Primary T-cell responses against SARS-CoV-2 in patients with hematological disorders

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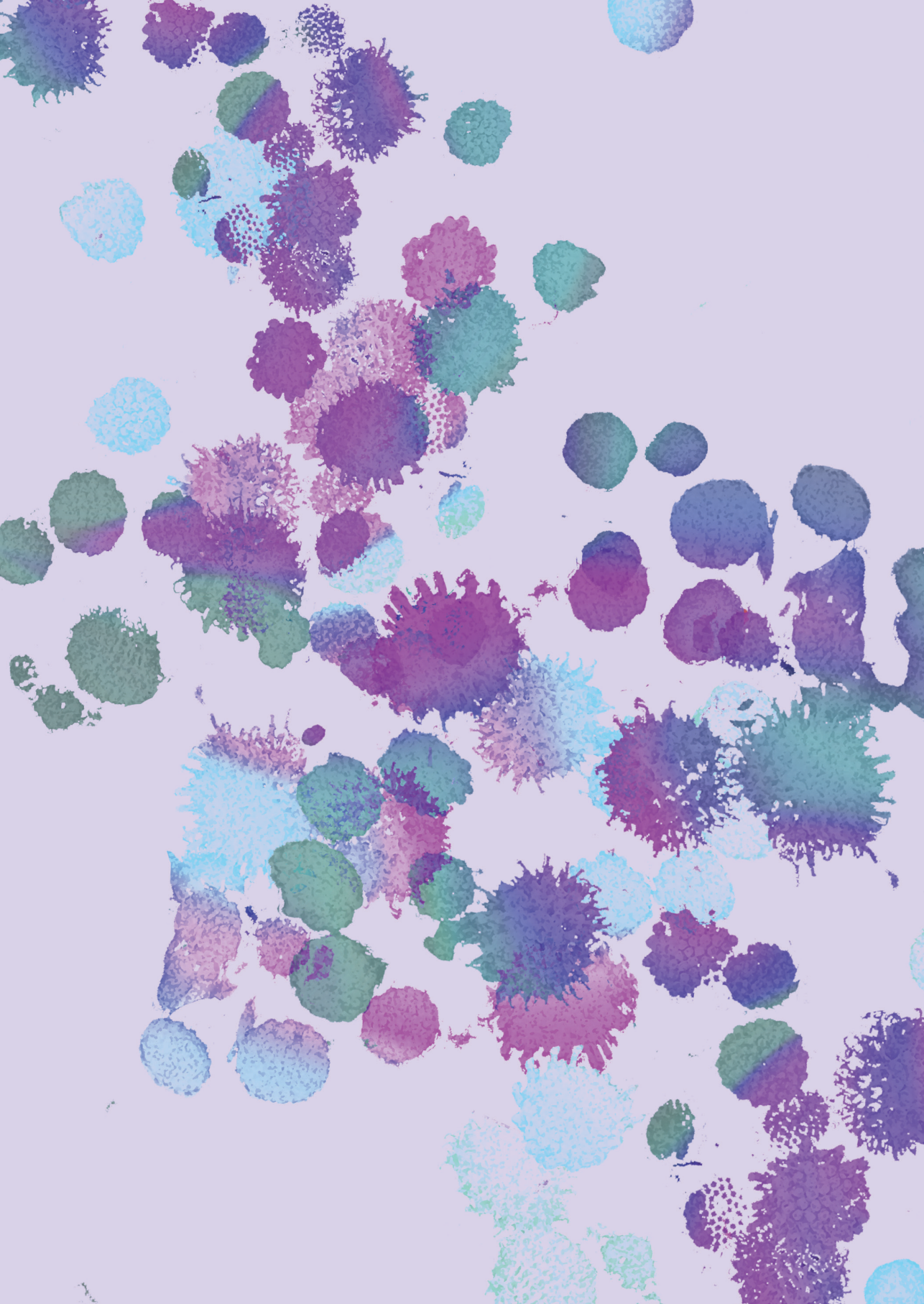

T-CELL AND ANTIBODY RESPONSES IN IMMUNOCOMPROMISED PATIENTS WITH HEMATOLOGIC MALIGNANCIES INDICATE STRONG POTENTIAL OF SARS-COV-2 MRNA VACCINES

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Chapter 5

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 seldom 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.[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 S1 IgG 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 Figure S1**). We performed an in-depth, combined analysis of the frequency, phenotype and functionality of spike-specific CD4+ and CD8+ T cells and spike (S1)-specific antibody responses before and four weeks after each mRNA-1273 vaccination (**Figure S2**). Methods are described previously.[2, 3] Study protocols were approved by the institutional review board of all participating centers.

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

	Included for T cell analyses	**Years of age**	**Female sex*[1]**	**WHO PS 0-1**
	n	***Median (IQR)***	***%***	***%***
All participants	193	61 (53-67)	44	96
Lymphoma				
During anti-CD20 therapy (αCD20)	13	64 (41-73)	46	100
Anti-CD20 therapy <12mo (< αCD20)	11	65 (51-73)	64	100
BEAM-autologous HCT <12mo (BEAM)	11	65 (58-66)	55	91
CD19 CAR T cell therapy (CAR T)	9	63 (57-67)	44	100
CLL				
Watch and wait (W&W)	10	64 (59-70)	60	100

	Included for T cell analyses	Years of age	Female sex*[1]	WHO PS 0-1
	n	*Median (IQR)*	*%*	*%*
Ibrutinib (BTKi)	11	67 (61-69)	64	100
Multiple myeloma (MM)				
Induction therapy (VTD)	17	59 (54-68)	35	94
Daratumumab (αCD38)	10	65 (57-71)	40	90
Immunomodulatory drug (IMiD)	8	60 (56-62)	13	100
HDM-autologous HCT <9mo (HDM)	9	63 (58-69)	11	100
AML and high-risk MDS (AML)				
High-dose chemotherapy (chemo)	14	58 (48-62)	29	92
Hypomethylating agents (HMA)*[2]	11	71 (65-73)	27	91
MPN				
Ruxolitinib (JAK2i)	9	55 (45-65)	33	100
CML				
Tyrosine kinase inhibitor (TKI)	10	51 (41-61)	60	90
Allogeneic HCT (alloHCT)				
<6 months (<6 mo)	11	59 (57-68)	36	91
Chronic graft-versus-host disease (GvHD)	9	52 (49-64)	22	100
Healthy individuals (HI)	20	58 (50-63)	70	N/A

All patients are part of the COBRA KAI study and received a three-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 G (IgG) 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 matched on age, type and number of vaccinations. For T cell assays, these were healthy participants of the RECOVAC study (NCT04741386), or healthcare workers from Erasmus MC (MEC 2020 0264). For spike-specific antibody concentrations, data from healthy participants 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 HMA as monotherapy, one patient also received venetoclax. 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. VTD = bortezomib-thalidomide-dexamethasone. HCT = hematopoietic cell transplantation. MDS = myelodysplastic syndrome. BEAM = carmustine-etoposide-cytarabine-melphalan.

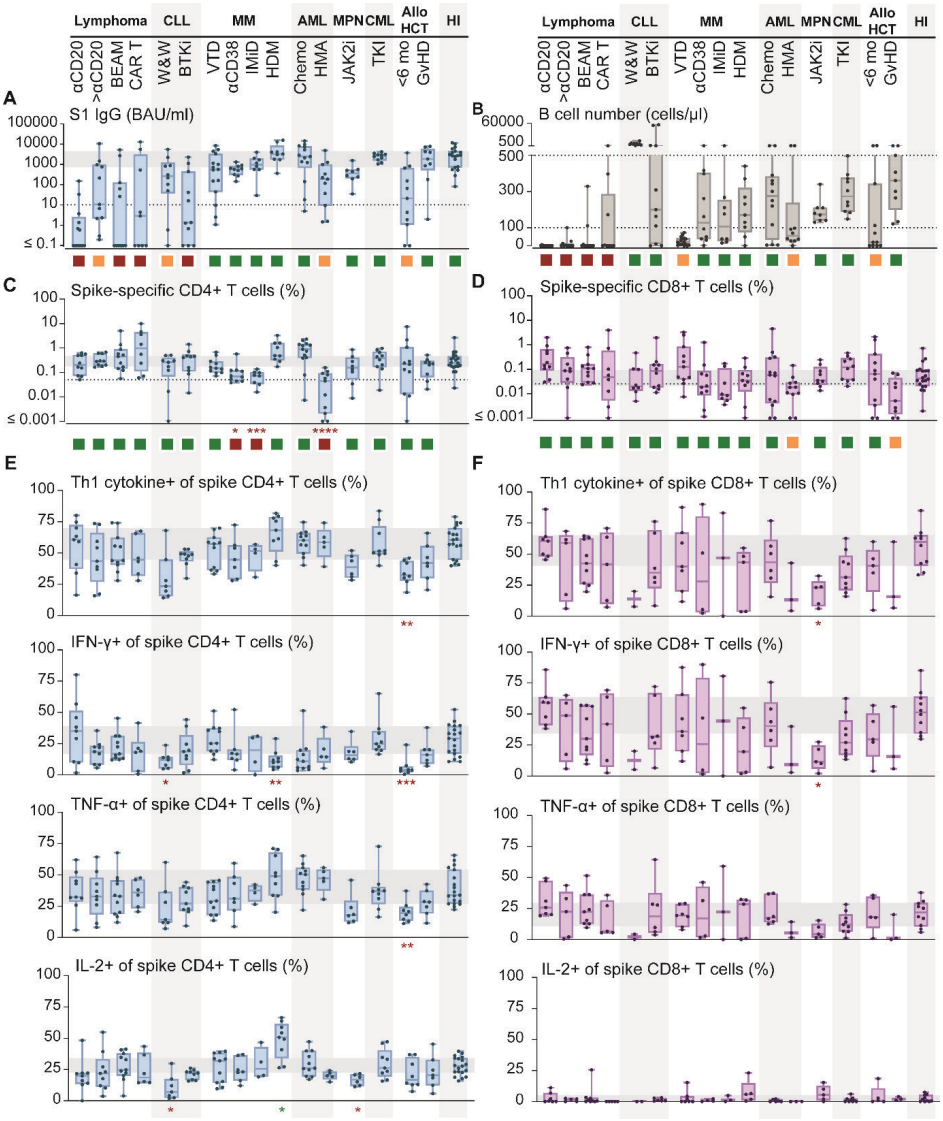


Figure 1 Vaccination-induced spike-specific antibody and T cell responses in patients with hematologic malignancies

