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Letter

Increased antibody titers and reduced seronegativity following fourth mRNA COVID-19 vaccination in patients with cancer

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Patients with cancer are at increased risk of severe COVID-19 disease because of immunosuppression caused by the cancer and/or cancer treatments (Ehmsen et al., 2021b; Tian et al., 2020). We and others have characterized the anti-SARS-CoV-2 immune response after two and three COVID-19 mRNA vaccinations in patients with solid and hematologic cancers and observed insufficient responses in a substantial portion following the second vaccination ((Ehmsen et al., 2021a; Gounant et al., 2022; Herishanu et al., 2022) but an improved response following the third vaccination (Ehmsen et al., 2022). We further showed that the anti-SARS-CoV-2 spike receptor binding domain (anti-S) IgG antibody titers declined rapidly within the first 3 months after both the second and third vaccination. This, in combination with high infectivity rate of COVID-19 in the population in the winter of 2021–2022, made the Health Authorities in several countries, including Denmark, recommend a fourth mRNA COVID-19 vaccination to boost the immune response in this patient group.

Here, we assess alterations in antibody titers (anti-S IgG) in blood samples following a fourth mRNA vaccination from patients with solid and hematologic malignancies, and we assess the waning antibody response at 3 months following the fourth vaccination.

Overall, 530 patients (316 with hematologic cancers and 214 with solid cancers) that had been included in our previous published study (Ehmsen et al., 2022) were also offered a fourth mRNA COVID-19 vaccination. Of these, 395 patients (256 with hematological and 139 with solid cancers) received the fourth vaccination and 94% had blood drawn at 1 month and 83% at 3 months after the fourth vaccination; these blood samples were analyzed for anti-S IgG levels. Clinical characteristics of the patients are provided in Table S1A. Patients with hematologic cancers who were included in the study were pre-selected based on an expected reduced immune response, and therefore the study primarily included patients with lymphoma (31%), chronic lymphocytic leukemia (CLL; 37%), and multiple myeloma (MM; 32%). At the time of fourth vaccination, 60% of patients with solid cancers were in active cancer treatment, e.g., chemotherapy or targeted therapy, whereas 35% of patients with hematologic cancers were in active cancer therapy, e.g., anti-CD20 therapy, BTK inhibitors, or targeted therapy. 6% received supportive immunoglobulin treatment. Steroid treatment ($\geq$50 mg/week) prior to the fourth vaccination was ongoing in 7% of patients with hematologic cancers.

Although many vaccines are administered three times to boost the immune system, limited information is available concerning the antibody response after four administrations of a vaccine (Munro et al., 2022), and none is available for an mRNA vaccine in potentially immunosuppressed patients with cancer. Thus, whether the antibody titer would reach markedly higher levels than those that were observed following the third vaccination or whether antibody response would level off is a question of great interest. Indeed, we observed a marked increase in mean anti-S IgG levels 1 month following a fourth mRNA vaccination (3,149 BAU/mL), and this was 1.7-fold higher than the levels observed 1 month after the third vaccination ($p < 0.0001$, Student's t test) (Table S1B). This was observed both for the whole group and for the solid cancer and hematologic cancer groups separately (Figure S1A).

For the total cohort, the mean anti-S IgG titer declined from 1 month (3,149 BAU/mL) to 3 months (2,642 BAU/mL) after a fourth vaccination, and this was similar to the decline observed in the same period following the second and the third vaccination (Figure S1B). However, because the starting IgG level was initially higher, the time to intersect the level for insufficient immune response became longer (Table S1C).

Some patients had blood drawn 6 months after the third vaccination, and 83% of those patients were from the group that declined the fourth vaccination. Analysis of the 6 months blood samples, as expected, showed a decline or equivalent anti-S IgG titers in 55% of patients compared to the 3 months blood sample. However, somewhat surprisingly, an increase in anti-S IgG titers was observed from the 1 month or 3 months blood samples to the 6 months blood sample in 45% of the patients after the third vaccination (mean of total cohort with increased anti-S IgG level: 1 month, 1,657 BAU/mL or 3 months, 1,136 BAU/mL to 6 months, 4,572 BAU/mL). Because the blood samples were drawn in the winter of 2022, when the Omicron variant was causing high level of infections, the

increased titers were likely caused by SARS-CoV-2 infection. This was confirmed through serological assays that detect IgG antibodies against the SARS-CoV-2 nucleocapsid antigen or through RT-PCR and/or antibody treatments to COVID-19 disease for 79% of the tested patients (n = 24).

