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RESEARCH

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Predictors of neutralizing antibody response to BNT162b2 vaccination in allogeneic hematopoietic stem cell transplant recipients

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ARBD Ab levels-HCT recipients and healthy adults, we observed signi cantly lower anti-RBD Ab levels in allo recipients at days 21, 28 and 49. Further, 49% of HCT patients versus 88% of healthy adults had detectable NT50 Ab at day 49 while all HCT recipients had signi cantly lower NT50 Ab titers than healthy adults ($P < 0.001$). Ongoing moderate/severe chronic GVHD ($P < 0.01$) as well as rituximab administration in the year prior to vaccination ($P < 0.05$) correlated with low anti-RBD and NT50 Ab titers at 49 days after the first vaccination in multivariate analyses. Compared to healthy adults, allo-HCT patients without chronic GVHD or rituximab therapy had comparable anti-RBD Ab levels and NT50 Ab titers at day 49. Flow cytometry analyses before vaccination indicated that Ab responses in allo-HCT patients were strongly correlated with the number of memory B cells and of naive CD4

$^{+}$ T cells ($r > 0.5$, $P < 0.01$)

and more weakly with the number of follicular helper T cells ($r = 0.4$, $P = 0.01$).

Background

Allogeneic hematopoietic stem cell transplantation (allo-HCT) has remained the best treatment option for many patients with life-threatening hematological disorders such as acute myeloid leukemia [1]. Unfortunately, the procedure induces severe immunosuppression persisting several months to several years after transplantation, particularly in patients su ering from chronic graft-versus-host disease (GVHD). is is due to defects in B-cell, T-cell, monocyte and dendritic cell compartments [2–4]. As a consequence, infection of allo-HCT recipients with

Terminology Criteria for Adverse Events (CTC) version 5.0.

Flow cytometry

After cell counting on pocH-100i automated whole blood counter (Sysmex, Kobe, Japan), PBMCs were isolated

t-SNE space of each t-SNE plot, a probability per data point was calculated following the same approach as in the t-SNE algorithm. From these point probabilities, the distribution of cross-entropy in the t-SNE space relative to the original space was obtained for each group represented in the plot. All pairwise comparisons between groups were evaluated with Kolmogorov–Smirnov tests on the di erence between the cross-entropy distributions. Dendrograms were obtained from hierarchical clustering, using as distance the Kolmogorov–Smirnov statistic, that is, the L-infinity distance between the cross-entropy distributions.

Statistical analyses

Comparisons of Ab titers between various groups were done with the Mann–Whitney test. Comparisons of frequencies (%) of FlowSOM clusters between various groups were also done with the Mann–Whitney test. Correlations between age, time from HCT to vaccination, absolute immune cell counts and Ab titers were done with the Spearman r test. Comparison of the proportion of responding patients according to chronic GVHD was done with the Fisher's exact test. Variances between samples were calculated using the F test. Analyses of clinical factors (i.e., the presence or not of moderate/severe chronic GVHD, delay from allo-HCT to vaccination, patient age and rituximab administration within 1 year before vaccination) associated with Ab response and Ab titers were performed using univariate and multivariate logistic regression (with Firth correction when indicated) and univariate and multivariate linear regression, respectively. For these analyses, delay from HCT to vaccination and Ab titers underwent logarithmic transformation. Multivariate linear regressions were also used to assess the associations between baseline counts of class-switched memory B cells, naive CD4+T cells and TFH cells and Ab levels at day 49. P values < 0.05 were considered as statistically significant and all P values were 2-sided. Statistical analyses were carried out with Graphpad Prism 9.0 (Graphpad Software, San Diego, CA, USA) and SAS version 9.4.

Results

Patients

We report here the data of the first 40 patients included in the study. eir characteristics are described in Table 1. Briefly, median age at vaccination was 60 years (range 26–76 years). Median time from allo-HCT to vaccination was 31 months (range 5–51 months). At the time of the first vaccination, 14 patients were still on systemic immunosuppressive treatment either as GVHD prevention ($n=5$) or as treatment of moderate/severe chronic GVHD ($n=9$). Seven patients were given rituximab in the year before the first vaccination, including 1 of the 9

Table 1 Characteristics of the patients ($n=40$)