Before and four weeks after each mRNA-1273 vaccination, serum and PBMCs were collected to measure antibodies and T cells. **A**) S1 IgG antibody concentrations after two SARS-CoV-2 mRNA vaccinations, categorized as no seroconversion (red; median S1 IgG <10 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 two independent prospective Dutch mRNA-1273 vaccination cohorts. Concentrations of S1 and N IgG were quantified in BAU/mL according to the WHO International Standard for COVID-19 serological tests. **B**) Number of B cells per microliter blood at start of vaccination (baseline). Dotted lines indicate range in healthy individuals (100-500 cells/

microliter blood). Squares indicate categorization of the cohorts based on the median. Cohorts with a median value below the arbitrary threshold of 10 cells/microliter 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 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 PBMCs were incubated with 15-mer spike peptides (SB peptide, France), DMSO negative control, or a CMV, EBV, Flu and extra (CEFX) peptide pool (JPT). After 1 hour, brefeldin A was added. All time points of one patient were measured simultaneously to minimize technical variance within one patient. Patients were measured and analyzed in random order across cohorts to minimize technical variance and bias between cohorts. 15 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, PBMCs 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 healthy individuals. 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 (ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$). 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, BEAM = BEAM-autologous HCT within 12 months, CAR T = CD19 CAR T cell therapy, CLL = chronic lymphocytic leukemia, W&W = watch and wait, BTKi = ibrutinib, MM = multiple myeloma, VTD = induction therapy, αCD38 = daratumumab, IMiD = immunomodulatory drugs, HDM = HDM-autologous HCT within 9 months, AML = acute myeloid leukemia and high-risk MDS, chemo = high-dose chemotherapy, HMA = hypomethylating agents, MPN = myeloproliferative neoplasms, JAK2i = ruxolitinib, CML = chronic myeloid leukemia, TKI = tyrosine kinase inhibitors, alloHCT = allogeneic hematopoietic cell transplantation, GvHD = chronic graft-versus-host disease, HI = healthy individuals.

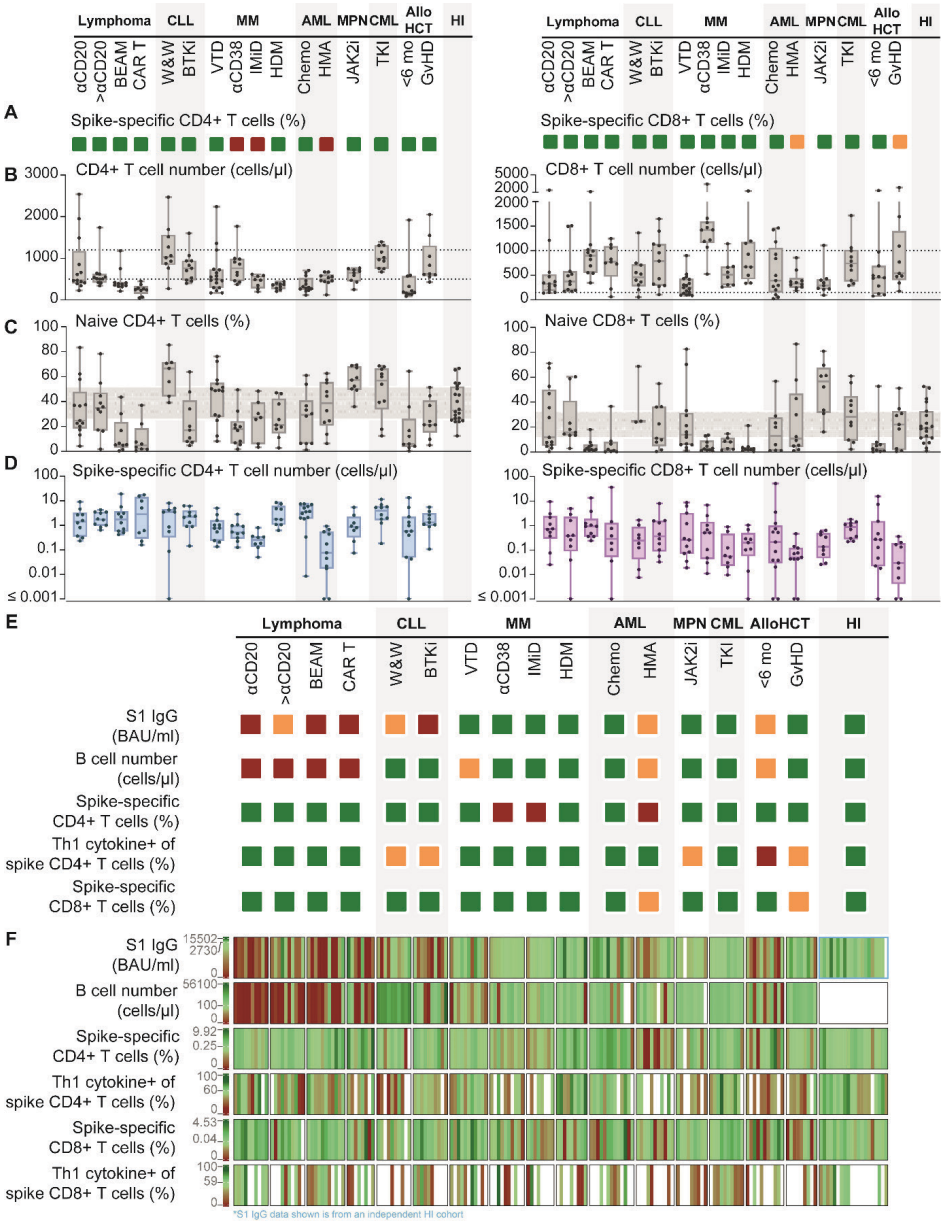
Generally, SARS-CoV-2 mRNA vaccination induced S1 IgG concentrations similar to HI in most patients except for patients that were B-cell depleted (**Figure 1A-B**). Spike-specific CD4+ and CD8+ T cells increased upon each vaccination, whilst other T cell specificities (CEFX; CMV, EBV, flu and more) remained constant in time (**Figure S3-7**). 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 (**Figure S28**). 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 (**Figure S9**). 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, S310A**). 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 (**Figure 1C-D, 2B-C, S310B-E**). 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 microliter blood, which showed comparable results (**Figure 2D**).

Figure 2 Baseline parameters, number of spike-specific T cells, and integrated analysis of cellular and humoral immune responses

A) Categorization of spike-specific CD4+ or CD8+ T cell frequencies as shown in figure 1. **B**) CD4+ or CD8+ T cell numbers directly measured in blood as cells per microliter. Absolute numbers of lymphocyte subsets were determined using fresh whole EDTA blood with Multitest 6-color reagents (BD Biosciences, San Jose, California) according to the manufacturer's instructions. The dotted lines indicate the clinically-accepted normal ranges in healthy individuals. **C**) Frequency of naïve (CCR7+CD45RA+) CD4+ or CD8+ T cells. Grey horizontal area corresponds to interquartile range in healthy individuals. **D**) Number of spike-specific CD4+ or CD8+ T cells in peripheral blood, calculated by multiplying the percentage of 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 cells frequencies is not depicted due to limited availability of data points. **F**) Summary heatmap of the data gathered from six 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 healthy individuals 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+ frequency of spike 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, BEAM = BEAM-autologous HCT within 12 months, CAR T = CD19 CAR T cell therapy, CLL = chronic lymphocytic leukemia, W&W = watch and wait, BTKi = ibrutinib, MM = multiple myeloma, VTD = induction therapy, αCD38 = daratumumab, IMiD = immunomodulatory drugs, HDM = HDM-autologous HCT within 9 months, AML = acute myeloid leukemia and high-risk MDS, chemo = high-dose chemotherapy, HMA = hypomethylating agents, MPN = myeloproliferative neoplasms, JAK2i = ruxolitinib, CML = chronic myeloid leukemia, TKI = tyrosine kinase inhibitors, alloHCT = allogeneic hematopoietic cell transplantation, GvHD = chronic graft-versus-host disease, HI = healthy individuals.


Lymphoma
CLL
MM
AML
MPN
CML
Allo HCT
HI
αCD20
>αCD20
BEAM
CAR T
W&W
BTKi
VTD
αCD38
IMiD
HDM
Chemo
HMA
JAK2i
TKI
<6 mo
GvHD
A
Spike-specific CD4+ T cells (%)
Spike-specific CD8+ T cells (%)
B
CD4+ T cell number (cells/µl)
CD8+ T cell number (cells/µl)
C
Naive CD4+ T cells (%)
Naive CD8+ T cells (%)
D
Spike-specific CD4+ T cell number (cells/µl)
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≤ 0.001
E
S1 IgG (BAU/ml)
B cell number (cells/µl)
Spike-specific CD4+ T cells (%)
Th1 cytokine+ of spike CD4+ T cells (%)
Spike-specific CD8+ T cells (%)
F
S1 IgG (BAU/ml)
B cell number (cells/µl)
Spike-specific CD4+ T cells (%)
Th1 cytokine+ of spike CD4+ T cells (%)
Spike-specific CD8+ T cells (%)
Th1 cytokine+ of spike CD8+ T cells (%)
*S1 IgG data shown is from an independent HI cohort

Analyzing the cohorts separately, the median S1 IgG concentration was <300 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 cell transplantation (alloHCT) <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 HCT (autoHCT) 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&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 IFN-γ and IL-2 compared to HI, which was partially reversed in CLL-depleted samples (**Figure 1E, S11**). In patients with 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 (**Figure S39**). 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 (**Figure S9D**). Patients who underwent alloHCT <6 months before vaccination had variable S1 IgG levels and spike-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 alloHCT could be related to corticosteroid use.[12] Patients that had developed chronic graft-versus-host-disease (cGvHD) after alloHCT 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 autologous (autoHCT), 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 (**Figure S12A**). This correlation, although not significant, was also observed when calculating the absolute number of spike-specific CD4+ T cells (**Figure S12B**). Patients treated with alloHCT within 6 months before vaccination had variable S1 IgG levels and spike-specific CD4+ T cell frequencies, which did not correlate with time since alloHCT (**Figure S12C**).

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 (**Figure 13**). 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 (**Figure S14A**). Frequencies of FOXP3+CD4+ T cells at the start of vaccination were low for all cohorts, yet in patients with MM who had received HDM, significantly increased frequencies were detected (**Figure S14B**).

