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Letter

Enhanced but variant-dependent serological and cellular immune responses to third-dose BNT162b2 vaccination in patients with multiple myeloma

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During the SARS-CoV-2 pandemic, patients with multiple myeloma (MM) are at increased risk of developing severe COVID-19 and succumbing to the disease (Chari et al., 2020). Since the introduction of different vaccines at the beginning of 2021, patients with cancer have been immunized with high priority (Ludwig et al., 2021). Because of therapy-induced immunosuppression and variations in humoral and cellular immune responses, early in the pandemic, patients with MM were hypothesized to require a more distinct approach to COVID-19 protection, including intensified and diversified vaccination strategies (Ludwig et al., 2021).

We and others previously reported variable vaccination responses in patients with MM after two doses of BNT162b2 (Aleman et al., 2021; Ehmsen et al., 2021; Enßle et al., 2022; van Oekelen et al., 2021). In our interim analysis, among patients with MM, we identified 53.2% serological responders (neutralization titer [NT] $\geq$ 1:20) to the wildtype (WT) and 43.6% serological responders to the B.1.1.7 (Alpha) variant, whereas 34.2% of the patients were classified as T cell responders (Enßle et al., 2022; Khoury et al., 2021). Factors that were predictive of poor response were older age, active treatment excluding maintenance, low $CD19^+$ B cell counts, and the presence of immunoparesis. Repetitive booster vaccinations were increasingly found to be necessary in some cases, and further clinical trials to examine the effects of booster vaccinations as well as observational studies were designed. Given the emergence of the novel virus strains B.1.617.2 (Delta) and B.1.1.529 (Omicron), the efficacy of first-generation vaccines against variants of concern (VOCs) must be assessed. Recently, augmented humoral and cellular response after the third dose of SARS-CoV-2 vaccination has been reported. These data highlight a suboptimal serological response against the Omicron variant, but they do not clarify the variant-dependent neutralization capacity and T cell response in detail after third-dose vaccination in patients with MM (Aleman et al., 2022).

In this follow-up analysis, we report on serological and T cell responses against the Delta and Omicron variants, in comparison to the WT strain, after a third dose of the BNT162b2 vaccine. The booster vaccination was administered 3–6 months after the second dose, and the serological assessment of the response was performed 21–28 days later (timepoint 5 [TP5]). A total of 100 patients and 23 healthy individuals as controls were included. In the core analysis, participants who were found to have anti-nucleocapsid IgG antibodies (indicative of previous natural infection) at any timepoint before TP5, as well as patients who had received heterologous vaccination (vector- and mRNA-based or different mRNA-based vaccines), were excluded, and the result was a study cohort of 71 patients with MM and 23 healthy controls.

First, we examined SARS-CoV-2 spike-binding IgG (anti-S IgG) levels at TP5 and compared them with those at TP3 (21–28 days after the second vaccination). We observed a significant increase in anti-S IgG levels in patients with MM and in healthy individuals (Figure S1A). Interestingly, we observed a 4-fold increase from a median of 193.2 BAU/ml at TP3 to 776.0 BAU/ml at TP5 in the MM cohort, and a 2-fold increase from a median of 1,367.9 BAU/ml at TP3 to 2,941.0 BAU/ml at TP5 in the control cohort. A total of 63.8% of MM patients and 100% of controls achieved Delta-specific NT at TP5 (Figure S1B, Table S1A). In contrast, we detected an Omicron-specific NT of $\geq$1:20 at TP5 in only 29.0% of MM patients and 39.1% of controls, and we observed no significant differences in NT and responder rates

between MM patients and controls (Figure S1B, Table S1A). The median Delta NT increased significantly from TP3 to TP5 (Figure S1C). Anti-S IgG levels and variant NT data showed a high correlation (Delta R = 0.917, $p < 0.01$; Omicron R = 0.819, $p < 0.01$). Through receiver operating characteristic analysis, we re-calculated the anti-S IgG levels necessary for achieving a variant-specific NT of $\geq$1:20. Whereas anti-S IgG levels of 280.8 BAU/ml were sufficient for Delta neutralization, anti-S IgG levels of 1763.15 BAU/ml were required for Omicron neutralization (Figure S1D). These data confirm the immune-escape of Omicron and thus support calls for rapid approval of second-generation vaccines that not only target the WT strain but also various novel SARS-CoV-2 strains (McCallum et al., 2022). Delta non-responders were undergoing active treatment significantly more often, and Omicron non-responders were significantly older than responders (Table S1B). However, the remission status, type of treatment, and line of treatment were not associated with serological response (Table S1B). $CD19^+$ B cell counts displayed a certain correlation with the serological data (Figure S1E). Interestingly, patients in complete remission (CR) or very good partial remission (VGPR) were able to seroconvert despite low $CD19^+$ cell counts, regardless of treatment status (Figure S1F). These findings show that patients should ideally be vaccinated either in a treatment-free interval or when deep remission is achieved.

