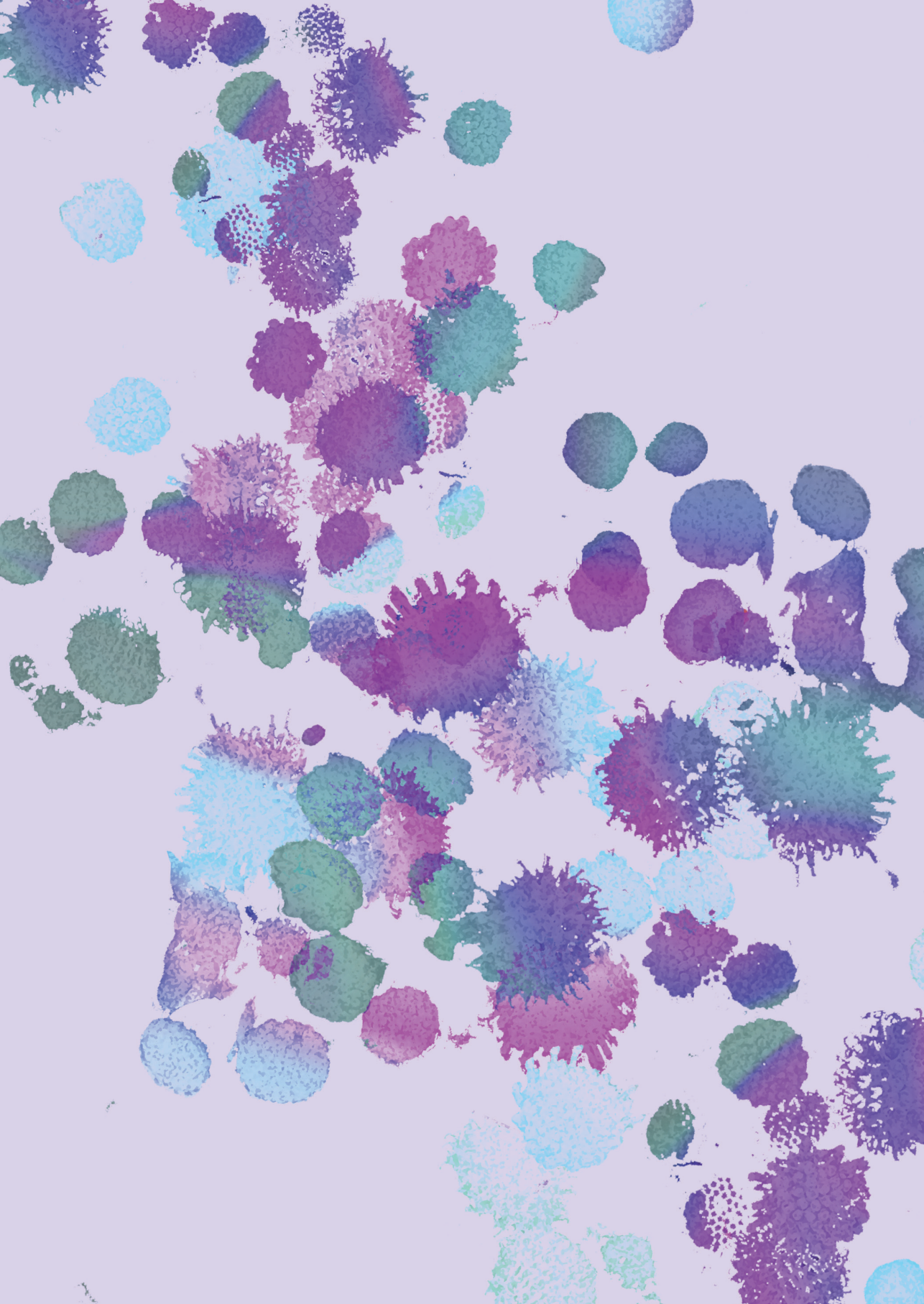
Primary T-cell responses against SARS-CoV-2 in patients with hematological disorders

Pothast, C.R.



INCREASED CD8 T-CELL IMMUNITY AFTER COVID-19 VACCINATION IN LYMPHOID MALIGNANCY PATIENTS LACKING ADEQUATE HUMORAL RESPONSE: AN IMMUNE COMPENSATION MECHANISM?

Boerenkamp, L. S.*, Pothast, C. R.*, Dijkland, R. C., van Dijk, K., van Gorkom, G. N. Y., van Loo, I. H. M., Wieten, L., Halkes, C. J. M., Heemskerk, M. H. M., & Van Elssen, C.

