

CORRESPONDENCE



Neutralizing antibody and T cell responses to SARS-CoV-2 vaccination in hematopoietic cell transplant recipients

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TO THE EDITOR:

Current availability of various vaccination platforms against SARS-CoV-2 generates optimism toward the development of robust herd immunity and end of the pandemic. For particular populations however, such as the hematopoietic cell transplant (HCT) recipients who represent a highly vulnerable population to COVID-19 with dismal prognosis and mortality higher than 20% [1–3], there is an urgent need for a prompt and effective protection.

Notwithstanding the prioritization of HCT recipients to COVID-19 vaccination, limited information is available on whether and to what extent, they could mount functional immune responses as they were generally excluded from vaccination trials [4]. Humoral responses post SARS-CoV-2 vaccination have been studied in solid organ transplant (SOT) [5, 6] or HCT [7] recipients, patients with hematologic malignancies [8–12] or/and after CAR T-cell therapy [13]. Nevertheless, information on both the B- and T-cell components of the adaptive immunity in terms of neutralizing antibody viral inhibition and interferon- γ secreting SARS-CoV-2-specific T-cells, after vaccination of HCT recipients is lacking. To gain insights in the adaptive immune responses post-HCT, we studied neutralizing antibody and T-cellular immune responses to SARS-CoV-2 vaccination of HCT recipients.

In our Institutional Review Board approved study, SARS-CoV-2 unexposed, adult HCT recipients scheduled to receive two doses of BNT162b2, were prospectively enrolled after providing written consent. Unexposed, fully vaccinated, health-care professionals served as control. Unexposed individuals were those reporting no close contact with known case, or/and none of the typical COVID-19 symptoms or/and negative testing since the pandemic initiation.

Blood samples were collected on day 1 and day 22 before the first and second BNT162b2 dose, respectively, and on day 50 post second dose. Neutralizing antibodies against SARS-CoV-2 (CoV-2-NAbs) were measured in serum using an FDA-approved methodology (ELISA, cPass™; GenScript) [14] where CoV-2-NAbs $\geq 30\%$ are considered positive and $\geq 50\%$ as providing clinically relevant viral inhibition [15].

T-cell responses were measured as previously described [16]. Briefly, peripheral blood mononuclear cells (PBMCs) were pulsed with spike pepmixes and interferon-gamma secretion was measured by Elispot and counted as Spot-forming cells (SFCs) on Eli.Scan Elispot scanner (A.EL.VIS) using the Eli.Analyse software V6.2.SFC. SARS-CoV-2 spike-specific T cells (spike-STs) were expressed as SFCs per number of input cells. Response was considered positive, if SFCs were ≥ 30 per 5×10^5 PBMCs.

Statistics were performed with GraphPad Prism. Descriptive statistics used median (range) values. Continuous variables were analyzed using one-way ANOVA with Bonferroni's correction or Kruskal–Wallis for multiple comparisons. Mann–Whitney or 2-tailed Student's *t* test were used for two group comparisons. Correlations between continuous variables were assessed using the Pearson's or Spearman's correlation coefficients. *P*-values ≤ 0.05 were considered significant.

Sixty-three eligible HCT patients (49 allo-, 14 auto-HCT) of median age 49 (21–71) years, at 2.8 (0.17–31) and 2.1 years (1.25–8) post allo- and auto-HCT respectively, who were vaccinated with the Pfizer-BioNTech were enrolled (Supplementary Table 1). No clinically significant adverse event related to SARS-CoV-2-vaccination was reported. CoV-2-NAb responses were studied in all patients, while T-cell responses were measured in 36/63 vaccinated patients (31 allo-HCT/5 auto-HCT). As control cohort, 17 unexposed, health-care vaccinees of median age 57 years (29–68) and without any known underlying disease, were included. CoV-2-NAbs and spike-STs were barely detectable before vaccination but a discernable activity was observed after the first dose, reaching highly protective levels after the second dose in 88% and 79% of all tested patients ($p < 0.0001$, $p = 0.002$, respectively; Fig. 1A and Supplementary Table 2). Notably, CoV-2-NAbs strongly correlated with T-cell responses in the tested patients (Pearson $r = 0.6009$; $p = 0.0024$; Fig. 1A).

All auto-HCT patients (including 2 patients receiving maintenance immunotherapy post-autoHCT) showed protective B- and T-cell responses, similar to healthy subjects, however, the long interval post transplantation (> 1.25 year) may have generated an unintended bias toward elevated immune responses (Fig. 1B). Post complete vaccination, neutralizing inhibition was similar among allo-HCT recipients, auto-HCT patients and healthy volunteers whereas circulating spike-STs were significantly lower – yet within protective levels –, in allo-HCT patients over healthy individuals ($p < 0.0001$) (Fig. 1B and Supplementary Table 2). This difference in spike-STs over the rather uniform CoV-2-Nabs levels across groups, practically reflects the different assay read-outs (SFCs/number of input cells vs % viral inhibition) and the broad dynamic range (2–906 SFCs/ 5×10^5 PBMCs) of the entire spike-ST pool (Supplementary Table 2), rather than critical differences in protective adaptive immunity across cohorts.