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 (**Figure S20A-D**).[13] Seven patients received an auto-HCT 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 autoHCT (**Figure S20E**). 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 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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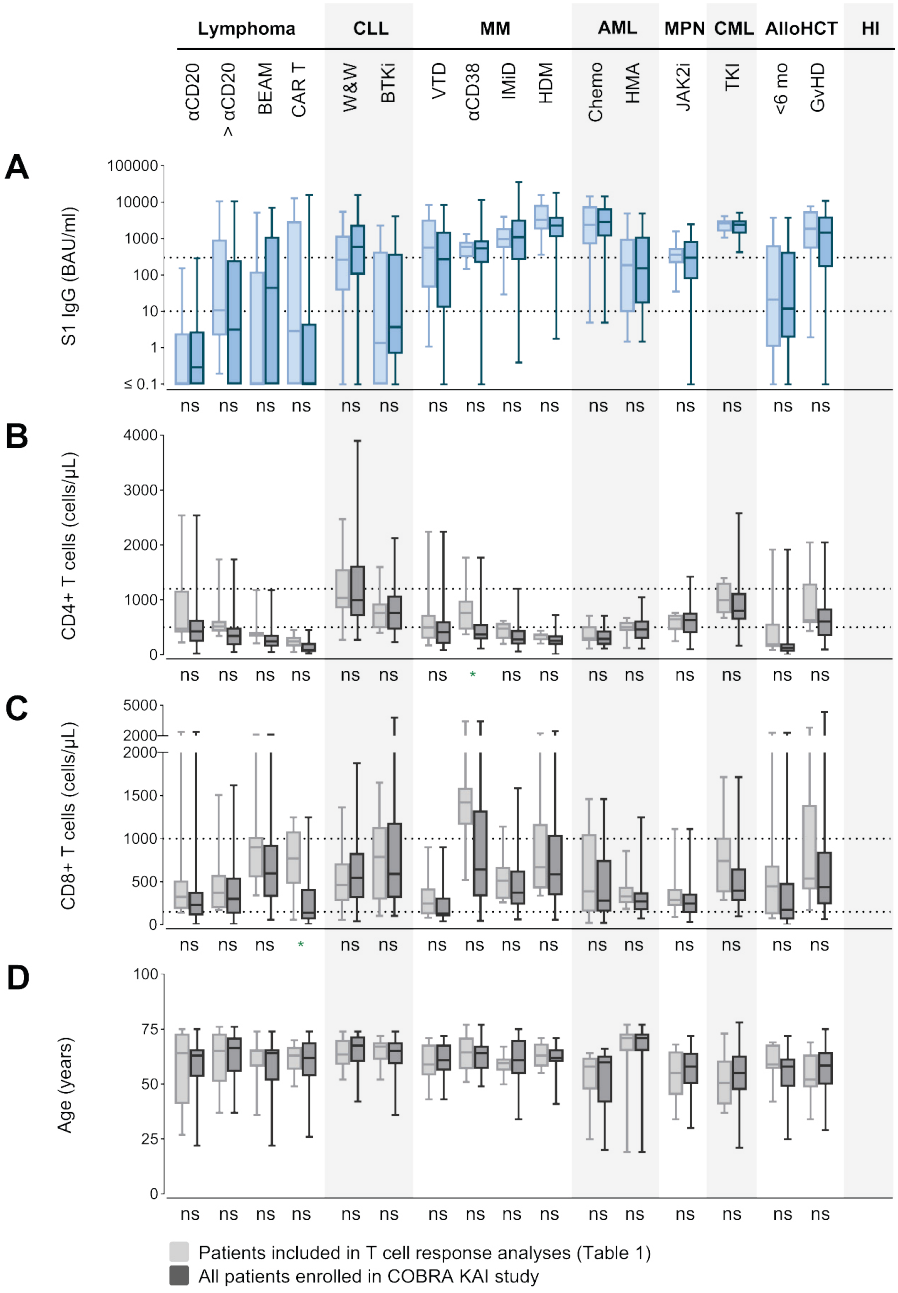
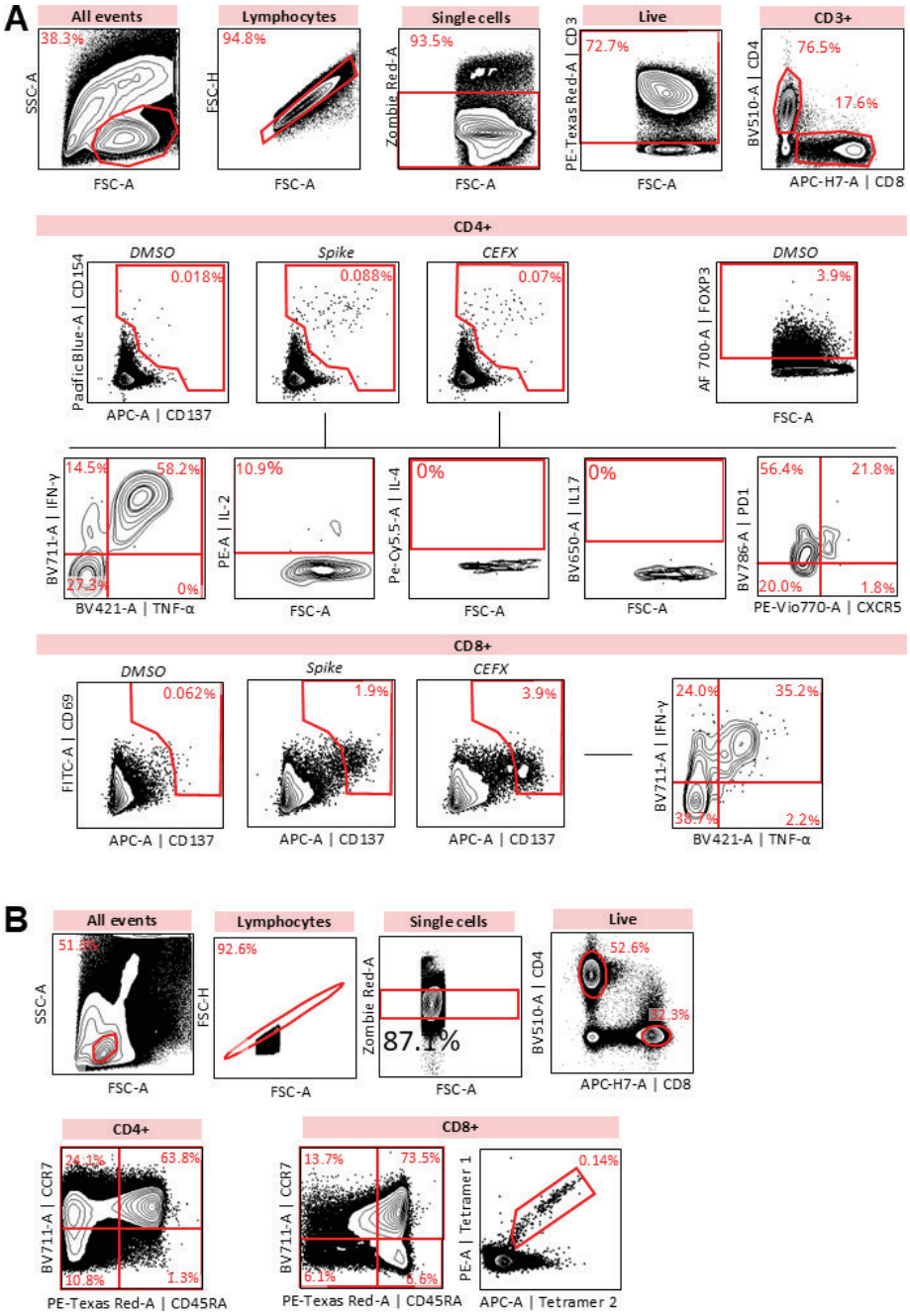
STUDY REGISTRATION

EudraCT 2021-001072-41



Supplementary figure 1 Clinical characteristics of patients included in T cell analyses in relation to full COBRA KAI study cohorts.

A) S1 IgG concentrations following the second mRNA vaccination. B) Number of CD4+ T cells per microliter blood. C) Number of CD8+ T cells per microliter blood. D) Age of the patients at start of vaccination. In A-D, values are compared to those in HI by Mann-Whitney U tests and significance corrected for multiple testing (times 16) is shown (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001).



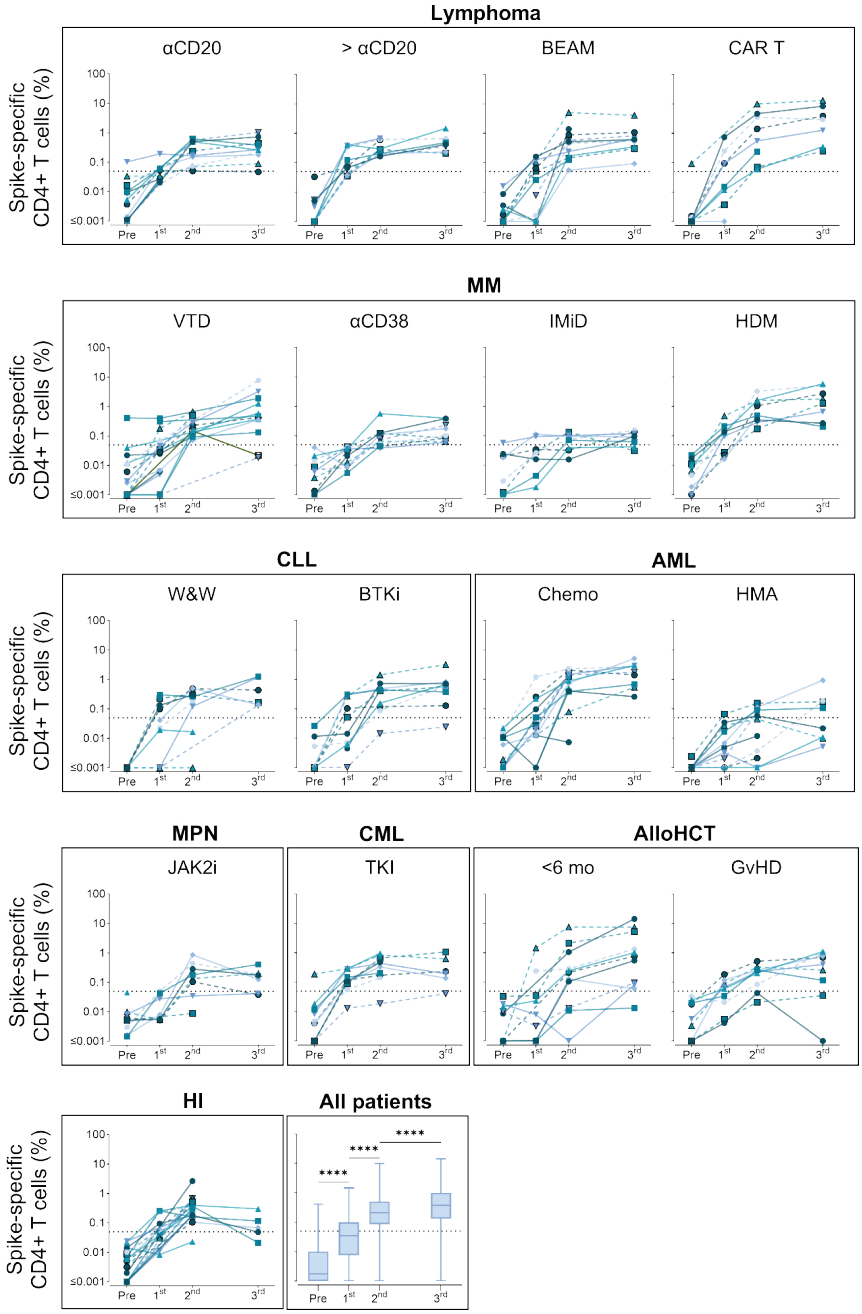
Supplementary figure 2 Flow cytometry gating example for the detection of antigen-specific T cells