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Chapter 4

TO THE EDITOR

SARS-CoV-2 vaccine immunogenicity is commonly evaluated by measuring antibody titers against the SARS-CoV-2 Spike (S) protein. Previously, inferior humoral vaccination responses in patients with lymphoid malignancies have been shown.[1] This can be attributed to immune defects caused by disease or treatment. NHL and CLL treated with CD20- and MM with CD38-directed therapies lead to long-lasting B- or plasma-cell depletion, respectively. CLL and MM are associated with hypogammaglobulinemia and aberrations in T-cell function. T-cell immunity is vital for viral clearance and long-lasting protection against COVID-19 after vaccination.[2] Moreover, high CD8+ T-cells contribute to COVID-19 survival in hematological patients.[3] Studies investigating T-cell responses after vaccination in patients with lymphoid malignancies are, however, scarce and results are conflicting. This leaves a knowledge gap, underlining the urgency of an in-depth and reproducible analysis of functional SARS-CoV-2-specific T-cell responses following vaccination in hematological patients.

Patients. Adult patients diagnosed with CLL, NHL, or MM at two tertiary care centers in the Netherlands undergoing SARS-CoV-2 vaccination were included in the study. Serological and T-cell responses were evaluated pre-vaccination and 2 weeks, 3- and 6-months post-vaccination. Patients were included between March and June 2021. All participants gave written informed consent and all procedures performed were in accordance with the ethical standards of the national research committee and the 1964 Helsinki declaration (NL76863.068.21/METC 21-014).

Vaccination responses. Antibody levels were measured with an anti-SARS-CoV-2 S immunoassay (Elecsys, Roche Diagnostics International Ltd). Adequate seroconversion was defined as an Ig serum concentration ≥250 BAU/ml. CD4+ and CD8+ T-cell responses were evaluated by stimulation of PBMCs using a 15-mer with 11 amino acid overlapping SARS-CoV-2 spike peptide pool (SB-peptide). T-cell activation and phenotype were measured by flow cytometry: CD154, CD137, CD69, IFN-γ, TNF-α, IL-2, IL-4, PD-1, IL-17, CXCR5, and FOXP3. In parallel, SARS-CoV-2-specific CD8+ T-cells were detected using spike peptide-HLA tetramer (spike/HLA-tetramer) staining combined with differentiation markers (CD45RA and CCR7). Stimulation with CEFX peptide pool (JPT/LUMC), containing peptides of CMV and other common pathogens, was used as a positive control. A response was considered to be positive if ≥0.05% of all CD4+ T-cells are spike reactive and if ≥0.005% of all CD8+ T-cells are spike reactive or binding spike-specific human leukocyte antigen (HLA)-peptide tetramers. These thresholds were based on the CD4 and CD8 T-cell responses measured in nine healthy individuals (data not shown). Additional information is provided in Supplemental Methods.

Statistics. Continuous variables are described as median (IQR) and categorical variables as number and percentage of total. Antibody and T-cell responses of diagnostic subgroups were compared to population proportions using one-sample binomial testing. Missing data varied across timepoints; therefore, the number of included measurements is mentioned in the figures and confidence intervals are provided. To evaluate factors associated with humoral and cellular responses, univariable logistic regression was performed. To compare groups Chi square, Wilcoxon or Mann–Whitney U tests were used when applicable. All tests were two-sided with an α of 0.05. Analyses were performed using IBM SPSS Statistics (version 25.0.0.2) and Graphpad Prism (version 9.3.1).

One hundred and sixty patients diagnosed with CLL (n = 31), aggressive NHL (n = 39), indolent NHL (n = 57), MM (n = 30), and other (acute lymphoblastic leukemia n = 2, autoimmune pancytopenia n = 1) were included (Table S1). All patients received two doses of an mRNA vaccine. Eight patients with pre-vaccination positive anti-SARS-CoV-2 S Ig were excluded from the analysis.

Adequate seroconversion rates 2 weeks post-vaccination were 31% in CLL, 62% in aggressive NHL, 60% in indolent NHL, and 93% in MM patients (Figure 1A). Compared to an age-adjusted population proportion of 99%, this was significantly lower in patients with all disease categories, except MM. Recent treatment with anti-CD20 containing regimens significantly reduces adequate seroconversion rate (Figure S1A). However, when treatment was >12 months before vaccination, this seroconversion rate increased significantly to 87% (Figure S1B), indicating that sufficient B-cell recovery to elicit seroconversion takes at least 12 months after B-cell depleting therapy. Next to disease and treatment, univariable logistic regression analysis showed that only a lymphocyte count below 1*109/L was a significant predictor of humoral outcome (Figure S2A), which is related to B-cell-depleting therapy. These findings are in line with previous studies.[1]

