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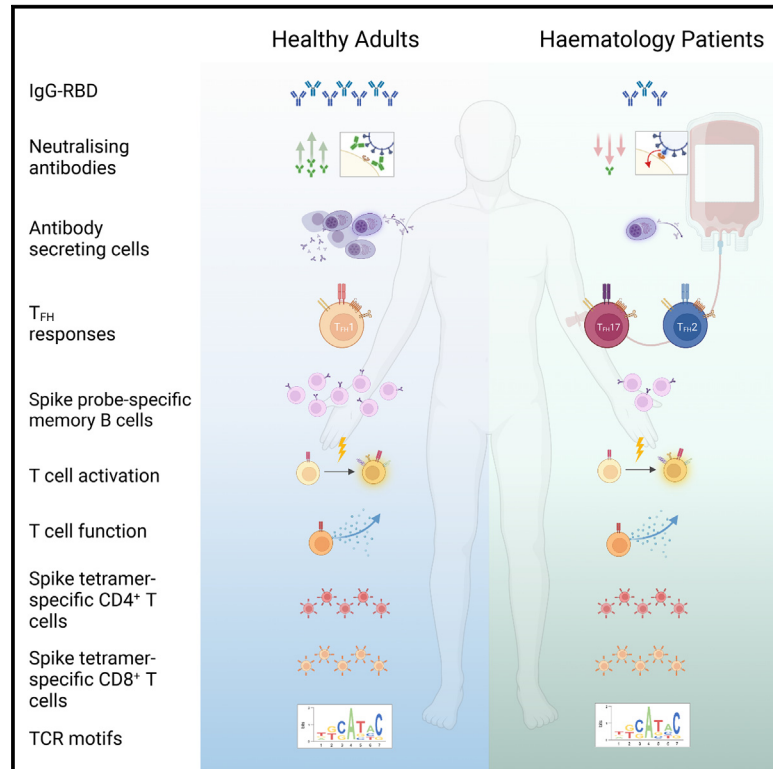
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Robust SARS-CoV-2 T cell responses with common TCR $\alpha\beta$ motifs toward COVID-19 vaccines in patients with hematological malignancy impacting B cells

Graphical abstract



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In brief

Nguyen et al. define antibody, B, and T cell responses following COVID-19 vaccination in patients with hematological malignancy that are immunocompromised and vulnerable to severe COVID-19 infection. COVID-19 vaccination induces robust T cell immunity in hematology patients with diseases and treatments impacting B cell immunity irrespective of B cell numbers and antibody responses.

Highlights

- COVID-19 vaccines elicit robust SARS-CoV-2-specific T cells in hematology patients
- Prevalent TCR $\alpha\beta$ motifs occur within tetramer⁺ T cells in hematological patients
- Conversely, perturbed antibody responses and skewing of T_{fh} and ASC/T_{fh} kinetics occur
- Breakthrough infection patients have higher antibodies and comparable T cell responses



Article

Robust SARS-CoV-2 T cell responses with common TCR $\alpha\beta$ motifs toward COVID-19 vaccines in patients with hematological malignancy impacting B cells

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SUMMARY

Immunocompromised hematology patients are vulnerable to severe COVID-19 and respond poorly to vaccination. Relative deficits in immunity are, however, unclear, especially after 3 vaccine doses. We evaluated immune responses in hematology patients across three COVID-19 vaccination doses. Seropositivity was low after a first dose of BNT162b2 and ChAdOx1 (~26%), increased to 59%–75% after a second dose, and increased to 85% after a third dose. While prototypical antibody-secreting cells (ASCs) and T follicular helper (Tfh) cell responses were elicited in healthy participants, hematology patients showed prolonged ASCs and skewed Tfh2/17 responses. Importantly, vaccine-induced expansions of spike-specific and peptide-HLA tetramer-specific CD4⁺/CD8⁺ T cells, together with their T cell receptor (TCR) repertoires, were robust in hematology patients, irrespective of B cell numbers, and comparable to healthy participants. Vaccinated patients with breakthrough infections developed higher antibody responses, while T cell responses were comparable to healthy groups. COVID-19 vaccination induces robust T cell immunity in hematology patients of varying diseases and treatments irrespective of B cell numbers and antibody response.

INTRODUCTION

Patients with hematological malignancies such as chronic lymphocytic leukemia (CLL) and multiple myeloma (MM) and those

in the early period following hematopoietic stem cell transplantation (HCT) and chimeric antigen receptor T (CAR-T) therapy are at higher risk for viral respiratory tract infections, including COVID-19.^{1–3} Up to half of hematology patients with COVID-19



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present with severe disease and require hospital admission, 15% require intensive care, and mortality rates can reach 30%–40%.^{1,2} Furthermore, immune suppression from underlying disease, immune reconstitution following cellular therapies (HCT, CAR-T), and ongoing treatments such as anti-CD20 monoclonal antibody therapies continue to drive risk for COVID-19 infection but concurrently impact protective responses from vaccination.^{4–6} To prevent severe SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection in this immunosuppressed high-risk group of patients, there is an urgent need to comprehensively profile their immune response to COVID-19 vaccination to better understand the interplay between underlying disease, treatment and optimal correlations of protection so that vaccination strategies can be enhanced.

Detection of circulating SARS-CoV-2-specific receptor-binding domains (RBDs) and neutralizing antibodies are widely utilized as surrogate endpoints for evaluation of vaccine efficacy in hematology patients,^{7,8} yet it is unclear whether these are appropriate endpoints, especially in the setting of B cell depletion. Patients with B cell hematological malignancies such as CLL, patients in the early period following HCT and CAR-T therapy, and those receiving active therapy and B cell-depleting therapies (anti-CD20, BTK inhibitors) within 12 months have low humoral response rates to SARS-CoV-2 vaccination.^{7,9}

Serological endpoints offer only a glimpse of the potential breadth of immune response to vaccination and may not be the best predictor of efficacy. Cellular responses reported to date relied on limited measurements of SARS-CoV-2-specific T cell responses.^{9–11} Early data suggest a lack of correlation between humoral and cellular responses in patients with hematological malignancy, and cellular responses tend to be higher in B cell-depleted patients than non-B cell-depleted patients.^{9,12} Furthermore, the majority of studies in hematology patients were performed following 2 COVID-19 vaccine doses.^{13–15}

In hematology patients hospitalized with COVID-19, robust CD8⁺ T cell responses correlated with better outcomes,

including among those treated with anti-CD20 therapy.¹⁶ Similarly, in patients with multiple sclerosis (MS), SARS-CoV-2-specific antibody and memory B cell responses were reduced in patients on monoclonal anti-CD20 treatment following SARS-CoV-2 mRNA vaccination; however, all patients generated robust spike-specific CD4⁺ and CD8⁺ T cell responses.¹⁷

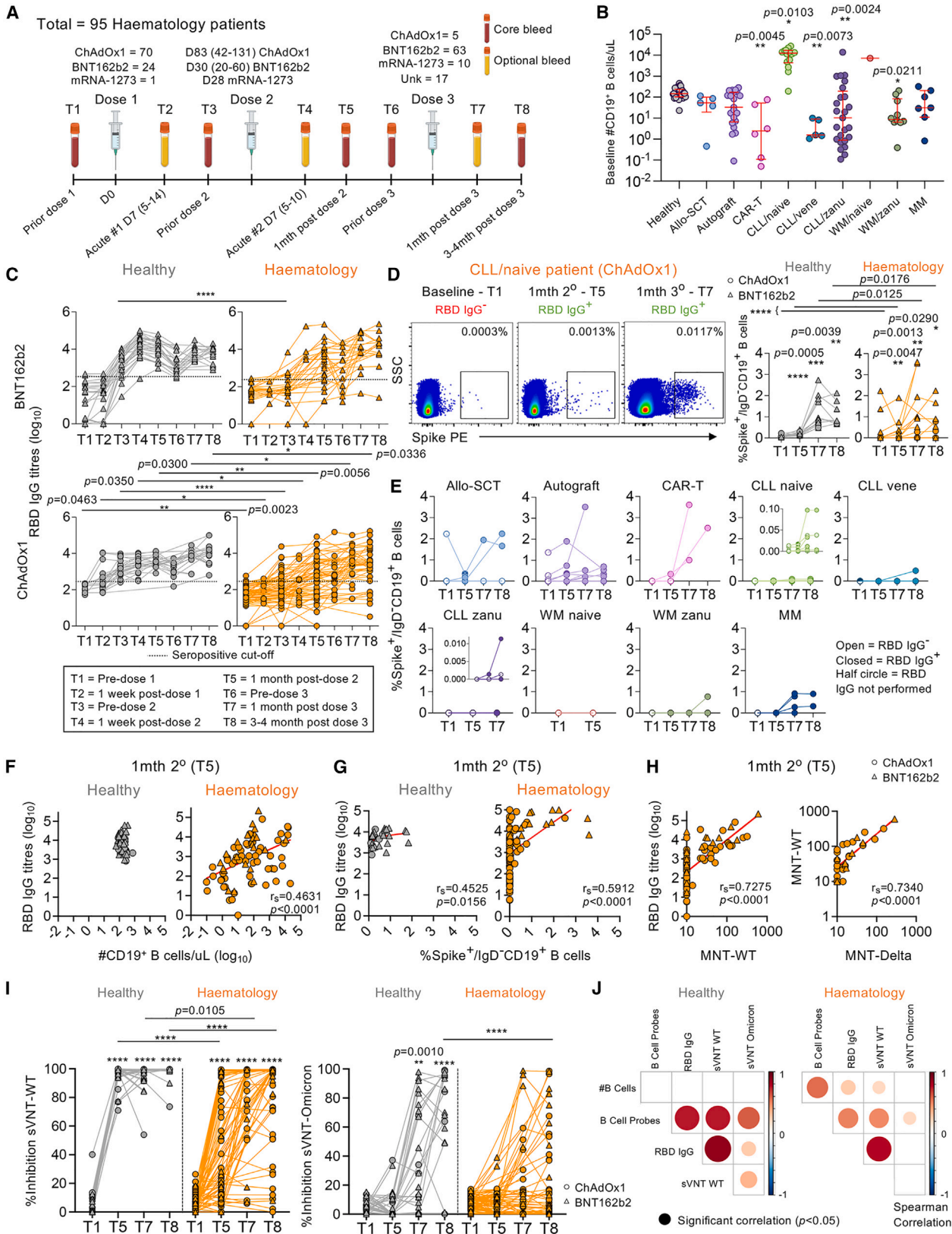
We have previously compared and contrasted *ex vivo* SARS-CoV-2-specific CD8⁺ and CD4⁺ T cell responses and their T cell receptor (TCR) repertoires in SARS-CoV-2-infected children and adults,^{18–21} pre-pandemic children and adults,^{18,19} and following COVID-19 mRNA vaccination versus infection,^{22,23} revealing diverse TCR repertoires with prominent TCR $\alpha\beta$ motifs that were shared between different individuals.^{18,20–24} Whether these prominent TCR $\alpha\beta$ signatures are observed in hematology patients following COVID-19 vaccination remains to be elucidated, particularly in those with B cell malignancies or following ChAdOx1 vaccination.