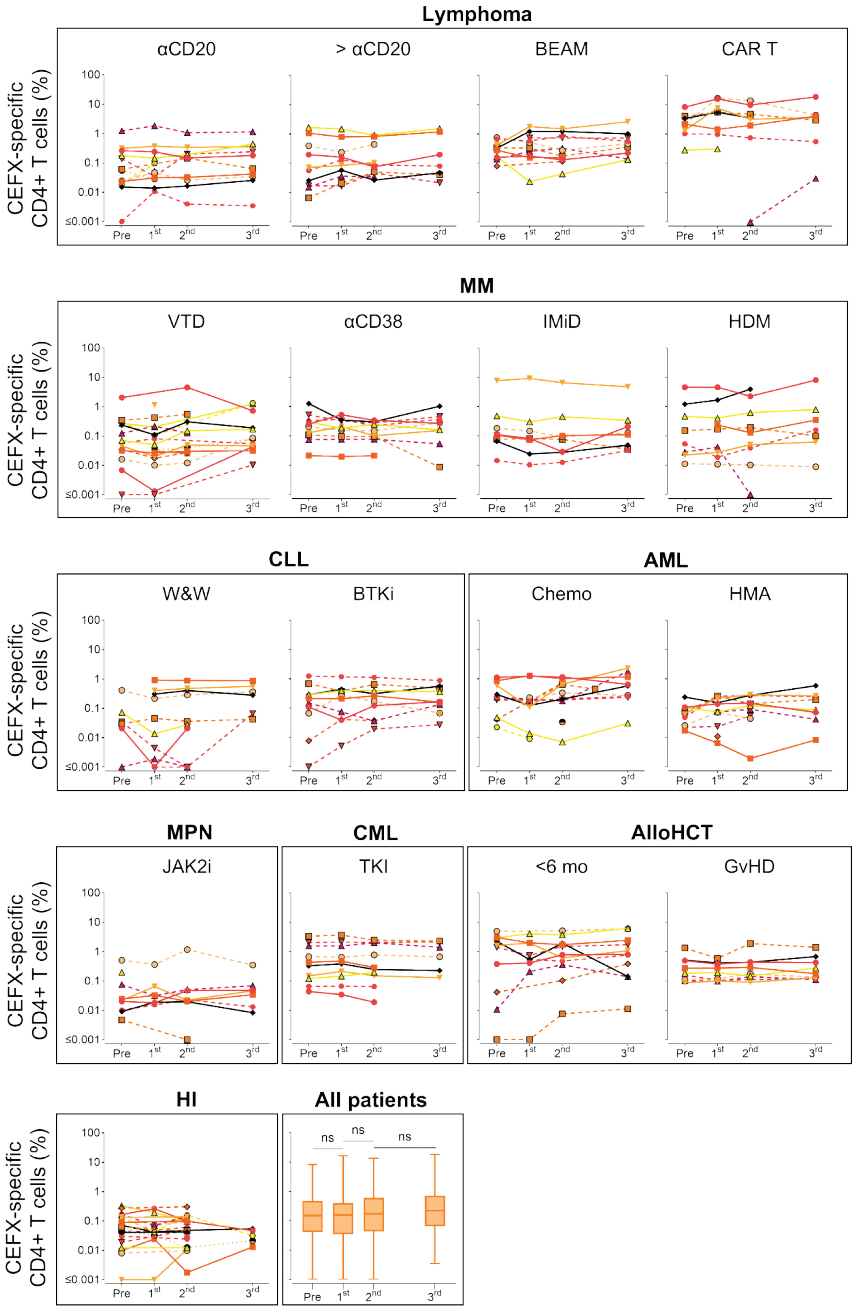
Supplementary figure 2 *Continued*

A) Representative example of flow cytometry gating strategy for antigen-reactive CD4+ and CD8+ T cells. Samples were measured on a three-laser Aurora (Cytek Biosciences) and analyzed using OMIQ (www.omiq.ai). All events were gated on lymphocytes, single cells, viable cells, CD3-positive, and either CD4- or CD8-positive. Only samples with more than 5,000 events in the CD4+ or CD8+ gates were analyzed. Activation was measured by upregulation of CD137 and CD154 by CD4+ T cells and CD137 and CD69 by CD8+ T cells, compared to DMSO. Response positivity thresholds were set at 0.05% for CD4+ T cells and 0.025% for CD8+ T cells based on results of an independent previous healthy cohort. Analysis of cytokine-producing spike-specific cells (IFN-γ, TNF-α, IL-2, IL-4, IL-17) and spike-specific Tfh cells (CD4+CXCR5+PD-1+) was only performed if the response positivity threshold was met and more than 25 events were measured in the CD154+/CD137+ gate for CD4+ T cells and in the CD137+CD69+ gate for CD8+ T cells. FOXP3+ cells were gated within total CD4+ T cell population in DMSO condition. B) Representative example of flow cytometry gating strategy for differentiation status of CD4+ and CD8+ T cells and for spike-specific CD8+ T cells using peptide- HLA tetramer technology. In addition to peptide-stimulation, 2 million unstimulated PBMCs were stained with a fixed pool of peptide-HLA tetramers to detect spike-specific CD8+ T cells. The tetramers consist of 23 peptides that were previously reported spike epitopes with strong (predicted) binding to 8 HLA-types common in The Netherlands. Tetramer staining was combined with antibodies directed against CD4, CCR7, and CD45RA followed by anti-CD8 staining. Samples were measured on a three-laser Aurora (Cytek Biosciences) and analyzed using OMIQ (www.omiq.ai). Only samples with more than 5,000 events in the CD4+ or CD8+ gates were analyzed, whilst 10,000 events were required in the CD8+ gate for the frequency of peptide-HLA tetramer-binding cells. All events were gated on lymphocytes, single cells, viable cells and CD4- or CD8-positive. Subsequently, the percentage of CD4+ or CD8+ T cells that express CCR7/CD45RA was determined. CD8+ T cells were gated on double positive tetramer binding for the detection of spike-specific CD8+ T cells.

Supplementary figure 3 Kinetics of spike-specific CD4+ T cell frequencies before and during the three-dose vaccination schedule

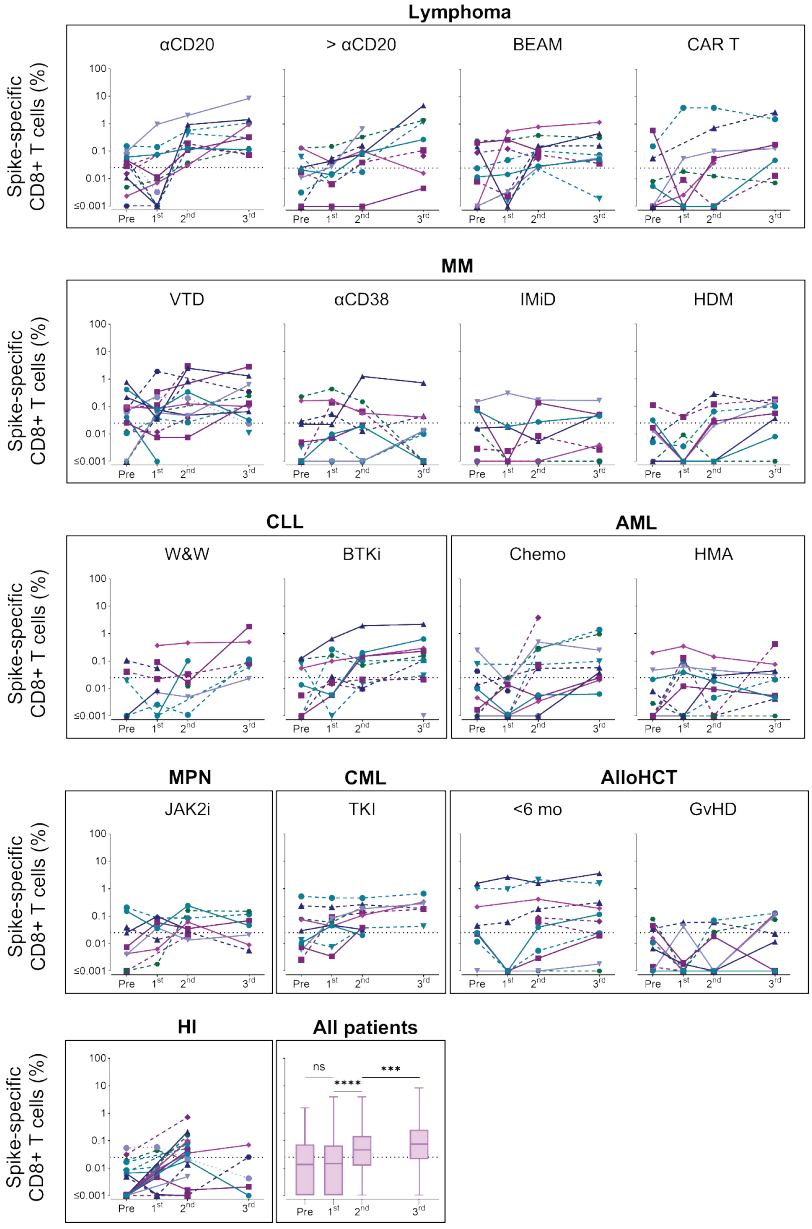
PBMC isolated prior to vaccination (Pre), four weeks after first vaccination (1st), four weeks after second vaccination (2nd) and four weeks after third vaccination (3rd) were incubated with SARS-CoV-2 spike peptide pool. Frequencies of CD4+ T cells positive for CD154 or CD137, corrected for DMSO, were plotted over time. Dotted line indicates response positivity threshold (0.05%). Each line represents one individual (ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$).