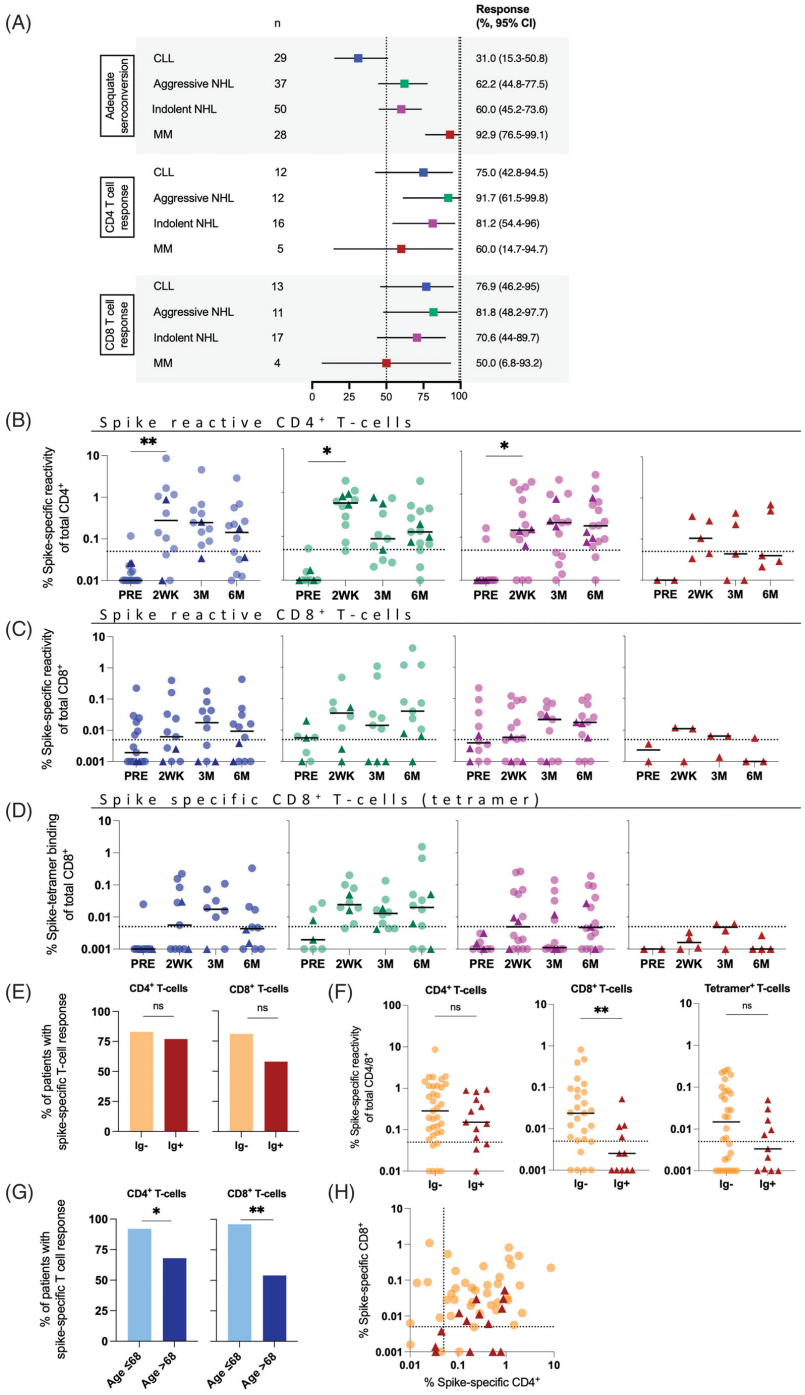
(A)
n
Response (%, 95% CI)
Adequate seroconversion
CLL 29 31.0 (15.3-50.8)
Aggressive NHL 37 62.2 (44.8-77.5)
Indolent NHL 50 60.0 (45.2-73.6)
MM 28 92.9 (76.5-99.1)
CD4 T cell response
CLL 12 75.0 (42.8-94.5)
Aggressive NHL 12 91.7 (61.5-99.8)
Indolent NHL 16 81.2 (54.4-96)
MM 5 60.0 (14.7-94.7)
CD8 T cell response
CLL 13 76.9 (46.2-95)
Aggressive NHL 11 81.8 (48.2-97.7)
Indolent NHL 17 70.6 (44-89.7)
MM 4 50.0 (6.8-93.2)
0 25 50 75 100
(B)
Spike reactive CD4+ T-cells
% Spike-specific reactivity of total CD4+
PRE 2WK 3M 6M
(C)
Spike reactive CD8+ T-cells
% Spike-specific reactivity of total CD8+
(D)
Spike specific CD8+ T-cells (tetramer)
% Spike-tetramer binding of total CD8+
(E)
CD4+ T-cells
CD8+ T-cells
% of patients with spike-specific T-cell response
ns
Ig- Ig+
(F)
CD4+ T-cells
CD8+ T-cells
Tetramer+ T-cells
% Spike-specific reactivity of total CD4/8+
(G)
CD4+ T-cells
CD8+ T-cells
% of patients with spike-specific T cell response
Age ≤68
Age >68
(H)
% Spike-specific CD8+
% Spike-specific CD4+

Figure 1 Humoral and spike-specific CD4+ and CD8+ T-cell responses after mRNA vaccination in patients with CLL (purple), aggressive NHL (green), indolent NHL (pink) or MM (red).