We evaluate the breadth of immune responses following COVID-19 vaccination in hematology patients with diseases and treatments impacting B cell immunity, where scant data exist after the third dose. Our study shows that hematology patients who fail to seroconvert and generate memory B cell responses post-vaccination can still generate robust SARS-CoV-2-specific T cell immunity to protect against severe and fatal COVID-19.

RESULTS

COVID-19 vaccination cohort

To assess immunological responses toward COVID-19 vaccines in hematology patients, 95 SARS-CoV-2-unexposed, seronegative patients were recruited between April and December 2021 (Figure 1A; Table S1). Patients were predominantly male compared with healthy individuals (71% versus 29%). For comparison, 58 healthy SARS-CoV-2-unexposed, seronegative participants were recruited during the same period (26 ChAdOx1, 32



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BNT162b2). During the study, 12 patients and 8 healthy individuals had breakthrough SARS-CoV-2 infection (Table S1). ChAdOx1 and BNT162b2 vaccine responses were analyzed separately or grouped together with different symbols. Subjects labeled as having BNT162b2 or ChAdOx1 were based on their two doses.

Hematology patients have reduced RBD-specific IgG antibodies, memory spike-specific B cells, and neutralizing antibodies following COVID-19 vaccination

Given the heterogeneity in the hematology cohort, blood B cell numbers varied in patients compared with healthy individuals (Figure 1B), with higher numbers in patients with CLL not on treatment but lower numbers in CAR-T patients, patients with CLL on venetoclax, patients with CLL on zanubrutinib, and patients with Waldenstrom macroglobulinemia on zanubrutinib. ELISA immunoglobulin G (IgG) antibody responses directed at RBDs corresponding to the ancestral vaccine strain (herein called RBD IgG) were lower in BNT162b2-vaccinated hematology patients compared with healthy individuals prior to the second vaccine dose (T3) but were comparable after the second and third doses (Figure 1C). RBD IgG antibody responses in ChAdOx1-vaccinated patients were lower than healthy individuals at all time points. Patient seropositivity was lower after first dose (26%, T3, $n = 87$), then increased to 65% 1 month after the second dose (T5, $n = 82$) and 87% 1 month after the third dose (T7, $n = 30$). Seropositivity remained stable at 84% ~3–4 months post-third dose (T8, $n = 56$), indicating that a small proportion of patients, particularly patients with CLL on zanubrutinib (CLL/zanu), still did not have any robust RBD IgG antibody responses after 3 COVID-19 vaccinations. Healthy individuals were 91% seropositive after the primary vaccination and 100% seropositive after the second- (T5) and third-dose vaccinations (T7 and T8). Heterogeneity of RBD IgG antibody responses were observed when hematology patients were grouped by low, normal, or high B cell numbers or by disease/treatment group (Figures S1A–S1C).

COVID-19 vaccination induced gradual significant increases in memory spike probe-specific IgD⁺ B cells in both healthy individuals and hematology patients of the same magnitude range (Figures 1D and S2A). However, hematology patients' spike-specific B cell responses were significantly lower than healthy individual responses after vaccination due to some of the patients

not having detectable memory spike-specific B cell responses, particularly those with B cell malignancies (CLL, Waldenstrom macroglobulinemia [WM], MM) (Figures 1D and 1E) or abnormal B cell numbers (Figure S1D). Total B cell numbers and frequencies of memory spike-specific B cells correlated with RBD IgG antibody titers in hematology patients ~1 month after the second vaccine, but only memory spike-specific B cell frequencies correlated with antibody titers in healthy individuals given the narrower range in B cell numbers (Figures 1F and 1G). Patients' RBD IgG antibody titers strongly correlated with microneutralizing titers (MNTs) against the ancestral strain (Figure 1H) and, to a lesser extent, the Delta strain ($r_s = 0.5994$, $p < 0.0001$, data not shown), whereas ancestral and Delta MNTs were strongly correlative (Figure 1H).

Ancestral surrogate virus neutralization test (sVNT) neutralizing antibodies increased following COVID-19 vaccination in both healthy individuals and hematology patients, but increases in Omicron (B.1.1.529; BA.1-like) sVNT antibodies were only observed in healthy individuals (Figure 1I). Neutralizing sVNT responses were lower in hematology patients compared with healthy individuals after the second and third doses using the ancestral strain and were also lower ~3–4 months after the third dose using the B.1.1.529 Omicron strain.

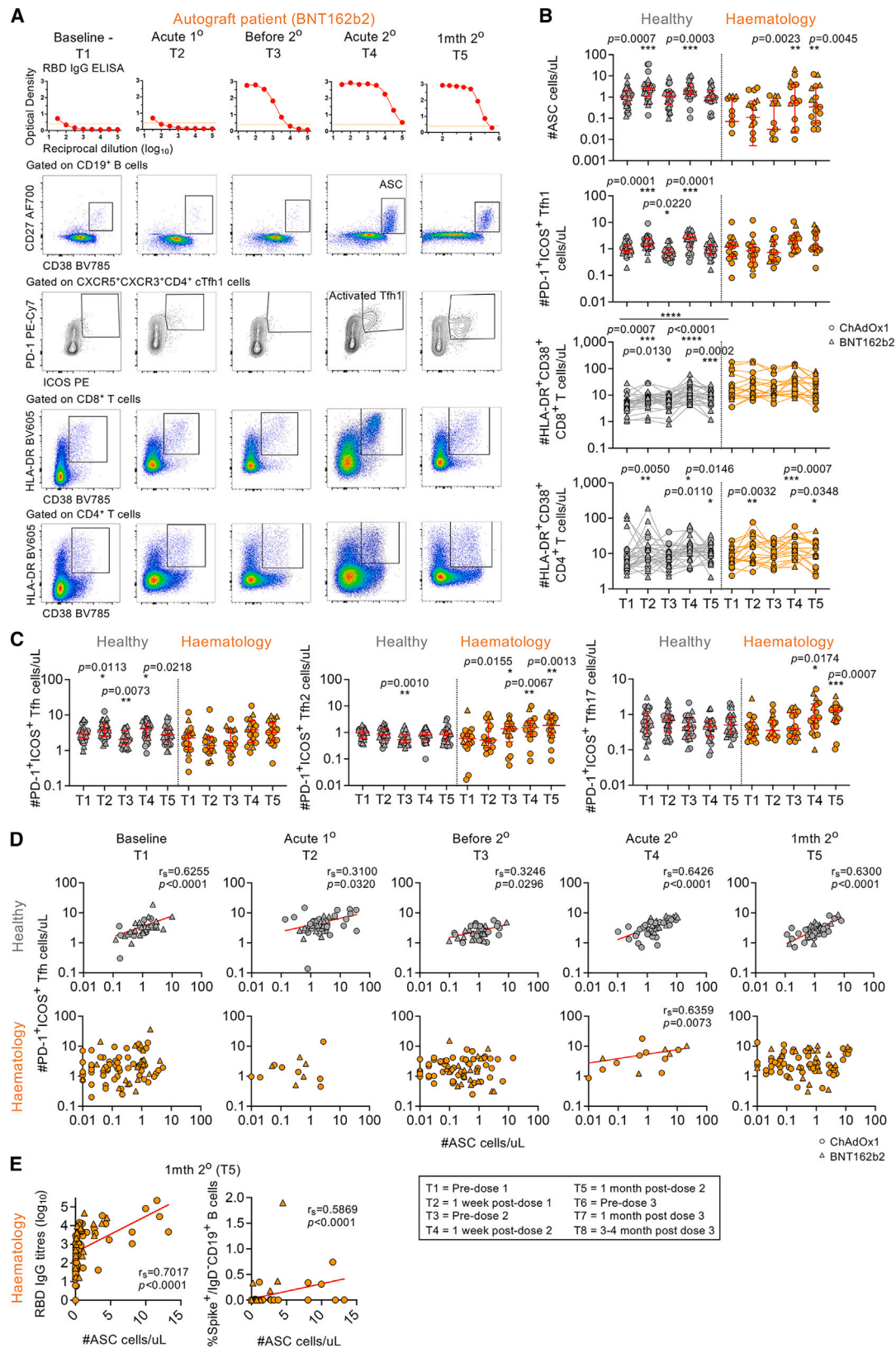
Correlation matrix of antibody and B cell responses showed B cell numbers only correlating with spike-specific memory B cells in hematology patients (Figure 1J). Correlations of spike-specific memory B cells with antibody responses were also stronger for healthy individuals compared with patients. Thus, antibody and B cell responses following COVID-19 vaccination were reduced compared with otherwise healthy individuals, likely due to their disease state and immunosuppressive treatment, thus explaining the low B cell numbers.

COVID-19 vaccination induces non-prototypical activation of ASCs, Tfh cells, and CD8⁺ T cells in hematology patients

Transient activation of CD27^{hi}CD38^{hi} antibody-secreting cells (ASCs) and circulating PD-1⁺ICOS⁺ CXCR5⁺ T follicular helper (Tfh) cells peak in the blood ~7–10 days after SARS-CoV-2 infection using whole-blood flow cytometry assays.^{25,26} Induction of activated Tfh cells also occurs in the blood and draining lymph nodes following COVID-19 mRNA vaccination.^{22,27} Dual ASC and Tfh cell responses have also been observed following

Figure 1. RBD-specific IgG antibodies, neutralizing antibodies, and memory spike-specific B cell responses following COVID-19 vaccination

(A) Study design and sampling timepoints.
(B) B cell numbers per μL with median and interquartile range (IQR) shown (hematology $n = 94$; healthy $n = 39$). Statistical significance determined by Dunn's multiple comparisons set on healthy versus all other disease groups.
(C) Endpoint IgG titers of ancestral RBD antibodies (hematology $n = 94$, 24 BNT162b2, 70 ChAdOx1; healthy $n = 53$, 27 BNT162b2, 26 ChAdOx1). Seropositive cutoff defined by baseline mean + 2 SD per group.
(D and E) Spike-probe staining of spike-specific memory B cells in (D) healthy ($n = 16$) and hematology groups ($n = 64$) and (E) per malignancy and treatment groups.
(F and G) Spearman's correlation (r_s) of RBD IgG titers with (F) B cell numbers (hematology $n = 82$; healthy $n = 45$) and (G) spike-specific memory B cells (hematology $n = 64$; healthy $n = 16$).
(H) r_s of RBD IgG titers with ancestral MNT and ancestral versus Delta MNT (hematology $n = 82$).
(I) Percentage sVNT inhibition assay against wild-type (WT) ancestral and Omicron strains (haematology $n = 63$; healthy $n = 29$).
(J) r_s matrix of B cell and antibody parameters at T1/T5/T7/T8.
Statistical significance determined by Wilcoxon test for time point comparisons against T1 or by Mann-Whitney for comparisons between healthy and patient time points. **** $p < 0.0001$. Experiments were performed once for each sample. Refer to Figure S2A.



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influenza virus infection²⁸ and influenza vaccination,²⁹ with the predominant Tfh subset being Tfh type 1 cells (Tfh1 cells; CXCR3⁺CCR6⁻). We performed whole-blood staining on a subset of 30 hematology patients that underwent intense blood sampling to capture transient acute responses following first and second COVID-19 vaccination doses (Figures 2A and S2B).