Age at vaccination (years); median (min, p25, p75, max)	60 (26, 54, 69, 76)
Sex (# males/# females)	19/21
Delay between vaccination and transplantation (months); median (min, p25, p75, max)	31 (6, 14, 42, 57)
Donor type (# MSD/MUD/ MMUD/Haplo)	8/26/1/5
Donor age at transplantation (years); median (min, p25, p75, max)	34 (18, 23, 46, 62)
Conditioning regimen (# patients)	
Fludarabine + 2 Gy TBI	5
Fludarabine + Melphalan	18
Fludarabine + busulfan	4
Cyclophosphamide + 12 Gy TBI	6
Thiotepa + busulfan + fludarabine	2
Sequential	3
Fludarabine + Cyclophosphamide + 2 or 4 Gy TBI	2
ATG (# yes/no)	29/11
PTCY (# yes/no)	6 /34
Chronic GVHD	
Never/only mild	29
Prior moderate/severe solved*	2
Ongoing moderate/severe	9
Rituximab (none or	

* and > 3 months out of systemic immunosuppression. MSD, HLA-identical sibling donor; MUD, 10/10 HLA-matched unrelated donor; MMUD, 1/10 HLA-mismatched unrelated donor; Haplo, HLA-haploidentical donor; TBI, total body irradiation; ATG, anti-thymocyte globulin; PTCY, post-transplant cyclophosphamide; MMF, mycophenolate mofetil; mPDN, methyl-prednisolone

equality of two variances yielded a $P=0.0005$ at day 49).

is prompted us to look for factors associated with Ab levels in allo-HCT recipients.

We first observed that patients with ongoing moderate/severe chronic GVHD had lower anti-RBD Ab levels than those with mild chronic GVHD or none. Specifically, 19 out of 28 patients without versus 1 out of 9 patients with ongoing moderate/severe chronic GVHD had detectable anti-RBD Ab at day 21 ($P=0.0055$). At day 49, the figures were 28 out of 28 patients versus 4 out of 9 patients ($P=0.0003$) (Additional file 1: Figure 3B, C). In addition, Ab titers were significantly lower in patients with than in those without moderate/severe chronic GVHD at days 21 ($P=0.002$), 28 ($P=0.002$) and 49 ($P<0.001$) (Fig. 1b).

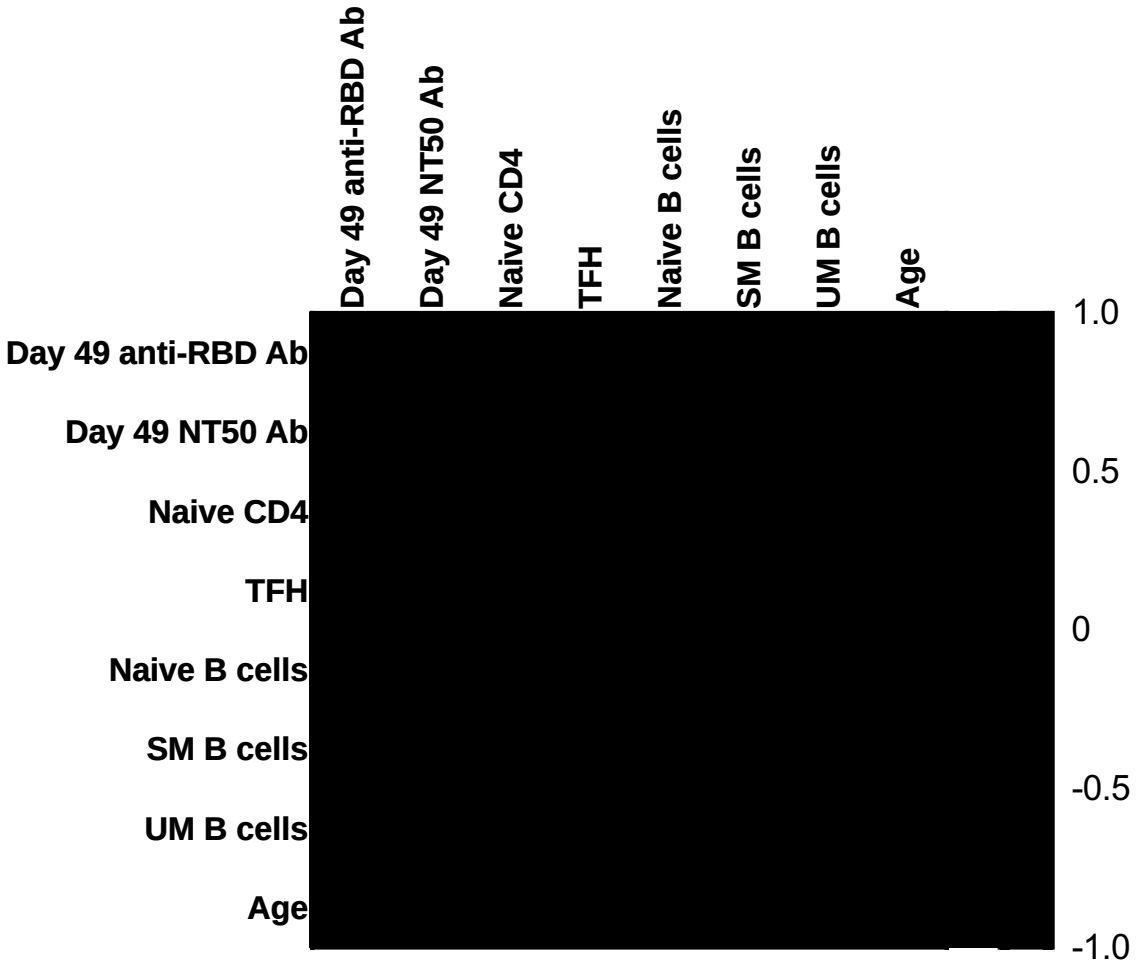
We then looked at the impact of rituximab on Ab responses in the cohort of naive allo-HCT patients without moderate/severe chronic GVHD ($n=28$). We observed that those given rituximab < 1 year before vaccination ($n=6$) had lower anti-RBD Ab levels than the remaining 22 patients on days 21, 28 and 49 after vaccination ($P<0.05$ at each time point) (Fig. 1b).

