



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

Letter

Antibody responses following third mRNA COVID-19 vaccination in patients with cancer and potential timing of a fourth vaccination

Sidse Ehmsen,^{1,2,3} Anders Asmussen,⁴ Stefan S. Jeppesen,^{1,2,3} Anna Christine Nilsson,^{2,5} Sabina Østerlev,¹ Amalie Kragh,¹ Henrik Frederiksen,^{2,3,4} and Henrik J. Ditzel^{1,2,3,6,7,*}

¹Department of Oncology, Odense University Hospital, Odense, Denmark

²Department of Clinical Research, University of Southern Denmark, Odense, Denmark

³Academy of Geriatric Oncology, Odense University Hospital, Odense, Denmark

⁴Department of Hematology, Odense University Hospital, Odense, Denmark

⁵Department of Clinical Immunology, Odense University Hospital, Odense, Denmark

⁶OPEN-Open Patient data Exploratory Network, Odense University Hospital, Odense, Denmark

⁷Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark

*Correspondence: hditzel@health.sdu.dk

<https://doi.org/10.1016/j.ccell.2022.02.011>

Patients with cancer are at risk of severe COVID-19 disease due to immunosuppression caused by cancer and/or cancer therapies (Ehmsen et al., 2021b; Tian et al., 2020). We and others have characterized the anti-SARS-CoV-2 immune response after two doses of COVID-19 mRNA vaccines in patients with solid and hematologic cancers and observed insufficient responses in a substantial portion (Ehmsen et al., 2021a; Gounant et al., 2022; Herishanu 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 the second vaccination, indicating the need to boost the immune response with a third vaccination.

To further assess the decay of the antibody response after a second mRNA vaccination and alteration in antibody titers following a third mRNA vaccination, we evaluated anti-S IgG titers in blood samples of patients with solid and hematologic malignancies. In addition, we analyzed the rate of decay of the antibody responses following the third mRNA vaccination to assess the possible optimal timing (based on antibody level) of a fourth mRNA vaccination.

Overall, 539 patients including 316 with hematologic and 223 with solid cancers had blood drawn after a mean of 36 days (93% of patients had blood samples drawn at this time), 3 months (91%), and 6 months (62%) following the second vaccination, and 39 days (86%) and 3 months (55%) after the third vaccination, and these blood samples were analyzed for anti-S IgG levels. Clinical characteristics of the pa-

tients are provided in Table S1A. Patients with hematologic cancers included in the study were pre-selected based on an expected reduced immune response and therefore primarily included patients with lymphoma (30%), chronic lymphocytic leukemia (CLL; 37%), and multiple myeloma (MM; 32%). At the time of second vaccination, 93% of patients with solid cancers were in active cancer treatment, e.g., chemotherapy, immunotherapy or radiotherapy, while 38% of patients with hematologic cancers were in active cancer therapy, the most prevalent of which were chemotherapy and anti-CD20 therapy, and 16% had received stem cell transplants. At time of third vaccination, 63% of patients with solid cancers were in active cancer therapy, e.g., chemotherapy or immunotherapy, and 28% of patients with hematologic cancers were in active cancer therapy, of whom 19% received anti-CD20 therapy. Seven percent received supportive immunoglobulin treatment. Steroid treatment (≥ 50 mg/week) prior to the third vaccination was ongoing in 4% of patients with solid cancers and in 9% of patients with hematologic cancers.

As previously demonstrated, the percentage of seropositivity for anti-S IgG (>54 BAU/mL) 36 days after a second vaccination in patients with solid cancers was significantly higher (92%) than in those with hematologic cancers (67%) ($p < 0.0001$, Fischer's exact test) (Table S1B). For the total cohort, the mean IgG titer declined from the blood sample drawn 36 days (912 BAU/mL) to 3 months (378 BAU/mL) and 6 months (165 BAU/mL) after a second vaccination (Figures S1A–S1C).

The majority of patients received their third vaccination 6 months after the second vaccination. A marked increase in mean anti-S IgG was observed 39 days following a third vaccination (1,903 BAU/mL), which was 2- to 5-fold higher than after the second vaccination ($p < 0.0001$, Student's *t* test) (Figure S1A). Not only were the titers higher following a third vaccination, but a larger percentage of patients with hematologic and solid cancers developed a sufficient antibody response. Among patients with hematologic cancers, only 26% were seronegative 3 months after the third vaccination, while 43% of this group were seronegative 3 months after the second vaccination (Table S1B). This improvement in anti-S IgG response was observed for several disease types (seronegative %; CLL, 54% versus 24%; and MM, 39% versus 10%).