Healthy individuals showed prototypical vaccine-induced responses with transient increases in CD27^{hi}CD38^{hi} ASCs, PD-1⁺ICOS⁺-activated Tfh1 cells, and HLA-DR⁺CD38⁺-activated CD8⁺ and CD4⁺ T cells acutely observed at ~7 days after the first (T2) and second COVID-19 vaccination doses (T4) when compared with baseline T1 levels (Figure 2B). In hematology patients, ASCs only increased at ~7 days after the second dose (T4) and remained high at ~1 month post-second dose (T5) (Figures 2A and 2B). PD-1⁺ICOS⁺ Tfh responses were skewed toward type 2 (Tfh2; CXCR3⁻CCR6⁻) and type 17 subsets (Tfh17; CXCR3⁻CCR6⁺), which similarly increased and remained high after the second dose (Figure 2C). Since hematology patients had higher baseline levels of activated HLA-DR⁺CD38⁺CD8⁺ T cells compared with healthy individuals, HLA-DR⁺CD38⁺CD8⁺ T responses were not further induced by two doses of COVID-19 vaccination (Figure 2B). In contrast, HLA-DR⁺CD38⁺ CD4⁺ T cell responses were prototypical of those observed in healthy individuals with acute transient increases after the first and second doses (Figure 2B).

Numbers of ASCs typically correlated with Tfh numbers across all time points measured (T1–T5) in healthy individuals, but this correlation was only observed at ~7 days post-second dose (T4) in hematology patients (Figure 2D). At T4, ASCs correlated with each Tfh subset in healthy individuals, whereas Tfh1 cells, but not Tfh2 or Tfh17 cells, correlated with ASCs in hematology patients (Figure S3A), even though we did not observe significant increases in Tfh1 responses but rather observed significant increases in the other Tfh2/17 subsets. HLA-DR⁺CD38⁺ CD8⁺ and CD4⁺ T cells correlated with each other across all time points in both healthy individuals and hematology patients except for ~7 days after the first vaccine dose (T2) in hematology patients (Figure S3B).

Overall, hematology patients needed two vaccination doses to generate ASC and Tfh responses, but there was prolonged activation of ASCs, skewing of Tfh2/17 responses, and high basal levels of activated CD8⁺ T cells.

Comparable spike-specific CD4⁺ and CD8⁺ T cell responses in hematology patients and healthy individuals post-COVID-19 vaccination

To evaluate COVID-19 vaccine-induced spike-specific T cell responses, we performed activation-induced marker (AIM) and intracellular cytokine staining (ICS) assays (Figures 3A, S2C,

S2D, and S4A). AIM frequency of CD134⁺CD137⁺CD4⁺ and CD69⁺CD137⁺ CD8⁺ cells increased at ~1 month post-second dose (T5) compared with baseline (T1) by a mean 32- and 31-fold for healthy individuals and a higher mean 87- and 75-fold for patients, respectively (Figure 3B), which was not due to any differences in baseline threshold levels at T1. These increases in AIM CD4⁺ and CD8⁺ T cell responses were maintained after the third dose (T7 and T8) in both healthy individuals and hematology patients. Unlike antibody and memory B cell responses, there was no difference in AIM responses between healthy individuals and hematology patients at any time point (Figure 3B).

Similarly, functional ICS assay showed increased interferon γ (IFN γ)⁺tumor necrosis factor α (TNF- α)⁺ CD4⁺ and CD8⁺ T cell responses for both healthy individuals and hematology patients at all time points post-COVID-19 vaccination (T5, T7, and T8) with mean 3- to 19-fold and 6- to 36-fold increases for healthy individuals and mean 11- to 31-fold and 10- to 44-fold for hematology patients, respectively (Figure 3C). Frequencies of IFN γ ⁺TNF- α ⁺ CD4⁺ and CD8⁺ T cells were higher for patients at baseline (T1) and ~1 month post-second dose (T5) compared with healthy individuals, but frequencies were comparable after the third dose. Importantly, T cell responses were observed across disease groups by AIM (Figure 3D) or ICS assay (Figure S4B). Autograft patients had overall higher CD134⁺CD137⁺ and IFN γ ⁺TNF- α ⁺ CD4⁺ T cell frequencies at ~1 month post-second dose (T5) when compared with healthy individuals, whereas CLL/naive patients had higher IFN γ ⁺TNF- α ⁺ CD4⁺ and CD8⁺ T cell frequencies (Figures 3E and S4C).

Correlations of CD4⁺ versus CD8⁺ T cell responses and of AIM versus ICS responses were more observed in healthy individuals compared with hematology patients (Figure S4D). AIM Tfh responses showed increases in CXCR5⁺ Tfh and CXCR5⁺CXCR3⁺ Tfh1 responses in both groups following COVID-19 vaccination, while CXCR5⁺CXCR3⁻ Tfh2/17 responses decreased (Figures 3F and S5A). Based on serostatus, RBD IgG⁺ patients generated more robust AIM and ICS CD4⁺ and CD8⁺ T cell responses compared with RBD IgG⁻ patients (Figure 3G). Correlations of T cell responses against antibody and B cell responses were stronger and more frequent in healthy individuals compared with hematology patients (Figure 3H).

Spike-specific CD4⁺ and CD8⁺ T cell responses were fairly similar between ChAdOx1- or BNT162b2-vaccinated patients and compared with healthy groups (Figures S5B and S5C). T cell responses were also comparable between hematology patients with low, normal, or high B cell numbers (Figure S1E). This was exemplified by volcano plot analyses combining antibodies with spike-specific memory B and T cell responses (Figures 3I and S6), where humoral responses were more enriched in

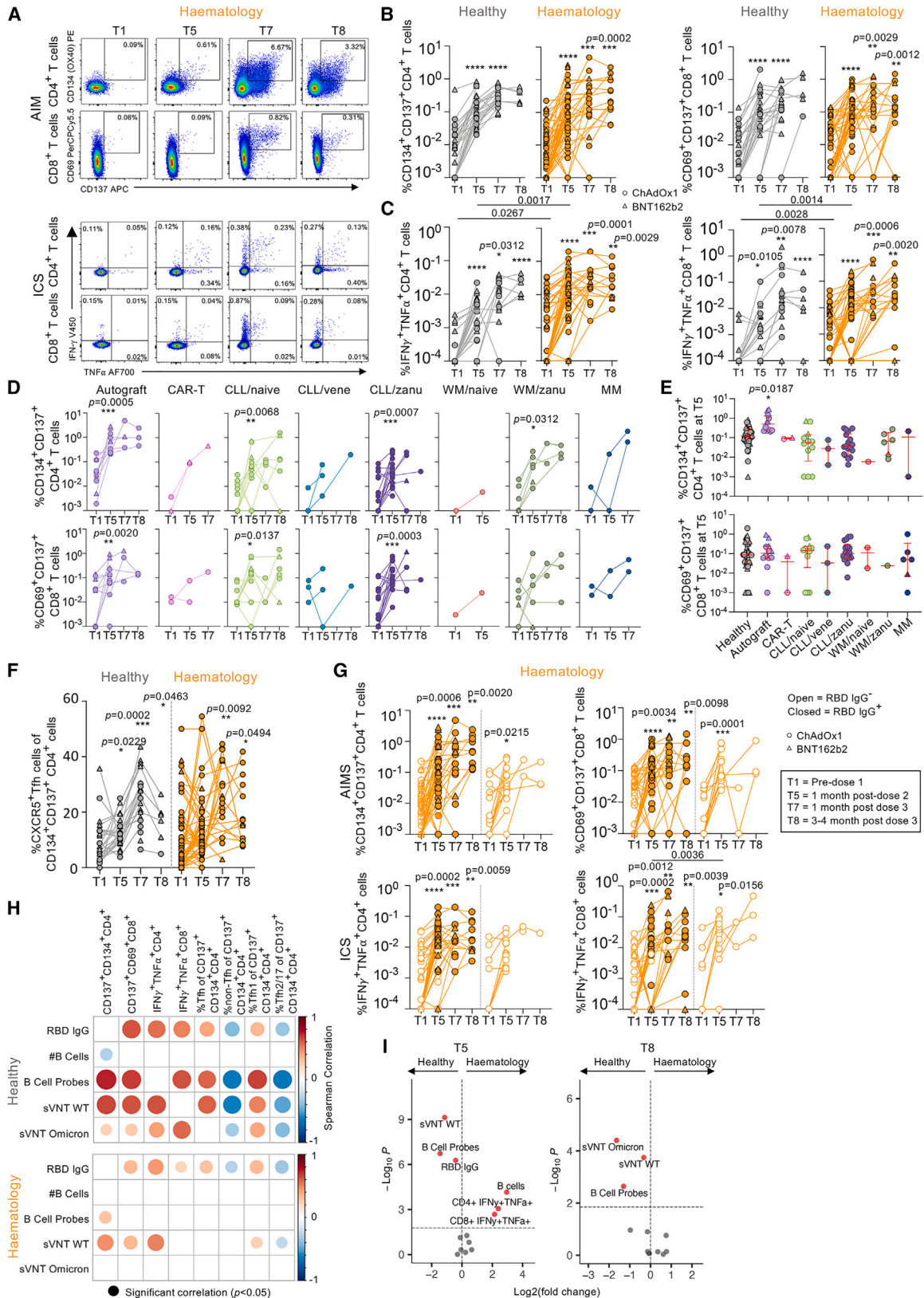
Figure 2. Whole-blood analyses of acute ASC, Tfh cell, and activated CD8⁺ and CD4⁺ T cell responses

(A) RBD IgG ELISA titration curves and fluorescence-activated cell sorting (FACS) plots of ASCs and activated Tfh1/CD8⁺/CD4⁺ T cells. Orange dotted lines indicate endpoint titer cutoffs.

(B) Numbers of ASCs and activated Tfh1 cells and CD8⁺ and CD4⁺ T cells per μ L (hematology n = 17; healthy n = 39).

(C) Numbers of Tfh, Tfh2, and Tfh17 subsets per μ L.

(D) r_s of ASCs and activated Tfh1 cells per time point T1–T5 (T1/T3/T5 hematology n = 94; T2/T4 hematology n = 17). Statistical significance determined by Wilcoxon test for time point comparisons against T1 (floating values) or by Mann-Whitney for comparisons between healthy and patient time points (connecting line). ****p < 0.0001. Experiments were performed once for each sample. Refer to Figures S2B and S3. Zero data points not shown but included in statistics.



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healthy individuals after the second and third doses compared with hematology patients, irrespectively of whether they had low or normal to high B cells.

Following three-dose COVID-19 vaccination, hematology patients of varying diseases and immunosuppressive treatments, including those with B cell deficiencies, can still generate robust and functional spike-specific T cell responses.