Lymphoma
αCD20
> αCD20
BEAM
CAR T
MM
VTD
αCD38
IMiD
HDM
CLL
W&W
BTKi
AML
Chemo
HMA
MPN
JAK2i
CML
TKI
AlloHCT
<6 mo
GvHD
HI
All patients
Spike-specific CD4+ T cells (%)
100
10
1
0.1
0.01
≤0.001
Pre
1st
2nd
3rd
****



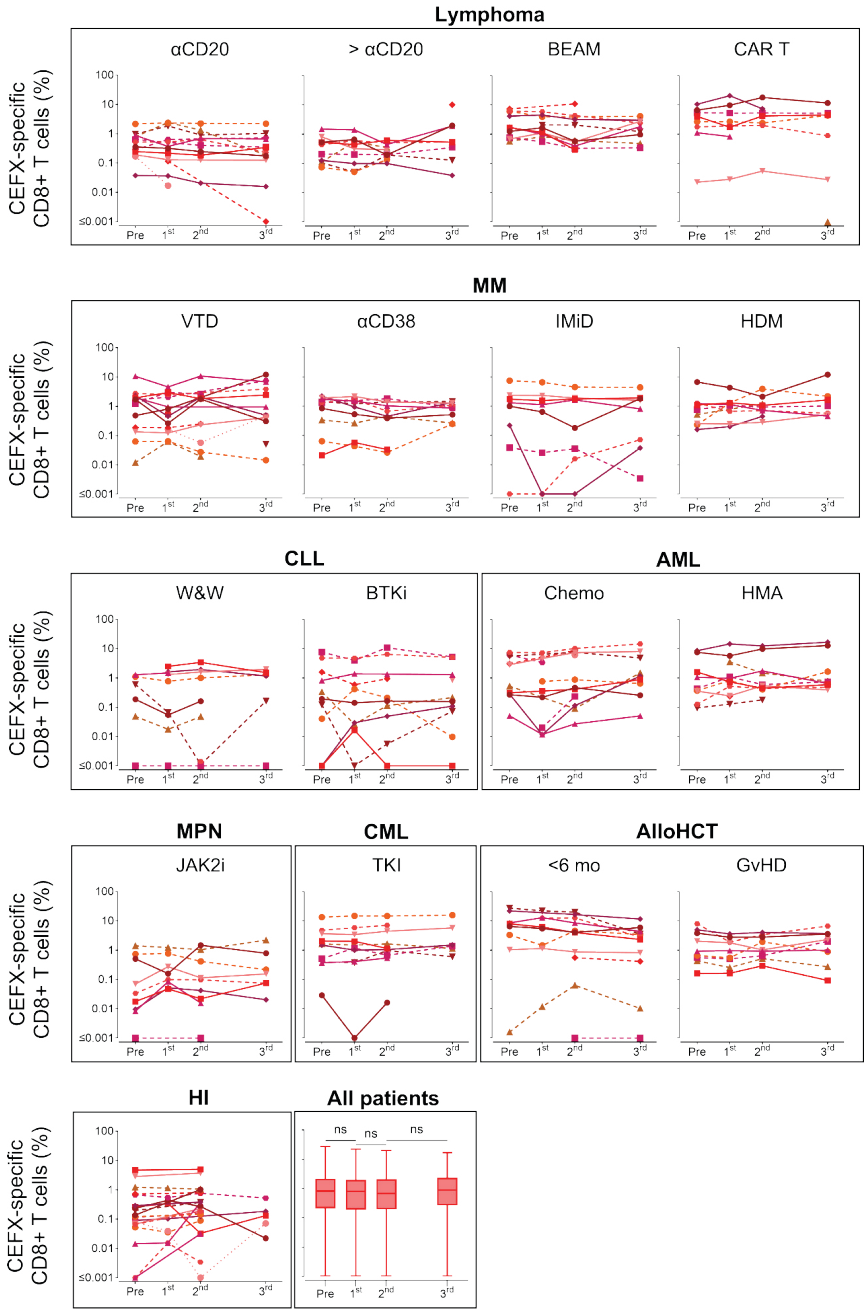
Supplementary figure 4 Kinetics of CEFX-specific CD4+ T cell frequencies before and during the three-dose vaccination schedule

PBMC isolated prior to vaccination (Pre), four weeks after first vaccination (1st), four weeks after second vaccination (2nd) and four weeks after third vaccination (3rd) were incubated with a CEFX peptide pool. Frequencies of CD4+ T cells positive for CD154 or CD137, corrected for DMSO, were plotted over time. Each line represents one individual (ns: p>0.055).



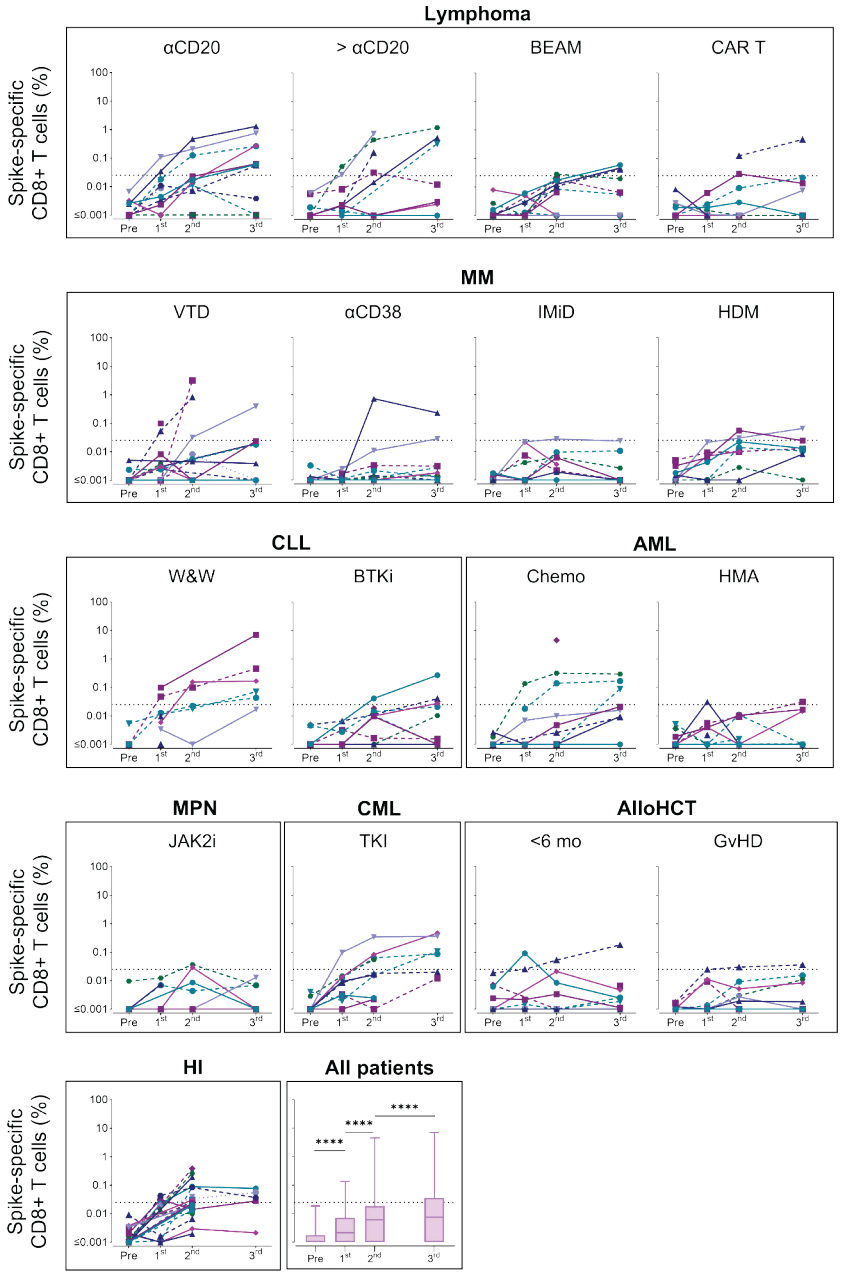
Supplementary figure 5 Kinetics of spike-specific CD8+ T cell frequencies before and during the three-dose vaccination schedule, determined by AIM

PBMC isolated prior to vaccination (Pre), four weeks after first vaccination (1st), four weeks after second vaccination (2nd) and four weeks after third vaccination (3rd) were incubated with SARS-CoV-2 spike peptide pool. Frequencies of CD8+ T cells positive for CD137 and CD69, corrected for DMSO, were plotted over time. Dotted line indicates response positivity threshold (0.025%). Each line represents one individual. Bottom right figure shows data points of all patients combined. Difference in frequency after each vaccination was tested by a Wilcoxon matched-pairs Signed-Rank test (ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$).



