV-2 at different time-points according to anticancer therapy received and in control subjects. All without previous COVID-19 infection. Chi-square test. Patients with IgG values \( \geq 880 \) ng/ml ( \( \geq 44 \) BAU/mL) were considered to have a high probability of immune protection against SARS-CoV-2. ChT – chemotherapy; ICI – immune checkpoint inhibitors; TT – targeted therapy; Controls – healthy controls.

Figure 4

Anti-SARS-CoV-2 S1 IgG antibody levels in previously infected patients according to anticancer therapies

Legend: (A) Differences in antibody (Ab) titres against spike (S1) protein of SARS-CoV-2 in cancer patients at different time-points according to anticancer therapy received and in control subjects. All with prior COVID-19 infection. Wilcoxon signed rank test & Mann Whitney test. (B) Proportion of cancer patients with antibodies against spike (S1) protein of SARS-CoV-2 at different time-points according to anticancer therapy received and in control subjects. All with prior COVID-19 infection. Chi-square test. Patients with IgG values \( \geq 880 \) ng/ml ( \( \geq 44 \) BAU/mL) were considered to have a high probability of immune protection against SARS-CoV-2. ChT – chemotherapy; ICI – immune checkpoint inhibitors; TT – targeted therapy; Controls – healthy controls.
Figure 5

Proportion of patients experiencing either local, systemic, or both types of adverse events after vaccination

Legend: Proportion of cancer patients experiencing either local, systemic, or both types of adverse events (AE) after (A) first vaccination, (B) second vaccination. Percentage of cancer patients, severity and types.
of adverse events experienced after (C) first vaccination (D) second vaccination; (E) frequency of AE reported after the first vaccination, second vaccination and the proportion of patients reporting AE after both vaccination doses for the entire group of cancer patients (left), those previously non-infected with SARS-CoV-s (middle) and those previously infected with SARS-CoV-2 (right).