SARS-CoV-2 peptide-HLA tetramer-specific T cell responses increase post-COVID-19 vaccination in hematology patients

To define SARS-CoV-2 epitope-specific CD4⁺ and CD8⁺ T cell responses, peptide-HLA tetramers combined with tetramer-associated magnetic enrichment (TAME)^{18,21} (Figures 4A and S2E) were used to measure directly *ex vivo* CD4⁺ T cell responses directed against the prominent DPB4/S₁₆₇ epitope^{21,22} and CD8⁺ T cell responses against 6 immunodominant CD8⁺ T cell epitopes (A1/S₈₆₅, A2/S₂₆₉, A3/S₃₇₈, A24/S₁₂₀₈, B15/S₉₁₉, and B35/S₃₂₁).^{18–20,23,30,31} These epitopes originated from the ancestral SARS-CoV-2 spike and are highly conserved across variant of concern (VOC) strains.^{21,32} B15/S₉₁₉ shares similar homology and is highly cross-reactive to common cold coronaviruses (HKU1/OC43).²³

Two doses of mRNA COVID-19 vaccination have previously been shown to induce robust CD4⁺ and CD8⁺ tetramer⁺ T cell responses.^{22,23,33} We observed robust expansions of pooled tetramer⁺ CD4⁺ and CD8⁺ T cell frequencies after the third dose with either vaccine (Figure 4B). Baseline tetramer precursor frequencies weakly correlated with tetramer responses at T5 and T7/T8 (Figure 4C). There was no correlation between tetramer-specific T cell responses and B cell numbers at T1 or T5, with weak correlations based on RBD serostatus (Figures 4D and S7A). Patients' tetramer⁺ T cell frequencies increased following three-dose COVID-19 vaccination, irrespectively of RBD IgG serostatus (Figure 4E) or B cell numbers (Figure S1F), similar to healthy individuals (Figure S7B). Cross-reactive B15/S₉₁₉ epitope had comparable or lower baseline frequency (mean 3.26×10^{-6}) than other CD8⁺ epitopes in patients and healthy individuals (4.47×10^{-5}), suggesting no numerical cross-reactive advantage. Importantly, increase and maintenance of SARS-CoV-2-epitope T cell responses following COVID-19 vaccination were observed across all malignancy and treatment groups, except for the WM/naive group, which was small in number and not followed up with after the second dose (Figure 4F). Most patient groups generated similar response magnitudes to healthy individuals at T5, except for CLL/venetoclax (vene) patients and patients with MM

(Figure 4G). CLL/vene patients also had lower tetramer⁺ T cell frequencies at T1 baseline and ~3 months post-third dose (T8) (Figure S7C). Venetoclax also targets T cells, which may explain the lower responses for CLL/vene patients.

Baseline tetramer⁺ phenotypes differed between healthy and hematology patients but converged post-vaccination (Figures 4H and S7D). This was attributed by overall baseline CD4⁺/CD8⁺ T cell phenotypes (Figure S7E) rather than individual epitope specificities (Figure S7F). Baseline tetramer⁺ T cell profiles from healthy individuals were more of a prototypical CD45RA⁺CD27⁺CD95⁻ naive-like phenotype compared with those of hematology patients, which gradually decreased post-vaccination. Tetramer⁺ cells from hematology patients displayed activated profiles, with elevated baseline CD45RA⁻CD27⁻ T effector memory (Tem)-like and CD45RA⁺CD27⁻ T effector memory CD45RA (Temra)-like features. Nevertheless, both groups had increasing CD45RA⁻CD27⁺ T central memory (Tcm)-like tetramer⁺ populations post-vaccination (Figure 4H), which is highly desirable for any T cell-based vaccine. Differences in phenotype profiles were also somewhat related to certain disease and treatment groups (Figure 4I).

Overall, hematology patients can generate robust SARS-CoV-2-specific T cell responses to a range of immunodominant spike epitopes following vaccination.

Hematology patients with COVID-19 breakthrough infections generate higher antibody responses than non-COVID-19 patients

During our study, 12 hematology patients had COVID-19 breakthrough infections (Figure 5). 2, 1, and 9 patients had COVID-19 after the first, second, and third vaccine doses, respectively. 3 patients were infected during the Delta period (June–July 2022), 6 during the emergence of Omicron (strain unknown, December 2021–January 2022), and 3 during the BA.1 Omicron wave (post-February 2022) where Delta was absent from circulation (Figure 5A). 8 healthy individuals had breakthrough COVID-19 after the third vaccine dose, which occurred during or after the emergence of BA.1 Omicron. All had mild COVID-19 infections, although 1 patient was hospitalized but not treated with any monoclonal antibodies, and 2 were treated with monoclonal antibody sotrovimab as outpatients (Figure 5B).

COVID-19⁺ patients had higher RBD IgG antibodies than COVID-19⁻ patients with 100% seropositivity at T8, consistent with an anamnestic response to the infection (Figure 5C). This was still significant when the 2 COVID-19⁺ patients treated with sotrovimab were excluded from the analysis ($p = 0.0077$, data not shown). There were no differences in MNT and

Figure 3. Comparable spike-specific CD4⁺ and CD8⁺ T cell responses between hematology patients and healthy individuals

(A) AIM and ICS FACS plots.

(B and C) AIM (B) and ICS (C) frequencies of CD4⁺ and CD8⁺ T cells in healthy ($n = 35$, 23 BNT162b2, 12 ChAdOx1) and hematology groups ($n = 56$, 8 BNT162b2, 48 ChAdOx1).

(D and E) AIM frequency (D) per malignancy and treatment group and (E) at T5 where median and IQR are shown. Statistical significance determined by Dunn's multiple comparisons set on healthy versus all other disease groups.

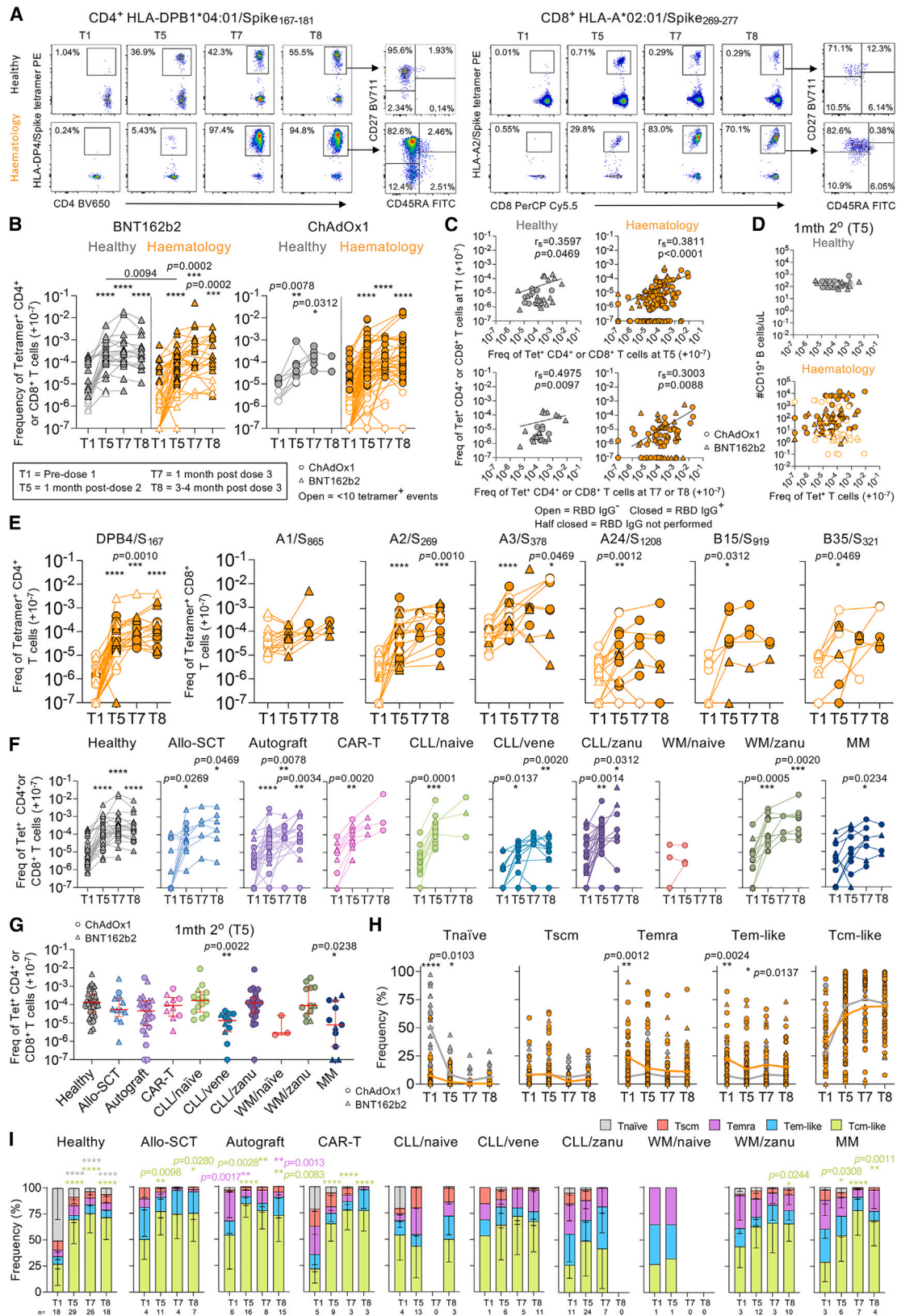
(F) CXCR5⁺CD4⁺ Tfh response of total CD134⁺CD137⁺ CD4⁺ T cells.

(G) AIM and ICS frequency between IgG RBD⁺ and RBD⁻ patients.

(H) r_s matrix of antibody/B cell and T cell responses.

(I) Volcano plots at T5 and T8 comparing healthy individuals and hematology patients.

Statistical significance determined by Wilcoxon test for time point comparisons against T1 (floating values) or by Mann-Whitney for comparisons between healthy and patient time points (connecting line). **** $p < 0.0001$. Experiments were performed once for each sample. Refer to Figures S2C, S2D, and S4–S6.



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sVNT neutralizing responses (Figures 5D and 5E), although COVID-19⁺ patients had higher sVNT neutralization toward the ancestral strain at T8 compared with COVID-19⁻ patients. Spike-specific T cell responses were comparable between COVID-19⁻ and COVID-19⁺ healthy individuals and hematology patients (Figures 5F, 5G, 5H, S8A, and S8B). Tetramer⁺ phenotypes were comparable between breakthrough COVID-19⁺ patients and healthy individuals, although the activation status of one patient sampled during acute infection showed increased PD-1 expression (Figures 5I and S8C). Our hematology patient data agree with previous reports in healthy individuals,²³ showing that vaccination or vaccination plus infection can elicit robust spike epitope-specific T cell responses.

We also performed tetramer staining directed against non-spike epitopes for 3 breakthrough patients and 3 healthy participants (Figure S9A). Spike, ORF1a, and nucleocapsid-specific CD8⁺ T cells increased in breakthrough patients, while spike CD8⁺ T cell frequencies were maintained in healthy participants (Figure S9B) with phenotype changes post-infection (Figure S9C) but similar activation profiles for spike and non-spike-specific CD8⁺ T cells (Figure S9D).

