T-cell and antibody responses in immunocompromised patients with hematologic malignancies indicate strong potential of SARS-CoV-2 mRNA vaccines

SARS-CoV-2 emergence combined with new mRNA vaccination provided a unique opportunity to investigate *de novo* mRNA vaccine-induced immune responses. In healthy individuals (HI) SARS-CoV-2 mRNA vaccines are effective, but immunocompromised patients are often understudied. In particular, patients with hematological malignancies are rarely stratified based on disease and treatment. Furthermore, the focus is usually on antibodies whilst T cells are underreported and seldomly studied in detail by flow cytometry. We therefore aimed to investigate the SARS-CoV-2 mRNA vaccine-induced humoral and T-cell responses in patients with hematological malignancies in a side-by-side comparison of different malignancies and treatments. We enrolled and categorized 723 patients with hematologic diseases in 16 pre-defined cohorts based on malignancy and therapy (study registration: EudraCT 2021-001072-41).[1] For the current study, we randomly selected 173 patients, representative for each cohort with respect to age, absolute baseline CD4$^+$ and CD8$^+$ T-cell numbers, and spike protein S1 subunit (S1) immunoglobulin (Ig)G concentrations. HI were age-matched to the overall patient cohort, except for patients treated with hypomethylating agents (HMA) where the median age was 71 years (Table 1; *Online Supplementary Figure S1A*). We performed an in-depth, combined analysis of the frequency, phenotype and functionality of spike-specific CD4$^+$ and CD8$^+$ T cells and spike-specific antibody responses before and 4 weeks after each mRNA-1273 vaccination (*Online Supplementary Figure S1B, C*). Methods are described previously.[2,3] Study protocols were approved by the institutional review board of all participating centers

Generally, SARS-CoV-2 mRNA vaccination induced S1 IgG concentrations similar to HI in most patients except for patients that were B-cell depleted (Figure 1A, B). Spike-specific CD4$^+$ and CD8$^+$ T cells increased upon each vaccination, whilst other T-cell specificities (CEFX, Cytomegalovirus, Epstein-Bar virus, Influenza [Flu] and more) remained constant in time (*Online Supplementary Figures S1D, E* and *S2A-C*). As antigen-specific CD8$^+$ T cells are challenging to detect, we performed activation-induced marker assays and peptide-HLA tetramer staining. Both methods correlated closely and were combined for further analysis (*Online Supplementary Figure S2D-F*). Most patients developed spike-specific CD4$^+$ (85%) and CD8$^+$ (65%) T-cell frequencies including Th1 cytokine production comparable to HI after the second vaccination (Figure 1C-F). Control CEFX-specific CD4$^+$ and CD8$^+$ T-cell responses including Th1 cytokine production were comparable to HI in most cohorts (*Online Supplementary Figure S3A-D*). Interestingly, patients with reduced antibody concentrations mostly did not have reduced T-cell frequencies, although antibody concentration and CD4$^+$ T-cell frequency did positively correlate (Figure 1A-D; *Online Supplementary Figure S3E*). Furthermore, reduced absolute T-cell numbers or lower percentage of naïve T cells at baseline were not associated with reduced spike-specific CD4$^+$ or CD8$^+$ T-cell frequencies (Figures 1C, D and 2B, C; *Online Supplementary Figure S3F, G*). Although counterintuitive, it demonstrates that limited baseline naïve T-cell frequencies could proliferate to adequate spike-specific frequencies. Since low numbers of circulating T cells can bias spike-specific frequencies, we calculated the absolute number of circulating spike-specific T cells per micro liter blood, which showed comparable results (Figure 2D).