The group of patients with hematologic cancers receiving active cancer therapies during the second and third mRNA vaccinations exhibited significantly lower seroconversion following the third vaccination compared to those who did not receive therapies (Table S1C; Figure S1D), in agreement with Herishanu et al. (2022). Interestingly, steroids at high doses (≥ 50 mg/week) did not affect seroconversion after a third vaccination (Table S1C). Comparing the medical history of patients with hematologic cancers who were seronegative after the second vaccination but became seropositive after the third vaccination with those that remained seronegative suggested that type of malignancy, type of treatment, and/or comorbidity could explain the change for some.



Patients treated with anti-CD20 therapy within the past 6 months did not seroconvert following the third vaccination (Table S1D), in agreement with [Debie et al. \(2021\)](#). However, for the majority of patients with hematologic cancers, seroconversion following the third vaccination could not be explained by type of malignancy, type of treatment, and/or comorbidity. This indicates that identification of cancer patients who would benefit from additional boosting by a third vaccination is complex and suggests that this entire immunocompromised population would need a third vaccination.

The improvement in seropositive rates following the third vaccination was even more pronounced in patients with solid cancers. One-fifth of the patients were seronegative 3 months after the second vaccination, while only one patient (<1%) was seronegative 3 months after the third vaccination (Table S1B). Part of this cohort had discontinued chemotherapy at the third vaccination and thus may have been able to elicit an immune response. Seroconversion from the first to the third blood samples after the second vaccination was more pronounced in patients with lung cancer and gastrointestinal cancers, and those patients benefitted from the boost of the third vaccination (Table S1E).

By formulating an equation based on the decay in antibody responses after the second vaccination, we could calculate when the anti-S IgG level declined below the level considered for seronegativity. Based on our data, some patients with hematologic cancers, e.g., those receiving BTK inhibitors and chemotherapy, already became seronegative 2 months after the second vaccination and should likely have received the third vaccination at that time, while those not receiving any active treatment could have waited up to a year for the third vaccination (Table S1F; Figures S1F and S1G). Finally, we also used the equation based on the decay in antibody responses after the second vaccination and the 39 day and 3 month titer values after the third vaccination to approximate when the titers declined below the level considered for seronegativity after the third vaccination (Table S1F; Figures S1F and S1G). Such estimates could be useful in planning optimal timing for the fourth vaccination should it become necessary, and the results again showed that the decay in antibody responses after the third

vaccination was significantly influenced by the disease type and treatment (Table S1F). Similar to the third vaccination, those receiving BTK inhibitors and chemotherapy should have already received the fourth vaccination 3–5 months after the third vaccination, while those receiving no active treatment could wait up to a year before a fourth vaccination. Our data support early administration of a fourth vaccination to selected groups of cancer patients, as has been initiated by some countries, including Denmark, where a fourth vaccination is offered to such patients approximately 4 months following their third vaccination. An alternative strategy would be to monitor anti-S IgG levels to determine the optimal time for a fourth vaccination for individual patients, as suggested by others ([Barrière et al., 2022](#)).

As limitations of this study, we have only evaluated the anti-S IgG levels, which often, but not always, correlate with virus neutralization and are predictive of immune protection ([Goldberg et al., 2021](#)). Although an absolute protective threshold has yet to be established for the different variants of the SARS-CoV-2 virus, we and others ([Eliakim-Raz et al., 2021](#)) defined seronegativity as ≤ 54 BA U/mL of anti-S IgG, since this level has been reported to confer an estimated 50% protective antibody level in standardized units ([Khoury et al., 2021](#)). Further, infection rate of this population and CD8 T cell immunity against COVID-19, which has been shown to be an important immune protective parameter, have not been evaluated. Studies are needed to verify the clinical effectiveness of repeated vaccination in these vulnerable patients.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.ccell.2022.02.011>.

