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Letter

Humoral and cellular immunity to delayed second dose of SARS-CoV-2 BNT162b2 mRNA vaccination in patients with cancer

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Patients with cancer are considered to be at high-risk for SARS-CoV-2 infection and severe COVID-19 (Bakouny et al., 2020). Our "SOAP-02" (Sars-CoV-2 fOr cAncer Patients) study has assessed their responses to COVID-19 vaccination. Our interim results provided safety and immune efficacy data for any COVID-19 vaccine in an immunocompromised patient population and showed that at 3 weeks following a single dose of BNT162b2 mRNA vaccine, seroconversion was conspicuously low (38%) in patients with solid cancer and very low (<20%) in patients with hematologic cancer (Monin et al., 2021). Importantly, however, a small sub-cohort of patients with solid cancer who received a second dose of vaccine at day 21 after the first shot showed substantially increased seroconversion (95%) as measured 2 weeks later. Conversely, too few patients with hematologic cancer had received a second shot at day 21 to permit their interim reporting. Recently, it was reported that a subset of patients with hematologic cancer failed to develop humoral responses despite receiving two vaccine doses 21 days apart (Addeo et al., 2021; Greenberger et al., 2021; Thakkar et al., 2021). However, no data are available regarding whether such patients might be seroconverted by delaying

the second dose, which became UK government policy on December 29, 2020 and as has been considered by other nations. The completed results of the SOAP-02 study presented here provide those data.

For study participants who received delayed second doses, the median days from the first vaccination were 74 for healthy controls (HC), 77 for patients with solid cancer (SC), and 70 for patients with hematologic malignancies (HM). Median days from the second vaccination to serum sampling (so-called time point [TP]4) were likewise comparable across cohorts: HC, 14; SC, 15; and HM, 15. At TP4, the primary endpoint of anti-SARS-CoV-2 Spike protein (S) specific IgG seroconversion following a delayed second dose could be assessed for 159 participant samples, from a total of 255 participants who consented to enroll in SOAP-02 (Table S1). Of these, 18 (5 HC, 8 SC, and 5 HM) were excluded from the analysis based on evidence of past or concurrent SARS-CoV-2 exposure (see Monin et al., 2021).

Of the remaining 141 individuals, vaccine responders comprised 100% (26/26) of HC, 84% (54/64) of SC, and 43% (22/51) of HM (Figure S1A). Anti-S IgG titers for SC and HM were comparable, but they were significantly lower than for

HC (Figure S1A). Anti-S IgG titers correlated strongly with age in HC (p = 0.00013) but not in SC or HM (Figure S1B). Likewise, age did not correlate with vaccine failure in SC or HM. Thus, other dominant factors influence B cell responsiveness in patients with cancer.

We assessed the immunoprotective potential of seroconversion by assessing neutralization of HIV1-based virus particles pseudotyped with Pango Lineage B (wild type [WT]), VOC.B.1.1.7 (alpha), or VOC.B.1.617.2 (delta) S proteins. All serological responders could neutralize WT except for 1 chronic lymphocytic leukemia (CLL) patient who received Bruton's tyrosine kinase inhibitor roughly coincident with the first and second vaccinations (Figure S1C). By paired analyses, all cohorts showed significantly greater neutralization (higher ID50) of WT than delta strains, and HC and SC showed greater neutralization of WT than alpha strains, although there were exceptions (Figure S1D).

Next, we compared TP4 titers with those taken at 3 weeks following first vaccination (TP2) for 24 HC, 28 SC, and 29 HM for whom matched samples existed. The second dose clearly induced significant increases in anti-S IgG titers for all three cohorts (Figure S1E). However,

whereas increased titers were mirrored by significantly increased WT and alpha neutralization for HC, this was not universally so for SC, who displayed heterogeneous behaviors (Figure S1E). Note that too few HM showed virus neutralization at TP2 to permit valid comparisons with TP4. Nonetheless, one can conclude that whereas delayed second vaccination could induce and/or enhance neutralizing antibodies effective against the three SARS-CoV-2 strains tested, the majority of patients with hematologic malignancies remained seronegative. The failure of several seroconverted patients with cancer to show boosted neutralization reflects yet another component of their vulnerability.