We next assessed the impact of age in allo-HCT recipients on anti-RBD Ab levels. We observed a negative correlation between COVID-19 naive allo-HCT patient age ($n=37$) and anti-RBD Ab levels at day 21 (Spearman $r=-0.36$, $P=0.029$), day 28 (Spearman $r=-0.38$, $P=0.019$) and day 49 (Spearman $r=-0.38$, $P=0.020$; univariate linear regression, $P=0.029$). In addition, there was a weak correlation between time from allo-HCT to vaccination and anti-RBD Ab levels at day 21 (Spearman $r=0.41$, $P=0.012$), day 28

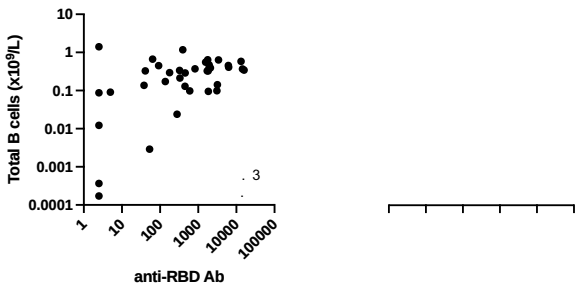
(Spearman $r = 0.38$, $P = 0.022$) and day 49 (Spearman $r = 0.31$, $P = 0.06$; univariate linear regression, $P = 0.13$).

In multivariate analysis, ongoing moderate/severe chronic GVHD ($n = 9$) was the only factor significantly associated with an absence of response to the vaccine

We next compared NT50 Ab in naive healthy adults and naive allo-HCT recipients at day 49 after vaccina-



	D y 49 t - BD A	D y 49N 50 A	N v CD4	FH	N v B lls	SMB lls	U MB lls	
D y 49 t - BD A		3.17108508166480 -015	0.0005	0.015	0.011	0.00003	0.000008	. 2
D y 49N 50 A	3.17108508166480 -015		0.0010	0.063	0.027	0.00002	0.000027	.
N v CD4	0.00052486791303	0.00103302710544		0.013	0.094	0.06787	0.049130	.
FH	0.01488436618793	0.06253567491882	0.0133		0.022	0.00759	0.013386	.
N v B lls	0.01137618734814	0.02669305788005	0.0941	0.022		5.47607 -008	0.000002	.
SMB lls	0.00002863463374	0.00001913847706	0.0679	0.008	5.476 -008		. 2 322 - 2	.
U MB lls	0.00000802931544	0.00002654706497	0.0491	0.013	2.189 -006	6.82932 -012		.
A	0.01966536622644	0.04533485800400	0.0768	0.018	0.904	0.78498	.	