Allo-HCT patients developed frequent (85% and 75%) and high (97% CoV-2-NAb inhibition and 125 SFCs/ 5×10^5 PBMCs-Supplementary Table 2) B- and T-cell responses post-second vaccination. Interestingly, early T-cellular protective immune responses were reached in 62% of allo-HCT pts after the 1st dose, whereas only 46% developed neutralizing humoral capacity at that time, in line with the detection of S-reactive CD4+ and CD8+ T-cells as early as Day7 and Day10 after the first BNT1622 dose [17]. The effect of the second dose was profound in this cohort where 69% of patients who had failed to mount any functional humoral response with the 1st dose, achieved protective immunity after the 2nd dose

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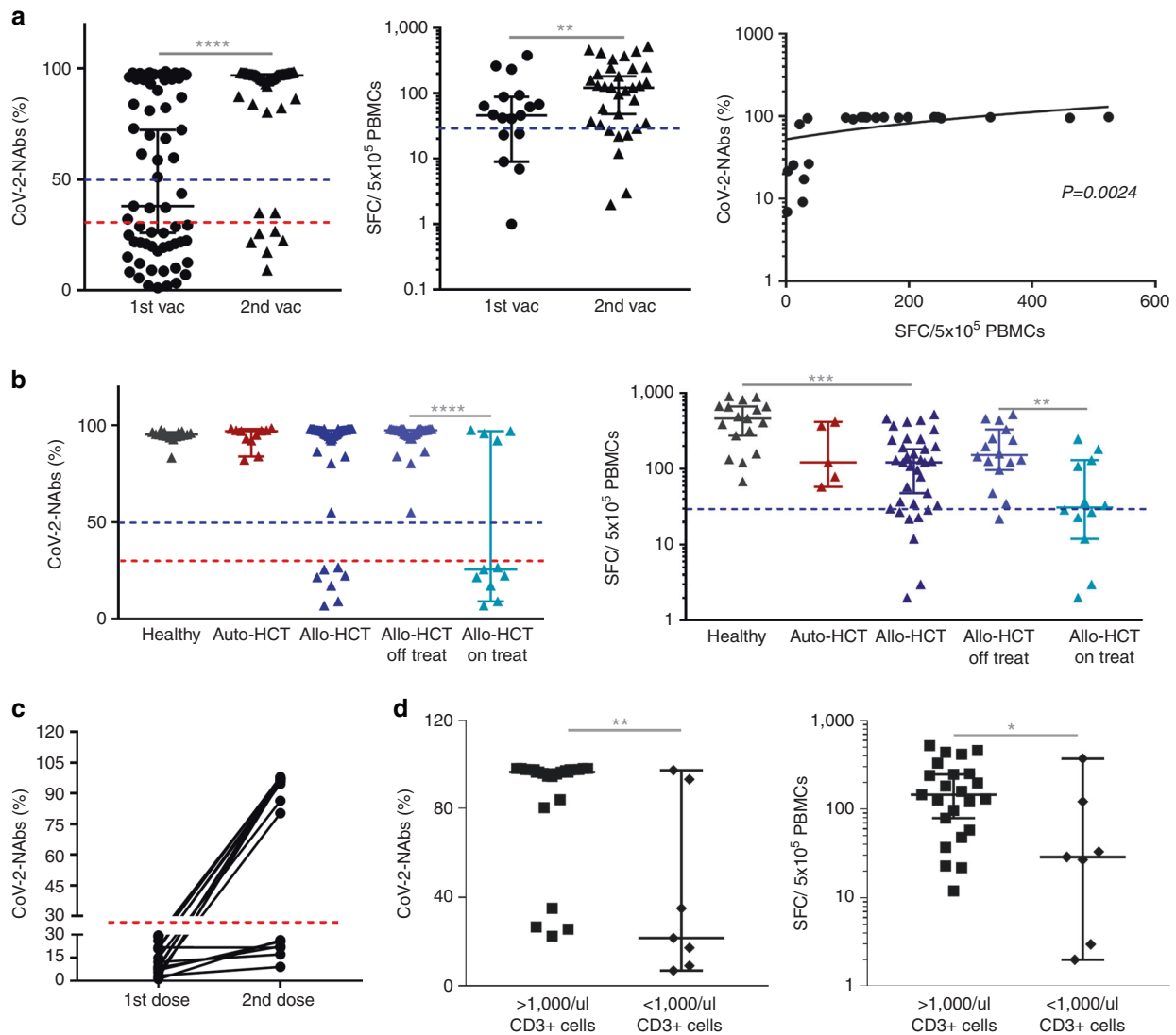