SARS-CoV-2-specific T cells in hematology patients display prominent gene segment usage

TCR $\alpha\beta$ repertoires of dominant SARS-CoV-2 T cell epitopes have been identified as sharing similar motifs and gene usage in immunocompetent healthy individuals following SARS-CoV-2 infection^{18,20–24} and COVID-19 mRNA vaccination.²³ To determine whether the molecular signatures underpinning epitope-specific T cell responses were conserved in our cohort of COVID-19-vaccinated hematology patients, we determined TCR $\alpha\beta$ repertoires for 3 of the most prominent spike-specific CD4⁺ and CD8⁺ T cell epitopes, DPB4/S₁₆₇, A2/S₂₆₉, and A24/S₁₂₀₈, using single-cell TCR $\alpha\beta$ multiplex-nested RT-PCR after *ex vivo* tetramer enrichment.³⁴ A total of 637 paired TCR $\alpha\beta$ clonotypes from up to 17 hematology patients were analyzed across DPB4/S₁₆₇ (n = 17), A2/S₂₆₉ (n = 17), and A24/S₁₂₀₈ (n = 12) epitopes in terms of their clonotype composition and clonal expansion. These TCR $\alpha\beta$ repertoires were compared with previously published TCR datasets from healthy SARS-CoV-2-infected (DPB4/S₁₆₇, A2/S₂₆₉, and A24/S₁₂₀₈) and COVID-19-vaccinated (A2/S₂₆₉ and A24/S₁₂₀₈ only) individuals.^{18,21–23}

In line with previous reports,^{21,22} DPB4/S₁₆₇-specific CD4⁺ T cells in hematology patients displayed a heavy bias for

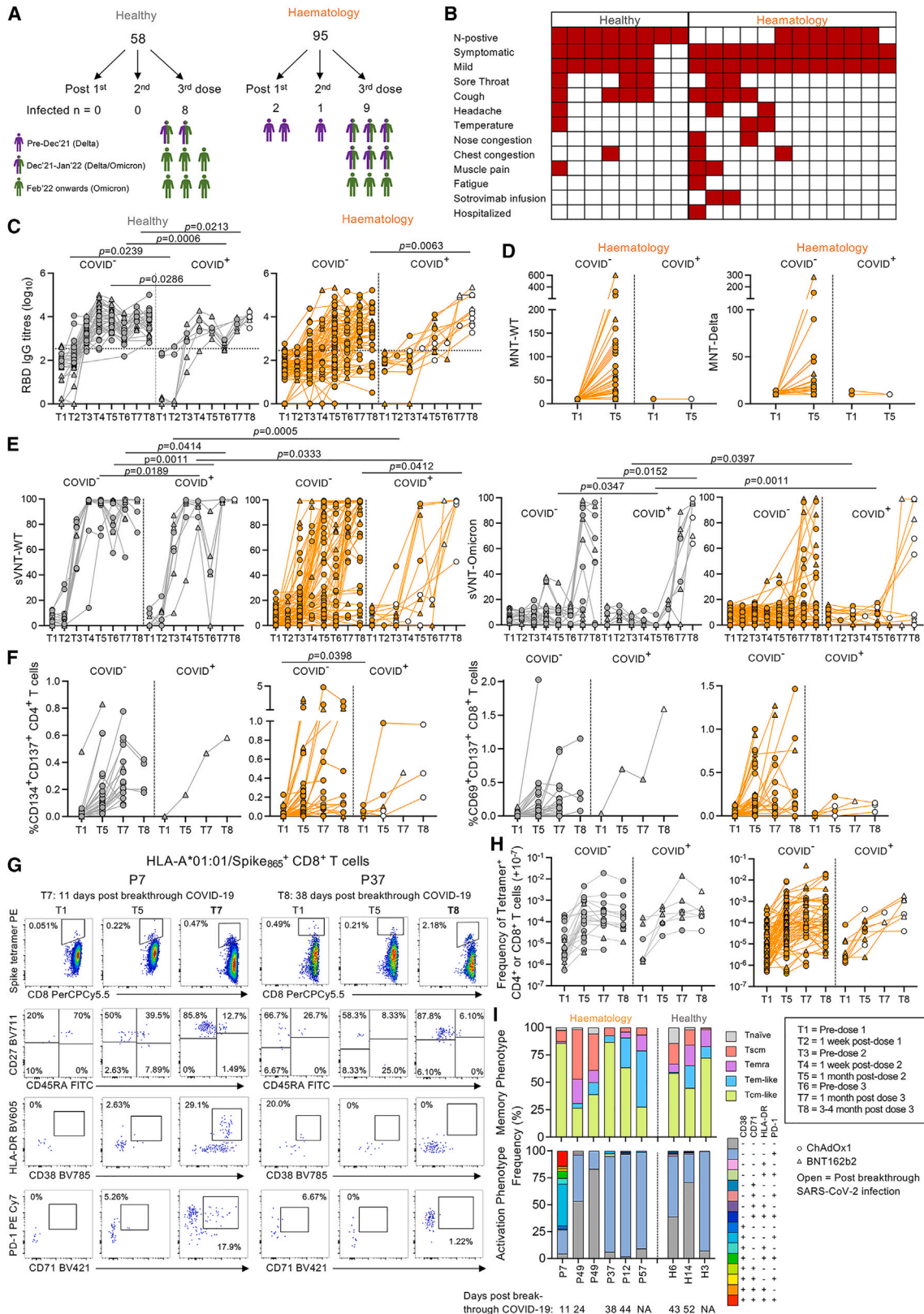
TRAV35/TRAJ42 gene segments (Figures 6A and S10; Table S2), which were paired with different TRBV/TRBJ genes. The A2/S₂₆₉-specific TCR repertoire in healthy infected and vaccinated cohorts is generally dominated by TRAV12-1 pairing with TRBV20-1 or TRBV7-9.^{18,21,23,24} In contrast, TRAV29 had the highest number of different clonotypes observed in hematology patients, followed by TRAV12-1, both of which were paired with TRBV2. In addition, both TRAV29 and TRAV12-1 were only observed in 4/17 donors tested with very little clonal expansions, while some hematology patients had very large clonal expansions of non-TRAV12-1 clonotypes pairing with the common TRBV20-1 gene (donor P46: 86% TRAV8-1; donor P51: 96% TRAV13-2) (Table S2). The A24/S₁₂₀₈-specific CD8⁺ TCR repertoire was more diverse in hematology patients, which has been observed previously²⁰; however, all 3 cohorts shared common TRAV19, TRAV21, and TRBV20-1 genes.

TCR sequence similarity network for each epitope identified prominent sharing of dominant motifs between vaccinated hematology and healthy infected cohorts for DPB4/S₁₆₇, followed by a smaller network for A2/S₂₆₉ connecting all 3 cohorts available, and the smallest network was for A24/S₁₂₀₈ (Figure 6B). The DPB4/S₁₆₇ network generated a common TRAV35/TRAJ42 sequence motif that represented both vaccinated hematology and healthy infected cohorts when motifs were analyzed separately per cohort (Figures 6B and S11), whereas TCR β motifs were very different between groups. Interestingly, the TCR α and TCR β network motifs for A2/S₂₆₉ were only observed in the healthy vaccinated and infected cohorts and not in vaccinated hematology patients, which mainly comprised of TRAV29/TRAJ45 and TRBV2-TRBJ2-2 motifs. The A24/S₁₂₀₈ TCR α and TCR β network motifs were only observed in vaccinated hematology patients, given the low number of sequences identified, while another TRBV5-6 motif was observed for healthy vaccinated individuals (Figures 6B and S11).

Strikingly, clonal expansions of diverse TCR clonotypes were most evident in hematology patients for A2/S₂₆₉ and A24/S₁₂₀₈ CD8⁺ T cells compared with healthy infected and healthy vaccinated cohorts. However, similarly to the network analyses, these clonotypes did not cluster as closely with each other compared with the DPB4/S₁₆₇ repertoire, which was closely clustered and sharing the same gene usage but had less evidence of clonal expansions from either vaccinated hematology and healthy infected cohorts (Figure 6C). Nevertheless, the probability to

Figure 4. COVID-19 vaccination induces expansion of SARS-CoV-2-specific tetramer⁺ T cell responses

(A) FACS plots of TAME-enriched CD4⁺ and CD8⁺ tetramer populations.
 (B) Tetramer CD4⁺ and CD8⁺ T cell frequencies of healthy (n = 16, ChAdOx1 = 4, BNT162b2 = 12) and hematology groups (n = 54, ChAdOx1 = 35, BNT162b2 = 19) per vaccine type. Any samples with <10 tetramer⁺ events are shown as open symbols. 1–3 tetramer responses shown per donor.
 (C) r_s of T1 tetramer frequencies versus T5 or T7/T8.
 (D) r_s of tetramer frequencies versus B cell numbers at T5.
 (E–G) Tetramer frequencies per (E) epitope, (F) malignancy and immunosuppressive treatment, or (G) at T5 where median and IQR are shown.
 (H) Phenotype frequencies of tetramer⁺ cells from healthy (gray line) and hematology patients (orange line).
 (I) Tetramer⁺ phenotype per malignancy and immunosuppressive treatment.
 The frequency of tetramer⁺ cells are right shifted by 10⁻⁷ (i.e., no detected tetramer⁺ events displayed as 10⁻⁷). Only samples with 10 or more tetramer⁺ events are included for (H) and (I). Statistical significance determined by Wilcoxon test for time point comparisons against T1 (floating values); Mann-Whitney for comparisons between healthy and patient timepoints (connecting line); (G) Dunn's multiple comparisons set on healthy versus all other disease groups; and (H) Sidak's multiple comparison test and (I) Dunnett's multiple comparison test for time point comparisons against T1. ****p < 0.0001. Experiments were performed once for each sample. Refer to Figures S2E and S7.



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generate TCRs were comparable across the cohort groups for each epitope (Figure 6D).

Overall, following COVID-19 vaccination, hematology patients could generate robust SARS-CoV-2-specific T cells that shared many common TCR signatures with healthy vaccinated and infected cohorts.

DISCUSSION

Our study provides comprehensive insights into humoral and cellular immune responses following COVID-19 vaccination in hematology patients with malignancies and treatments expected to eradicate or profoundly impair B cell function. We demonstrate that hematology patients mount effective SARS-CoV-2-specific T cell responses to COVID-19 vaccines irrespective of their B cell malignancy or B cell-depleting therapies, with which B cell numbers are greatly affected. Importantly, these T cell responses, detected directly *ex vivo* with peptide-HLA tetramers, are comparable to healthy individuals with respect to the magnitude, phenotype, TCR $\alpha\beta$ diversity, clonal composition, and motifs. Conversely, B cell numbers relating to disease status markedly impacted SARS-CoV-2-specific antibody levels and memory spike-specific B cell responses, together with ASC and Tfh skewing.

Our cohort is representative of a hematological malignancy population with B cell deficiencies arising from disease and/or therapies. Apart from patients with CLL, WM, and myeloma, other hematological malignancies and prior treatments are well represented in our post-cellular therapies cohort (allogeneic transplant, autologous transplant, and CAR-T therapy), including patients with non-Hodgkin's lymphoma treated with prior anti-CD20 antibody exposure. While our patient group is heterogeneous, breakdown of immune response is provided by individual disease/treatment group in the individual figures to allow a reader to consider/assess responses by a particular group of interest (Figure S12).

Consistent with previous studies, hematology patients had lower seroconversion rates following the first and second doses compared with healthy individuals. We highlight that the third dose observed close to 90% seropositivity by RBD IgG in hematology patients, which is an excellent outcome for immunocompromised patients and comparable to the seropositivity rates observed after 2 doses in healthy individuals.