Supplementary figure 6 Kinetics of CEFX-specific CD8+ T cell frequencies before and during the three-dose vaccination schedule

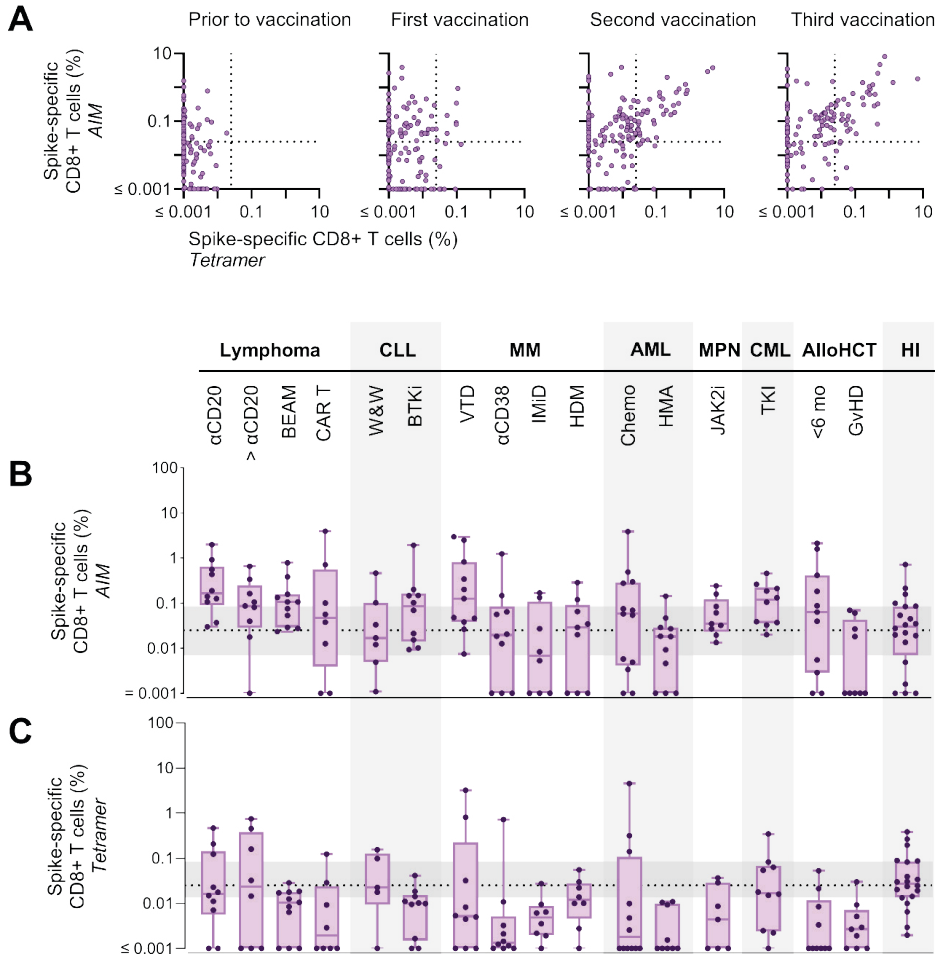
PBMC isolated prior to vaccination (Pre), four weeks after first vaccination (1st), four weeks after second vaccination (2nd) and four weeks after third vaccination (3rd) were incubated with a CEFX peptide pool. Frequencies of CD8+ T cells positive for CD137 and CD69, corrected for DMSO, were plotted over time. Each line represents one individual. Difference in frequency after each vaccination was tested by a Wilcoxon matched-pairs Signed-Rank test (ns: $p>0.05$; *: $p\leq0.05$; **: $p\leq0.01$; ***: $p\leq0.001$; ****: $p\leq0.0001$).



Supplementary figure 7 Supplementary figure 10: Kinetics of spike-specific CD8+ T cell frequencies before and during the three-dose vaccination schedule, determined by peptide-HLA tetramer technology PBMC isolated prior to vaccination (Pre), 4 weeks after first vaccination (1st), 4 weeks after second vaccination (2nd) and 4 weeks after

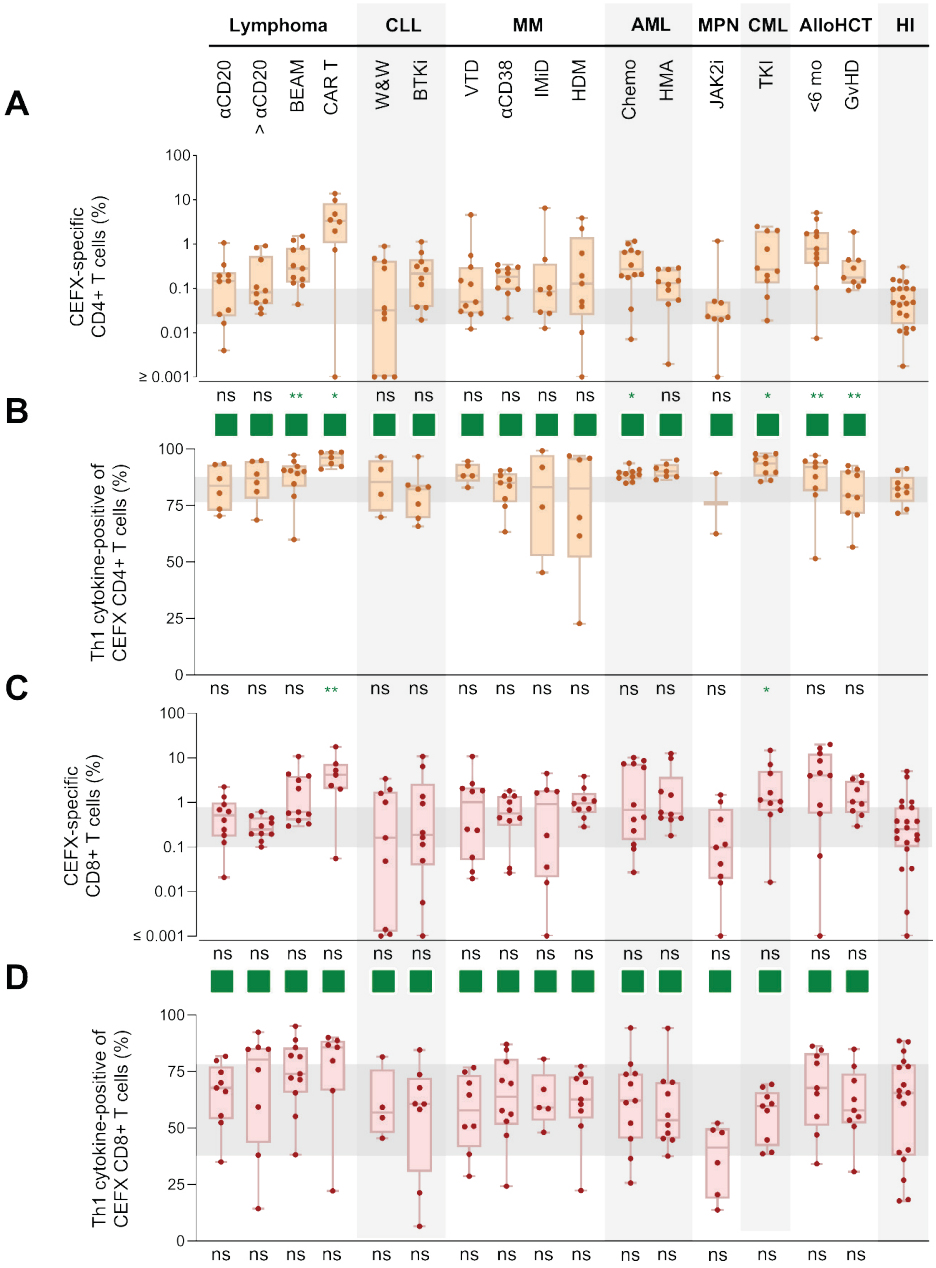
Supplementary figure 7 *Continued*

third vaccination (3rd) were incubated with peptide-HLA tetramers containing SARS-CoV-2 spike peptides bound to common HLA-types. Frequency of CD8+ T cells that bound to the tetramers were plotted over time. Dotted line indicates response positivity threshold (0.025%). Each line represents one individual. Difference in frequency after each vaccination was tested by a Wilcoxon matched-pairs Signed-Rank test (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001).



Supplementary figure 8 Supplementary figure 8: Detection of spike-specific CD8+ T cells through peptide-stimulation assays and peptide-HLA tetramer staining

A) Frequency of spike-specific CD8+ T cells measured by activation induced markers (AIM) plotted against frequency of spike-specific CD8+ T cells measured by peptide-HLA tetramer technology. Spike-specific CD8+ T cell frequencies are shown before vaccination and four weeks after the first, second, and third mRNA vaccine dose. B) Frequency of spike-specific CD8+ T cells measured by AIM assay four weeks after the second mRNA vaccination. C) Frequency of spike-specific CD8+ T cells measured by peptide-HLA tetramer technology four weeks after the second mRNA vaccination. In B and C, horizontal grey area shows interquartile range of HI. Dotted line indicates response positivity threshold (0.025%). Each dot represents one individual.

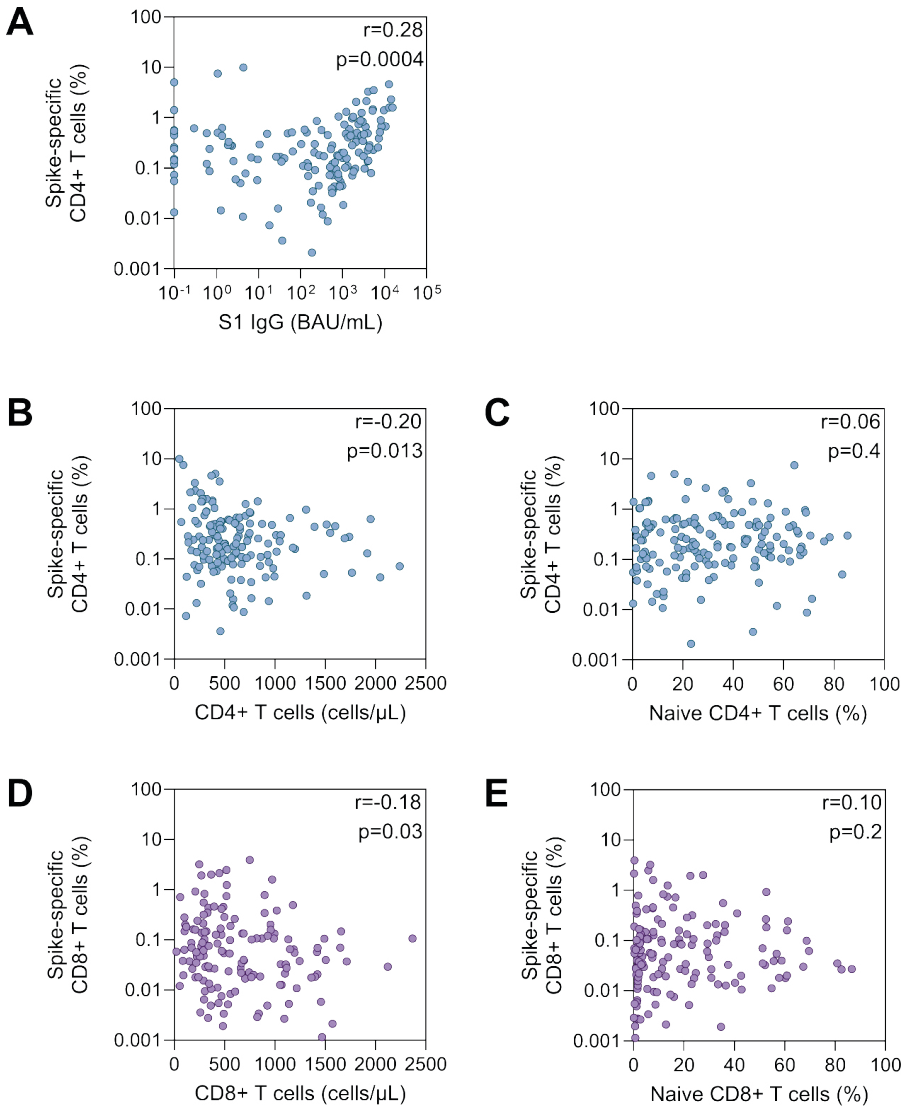


Supplementary figure 9 Supplementary figure 7: Frequency of CEFX-specific CD4+ and CD8+ T cells