ACKNOWLEDGMENTS

The study was supported by a grant from the Odense University Research Council. The funder had no role in study design, patient recruitment, data collection, data analysis, data interpretation, or writing of the report. We want to express our gratitude to the participants of the study; to laboratory technicians at the Odense University Department of Oncology for technical assistance with the blood samples; and to the Clinical Experimental Unit, Department of Oncology, Odense University Hospital for assistance with the recruitment of patients. We thank M.K. Occhipinti for editorial assistance.

DECLARATION OF INTERESTS

All authors declare no competing interests.

REFERENCES

- [Barrière, J., Carles, M., Audigier-Valette, C., Re, D., Adjtouth, Z., Seitz-Polski, B., Gounant, V., Descamps, D., and Zalcman, G. \(2022\).](#) Third dose of anti-SARS-CoV-2 vaccine for patients with cancer: should humoral responses be monitored? A position article. *Eur. J. Cancer* **162**, 182–193.
- [Debie, Y., Vandamme, T., Goossens, M.E., van Dam, P.A., and Peeters, M. \(2021\).](#) Antibody titres before and after a third dose of the SARS-CoV-2 BNT162b2 vaccine in patients with cancer. *Eur. J. Cancer* **163**, 177–179.
- [Ehmsen, S., Asmussen, A., Jeppesen, S.S., Nilsson, A.C., Østerlev, S., Vestergaard, H., Justesen, U.S., Johansen, I.S., Frederiksen, H., and Ditzel, H.J. \(2021a\).](#) Antibody and T cell immune responses following mRNA COVID-19 vaccination in patients with cancer. *Cancer Cell* **39**, 1034–1036.
- [Ehmsen, S., Jakobsen, L.H., Lendorf, M.E., Eefsen, R.L., Bentsen, L., Knoop, A.S., Aggerholm-Pedersen, N., Skuladottir, H., Herrstedt, J., Jensen, L.H., et al. \(2021b\).](#) Severity and 1-month outcome of SARS-CoV-2 infection in patients with solid cancers: a Danish nationwide cohort study. *Acta Oncol.* **60**, 859–865.
- [Eliakim-Raz, N., Leibovici-Weisman, Y., Stemmer, A., Ness, A., Awwad, M., Ghantous, N., and Stemmer, S.M. \(2021\).](#) Antibody titers before and after a third dose of the SARS-CoV-2 BNT162b2 vaccine in adults aged ≥ 60 years. *JAMA* **326**, 2203–2204.
- [Goldberg, Y., Mandel, M., Bar-On, Y.M., Bodenheimer, O., Freedman, L., Haas, E.J., Milo, R., Alroy-Preis, S., Ash, N., and Huppert, A. \(2021\).](#) Waning immunity after the BNT162b2 vaccine in Israel. *N. Engl. J. Med.* **385**, e85.
- [Gounant, V., Ferré, V.M., Soussi, G., Charpentier, C., Flament, H., Fidouh, N., Collin, G., Namour, C., Assoun, S., Bizot, A., et al. \(2022\).](#) Efficacy of severe acute respiratory syndrome coronavirus-2 vaccine in patients with thoracic cancer: a prospective study supporting a third dose in patients with minimal serologic response after two vaccine doses. *J. Thorac. Oncol.* **17**, 239–251.
- [Herishanu, Y., Rahav, G., Levi, S., Braester, A., Itchaki, G., Bairey, O., Dally, N., Shvidel, L., Ziv-Baran, T., Polliack, A., et al.; Israeli CLL Study Group \(2022\).](#) Efficacy of a third BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard 2-dose vaccination. *Blood* **139**, 678–685.
- [Khoury, D.S., Cromer, D., Reynaldi, A., Schlub, T.E., Wheatley, A.K., Juno, J.A., Subbarao, K., Kent, S.J., Triccas, J.A., and Davenport, M.P. \(2021\).](#) Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* **27**, 1205–1211.
- [Tian, J., Yuan, X., Xiao, J., Zhong, Q., Yang, C., Liu, B., Cai, Y., Lu, Z., Wang, J., Wang, Y., et al. \(2020\).](#) Clinical characteristics and risk factors associated with COVID-19 disease severity in patients with cancer in Wuhan, China: a multicentre, retrospective, cohort study. *Lancet Oncol.* **21**, 893–903.