Next, we focused on variant-specific T cell responses 1–3 months after the booster vaccination. Previously, we hypothesized that T cell responses might be necessary in patients with B cell deficiency to achieve a maximal vaccination response (von Metzler et al., 2021). However, after the second dose of vaccine, we reported abrogated T cell responses in patients with MM compared with healthy individuals (Enßle et al., 2022). After the third vaccination, we expanded our analysis and used flow cytometry to examine WT-, Delta-, and Omicron-specific T cell subsets and activation. To account for general differences in $CD4^+$/$CD8^+$ T cell counts between MM patients and controls (Figure S1G), we measured the absolute counts of IFN-γ-, IL-2-, TNF-α-, and CD154-positive $CD4^+$ or $CD8^+$ T cells after variant-specific stimulation. Unexpectedly, despite significantly lower $CD4^+$ T cell counts (Figure S1G), patients with MM mounted a strong $CD4^+$ T cell response against the WT strain, and this indicates well-preserved functionality of the $CD4^+$ T cell fraction (Figures S1H–S1J). No differences were observed between MM and controls (Figures S1H–S1J). Importantly, most patients with MM achieved a T cell response against WT (Figure S1H, Table S1C). For the Delta variant, patients with MM achieved T cell responses comparable to those observed in controls. A trend toward lower medians of SARS-CoV-2-specific T cell counts and lower T cell responder fractions in MM compared with controls was observed for Omicron (Figures S1H–S1J). Patients not responding to WT stimulation were also T cell non-responders to Delta and Omicron. The T cell response rates against both recent VOCs were lower than those against WT, although we note that the commercially available peptides for stimulation might differ in their response induction. No association between T cell response status and the patients' clinical characteristics was observed (Table S1D). Furthermore, we investigated the functional T cell response by measuring the IFN-γ release via enzyme-linked-immuno-spot assay (ELISpot). In contrast to our previous data, after two doses of BNT162b2, no differences in the response against WT and receptor-binding-domain peptide were observed between MM and controls (Figure S1K) (Enßle et al., 2022). Diminished responses were observed toward Delta and Omicron peptides, but no differences between groups were observed (Figure S1K). Patients with MM without a response in SARS-CoV-2-specific T cell counts trended toward decreased IFN-γ spot-forming-units (SFUs) after variant-specific peptide stimulation, and this further illustrates a reduced functional IFN-γ response in T cell non-responders (Figures S1L–S1N). Interestingly, no general correlation between serological and T cell response levels was observed (Figures S1O–S1Q).

We also analyzed patients who were infected with SARS-CoV-2 before (n = 11, initially excluded) and after (n = 7) the third vaccination (Table S1E). Generally, patients who were infected with COVID-19 before the third vaccination showed higher neutralization levels than those without a history of infection at TP5 (Table S1E). Patients who were infected after the third vaccination showed lower serological response levels than previously infected patients did (Table S1E), but no differences were observed regarding SARS-CoV-2-specific T cell counts for all variants (Table S1E). Furthermore, no differences in clinical characteristics were found between both groups and patients without infection (Table S1E).

Together, our findings indicate that after the third BNT162b2 vaccination, patients with MM mounted greater serological responses than did those who had received two vaccinations. However, they presented poor neutralization capacity against the Omicron VOC. Most patients with MM were T cell responders toward the initial WT strain. T cell responses against Delta and Omicron were trending to be reduced in comparison to WT but did not differ between patients with MM and healthy controls. Given the diminished immune responses against Omicron despite the general immunogenicity observed for WT, we recommend variant-adapted vaccine trials in immunocompromised patients. Such approaches may prevent severe infections in immunosuppressed patients, as well as the continued emergence and spread of novel variants in the general population.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2022.05.003.

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AUTHOR CONTRIBUTIONS

J.C.E., J.C., S.B., A.M., F.S., K.G., M.B., S.H., and M.W. performed experiments; J.C.E., J.C., A.M., F.S., S.B. M.A.R., and S.W. analyzed data; I.v.M., E.U., and J.C.E. designed the study; I.v.M. and E.U. directed the study; J.C.E. and S.W. performed statistical analyses; I.v.M., E.U., J.C.E., J.C., S.B., S.H., M.B., H.F.R., S.C., O.B., H.S., M.W., and B.S. discussed the results and interpreted the data together with all co-authors. J.C.E., I.v.M., and E.U. wrote the manuscript with contributions from all authors.

DECLARATION OF INTERESTS

S.C. received honoraria for advising Pfizer/BioNTech. I.v.M. received honoraria for advising Pfizer, Sanofi, BMS, GSK, Amgen, Janssen, Takeda, and AstraZeneca. E.U. received honoraria for advising Phialogics and BMS. Other authors declare no conflict of interest to the present study.

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