A) Frequency of CEFX-specific CD4+ T cells after two COVID-19 mRNA vaccinations. B) Frequency of CEFX-specific CD4+ T cells that produce IFN-γ, TNF-α and/or IL-2. Frequency was calculated by subtracting the frequency of cells that do not produce any cytokines from 100%. C) Frequency of CEFX-

Supplementary figure 9 *Continued*

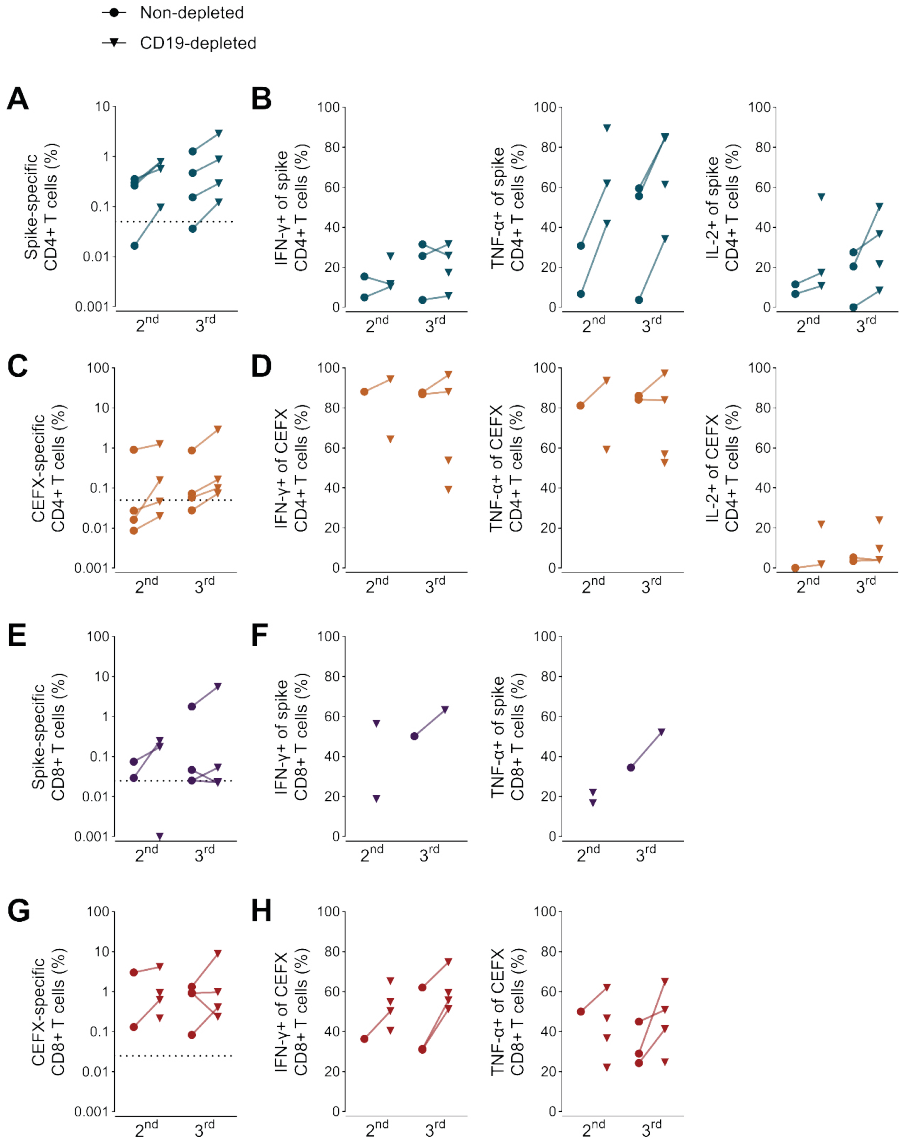
specific CD8+ T cells after two COVID-19 mRNA vaccinations. D) Frequency of CEFX-specific CD8+ T cells that produce IFN-γ, TNF-α and/or IL-2. Frequency was calculated by subtracting the frequency of cells that do not produce any cytokines from 100%. The grey horizontal area corresponds to interquartile range in healthy individuals. Each dot represents one individual. T cell frequencies are compared to those in HI by Mann-Whitney U tests and significance corrected for multiple testing (times 16) is shown (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001). T cell responses are categorized based on p value prior to and after correction for multiple testing (green when not significantly lower prior to correction; orange when significantly lower prior to, but not after correction; red when significantly lower after correction).



Supplementary figure 10 Supplementary figure 5: Correlations between spike-specific T cell frequencies and other immune parameters

A) Correlation between spike-specific CD4+ T cell frequencies and S1 IgG concentration after two

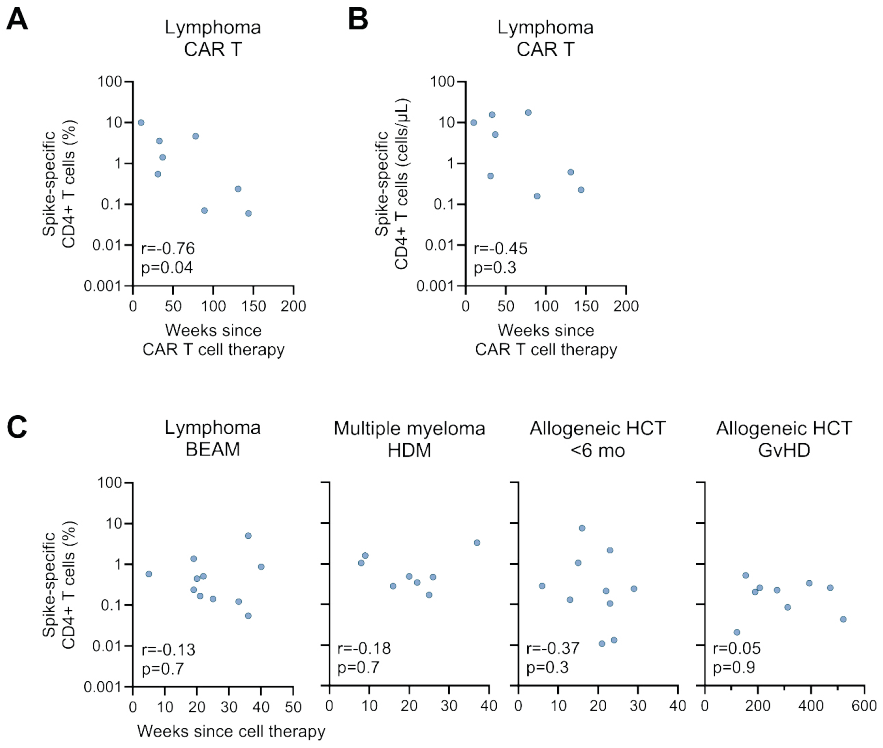
vaccinations. B) Correlation between spike-specific CD4+ T cell frequencies after two vaccinations and CD4+ T cell numbers in blood at start of vaccination. C) Correlation between spike-specific CD4+ T cell frequencies after two vaccinations and naïve CD4+ T cell frequencies (CCR7+CD45RA+) at start of vaccination. D) Correlation between spike-specific CD8+ T cell frequencies after two vaccinations and CD8+ T cell numbers in blood at start of vaccination E) Correlation between spike-specific CD8+ T cell frequencies after two vaccinations and naïve CD8+ T cell frequencies (CCR7+CD45RA+) at start of vaccination. Each dot represents one individual. Statistics show result of Spearman's correlation.



Supplementary figure 11 Supplementary figure 13: Spike-specific T cell frequencies, including cytokine production, in CD19-depleted and non-depleted PBMC from patients with CLL

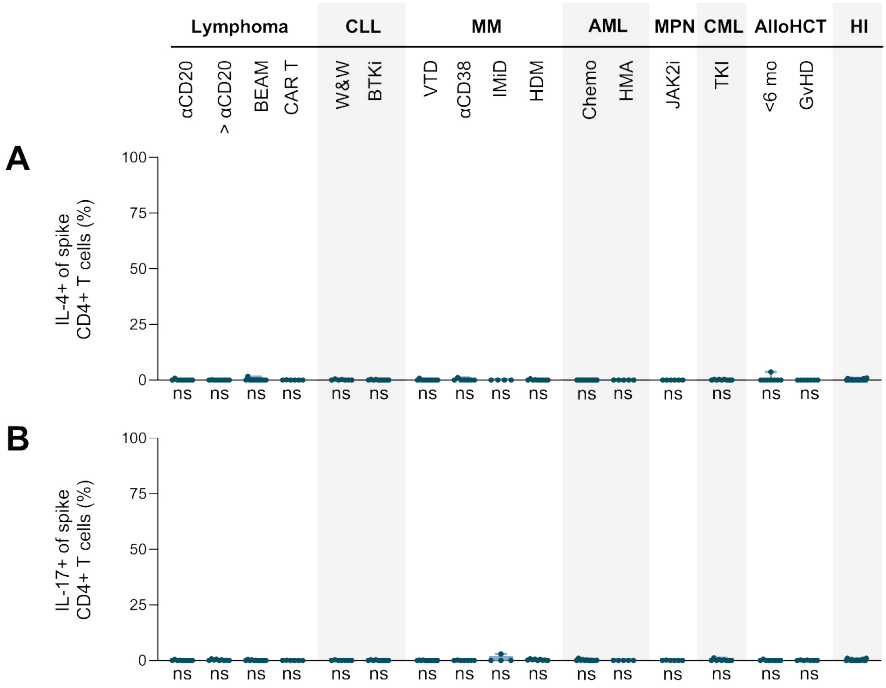
Supplementary figure 11 *Continued*

PBMCs from four patients with CLL with B cell counts >5000/μl were randomly selected and depleted from CD19+ cells using QuadroMACS™ (Miltenyi) before incubation with peptides and measurement. A) Spike-specific CD4+ T cells frequencies. B) Frequency of spike-specific CD4+ T cells positive for IFN-γ, TNF-α or IL-2. C) CEFX-specific CD4+ T cells frequencies. D) Frequency of CEFX-specific CD4+ T cells positive for IFN-γ, TNF-α or IL-2. E) Spike-specific CD8+ T cells frequencies. F) Frequency of spike-specific CD8+ T cells positive for IFN-γ or TNF-α. G) CEFX-specific CD8+ T cell frequencies. H) Frequency of CEFX-specific CD4+ T cells positive for IFN-γ or TNF-α. In A-H, PBMC were either non-depleted (circles) or depleted from CD19+ cells (triangles). Dotted line indicates response positivity threshold. Each line or dot represents one individual.