Analyzing the cohorts separately, the median S1 IgG concentration was <300 binding antibody units (BAU)/mL in patients with lymphoma receiving B-cell depleting therapy, patients with chronic lymphocytic leukemia (CLL), patients treated with HMA for acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS), and patients who had received allogeneic hematopoietic stem cell transplantation (alloHSCT) <6 months before vaccination (Figure 1A). Despite the absence or low seroconversion, patients in the lymphoma groups had adequate spike-specific T-cell responses, as described previously (Figure 1C, D).[4,5] These cohorts included patients on active anti-CD20 therapy, shortly after anti-CD20 therapy, post BEAM-autologous HSCT (autoHSCT) or after chimeric antigen receptor (CAR) T-cell therapy (Table 1). These T-cell responses may explain our previous observation that vaccination in B-cell-depleted patients was associated with rapid antibody maturation in future humoral responses once B cells are reconstituted.[6] Patients with untreated (watch and wait [W&W]) CLL or treated with BTK inhibitors (BKTi) also showed spike-specific CD4$^+$ and CD8$^+$ T-cell frequencies comparable to HI, which is in contrast to other reports and could be related to vaccine type.[7,8] In patients treated with BTKi, S1 IgG concentrations were lower compared to patients with untreated CLL, possibly related to impairment of non-malignant B cells induced by BTKi.[9] Furthermore, spike-specific CD4$^+$ T cells showed significantly lower production of interferon (IFN)-γ and interleukin (IL)-2 compared to HI, which was partially reversed in CLL-depleted samples (Figure 1E; *Online Supplementary Figure S3H, O*). In patients with