Fig. 1 Humoral and T cell responses in HCT recipients. **A** Humoral and T cell responses after the second vaccination dose as compared to the first dose in HCT recipients. The red and blue dotted lines in the left graph indicate the 30% and 50% threshold above which CoV-2-NAbs are considered positive or highly protective, respectively. The blue dotted line in the middle graph indicates the threshold above which T cell response was considered positive. Median with 95% CI is shown. **** $p < 0.0001$, ** $p = 0.002$. **B** CoV-2-NAbs and spike-STs after the second dose in healthy subjects, auto- and allo-HCT recipients, as well as the allo-HCT subgroups, on and off immunosuppression. The red and blue dotted lines in the left graph indicate the 30% and 50% threshold above which CoV-2-NAbs are considered positive and have been associated with clinically relevant viral inhibition, respectively. The blue dotted line in the right graph indicates the threshold above which T cell response was considered positive. Median with 95% CI is shown. **** $p < 0.0001$, *** $p = 0.0005$, ** $p = 0.0044$. **C** Humoral protective immune responses after the 1st and 2nd vaccination dose in allo-HCT patients. Each line represents a single allo-HCT patient. The red dotted line indicates the 30% threshold above which CoV-2-NAbs are considered positive. **D** Association of circulating CD3+ cells <1000/ μ l with neutralization capacity and levels of spike-STs. Median with 95% CI is shown. ** $p = 0.0029$, * $p = 0.0292$. HCT hematopoietic cell transplantation, Auto-HCT autologous HCT, Allo-HCT allogeneic HCT, CoV-2-NAbs neutralizing antibodies against SARS-CoV-2, spike-STs SARS-CoV-2 spike-specific T cells.

(Fig. 1C). This underscores the importance of second immunization in vulnerable patients who may remain otherwise, sub-optimally protected while serve as a viral reservoir for reactivations and novel mutations.

Importantly, a significant proportion of allo-HCT recipients under active immunosuppression failed to reach protective levels of CoV-2-NAbs and spike-STs, demonstrating lower immunogenicity to vaccination, compared to patients off-treatment (Fig. 1B, $p < 0.0001$, $p = 0.0044$ respectively) whose adaptive immune responses were similar to auto-HCT recipients and healthy subjects. Only 36% and 50% of patients on systemic immunosuppression reached protective (>50%) CoV-2-NAb inhibition and spike-ST (≥ 30 SFCs/ 5×10^5 PBMCs) levels, compared to 100% and 93% of immunosuppression-


free patients, respectively (Supplementary Table 2). One patient under immunosuppression who mounted marginal adaptive immune responses post-second vaccination (CoV-2-NAbs: 40%, CoV-2-STs: 33 SFC/ 5×10^5 PBMCs), succumbed to COVID-19 infection 40 days later. As others have shown, circulating CD3+ cells <1000/ μ l were associated with impaired neutralization capacity [7], but also with suboptimal spike-ST levels (Fig. 1D; $p = 0.003$, $p = 0.03$ respectively).

The majority of available literature on SARS-CoV-2-vaccination immune responses in immunocompromised patients relies on the B-cell component of adaptive immunity and in particular, serological responses rather than functional neutralizing capacity [7, 9–11, 18]. T-cellular immune responses however, are an indispensable

component of protection, especially early post-vaccination [17], in the absence yet of optimal CoV-2-Nabs [19], while unlike the relatively short-lived humoral response, T-cell immunity against SARS-CoV-2 may be heightened and long-lasting [20, 21].

In transplantation, compound B- and T-cell immune responses post SARS-CoV-2-vaccination have been only studied in SOT patients demonstrating poor immune reactivity [5]. Our study provides insights in the whole spectrum of adaptive immune responses in terms of functional immune protection of HCT patients following SARS-CoV-2 vaccination. Limitations of the study include the wide range of post-HCT interval, lack of matching between controls and cases and the relatively small sample size of control group. Results from trials investigating the immunogenicity of vaccines in this vulnerable population, such as the NCT04723706, will solidify the vaccination effect in establishing protective immunity in HCT patients.

In conclusion, active immunosuppression emerged as the major determinant of poor/suboptimal adaptive responses. Immunosuppression-free HCT patients may elicit powerful humoral neutralizing and T-cell responses, whereas it seems highly unlikely that those on systemic immunosuppression, could be protected by full vaccination. Limited data from patients receiving a third dose show humoral responses in almost half of the allo-HCT subjects who failed to respond after two doses [22]. The current, yet dynamically formulated, guidance in HCT recipients, recommends a fourth vaccine dose for those within the first 2 years post-HCT or under systemic immunosuppression [23], however, further studies are needed to find robust immune correlates and evaluate additional vaccine doses or alternative therapeutic platforms, such as adoptive immunotherapy with convalescent-donor CoV-2-STs [16, 24, 25].

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DATA AVAILABILITY

Data are available upon request.

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AUTHOR CONTRIBUTIONS

EG, AP, EY, IS, AA designed the study. EG, AP and EY analyzed data and wrote the manuscript. TT, FS, EEK, MG, AP, AK, FC collected samples and performed laboratory

analysis. GK and IB collected samples and clinical data. DC, DS, EY, IS, AA edited and approved the final draft.

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COMPETING INTERESTS

EG has consulted for Amyndas, Alexion, Omeros, and Sanofi Pharmaceuticals. EY has consulted for BlueBirdBio and Vertex/CrispRTherapeutics. Remaining authors declare no competing interests.

ADDITIONAL INFORMATION

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