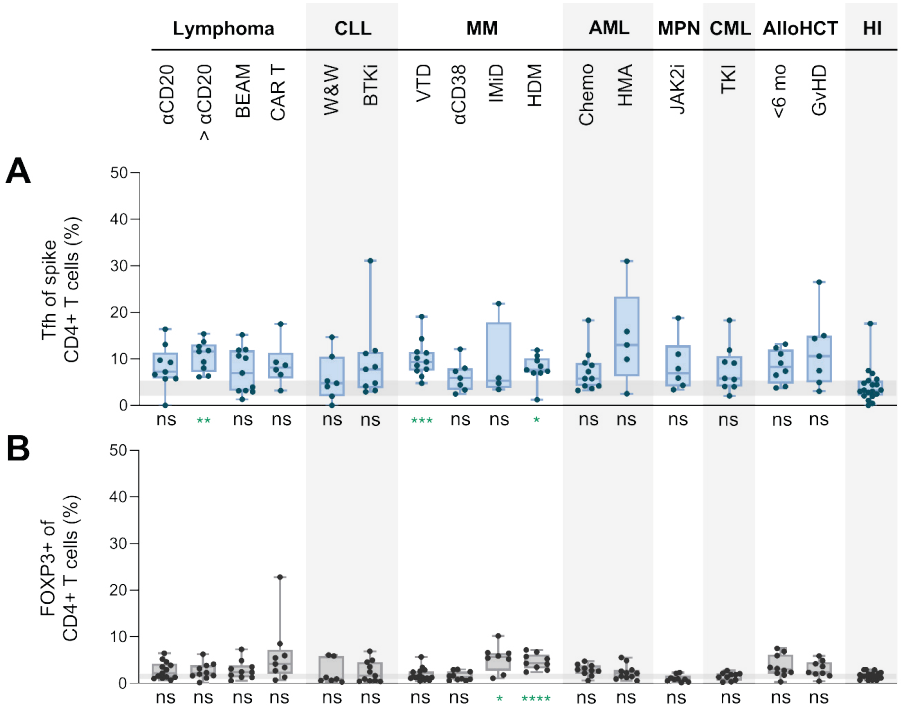
Supplementary figure 12 Supplementary figure 6: Correlation between spike-specific CD4+ T cell frequencies/numbers and duration since cell therapy

A) Correlation between spike-specific CD4+ T cell frequency and weeks since CAR T cell infusion. B) Correlation between number of spike-specific T cells per microliter blood and weeks since CAR T cell infusion. C) Correlation between spike-specific T cell frequency and weeks since hematopoietic cell transplantation. Each dot represents one individual. Statistics are shown for Spearman's correlation test.



Supplementary figure 13 Supplementary figure 12: IL-4 and IL-17 production by spike-specific CD4+ T cells

A) Frequency of spike-specific CD4+ T cells that produce IL-4. B) Frequency of spike-specific CD4+ T cells that produce IL-17. Each dot represents one individual. 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 (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001).

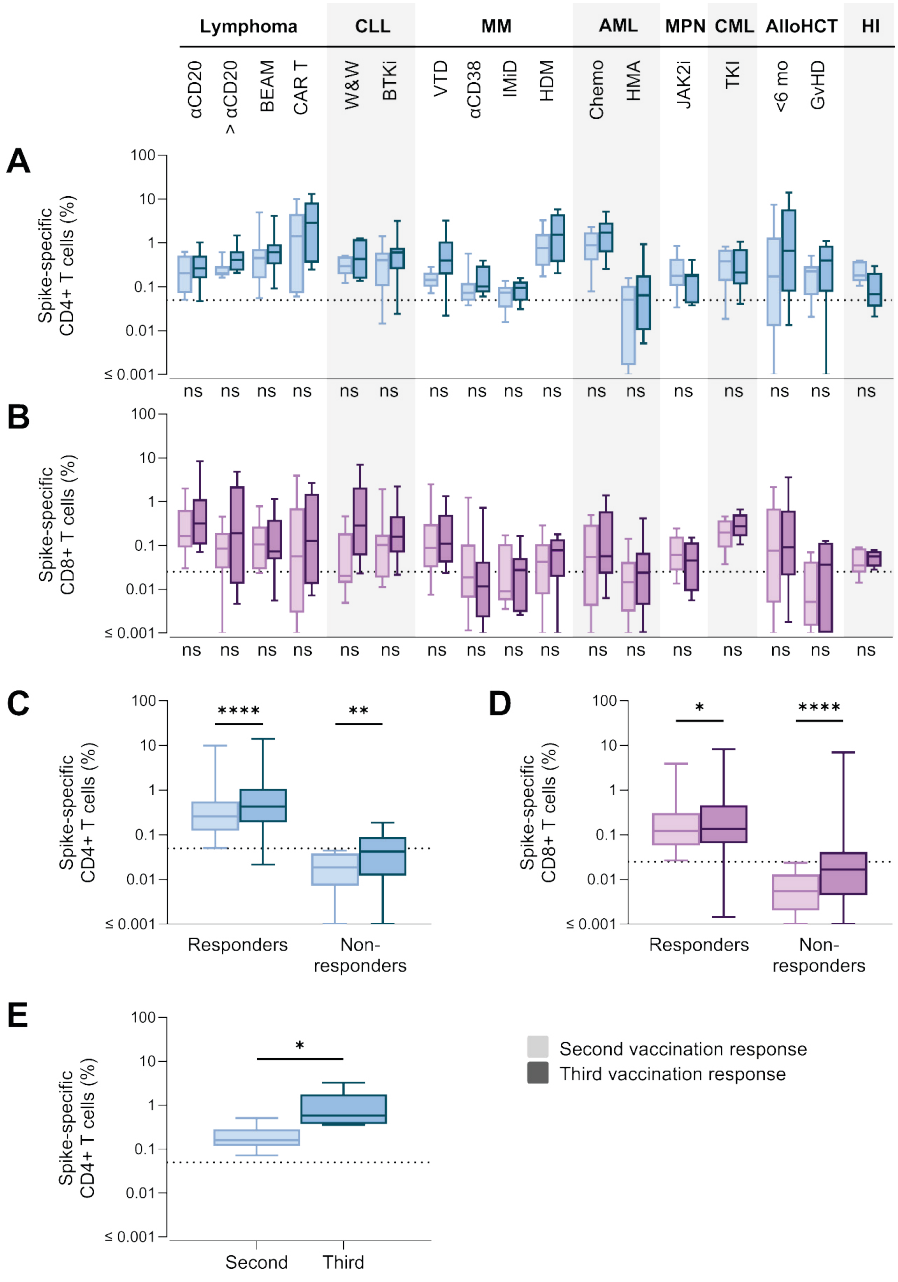


Supplementary figure 14 Supplementary figure 18: FOXP3+ expression by CD4+ T cells, and PD-1+CXCR5+ expression by spike-specific CD4+ T cells

A) Frequency of FOXP3+ CD4+ T cells at start of vaccination. B) Frequency of spike-specific CD4+ T cells that are follicular helper T cells (Tfh; CXCR5+PD-1+) after two mRNA vaccinations. In A and B, horizontal grey area indicate interquartile range of HI. Each dot represents one individual. 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 (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001).

Supplementary figure 15 Supplementary figure 20: Spike-specific CD4+ and CD8+ T cell frequencies after second and third vaccination.

A) Spike-specific CD4+ T cell frequencies after two (light blue) or three (dark blue) COVID-19 mRNA vaccinations. B) Spike-specific CD8+ T cell frequencies after two (light purple) or three (dark purple) COVID-19 mRNA vaccinations. C) Spike-specific CD4+ T cell frequencies from all patients, separated in responders and non-responders based on spike-specific CD4+ T cell frequencies above (responders) or below (non-responders) the response positivity threshold (0.05%) after two vaccinations. D) Spike-specific CD8+ T cell frequencies from all patients, separated in responders and non-responders based on spike-specific CD8+ T cell frequencies above (responders) or below (non-responders) the response positivity threshold (0.025%) after two vaccinations. E) Comparing the spike-specific CD4+ T cell frequencies after the second and third vaccination of patients that received an autoHCT between second and third vaccination. Dotted lines indicate response positivity thresholds. T cell frequencies are compared between four weeks after second and third vaccination using a paired t-test and p values are shown (ns: p>0.05; *: p≤0.05; **: p≤0.01; ***: p≤0.001; ****: p≤0.0001). Correction for multiple testing was performed (p-value times 16).


Lymphoma
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AML
MPN
CML
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HI
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> αCD20
BEAM
CAR T
W&W
BTKi
VTD
αCD38
IMiD
HDM
Chemo
HMA
JAK2i
TKI
<6 mo
GvHD
A
Spike-specific CD4+ T cells (%)
B
Spike-specific CD8+ T cells (%)
100
10
1
0.1
0.01
≤ 0.001
ns
C
D
****
**
*
Responders
Non-responders
E
Second
Third
Second vaccination response
Third vaccination response

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