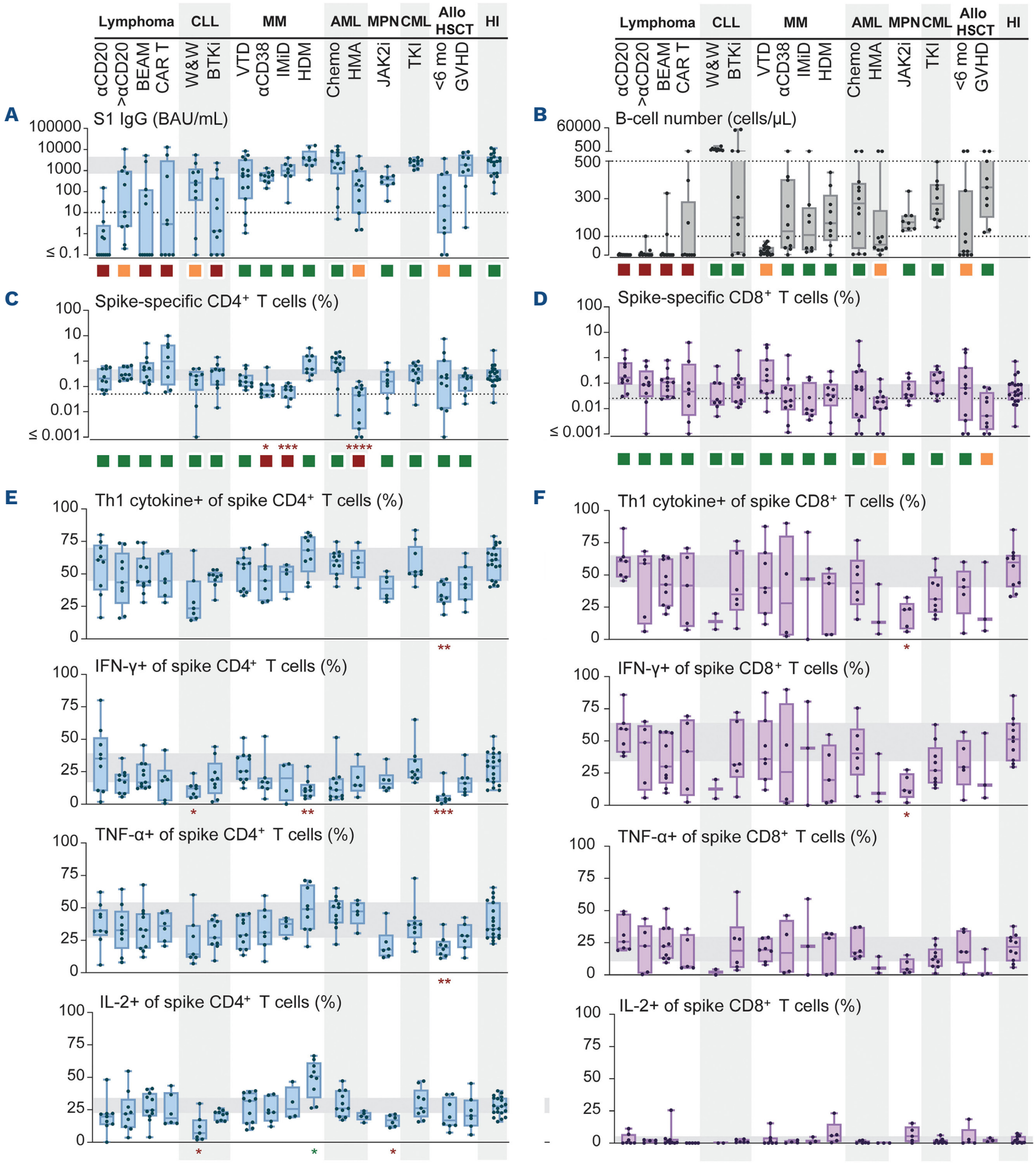

Figure 1. Vaccination-induced spike-specific antibody and T-cell responses in patients with hematologic malignancies. Before and 4 weeks after each mRNA-1273 vaccination, serum and peripheral blood mononucelar cells (PBMC) were collected to measure antibodies and T cells. (A) Spike protein S1 subunit (S1) immunoglobulin (Ig)G antibody concentrations after 2 SARS-CoV-2 mRNA vaccinations, categorized as no seroconversion (red; median S1 IgG <10 binding antibody units [BAU]/mL), low concentration (orange; median S1 IgG 10-300 BAU/mL), or adequate concentration (green; median S1 IgG >300 BAU/mL). Dotted line indicates seroconversion threshold. S1 IgG >300 BAU/mL was considered an adequate antibody response against the ancestral SARS-CoV-2, since this IgG concentration corresponded with a 50% plaque reduction neutralization titer of 40 or higher in 2 independent prospective Dutch mRNA-1273 vaccination cohorts. Concentrations of S1 and N IgG were quantified in BAU/mL according to the World Health Organization International Standard for COVID-19 serological tests. (B) Number of B cells/µL blood at start of vaccination (baseline). Dotted lines indicate range in healthy individuals (HI) (100-500 cells/µL blood). Squares indicate categorization of the cohorts based on the median. Cohorts with a median value below the arbitrary threshold of 10 cells/µL are depicted as red, with a median between 10 and 100 are depicted as orange, and with a median above 100 as green. (C, D) Frequency of