RBD-specific IgG antibody responses toward two doses of ChAdOx1 vaccine in hematology patients were lower than BNT162b2-vaccinated patients, when compared with healthy individuals, and remained lower after the third mRNA dose. This could partially be explained by the age difference, where ChAdOx1-vaccinated patients were older than BNT162b2-vaccinated patients or healthy individuals. Another explanation could be related to the type of vaccine, as it has been reported that two-dose ChAdOx1 vaccination provides lower protective efficacy (~60%) in terms of neutralizing antibodies compared with BNT162b2 (~90%).³⁵ A recent study comparing antibody and memory B cell responses following two-dose ChAdOx1, two-dose mRNA, or combined ChAdOx1/mRNA vaccination also observed inferior antibody responses with the ChAdOx1 vaccine.³⁶ Importantly, there were no differences in T cell responses between vaccine type.

Spike-specific CD4⁺ and CD8⁺ T cell responses following COVID-19 vaccination were also measured by AIM and ICS assays, as previously used in other SARS-CoV-2 infection studies.^{37,38} The skewing of PD-1⁺ICOS⁺-activated Tfh2/Tfh17 in the whole-blood assay aligned with our AIM data, where the majority of the AIM Tfh response was made up of Tfh2/Tfh17 cells rather than Tfh1 cells. Our data support Apostolidis et al.'s study¹⁷ where all patients with MS on anti-CD20 monoclonal antibody treatment (n = 20) generated robust spike-specific CD4⁺ and CD8⁺ T cell responses following BNT162b2 or mRNA-1273 mRNA vaccination in the absence of B cells.

Two doses of mRNA COVID-19 vaccination can induce robust CD4⁺ and CD8⁺ tetramer⁺ T cell responses.^{22,23,33} Here, both hematology patients and healthy individuals can generate robust CD4⁺ and CD8⁺ T cell responses to 7 immunodominant spike-specific epitopes after two doses of ChAdOx1 or BNT162b2 vaccines and after the third dose. Importantly, patients lacking RBD-specific IgG antibodies could still generate robust epitope-specific T cell responses. Furthermore, TCR repertoires from hematology patients shared common TCR signatures with healthy vaccinated and infected cohorts.

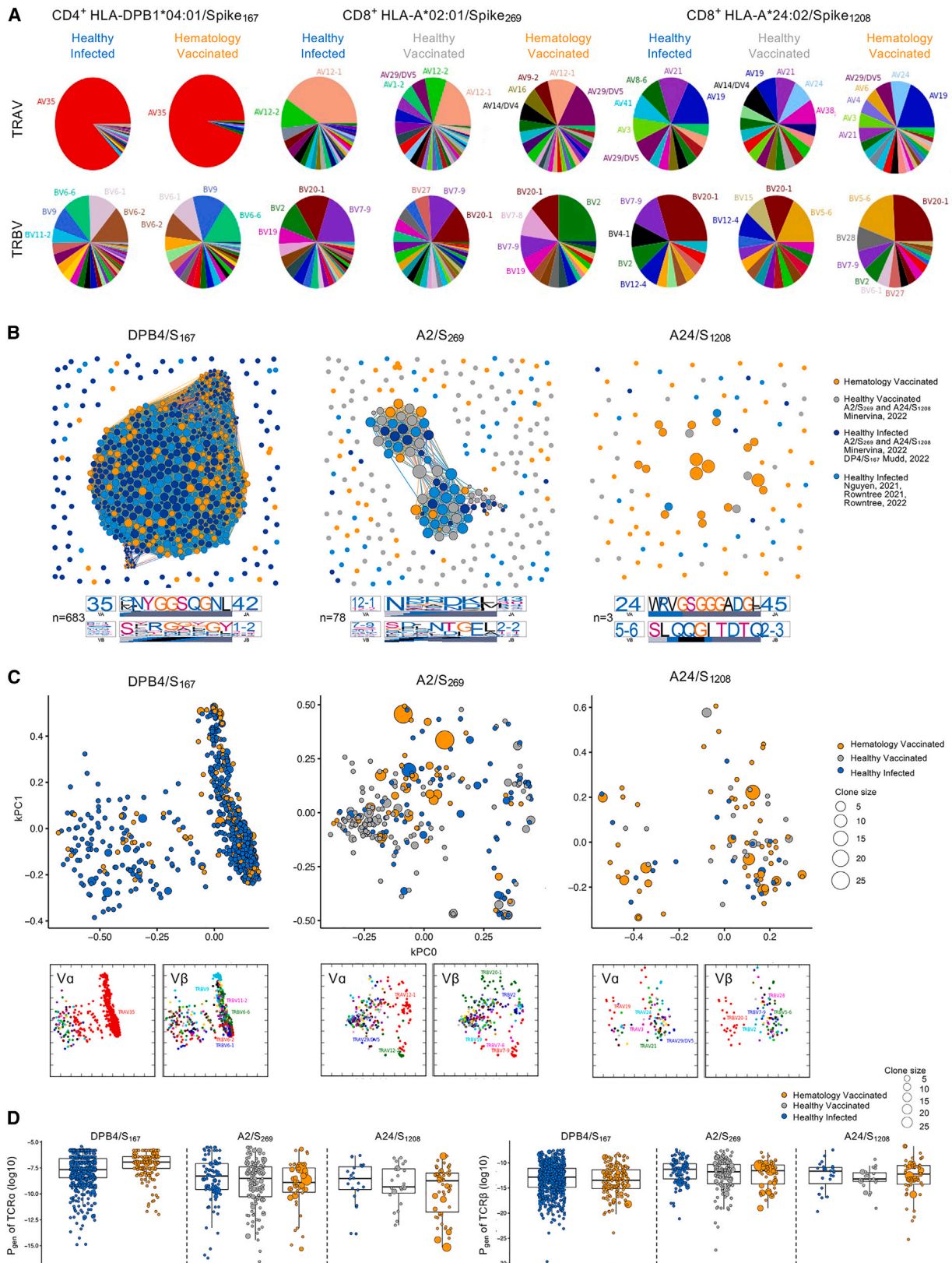
Breakthrough SARS-CoV-2 infections are increasingly becoming more common in the vaccinated community. Although SARS-CoV-2-infected patients had higher RBD-specific IgG antibody responses, T cell responses were

Figure 5. Vaccine responses between non-COVID-19 and breakthrough COVID-19

- (A) COVID-19 following SARS-CoV-2 vaccination.
 (B) Clinical symptoms and monoclonal antibody treatment for SARS-CoV-2 breakthrough infections.
 (C) Endpoint IgG titers of ancestral RBD antibodies (44 COVID⁻ and 8 COVID⁺ healthy individuals; 83 COVID⁻ and 12 COVID⁺ patients). Seropositive cutoff defined by baseline mean + 2 SD per group.
 (D) MNT titers at T5 against WT ancestral and Delta strains (74 COVID⁻ and 2 COVID⁺ patients).
 (E) Percentage of inhibition by sVNT assay against WT ancestral and Omicron strains (44 COVID⁻ and 8 COVID⁺ healthy individuals; 83 COVID⁻ and 12 COVID⁺ patients).
 (F) AIM between COVID⁻ and COVID⁺ groups (33 COVID⁻ and 1 COVID⁺ healthy individuals; 51 COVID⁻ and 5 COVID⁺ patients).
 (G) TAME-enriched FACS plots depicting tetramer and memory and activation phenotypes.
 (H) Tetramer frequencies between COVID⁻ and COVID⁺ groups (12 COVID⁻ and 3 COVID⁺ healthy individuals; 49 COVID⁻ and 5 COVID⁺ patients). 1–2 tetramer responses per donor.
 (I) CD8⁺tetramer⁺ phenotypes from individuals with breakthrough COVID-19.

The frequency of tetramer⁺ cells has been right shifted by 10⁻⁷ (i.e., no detected tetramer⁺ events displayed as 10⁻⁷).

Statistical significance determined by Mann-Whitney for comparisons between COVID⁻ and COVID⁺ time points (connecting line). ****p < 0.0001. Experiments were performed once for each sample. Refer to Figures S8 and S9.



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indistinguishable between infected and non-infected individuals in both patient and healthy groups, which aligns with previous reports in healthy individuals,²³ showing that vaccination alone or vaccination followed by subsequent infection can elicit comparable spike epitope-specific T cell responses.

Overall, our study shows that hematology patients who fail to seroconvert and generate memory B cell responses post-vaccination can nevertheless generate robust SARS-CoV-2-specific T cell immunity. Our findings are particularly relevant for reducing disease severity in hematology patients who acquire breakthrough infections since robust CD8⁺ T cell responses correlate with better outcomes in hematology patients hospitalized with COVID-19, including those treated with anti-CD20 therapy, while B cells had no impact on survival.¹⁶ Our data also support development of vaccines targeted to immunocompromised patients, such as those that have shown to induce potent and prolonged T cell responses to multiple SARS-CoV-2 antigens in healthy individuals.³⁹ Therefore, COVID-19 vaccination can still be immunogenic, especially with respect to SARS-CoV-2-specific CD4⁺ and CD8⁺ T cell responses, in hematology patients of varying diseases and treatment.

Limitations of the study

Our patient cohort is heterogeneous, and so we were not always powered to statistically analyze the data per disease, treatment, or vaccine group for all assays. Some assays were not performed for all patients, but patient numbers per assay are included in figure legends. Larger prospective studies are needed to demonstrate protection against severe and fatal disease with patient stratification based on antibody/B cell and T cell responses. SARS-CoV-2 infections occurred after 1/2/3 vaccinations in patients but only after 3 vaccinations in healthy individuals, although numbers are small. ChAdOx1-vaccinated patients were older than BNT162b2-vaccinated or healthy individuals.

STAR★METHODS

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- Assessment of SARS-CoV-2-specific antibodies and memory B cells
- 24-H stimulation with spike overlapping peptide pools
- Spike and non-spike epitope-specific tetramer⁺ T cell responses

QUANTIFICATION AND STATISTICAL ANALYSIS

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STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2023.101017>.

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AUTHOR CONTRIBUTIONS

K.K. led the study. K.K., B.W.T., C.S.T., M.A.S., and T.H.O.N. supervised the study. K.K., T.H.O.N., L.C.R., L.F.A., B.Y.C., L.K., L.H., J.A.N., F.L.M., H.-X.T., K.S., T.K., S.N., and D.A.W. designed the experiments. T.H.O.N., L.C.R., L.F.A., B.Y.C., L.K., E.B.C., W.Z., S.Y.C., J.R.H., X.J., L.H., J.A.N., F.L.M., H.-X.T., A.F.C., and T.K. performed and analyzed experiments. H.A.M., T.M., A.A.M., M.V.P., A.F.C., J.C.C., and P.G.T. analyzed data. P.C., J.P., A.K.W., S.J.K., K.S., and J.R. provided crucial reagents. C.L., M.L.,

Figure 6. TCR sequence similarity network identifies sharing of dominant motifs between the groups

(A) Pie charts of TRAV and TRBV usage for TCRαβ clonotypes specific to DPB4/S₁₆₇ (n = 18 patients), A2/S₂₆₉ (n = 18 patients), and A24/S₁₂₀₈ (n = 13 patients). Clonally expanded TCRs were reduced to a single data point for this analysis.