An additional question of interest was whether only patients with cancer who had already exhibited a sufficient antibody response after the third vaccination had boosted anti-S IgG levels or whether there was an increase in the percentage of patients who developed a sufficient antibody response 1 month after a fourth vaccination (defined as anti-S IgG>54 BAU/mL). Among patients with hematologic cancers, only 13% were seronegative 3 months after the fourth vaccination, whereas 24% and 43% of this group were seronegative 3 months after the third and second vaccination, respectively (Table S1B, Figure S1C). This improvement in anti-S IgG response was observed for several disease types (seronegative % after the fourth and third vaccinations: CLL, 18% versus 34% and multiple myeloma, 4% versus 12%). The seronegative patients with hematologic cancer whose blood was sampled 3 months after the fourth vaccination were diagnosed with Mantle cell lymphoma (n = 4/7 = 50%) and CLL (n = 13/72 = 18%), and they were treated with BTK inhibitors (n = 5/10 = 50%) or anti-CD20 therapy (n = 7/19 = 37%). The patients who were seronegative after the third vaccination but became seropositive after the fourth vaccination included a few treated with BTK inhibitors (1/7 = 14%) and anti-CD20 therapy (2/18 = 11%), as well as several treated with steroid (7/13 = 54%) before the fourth vaccination.

For patients with solid cancers, nearly 100% had sufficient antibody responses after the third vaccination. Although the mean anti-S IgG titer declined from 2,464 BAU/mL at 1 month to 1,951 BAU/mL 3 months after the third vaccination, all patients with solid cancers continued to have sufficient antibody responses. Following the fourth vaccination, the anti-S IgG titer increased 1.6-fold compared to 1 month after the third vaccination, and all patients with solid cancers continued to have sufficient antibody responses even 3 months after the fourth vaccination (Figure S1C).

The majority of patients received the fourth vaccination 4.7 months after the third vaccination. Because patients in the study were informed about their anti-S IgG titers during the study, some patients likely declined the fourth vaccination because they had high anti-S IgG titers. This is supported by the observation that patients not accepting a fourth vaccination had a significantly higher mean IgG titer 3 months after the third vaccination compared to those who received the fourth vaccination (mean IgG[std]: 2,086[1,948] versus 1,326 [1,648], p = 0.0002). Other patients may have declined the fourth vaccination because they were infected with the Omicron variant.

Our data indicate that administration of a fourth vaccination to selected groups of cancer patients effectively maintains high anti-S IgG levels. As a limitation of this study, we have only evaluated the level of anti-nucleocapsid antigen IgG to identify natural SARS-CoV-2 infections in blood samples after the third vaccination, where the anti-S IgG level increased dramatically. The increase in anti-S IgG level after the fourth vaccination could, in some cases, also be caused by natural SARS-CoV-2 infections.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2022.06.012.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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Cancer Cell, Volume *40*

Supplemental information

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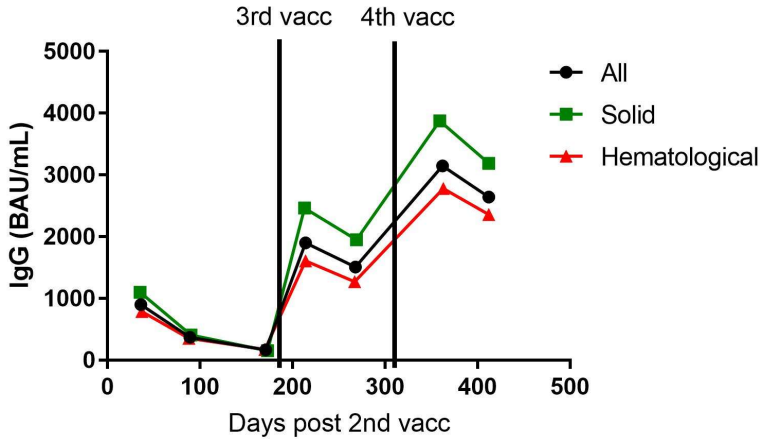
A


3rd vacc
4th vacc
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4000
3000
2000
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0
IgG (BAU/mL)
0
100
200
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400
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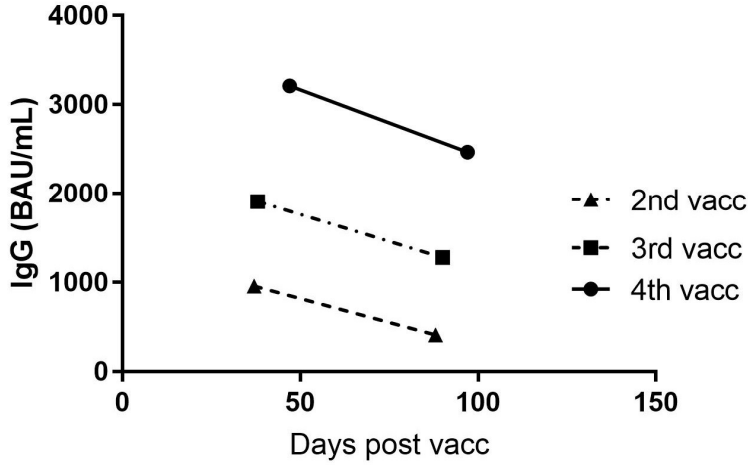
Days post 2nd vacc
All
Solid
Hematological