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spike-specific CD4+ or CD8+ T cells after two SARS-CoV-2 mRNA vaccinations as determined by activation-induced marker (AIM) assay and, for CD8+ T cells, in combination with peptide-HLA tetramer staining. For the AIM assay, 2 million thawed PBMC were incubated with 15-mer spike peptides (SB peptide, France), dimethyl sulfoxide (DMSO)-negative control, or a Cytomegalovirus, Epstein-Barr virus, Influenza (Flu) and extra (CEFX) peptide pool (JPT). After 1 hour, brefeldin A was added. All time points of 1 patient were measured simultaneously to minimize technical variance within 1 patient. Patients were measured and analyzed in random order across cohorts to minimize technical variance and bias between cohorts. Fifteen hours after adding brefeldin A, cells were stained for viability, fixated, permeabilized and incubated with antibodies directed against CD3, CD4, CD8, CD154, CD137, CD69, IFN-γ, TNF-α, IL-2, IL-4, IL-17, PD-1, FOXP3 and CXCR5. In parallel, PBMC were incubated with a viability dye, peptide-HLA tetramers and antibodies directed against CD4, CD8, CCR7 and CD45RA. Dotted lines indicate response positivity threshold (0.05% for CD4+, 0.025% for CD8+). (E, F) Frequency of spike-specific CD4+ (E) or CD8+ (F) T cells that produce IFN-γ, TNF-α and/or IL-2. Th1 cytokine-positive frequency was calculated by subtracting the frequency of cells that do not produce any of these cytokines from 100%. For all panels, grey horizontal area corresponds to interquartile range in HI. T-cell frequencies from each cohort are compared to those in HI by Mann-Whitney U tests and significance corrected for multiple testing (times 16) is shown (not significant $P>0.05$; *$P\leq0.05$; **$P\leq0.01$; ***$P\leq0.001$; ****$P\leq0.0001$). Squares indicate categorization of the T-cell responses based on *P* value prior to and after correction for multiple testing (green when not significantly lower prior to correction; orange when significantly lower before, but not after correction; red when significantly lower after correction). αCD20: during anti-CD20 therapy; >αCD20: within 12 months after anti-CD20 therapy; HSCT: hematopoietic stem cell transplantation; BEAM: BEAM-autologous HSCT within 12 months; CAR T: CD19 chimeric antigen receptor T-cell therapy; CLL: chronic lymphocytic leukemia; W&W: watch and wait; BTKi: Bruton's tyrosine kinase inhibitor ibrutinib; MM: multiple myeloma; VTD: bortezomib-thalidomide-dexamethasone induction therapy; αCD38: daratumumab; ImiD: immunomodulatory drugs; HDM: high-dose melphalan and autologous HSCT within 9 months; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia and high-risk MDS; chemo: high-dose chemotherapy; HMA: hypomethylating agents; MPN: myeloproliferative neoplasms; JAK2i: Janus 2 kinase inhibitor ruxolitinib; CML: chronic myeloid leukemia; TKI: tyrosine kinase inhibitors; alloHSCT: allogeneic hematopoietic stem cell transplantation; cGVHD: chronic graft-*versus*-host disease.

multiple myeloma treated with induction therapy (VTD), daratumumab (α-CD38), immune modulatory drugs (IMiD) or high-dose melphalan (HDM) humoral and cellular spike-specific immune responses were generally detected. However, S1 IgG concentrations were reduced in patients treated with daratumumab, probably caused by depletion of plasma cells by daratumumab. Patients treated with daratumumab or IMiD had reduced spike-specific CD4+ T-cell frequencies compared to HI. Interestingly, patients treated with HDM demonstrated a skewing towards IL-2- and TNF-α-producing CD4+ T cells with reduced IFN-γ production, indicating a change in cytokine profile. Patients with AML/MDS treated with HMA had both low S1 IgG concentrations and low frequencies of spike-specific CD4+ and CD8+ T cells which is consistent with previous reports of impaired vaccine responses in patients with AML/MDS.[10] The observation that patients with AML receiving high-dose chemotherapy were able to generate immune responses comparable to HI suggests that the therapy, rather than disease, hampered the vaccination responses. Since HMA preferentially targets replicating cells, it may suppress active, vaccine-induced T cells rather than resting T cells.[11] Indeed, CEFX-specific T cells were unaffected (*Online Supplementary Figure S3A-D*). Notably, the reduced immune responses may also be related to the higher median age of patients in the HMA cohort. Patients with MPN, including chronic myeloid leukemia (CML), showed humoral- and T-cell responses similar to HI. However, patients with MPN treated with JAK2-inhibitors demonstrated a lower frequency of IL-2-producing spike-specific CD4+ T cells and IFN-γ-producing spike-specific CD8+ T cells (Figure 1E, F). CEFX-specific CD8+ T cells showed a similar trend (*Online Supplementary Figure S3D*). Patients who underwent allo HSCT <6 months before vaccination had variable S1 IgG levels and S1-specific CD4+ T-cell frequencies, and production of IFN-γ and TNF-α by the CD4+ T cells was reduced. Others have suggested that impaired mRNA vaccination-induced T-cell responses after allo HSCT could be related to corticosteroid use.[12] Patients that had developed chronic graft-*versus*-host-disease (cGVHD) after alloHSCT tended to have reduced spike-specific CD8+ T-cell frequencies compared to HI, while this was not observed for antibody concentrations and spike-specific CD4+ T-cell frequencies. In patients who were vaccinated shortly after autoHSCT, spike-specific antibodies (HDM) and T-cell frequencies (BEAM and HDM) were comparable to HI.