us to examine whether absolute B cell and B cell subset counts calculated using manual gating correlated with anti-RBD Ab levels. We observed a weak correlation between absolute B cell counts and anti-RBD Ab levels: at day 21 (Spearman $r=0.34$, $P=0.039$), day 28 (Spearman $r=0.39$, $P=0.02$) and day 49 (Spearman $r=0.43$, $P=0.007$). A weak correlation was also observed with naive B cells: at day 21 (Spearman $r=0.31$, $P=0.06$), day 28 (Spearman $r=0.37$, $P=0.02$) and day 49 (Spearman $r=0.41$, $P=0.01$). ere was however a much stronger correlation between class-switched memory B cell counts and anti-RBD Ab levels at day 21 (Spearman $r=0.56$, $P=0.0003$), day 28 (Spearman $r=0.53$, $P=0.0007$) and day 49 (Spearman $r=0.63$, $P<0.0001$). Unswitched memory B cell counts correlated also with anti-RBD Ab levels: at day 21 (Spearman $r=0.51$, $P=0.0011$), day 28 (Spearman $r=0.53$, $P=0.0008$) and day 49 (Spearman $r=0.66$, $P<0.0001$) (Fig. 3). Finally, looking at associations between B cell subset frequencies among absolute lymphocytes and anti-RBD Ab levels at day 49, we observed

anti-RBD Ab 28 days after the second dose [22]. Comparing anti-RBD Ab levels in allo-HCT patients and in adult controls, we observed significantly lower Ab levels in allo-HCT patients at each time point. In addition, only half of allo-HCT patients had detectable neutralizing Ab against WT SARS-CoV-2 at day 49 while allo-HCT recipients had significantly lower neutralizing Ab titers than healthy controls at that time point. Restricting the analyses to allo-HCT patients without GVHD and without rituximab administration in the year before vaccination, we still observed significantly lower Ab levels in allo-HCT recipients at day 21 but Ab levels were comparable to healthy controls at day 49. e same was true for neutralizing Ab titers. ese results emphasize the importance of a timely second vaccination in this population.

A second important observation was that ongoing moderate/severe chronic GVHD was associated with a lower Ab response to the vaccine both after 1 and 2 vaccine doses. Indeed, 5 out of 9 patients with moderate/severe chronic GVHD failed to develop anti-RBD Ab following the 2 vaccine doses, while 3 additional patients had anti-RBD Ig titers below 200 IU/mL on day 49. Accordingly, none of the patients with ongoing moderate/chronic GVHD had neutralizing antibodies against the WT SARS-CoV-2 at day 49. e impaired response to mRNA vaccine in patients with chronic GVHD is likely due to delayed/disrupted return to immune homeostasis in chronic GVHD patients leading to defects in key cell populations. Indeed, it is well known that chronic GVHD (and its treatment) has a profound impact on immunity after allo-HCT, a ecting many cell subtypes such as B cells, $CD4^+$ T cells, naive $CD4^+$ T cells, TfH and $CD8^+$ T cells [8, 23, 24]. Alternatively, ongoing chronic GVHD might distract from coordinated immune response to mRNA vaccine by driving concurrent immune responses against host antigens. Previous clinical studies have observed lower response rates to pneumococcal conjugate vaccine, hepatitis B vaccine, tetanus vaccine and influenza vaccine in patients with chronic GVHD [25–28]. However, chronic GVHD had a modest or no impact on the response to diphtheria and hemophilus influenza type B vaccination [27].

In our cohort, all allo-HCT patients without chronic GVHD had detectable Ab on day 49 after vaccination. However, only 64% of them had neutralizing antibodies against the WT SARS-CoV-2 at day 49. Looking at factors associated with Ab levels in the subgroup of naive patients without moderate/severe chronic GVHD, we observed that patients given rituximab 6 months to 1 year before vaccination had lower Ab titers. is is in line with what was observed in patients with chronic lymphocytic leukemia [14], B-cell non-Hodgkin lymphoma [29], and multiple sclerosis [30]. We also observed a negative correlation between Ab levels and age, particularly in the subgroup of patients without chronic GVHD and without recent rituxi-

poor responses to the vaccine in aged and in allo-HCT (and particularly those with chronic GVHD) patients [33].

Conclusions

In summary, we observed that allo-HCT patients without moderate/severe chronic GVHD and not given rituximab within 1 year before vaccination had comparable anti-RBD Ab levels to those of healthy adults following two doses of the vaccine. However, moderate/severe chronic GVHD and rituximab administration were associated with lower Ab levels in allo-HCT recipients. Administra

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