To measure functional T cell responses to delayed second vaccination, sub-cohorts (17 HC, 32 SC, 33 HM) were assessed through the use of fluorospot (Monin et al., 2021). SARS-CoV-2-specific interferon γ (IFNγ) or interleukin-2 (IL-2 T) cell responses to Spike protein 2 (S2) and/or to receptor binding domain (RBD) were evident for 88% (15/17) of HC, 94% (30/32) of SC, and 70% (23/33) of HM (Figure S1F). The failures of some HM to make T cell responses to S2 or RBD contrasted with almost invariably robust recall responses to control peptides derived from Cytomegalovirus (CMV), Epstein-Barr virus (EBV) , flu and tetanus (CEF; CEFT), to which most adults will have been exposed and/or vaccinated (Figure S1F). Moreover, bi-variate representation (Figure S1G) showed that the percentages of individuals who made "dual responses"—i.e., displayed seroconversion and at least one type of RBD-specific or S-specific T cell response— were 88% for HC and 78% for SC, but only 36% for HM. Thus, patients with hematologic malignancies showed very poor seroconversion rates following primary vaccination (< 20%) and relatively poor seroconversion rates following delayed second vaccination (< 50%), and they failed to establish a prototypic correlation of B and T cell responses. Interestingly, when TP4 T cell responses were compared with TP2 responses, there was no evidence that delayed second vaccination increased IL-2 responses in any cohort, and for IFNγ responses in HC, there was merely an upward trend (Figure S1H). Although the second vaccination did increase serological responses,

the endpoint titers and fold-change increases were comparable for day 21 and delayed second doses, respectively (Figure S1I); note that <1 fold-change values reflect rare individuals whose anti-S IgG titers to first-dose vaccination had declined thereafter and were not fully restored by a second dose (Figure S1I).

Next, we investigated whether failures to make dual responses were associated with specific clinical phenotypes. T cell response data for double-vaccinated individuals were available for 48 SC (16 of whom received their second vaccination at day 21) and 37 HM (4 of whom received their second vaccination at day 21) (Monin et al., 2021). 10 SC showed dual response failures, majority of these were patients who had received steroid treatments within 15 days of either the first and/or the second dose (Figure S1J). Additionally, 8 of these 10 non-responders received chemotherapy within this period, 7 of whom were on concomitant high-dose steroids.

The heterogeneity of the HM cohort undermined statistical power for most subgroup discriminations (Table S1). Nonetheless, we noted that of 19 plasma cell myeloma patients for whom B and T cell data existed, 9 (47%) were non-responders (Figure S1K), 8 of whom had received anti-cancer treatment within 15 days of the prime and/or the second vaccination. When seroconversion alone was assessed for the 56 HM patients who received either a day 21 (n = 5) or delayed second dose (n = 51), non-response was significantly associated with receiving anti-cancer treatment within 15 days of the second vaccination (Figure S1L).

In summary, our study re-emphasizes the vulnerabilities of patients with cancer, especially those with hematologic malignancies, vis-à-vis the COVID-19 pandemic. Whereas the second vaccination significantly increased seroconversion rates in patients with cancer, most patients with hematologic cancer remained without serological protection. Moreover, delayed second vaccination showed no obvious advantage, highlighting the importance of early (and possibly repeated) boosting of cancer patients as an attempt to rescue their very poor single-dose vaccine immunogenicity. Likewise, the data compose a strong case for continued transmission

mitigation measures in community and healthcare settings, e.g., protective measures in crowded areas, such as public transport, and the inclusion of young persons in ring vaccinations of patients' contact groups.

Our data stress the need for customizing vaccination schedules according to data-driven assessments of need. Specifically, they emphasize the importance, where possible, of completing a two-dose schedule prior to commencing immunosuppressive therapies, and of withholding chemotherapy regimens (especially those which use concomitant high-dose steroids) across 30-day windows spanning first- and subsequent-dose vaccinations, respectively. Clearly, implementing such measures requires thoughtful assessment, given that the SOAP-01 study of cancer patients with COVID-19 reported that most increased deaths were attributable to altered cancer treatment schedules rather than to COVID-19 (Abdul-Jawad et al., 2021).

Our study has also highlighted several aspects of human immunology which are possibly germane to preparedness for future pandemics. First, the frequencies of functional (cytokine-releasing) T cell responses exceeded seroconversion rates for cancer patients who received the delayed second vaccination. This might reflect some degree of vaccine boosting of pre-existing memory T cells induced by seasonal coronaviruses, particularly those reactive to S2 antigens, although the protective utility of these against SARS-CoV-2 is not clear. Given that anti-S IgG reflects only part of the serological response, some patients who make T cell responses might have developed IgM responses that did not efficiently progress to IgG. Strikingly, T cell response magnitudes were not increased by second-dose vaccination in any cohort, although it is conceivable that boosting enhanced the frequency and/or durability of memory T cells. Indeed, routine practical means for population-scale assessment of the pleiotropic immunoprotective potentials of T cells would clearly be a profound addition to the clinical armamentarium. Finally, it is noteworthy that vaccine non-responsiveness in patients with cancer does not reflect broad immunodeficiencies, since extremely robust recall responses were made to CEFT peptides. Rather, their

responsiveness to neo-antigens seems to be particularly impaired.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2021.10.003.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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