Since cellular therapy can affect T-cell counts and function, we investigated the correlation between time since therapy and spike-specific CD4+ T-cell frequencies. The frequency of spike-specific CD4+ T cells was negatively correlated with time since CAR T-cell therapy (*Online Supplementary Figure S3P*). This correlation, although not significant, was also observed when calculating the absolute number of spike-specific CD4+ T cells (*Online Supplementary Figure S3Q*). Patients treated with alloHSCT within 6 months before vaccination had variable S1 IgG levels and spike-specific CD4+ T-cell frequencies, which did not correlate with time since alloHSCT (*Online Supplementary Figure S3R*).

The SARS-CoV-2 mRNA vaccines are designed to especially induce Th1 responses, indeed, IL-4 or IL-17 was not produced by spike-specific CD4+ T cells (*data not shown*). Frequencies of circulating spike-specific follicular helper T cells (Tfh; PD-1+CXCR5+) were significantly increased in patients with lymphoma shortly after anti-CD20 therapy and in patients with multiple myeloma treated with VTD (*Online Supplementary Figure S3S*). Frequencies of FOX-

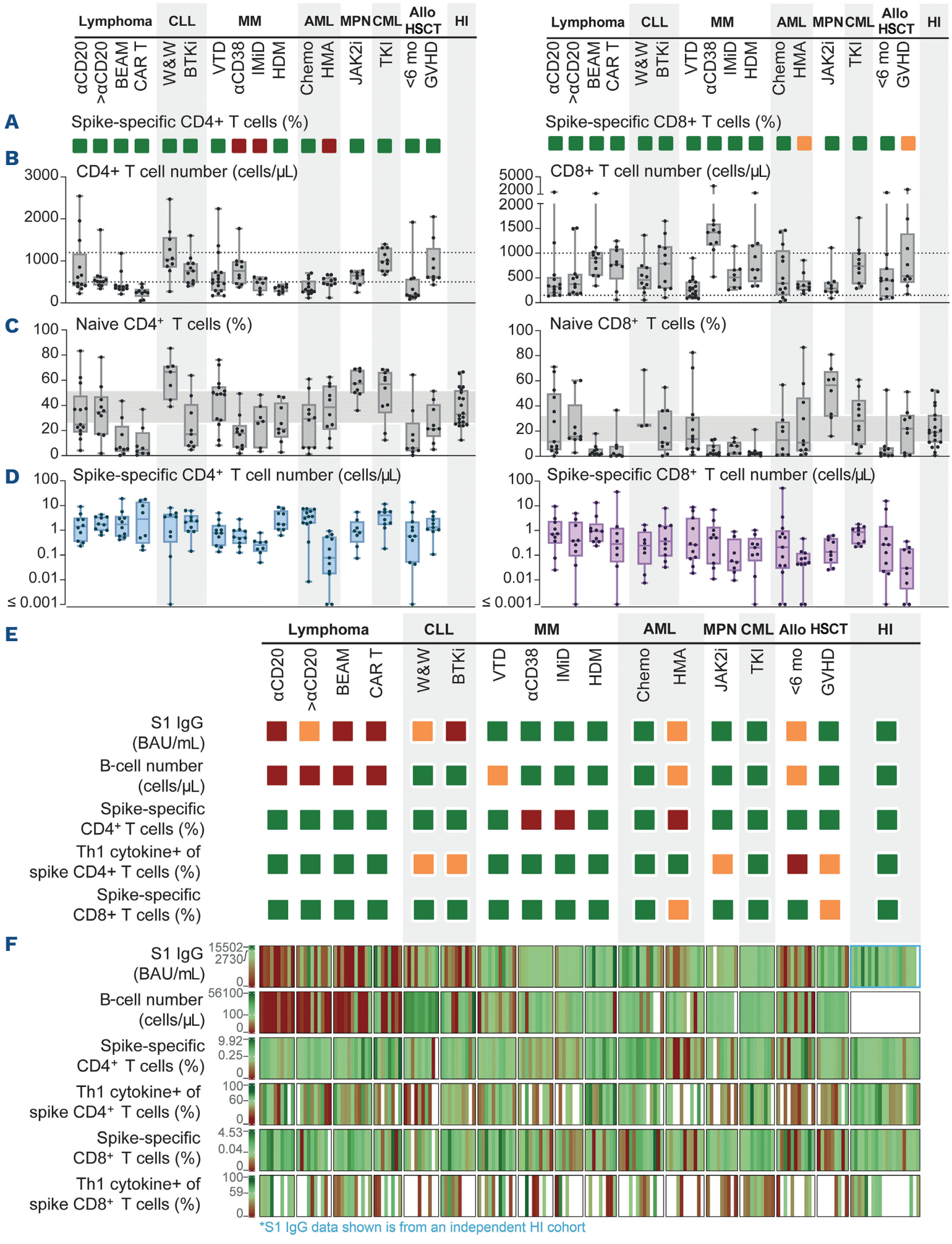

Figure 2. Baseline parameters, number of spike-specific T cells, and integrated analysis of cellular and humoral immune responses. (A) Categorization of spike-spike specific CD4+ or CD8+ T cell frequencies as shown in Figure 1. Spike protein S1 subunit (S1) immunoglobulin (Ig)G antibody concentrations after 2 SARS-CoV-2 mRNA vaccinations, categorized as no seroconversion (red; median S1 IgG <10 binding antibody units [BAU]/mL), low concentration (orange; median S1 IgG 10-300 BAU/mL), or adequate concentration (green; median S1 IgG >300 BAU/mL). (B) CD4+ or CD8+ T-cell numbers directly measured in blood as cells/µL. Absolute numbers of lymphocyte subsets were determined using fresh whole EDTA blood with Multitest 6-color reagents (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. The dotted lines indicate the clinically-accepted normal ranges in healthy individuals (HI). (C) Frequency of naïve (CCR7+CD45RA+) CD4+ or CD8+ T cells. Grey horizontal area corresponds to interquartile range in HI. (D) Number of spike-specific CD4+ or CD8+ T cells in peripheral blood, calculated by multiplying the percentage of