(B) TCRαβ sequence similarity networks. Each vertex is a different clonotype, and edges connect clonotypes with highly similar amino acid TCRαβ sequences (TCR distance [TCRdist] ≤ 120). Size of the vertex is proportional to number of neighbors. TCRdist sequence logos for TCRα and TCRβ chains are shown for central largest cluster for each panel. Each TCR motif depicts the V (left side) and J (right side) gene frequencies, the CDR3 amino acid sequence (middle), and the inferred rearrangement structure (bottom bars colored by source region; V-region, light gray; insertions, blue; diversity [D]-region, black; and J-region dark gray).

(C) TCR landscapes displayed using kernel principal-component analysis (PCA) projections. Vα/Vβ usage shown below.

(D) P_{gen} for TCRα and TCRβ. Boxplots represent the median (middle bar), 75% quantile (upper hinge), and 25% quantile (lower hinge), with whiskers extending 1.5 times the IQR.

Experiments were performed once for each sample. Refer to [Figures S10](#) and [S11](#).

G.S.T., N.R.S., M.C., L.C., J.F.S., M.A.A., A.W., K.A.T., M.A.S., C.S.T., and B.W.T. recruited the vaccinated hematology patients. G.G., F.J., J.M.T., J.A.T., J.M., B.C., K.A.B., and D.A.W. recruited the healthy vaccinated cohorts. T.H.O.N., L.C.R., L.F.A., B.W.T., and K.K. wrote the manuscript. All authors reviewed and approved the manuscript.

DECLARATION OF INTERESTS

The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines, which list F.K. as co-inventor. Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. F.K. has consulted for Merck and Pfizer (before 2020) and is currently consulting for Pfizer, Seqirus, and Avimex. The Krammer laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. H.A.M. and B.Y.C. are currently consulting for Ena Respiratory. B.W.T. has received research funding from MSD, Seqirus, and Sanofi and is on the advisory board for Moderna, CSL-Behring, and Takeda.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
CD71 M-A712 BV421	BD Biosciences	Cat#562995; RRID: AB_2737939
CD4 SK3 BV650	BD Biosciences	Cat#563875; RRID: AB_2744425
CD27 L128 BV711	BD Biosciences	Cat#563167; RRID: AB_2738042
CD38 HIT2 BV786	BD Biosciences	Cat#563964; RRID: AB_2738515
CCR7 150503 AF700	BD Biosciences	Cat#561143; RRID: AB_10562031
CD14 MφP9 APC-H7	BD Biosciences	Cat#560180; RRID: AB_1645464
CD19 SJ25C1 APC-H7	BD Biosciences	Cat#560177; RRID: AB_1645470
CD45RA HI100 FITC	BD Biosciences	Cat#555488; RRID: AB_395879
CD8a SK1 PerCP-Cy5.5	BD Pharmingen	Cat#565310; RRID: AB_2687497
CD95 DX2 PE-CF594	BD Biosciences	Cat#562395; RRID: AB_11153666
PD-1 EH12.1 PE-Cy7	BD Biosciences	Cat#561272; RRID: AB_10611585
CD3 OKT3 BV510	BioLegend	Cat#317332; RRID: AB_2561943
HLA-DR L243 BV605	BioLegend	Cat#307640; RRID: AB_2561913
CXCR5 RF8B2 BV421	BD Biosciences	Cat#562747; RRID: AB_2737766
CD19 SJ25C1 BV510	BD BioSciences	Cat#562947; RRID: AB_2737912
CCR6 11A9 BV650	BD Biosciences	Cat#563922; RRID: AB_2738488
CD20 2H7 BV711	BD Biosciences	Cat#563126; RRID: AB_2313579
CD27 M-T271 AF700	BD Biosciences	Cat#560611; RRID: AB_1727454
CD4 RPA-T4 APC-H7	BD Biosciences	Cat#560158; RRID: AB_1645478
CD3 UCHT1 PE-CF594	BD Biosciences	Cat#562280; RRID: AB_11153674
CXCR3 (CD183) 1C6 APC	BD Biosciences	Cat#550967; RRID: AB_398481
CD8 SK1 FITC	BD Biosciences	Cat#555634; RRID: AB_395996
CD45 HI30 PerCPCy5.5	BD Biosciences	Cat#340953; RRID: AB_400194
ICOS (CD278) DX29 PE	BD Biosciences	Cat#557802; RRID: AB_396878
CD8a SK1 BV605	BD Biosciences	Cat#564116; RRID: AB_2869551
CD25 2A3 BV711	BD Biosciences	Cat#563159; RRID: AB_2738037
CXCR3 G025H7 BV785	BioLegend	Cat#353738; RRID: AB_2565924
APC (4-1BB) 4B4-1 CD137	BioLegend	Cat#309810; RRID: AB_830672
CD69 FN50 PerCPCy5.5	BioLegend	Cat#310926; RRID: AB_2074956
CD134 L106 PE	BD Biosciences	Cat#340420; RRID: AB_400027
CD45RA L48 PECy7	BD Biosciences	Cat#337167; RRID: AB_647424
CD19 J4.119 ECD	Beckman Coulter	Cat#IM2708U; RRID:AB_130854
IgM G20-127 BUV395	BD Biosciences	Cat#563903; RRID:AB_2721269
CD21 B-ly4 BUV737	BD Biosciences	Cat#564437; RRID:AB_2738807
IgD IA6-2 PE-Cy7	BD Biosciences	Cat#561314; RRID:AB_10642457
IgG G18-145 BV786	BD Biosciences	Cat#564230; RRID:AB_2738684
CD27 O323 BV605	BioLegend	Cat#302829; RRID:AB_11204431
Streptavidin PE	BD Biosciences	Cat#554061; RRID:AB_10053328
Streptavidin APC	BD Biosciences	Cat#554067; RRID:AB_10050396
Peroxidase AffiniPure goat anti-human IgG, Fcγ fragment specific	Jackson ImmunoResearch	Cat#109-035-098; RRID: AB_2337586

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Blood samples (peripheral blood mononuclear cells (PBMCs) and plasma samples) from COVID-19-vaccinated adult haematological malignancy patients and healthy control individuals	The Royal Melbourne Hospital, The Austin Hospital, St Vincent's Hospital, and The Peter McCallum Cancer Center	N/A
Chemicals, peptides, and recombinant proteins		
3,3',5,5'-Tetramethylbenzidine (TMB) Liquid Substrate System for ELISA, peroxidase substrate	Sigma	Cat#T0440-1L
Alkaline phosphatase yellow (pNPP) liquid substrate for ELISA	Sigma	Cat#P7998-100ML
Pierce High Sensitivity Streptavidin-HRP	Thermo Fisher Scientific	Cat#21130
SARS-CoV-2 RBD protein	Amanat et al., ⁴⁰	N/A
SARS-CoV-2 Spike protein	Juno et al., ³⁷	N/A
SARS-CoV-2 peptides – A1/ORF1a ₁₆₃₇ TTDPSTFLGRY; A1/S ₈₆₅₋₈₇₃ LTDEMIQY; A2/S ₂₆₉ YLQPRTFLL; A3/N ₃₆₁ KTFPPTEPK; A3/S ₃₇₈ KTFPPTEPK; A24/S ₁₂₀₈ QYIKWPWYI; B7/N ₁₀₅₋₁₁₃ SPRWYFYLL; B15/S ₉₁₉₋₉₂₇ NQKLIANQF; B35/S ₃₂₁₋₃₂₉ QPTESIVRF; and DPB4/S ₁₆₇ TFEYVSPFLMDLE	GenScript	N/A
HLA-A*01:01/S ₈₆₅ monomer (SARS-CoV-2, S ₈₆₅ , LTDEMIQY)	Peptide sequence ³⁰ , monomer [Rossjohn Laboratory]	N/A
HLA-A*01:01/ORF1a ₁₆₃₇ monomer (SARS-CoV-2, ORF1a ₁₆₃₇ , TTDPSTFLGRY)	Peptide sequence ⁴¹ , monomer ²¹	N/A
HLA-A*03:01/S ₃₇₈ monomer (SARS-CoV-2, S ₃₇₈ , KCYGVSPK)	Peptide sequence ⁴¹ , monomer [Rossjohn Laboratory]	N/A
HLA-A*03:01/N ₃₆₁ monomer (SARS-CoV-2, N ₃₆₁ , KTFPPTEPK)	Peptide sequence ⁴² , monomer ²¹	N/A
HLA-B*07:02/N ₁₀₅ monomer (SARS-CoV-2, N ₁₀₅ , SPRWYFYLL)	Peptide sequence ⁴² , monomer ¹⁸	N/A
HLA-B*15:01/S ₉₁₉ monomer (SARS-CoV-2, S ₉₁₉ , NQKLIANQF)	Peptide sequence ²³ , monomer [Rossjohn Laboratory]	N/A
HLA-A*02:01/S ₂₆₉ monomer (SARS-CoV-2, S ₂₆₉ , YLQPRTFLL)	Peptide sequence/monomer ¹⁹	N/A
HLA-B*35:01/S ₃₂₁ monomer (SARS-CoV-2, S ₃₂₁ , QPTESIVRF)	Peptide sequence ³³ , monomer [Rossjohn Laboratory]	N/A
HLA-A*24:02/S ₁₂₀₈ monomer (SARS-CoV-2, S ₁₂₀₈ , QYIKWPWYI)	Peptide sequence ³¹ , monomer ²⁰	N/A
HLA-DPA1*01:03/DPB1*04:01/S ₁₆₇ monomer (SARS-CoV-2, S ₁₆₇ , TFEYVSPFLMDLE)	Peptide sequence/monomer ²²	N/A
Software and algorithms		
R v4.2.1	R Core Team, ⁴³	https://cran.r-project.org
conga package	Schattgen et al. ⁴⁴	https://github.com/phbradley/conga
igraph R package v1.3.2	Csárdi and Nepusz ⁴⁵	https://igraph.org/r/
TCRdist pipeline	Dash et al. ⁴⁶	https://github.com/phbradley/tcr-dist
Gephi v0.9.7	Jacomy et al. ⁴⁷	https://gephi.org/
Corrplot v0.92	Wei and Simko ⁴⁸	https://github.com/taiyun/corrplot
rstatix package v0.7.0	Kassambara ⁴⁹	https://CRAN.R-project.org/package=rstatix
EnhancedVolcano v1.14.0	Blighe et al. ⁵⁰	https://github.com/kevinblighe/EnhancedVolcano
FlowJo v10.5.3	FlowJo	https://www.flowjo.com
Prism v8.3.1 or v9.1.0	GraphPad	https://www.graphpad.com

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
BD FACS Diva v8.0.1	BD Biosciences	https://www.bdbiosciences.com/en-us/instruments/research-instruments/research-software/flow-cytometry-acquisition/facsdiva-software
Other		
Anti-PE Micro-Beads	Miltenyi Biotec	Cat# 130-048-801, RRID:AB_244373
Anti-APC Micro-Beads	Miltenyi Biotec	Cat# 130-090-855, RRID:AB_244367

