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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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