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spike-specific T cells by the number of T cells in peripheral blood. (E) Categorization of mRNA vaccine-induced B- and T-cell immune responses, and number of circulating B cells at start of vaccination per cohort. Categorization was based on median (S1 IgG), clinically accepted threshold (B cells), or statistics (T-cell responses). T-cell responses are categorized based on significance before and after correction for multiple testing (green when not significantly lower before correction; orange when significantly lower before, but not after correction; red when significantly lower after correction). Categorization of cytokine-producing spike-specific $CD8^+$ T-cell frequencies is not depicted due to limited availability of data points. (F) Summary heatmap of the data gathered from 6 variables of all cohorts, generated using RStudio (R-4.3.0, packages: circlize-0.4.15, ComplexHeatmap-2.15.4). Each vertical line represents the same individual. However, S1 IgG concentrations were obtained from an independent HI cohort (blue box), therefore, the vertical lines of the S1 IgG in HI do not represent the same vertical lines as the HI cohort of the T-cell data. Values are color-coded by relative abundance within each variable. The minimum value (red) was set to zero, the maximum (dark green) to the highest measured value, and the median (light green) to the median value in healthy individuals. B cells were not measured in HI and therefore the light-green median is set to the clinically-accepted minimal normal value of 100 cells/μL. Unavailable data are shown as white-colored bars. Cytokine-postive frequency of S1 $CD4/8^+$ indicates frequency of spike-specific $CD4/8^+$ T cells that produce IFN-γ, TNF-α and/or IL-2. αCD20: during anti-CD20 therapy; >αCD20: within 12 months after anti-CD20 therapy; HSCT: hematopoietic stem cell transplantation; BEAM: BEAM-autologous HSCT within 12 months; CAR T: CD19 chimeric antigen receptor T-cell therapy; CLL: chronic lymphocytic leukemia; W&W: watch and wait; BTKi: Bruton's tyrosine kinase inhibitor ibrutinib; MM: multiple myeloma; VTD: bortezomib-thalidomide-dexamethasone induction therapy; αCD38: daratumumab; ImiD: immunomodulatory drugs; HDM: high-dose melphalan and autologous HSCT within 9 months; MDS: myelodysplastic syndrome; AML: acute myeloid leukemia and high-risk MDS; chemo: high-dose chemotherapy; HMA: hypomethylating agents; MPN: myeloproliferative neoplasms; JAK2i: Janus 2 kinase inhibitor ruxolitinib; CML: chronic myeloid leukemia; TKI: tyrosine kinase inhibitors; alloHSCT: allogeneic hematopoietic cell transplantation; cGVHD: chronic graft-*versus*-host disease.

Table 1. Patient characteristics, stratified by disease and treatment at time of first COVID-19 vaccination.

Characteristics	Included for T-cell analyses, N	Age, years, median (IQR)	Female sex[1*], %	WHO PS 0-1, %
All participants	193	61 (53-67)	44	96
Lymphoma				
During anti-CD20 therapy (αCD20)	13	64 (41-73)	46	100
Anti-CD20 therapy <12 mo (<αCD20)	11	65 (51-73)	64	100
BEAM-autologous HSCT <12 mo (BEAM)	11	65 (58-66)	55	91
CD19 CAR T-cell therapy	9	63 (57-67)	44	100
CLL				
Watch and wait	10	64 (59-70)	60	100
Ibrutinib (BTKi)	11	67 (61-69)	64	100
Multiple myeloma				
Induction therapy (VTD)	17	59 (54-68)	35	94
Daratumumab (αCD38)	10	65 (57-71)	40	90
Immunomodulatory drug	8	60 (56-62)	13	100
HDM-autologous HSCT <9 mo	9	63 (58-69)	11	100
AML and high-risk MDS				
High-dose chemotherapy	14	58 (48-62)	29	92
Hypomethylating agents*[2]	11	71 (65-73)	27	91
MPN				
Ruxolitinib (JAK2i)	9	55 (45-65)	33	100
CML				
Tyrosine kinase inhibitor	10	51 (41-61)	60	90
Allogeneic HSCT				
<6 mo	11	59 (57-68)	36	91
Chronic graft-*versus*-host disease	9	52 (49-64)	22	100
Healthy individuals	20	58 (50-63)	70	NA