B


4000
3000
2000
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IgG (BAU/mL)
0
50
100
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Days post vacc
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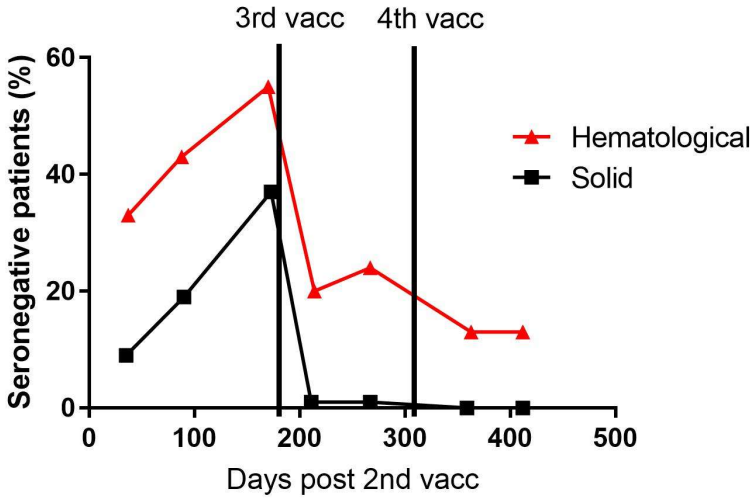
C


3rd vacc
4th vacc
60
40
20
0
Seronegative patients (%)
0
100
200
300
400
500
Days post 2nd vacc
Hematological
Solid

Supplemental figure legend

Figure S1. SARS-CoV-2-specific IgG (anti-S IgG) titers over time in blood samples from patients with cancer after two, three and four doses of mRNA COVID-19 vaccine.

A. Mean anti-S IgG levels in blood samples from patients with hematologic or solid cancers. The graph shows results from blood samples collected 1, 3 and 6 months after the 2nd vaccination, 1 and 3 months after 3rd and 4th vaccinations. The 4th vaccination boosted anti-S IgG levels significantly (Patients received the 4th vaccination, all: n=395, solid cancers: n=139, hematological cancers: n=256).

B. Decreasing mean anti-S IgG titers of the total cohort comparing blood samples collected 1 and 3 months after the 2nd (n=466), 3rd (n=325) and 4th (n=281) vaccinations. Patients with IgG titers that increases between the analyzed blood samples were withdrawn from the analysis.

C. Percentage of seronegative patients (anti-S IgG ≤ 54BAU/mL) with hematological or solid cancers. The number of seronegative patients were calculated for blood samples collected 1, 3 and 6 months after the 2nd vaccination, 1 and 3 months after 3rd and 4th vaccinations. The 4th vaccination reduced the number of seronegative patients with hematological and solid cancers to 13% and 0%, respectively.

Supplemental Table S1. Patient characteristics and association with anti-SARS-CoV-2 immune status.

Supplemental Materials and Methods

Study Design

This is an analytical, observational prospective, single-center cohort study of immune responses after SARS-CoV-2 vaccination among patients with solid and hematologic cancers. Patients eligible for inclusion were adults (age >18 years) diagnosed either with solid tumors (histologically or cytologically verified) and undergoing active systemic cancer treatments, or selected hematologic malignancies either in active systemic cancer treatment or not receiving treatment, during the period of vaccination with two, three and, in most cases, four COVID-19 mRNA vaccinations. The included patients with hematologic cancers were pre-selected based on the expectation of a poor immune response and therefore primarily included patients with lymphoma, chronic lymphocytic leukemia (CLL), or multiple myeloma; patients with acute leukemia, myelodysplastic syndrome or chronic myeloproliferative neoplasms were not included. All included patients were followed and treated at the Departments of Oncology or Hematology at Odense University Hospital, and all were able to provide written and oral informed consent. A total of 2,601 patients were invited to participate in the study. Of those, 715 provided electronic written consent, of which 590 also provided oral consent and thus met the eligibility criteria and were enrolled in the study. Of the included patients, 530 completed the recommended three mRNA vaccinations at time of data interpretation and 395 of those completed the fourth vaccination. Of the included patients, 24 withdrew their consent but allowed us to use the results from the blood samples already taken. Patients were excluded from the study if they did not have a personal digital mailbox or mobile phone, as the communication regarding blood samples was relayed via these means. No patients were lost to follow-up. Twelve patients died of their disease after receiving only two vaccinations, 20 patients died after receiving only three vaccinations, and 6 died after the 4^{th} vaccination (end of study June 10, 2022).