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Katherine Kedzierska (kkedz@unimelb.edu.au).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- The published article includes all datasets generated or analyzed during the study.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the [lead contact](#) upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Victorian blood cancer patients and healthy volunteers that were scheduled for the COVID-19 vaccine, as part of Australia's COVID-19 vaccine rollout in 2021, were recruited to the study. Patients with various haematological malignancies were enrolled at the Peter MacCallum Cancer Centre or St Vincent's Hospital following ethics approval by the Peter MacCallum Cancer Centre Human Research Ethics Committee (HREC/74271/PMCC-2021, HREC/74260/PMCC-2021). Healthy volunteers were enrolled at the Royal Melbourne Hospital or the Austin Hospital with approvals from Melbourne Health (HREC/68355/MH-2020) and Austin Health (HREC/73256/Austin-2021), respectively. Human ethics was also approved by the University of Melbourne HREC (21817, 21711, 21626, 21560, 13344). All participants provided written informed consent. Patient and healthy cohort demographics are summarised in [Table S1](#). Clinical information and limited immune data have been described for a subset of CLL patients.⁵¹

Participants were vaccinated with 2 doses of the BNT162b2 Comirnaty (Pfizer) vaccine scheduled ~3 weeks apart or the ChAdOx1 (AstraZeneca) vaccine scheduled ~8–12 weeks apart. One patient also received 2 doses of the mRNA-1273 (Moderna) vaccine. Heparinated peripheral blood and serum were collected prior to vaccination (T1), ~1 week following the first dose (T2, optional bleed), just prior to the second dose (T3), ~1 week following the second dose (T4, optional bleed), and ~1 month (T5) following the second dose. Additionally, a subset of patients were bled prior to receiving a third dose of BNT162b2 Comirnaty or mRNA-1273 (T6), 1 month following the third dose (T7) and 3–4 months following the third dose (T8). Healthy vaccinated adults were recruited as controls. PBMCs were isolated by Ficoll-Paque separation for cellular assays, plasma was collected for measuring antibodies, serum for microneutralisation assays and DNA isolated from granulocytes for HLA typing by VTIS (Melbourne, Australia), essentially as described.²⁸

The study was conducted in compliance with the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007) and the Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95).

METHOD DETAILS

Cellular activation in whole blood

The kinetics of ASCs and activation of Tfh/CD8⁺/CD4⁺ T cell subsets were measured at T1–T5 timepoints by directly staining whole blood with antibodies for flow cytometry, essentially as described.^{25,28,52}

Assessment of SARS-CoV-2-specific antibodies and memory B cells

Spike and nucleocapsid antibodies were measured using Elecsys Anti-SARS-CoV-2 kit and Roche e601 analyser according to manufacturer's instructions. Plasma antibodies against wildtype SARS-CoV-2 RBD protein (vaccine strain) were assessed by IgG ELISA as previously described in detail.^{18,40,53} MNT activity of serum samples (T1 and T5 only) was assessed as previously described with the wildtype and delta strains.^{26,37,54} The sVNT assay was performed with wildtype and B.1.1.529 omicron (Genscript Z03730) strains essentially as described.⁵³ Spike-specific B cell responses from the vaccine strain were measured on thawed PBMCs or TAME-flow through fractions. Cells were stained with wildtype Spike recombinant probes conjugated to PE, fixed and acquired on a BD LSRII Fortessa, essentially as described.^{18,37}

24-H stimulation with spike overlapping peptide pools

Thawed PBMCs were plated into a 96-well plate at 1e6 PBMCs/well. For AIM assay, cells were stimulated in complete-RPMI with 10 µg/ml SARS-CoV-2 Spike peptide pool (181 peptides, 0.06 µg/ml per peptide; BEI Resources, NIAID, NIH, SARS-Related Coronavirus 2 Spike (S) Glycoprotein, NR-52402) or DMSO (0.1%; Sigma), as a negative control, and cultured at 37°C/5% CO₂ for 24 h. Cells were washed and stained with CXCR5-BV421 (562747; BD Biosciences), CD3-BV510 (317332; BioLegend), CD8-BV605 (564116; BD Biosciences), CD4-BV650 (563875; BD Biosciences), CD25-BV711 (563159; BD Biosciences), CXCR3-BV786 (353738; BD Biosciences), CD137-APC (309810; BioLegend), CD27-AF700 (560611; BD Biosciences), CD14/CD19-APC-H7 (560180/560252; BD Biosciences), Live/Dead NIR (L34976; Invitrogen), CD69-PerCPCy5.5 (310925; BioLegend), CD134-PE (340420; BD Biosciences), CD95-PE-CF594 (562395; BD Biosciences), CD45RA-PeCy7 (337167; BD Biosciences) before fixing with 1% PFA.

For ICS, cells were stimulated in complete-RPMI with 100 µg/mL overlapping Spike peptide pool (181 peptides, 0.6 µg/ml per peptide; BEI Resources, NR-52402) or DMSO (1%), as a negative control, in combination with anti-CD28/CD49d (1:100, 347690; BD Biosciences) and 10U/ml IL-2 (11147528001; Roche), with Brefeldin A (½000 dilution; 555029; BD Biosciences) added after 6 h. Following further 18 h of the stimulation, cells were washed twice with MACS buffer (PBS/0.5% BSA, 2mM EDTA), then stained with surface antibodies: CD3-BV510 (317332; BioLegend), CD4-BV650 (563875; BD Biosciences), CD8-PerCPCy5.5 (565310; BD Biosciences) and Live/Dead NIR (L34976; Invitrogen) for 30 min on ice. Cells were washed twice, then fixed using the BD Cytotfix/Cytoperm kit (554723; BD Biosciences) according to the manufacturer's instructions, washed twice and intracellularly stained with IFNγ-v450 (560371; BD Biosciences), MIP-1β-APC (560656; BD Biosciences) and TNFα-AF700 (557996; BD Biosciences) for 30 min on ice. Following two further washes, lymphocytes were resuspended in MACS buffer and acquisition was on an LSRII Fortessa. Data were analyzed using FlowJo v10. Values obtained for PBMCs cultured with DMSO under the same conditions (negative controls) were subtracted from peptide-stimulated values. As a negative control, PBMCs cultured with DMSO also had IL-2, thus this control accounts for any background cytokine production triggered by IL-2 by CD4⁺ and CD8⁺ T cells.

Spike and non-spike epitope-specific tetramer⁺ T cell responses

HLA class I tetramers HLA-A*01:01/S₈₆₅³⁴ (LTDEMIAQY), HLA-A*01:01/ORF1a₁₆₃₇⁴¹ (TTDPSFLGRY), HLA-A*02:01/S₂₆₉¹⁹ (YLQPRFTLL), HLA-A*03:01/S₃₇₈⁴¹ (KCYGVSPVK), HLA-A*03:01/N₃₆₁⁴² (KTFPPTPEPK), HLA-A*24:02/S₁₂₀₈³⁵ (QYIKWPWYI), HLA-B*07:02/N₁₀₅⁴² (SPRWYFYLL), HLA-B*15:01/S₉₁₉²³ (NQKLIANQF) and HLA-B*35:01/S₃₂₁³³ (QPTEISIVRF) were generated by Rossjohn laboratory and validated as previously described.^{18–20} HLA class II tetramer HLA-DPA1*01:03/DPB1*04:01/S₁₆₇ (TFEYVSQPFLMDLE) was generated by Rossjohn laboratory and validated as previously described.²²

Cryopreserved PBMCs (5–10x10⁶) underwent tetramer-associated magnetic enrichment (TAME) following staining with a class I and/or DP4 class II Spike tetramer on PE and/or another class I tetramer on APC as described.¹⁸ Class I and class II tetramers on PE were exclusively stained on CD8⁺ or CD4⁺ T cells, respectively, with minimal to zero non-specific binding. Flow-through fractions were cryopreserved for Spike-specific B cell probe analysis, as previously described²¹ which were negative for tetramer⁺ cells. Samples were acquired on an LSRII Fortessa using the software BD FACS DIVA v8.0.1 and flow cytometry data were analyzed using FlowJo v10 software.

Enriched HLA-A*02:01/S₂₆₉, HLA-A*24:02/S₁₂₀₈ and HLA-DPB1*04:01/S₁₆₇ tetramer⁺ T cells were indexed single-cell sorted on a BD FACSAria III for TCR analysis essentially as described.^{18,21} CDR3α and CDR3β regions from single cells were amplified using multiplex-nested RT-PCR^{18,34} analyzed by IMGIT/V-QUEST.

As in our study, we had a limited number of PBMCs from hematology patients (and healthy controls), our baseline T1 tetramer-positive T cell population correspond to a few events (<10 events) for some individuals. For transparency, we show any samples with <10 tetramer⁺ events as open symbols. Only samples with more than 10 tetramer⁺ events are included in the phenotypic analysis (Figures 4H and 4I).

QUANTIFICATION AND STATISTICAL ANALYSIS

TCRαβ statistical analysis

Single-chain alpha and beta TCR sequences were analyzed by TCRdist for modeling amino acid motifs, TCR landscapes, neighbor distance distribution and probabilities of generation (P_{gen}).⁴⁶ For comparisons, published TCR datasets from unvaccinated SARS-CoV-2-infected individuals in the blood were sourced from Rowntree et al.,²¹ Nguyen et al.,¹⁸ Rowntree

et al.²⁰ (HLA-A*02:01/S₂₆₉, HLA-A*24:02/S₁₂₀₈ and HLA-DPB1*04:01/S₁₆₇), Minervina et al.²³ (HLA-A*02:01/S₂₆₉ and HLA-A*24:02/S₁₂₀₈) and Mudd et al.²² (HLA-DPB1*04:01/S₁₆₇). Published TCR datasets from COVID-19-vaccinated uninfected individuals were sourced from Minervina et al.²³ (HLA-A*02:01/S₂₆₉ and HLA-A*24:02/S₁₂₀₈). TCRdist⁴⁶ was used to calculate pairwise distances between clonotypes, sequence logos was created with *conga* python package⁴⁴ network was generated with igraph R package (v. 1.3.2)⁴⁵ and visualized with Gephi (v.0.9.7).⁴⁷

STATISTICAL ANALYSIS

Statistical significance of nonparametric datasets (two-tailed) were determined using GraphPad Prism v9 software. Mann-Whitney U-test (unpaired) and Wilcoxin signed-rank test (paired) were used for comparisons between two groups. Kruskal-Wallis test (unmatched) with Dunn's multiple comparisons was used to compare more than two groups. Tukey's multiple comparison test compared row means between more than two groups, while Sidak's multiple comparison test compared column means between multiple groups. Correlation matrices were prepared in R using corrplot version 0.92.⁴⁸ Volcano plots were generated within healthy individuals and haematology patients, and in patients with differing B cell ranges by Wilcoxon Rank-Sum test with Benjamini-Hochberg adjustment for multiple comparisons, using the rstatix version 0.7.0 package⁴⁹ within R version 4.2.1,⁴³ and plotted using EnhancedVolcano version 1.14.0.⁵⁰ Volcano plots display log₂(fold change) vs. -log₁₀(unadjusted p value), with horizontal dashed line representing the adjusted p threshold. Parameters used for the volcano plots included sVNT, AIM, IgG RBD; log MNT (T5 only); B cell number and B cell probes (all participants and med-high B cell participants only).