All patients are part of the COBRA KAI study and received a 3-dose mRNA-1273 vaccination series, according to the Dutch National Institute for Public Health and the Environment (RIVM) guidelines. SARS-CoV-2-naïve patients were randomly selected from each cohort, with SARS-CoV-2-naïve defined as spike protein S1 subunit (S1) immunoglobulin (Ig) G concentration <10 binding antibody units (BAU)/mL before vaccination, nucleocapsid (N) antibodies <14.3 BAU/mL in all measurements, and absence of patient-reported SARS-CoV-2 infection. Patients were compared to healthy individuals (HI) matched on age, type and number of vaccinations. For T-cell assays, these were healthy participants of the RECOVAC study (*clinicaltrials gov. Identifier: NCT04741386*), or healthcare workers from Erasmus MC (MEC 2020 0264). For S1-specific antibody concentrations, data from HI from the PIENTER Corona (PICO) cohort (*clinical trial registration: TR8473*) were used. All participants involved provided written informed consent. *[1]All patients were female or male. *[2]Patients received hypomethylating agents (HMA) as monotherapy, 1 patient also received venetoclax. IQR: interquartile range; WHO PS: World Health Organization Performance Status; BEAM: carmustine-etoposide-cytarabine-melphalan; HDM: high-dose melphalan; CAR: chimeric antigen receptor; BTKi: Bruton's tyrosine kinase inhibitor; JAK2i: Janus 2 kinase inhibitor; VTD: bortezomib-thalidomide-dexamethasone; HSCT: hematopoietic stem cell transplantation; MDS: myelodysplastic syndrome; mo: months; CLL: chronic lymphocytic leukemia; AML: acute myeloid leukemia; NA: not available.

P3$^+$CD4$^+$ T cells at the start of vaccination were low for all cohorts, yet in patients with multiple myeloma who had received HDM, significantly increased frequencies were detected (*Online Supplementary Figure S3T*).

A third vaccination significantly increased S1 IgG concentrations and frequencies of spike-specific T cells but the T-cell frequencies of non-responders remained low (*Online Supplementary Figure S3U-X*).[13] Seven patients received an autoHSCT between the second and third vaccination. Frequencies of spike-specific CD4$^+$ T cells in these patients increased further after the third vaccination, suggesting that pre-existing immunity was not fully eliminated by autoHSCT (*Online Supplementary Figure S3Y*). A similar pattern was observed for humoral responses in these patients.[13]

This study showed that humoral and cellular immune responses to SARS-CoV-2 vaccination, summarized per cohort (Figure 2E) or per individual (Figure 2F), were differently affected depending on the hematological malignancy and treatment. A limitation of our study is the small size per cohort, which especially applies to the cohorts where heterogeneous responses were found. Yet our results depict some patient cohorts that may respond inadequately to mRNA vaccination which warrants further research. Importantly, it remains to be determined to what extent humoral and cellular responses correlate to protection against severe disease. A large, population-based COVID-19 outcome study, including patients with comparable immunodeficiency states, is ongoing and may identify cohorts that are more susceptible to severe disease.[14] Both studies combined may provide further insight in the contribution of each component of the immune system in the protection against severe COVID-19. In conclusion, most patients with hematologic malignancies receiving immunosuppressive therapies generated antibody and/or T-cell responses after a two-dose SARS-CoV-2 mRNA vaccination. While all study participants were considered immunodeficient, the combination of reduced cellular and humoral SARS-CoV-2-specific immune responses was rare. These findings emphasize the potential of mRNA vaccines in generating humoral and cellular immune responses in patients with hematologic malignancies.

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Contributions

CRP, MHMH, CER, AG, MDH and ISN initiated and designed the study.

AECB, JAvD, TvM, PGNJM, CER, MDH and ISN recruited patients, and together with QH, SH, AG and SV-N collected clinical data. CRP, RCD, MvdM, KvD, CER, QH, SH, MSB, NJEH, JvM, JAvD, GPS, ISN, MDH, RDdV, DvB, RSvB, GdH, DW, EMMvL, HJB, NAK, AG collection of samples and experimental data. CRP, QH, SH, JHFF, ISN, MDH, MHMH and CER interpreted experimental data. CRP, QH and BIL-W performed statistical analyses. CRP, QH, MHMH and CER wrote the first drafts of the manuscript and verified the data. All authors contributed to and approved the final version and had access to primary clinical trial data.

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Data-sharing statement

For original participant data, please contact the corresponding author. The study protocol is included as a data in the *Online Supplementary Appendix* available with the online version of this article.

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