The primary outcomes that were reported in our initial study (Ehmsen et al., 2021a) were the rate of SARS-CoV-2-specific IgG seropositivity and the degree of SARS-CoV-2-specific T cell responses after vaccination. Secondary outcomes included comparisons of IgG titer and interferon (IFN)-γ release and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity. The primary outcomes of our previous follow-up study (Ehmsen et al., 2022) were the rate of SARS-CoV-2-specific IgG seropositivity after the 3rd vaccination and the decay of SARS-CoV-2-specific IgG titer over time after the 2nd and 3rd vaccinations. Secondary outcomes included comparisons of IgG titers and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity. The primary outcomes of this follow-up study were the rate of SARS-CoV-2-specific IgG seropositivity after the fourth vaccination and the decay of SARS-CoV-2-specific IgG titer over time after the 2nd, 3rd and 4th vaccinations. Secondary outcomes included comparisons of IgG titers and identifying factors associated with vaccine response, including anti-cancer therapies and cancer types associated with seropositivity.

Participants

Clinical information regarding patient medical histories was obtained from the hospital digital medical file system using the patient's Danish Civil Registration numbers (CPR), allowing for follow-up with accurate censoring at emigration or death. All participants received vaccination as part of the national COVID-19 vaccination program. Participants were included from March 7 to November 8, 2021. The first blood sample was drawn 36 days (mean, standard deviations: 10 days) after the 2nd vaccination, while the second and third blood samples were drawn 89 and 171 days (mean, std. dev: 12 days and 12 days) after the 2nd vaccination, respectively. After the 3rd vaccination, the first blood samples were drawn at 39 days (mean, std. dev: 9 days), while the second and third blood samples

were drawn at 93 and 186 days (mean, std. dev: 11 days and 14 days) after 3rd vaccination, respectively. After the 4th vaccination, the first and second blood samples were drawn at 47 days and 97 days (mean, std. dev: 10 days and 10 days) respectively after the 4th vaccination. The last blood sample included in this study was drawn June 10, 2022, 119 days after 4th vaccination.

Baseline

Baseline data included age (at time of 3rd vaccination), sex, cancer type, dates of vaccinations, type of vaccine, other malignancies, and cancer therapy (at time of 4th vaccination). At time of 4th vaccination, most recent cytotoxic drugs were registered during the past three months for cytotoxic drugs and immunotherapy and during the past 12 months for anti-CD20 antibody therapy. Immunoglobulin treatments up to three months and steroid treatment (≥ 50mg) given up to one week prior to 4th vaccine dose were registered.

Cancer types were defined according to the International Classification of Disease (10th revision) diagnostic codes. The registered data was abstracted from medical files by local investigators and entered into the research electronic data capture (REDCap) application and analyzed using Stata, version 17.

Anti-SARS-CoV-2 S RBD and N IgG antibody analysis

For antibody quantification, the samples were separated by centrifugation and IgG antibodies against SARS-CoV-2 spike receptor binding domain (RBD), the main target for virus neutralization, (anti-S IgG) and quantified using a chemiluminescent microparticle immunoassay (SARS-CoV-2 IgG II Quant assay; Abbott Laboratories). The resulting chemiluminescence in relative light units following the addition of anti-human IgG acridinium-labeled conjugate in comparison with the IgG II

calibrator/standard indicates the strength of response, which reflects the quantity of IgG present. This FDA Emergency Use Authorization approved assay has shown excellent correlation with the first WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC code 20/136), enabling the issuing of immunogenicity results in standardized units; binding antibody units (BAU)/mL. The relationship between the Abbott arbitrary units (AU)/mL unit and the WHO BAU/mL unit follows the equation BAU/mL = 0.142 × AU/mL. Cut-off was set at 9 BAU/mL and considered as negative IgG response (IgG negative) and a result of >9 to ≤54 BAU/mL was considered weak IgG antibody response.

In this study, we used the Abbott SARS-CoV-2 IgG (N; Abbott Laboratories) serological assay, which is a two-step chemiluminescent microparticle immunoassay designed to detect IgG antibodies against the SARS-CoV-2 nucleocapsid antigen. A value >1.4 AU was interpreted as positive (manufacturer defined).

Statistical Analysis

Baseline characteristics and immune responses were described proportionately across the pre-defined diagnosis groups. Students t-test was used to investigate the significance of the relationship between SARS-CoV-2 IgG in patients receiving or not receiving the 4^{th} vaccination. A p value < 0.05 was considered statistically significant. Statistical analysis and figures were performed using GraphPad Prism, version 7.

Ethics

This study was approved by the Danish ethics committee (record no. S-20210008C) and data handling approved by Region of Southern Denmark (record no. 21/14199). Data are reported according to STROBE (Strengthening the reporting of observational studies in epidemiology) guidelines.