Response evaluation at different time points (2 weeks, 3 months, and 6 months) after initial complete vaccination, which for mRNA-1273 and BNT162b consisted of two vaccination doses, administered within a 4–5 week interval. Data for patients with CLL are shown in blue, for aggressive NHL in green, for indolent NHL in purple and, for MM in red. Anti-SARS-CoV-2 Spike antibodies were measured using anti-SARS-CoV-2 S immunoassay. Spike-specific T-cell responses were measured by thawing PBMCs and stimulating them with a spike peptide pool for 16 h, and afterwards, samples were analyzed by flow cytometry using various markers. Values were corrected for background measured in DMSO. In (B–H), each dot represents a patient; seronegative patients are depicted as circles and seropositive patients are depicted as darker triangles. (A) Response rates 2 weeks after complete vaccination, serological response rate was based on the percentage of patients with adequate anti-SARS-CoV-2 spike Ig (>250 BAU/ml). CD4+ T-cell response was the percentage of patients with >0.05% of spike-specific CD4+ T-cells (CD137+ and/or CD154+) within the total CD4+ population and CD8+ T-cell response was the percentage of patients with >0.005% of spike-specific CD8+ T-cells (CD69+ and/or CD137+ and IFN-γ+ and/or TNF-α+) and/or Spike/HLA-tetramer positive CD8+ T-cells within the total CD8+ population. All proportions were stratified based on hematological disorder and compared to expected population proportions of 99% for serological response, 100% for CD4+ T-cell response, and 50% for CD8+ T-cell response (shown as dotted lines in the figure) using one sample binomial testing. Clopper-Pearson method was used to estimate 95% confidence intervals. (B) Percentage of spike-specific CD4+ T-cells of total CD4+ T-cells measured before and after vaccination. Spike-specific CD4+ T-cells were defined as CD4+ T-cells expressing CD137+and/or CD154+ after stimulation with SARS-CoV-2 Spike peptides. Dotted line represents threshold of 0.05%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months (p = .526, p = .319, .227). Wilcoxon test shows a significant increase in percentage of spike-specific CD4+ T-cells 2 weeks after vaccination for CLL, aggressive NHL and indolent NHL (p = .0078, .0156, .0313) compared to pre vaccination, MM was not tested due to lack of sufficient samples. (C) Percentage of spike-specific CD8+ T-cells of total CD8+ T-cells measured before and after vaccination. Spike-specific CD8+ T-cells were defined as CD8+ T-cells having a expressing CD69+and/or CD137+ and IFN-γ+ and/or TNF-α+ after SARS-CoV2 spike peptide stimulation. Dotted line represents threshold of 0.005%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months (p = .369, .921, .082). Wilcoxon test shows no significant increase in spike-specific CD8+ T-cell response 2 weeks after vaccination for CLL, aggressive and indolent NHL (p = .195, .250, .922) compared to pre-vaccination, MM was not tested due to lack of sufficient samples. (D) Percentage of Spike/HLA-tetramer positive CD8+ T-cells of total CD8+ T-cells measured before and after vaccination. Dotted line represents threshold of 0.005%. Black horizontal line represents median. Kruskal–Wallis testing does not show significant difference between hematologic disorders at 2 weeks, 3, or 6 months (p = .146, .225, .188). Wilcoxon test shows no significant increase in tetramer positive CD8+ T-cell responses 2 weeks after vaccination for CLL, aggressive and indolent NHL (p = .063, .063, .094) compared to pre-vaccination, MM was not tested due to lack of sufficient samples. (E) Percentage of patients with spike-specific CD4+ or CD8+ T-cell responses in adequately seroconverted (Ig+) or seronegative (Ig–) patients. Significance was tested by Chi square test (CD4+ p = .640, CD8+ p = .143). (F) Frequency of spike-specific CD4+ or CD8+ T-cells in adequately seroconverted (Ig+) or seronegative (Ig–) patients. Significance was tested by Mann–Whitney U tests (CD4+ p = .332, CD8+ p = .007, tetramer p = .21). (G) Percentage of patients with a spike-specific CD4+ or CD8+ T-cell response split by age under or above 68 years. Significance was tested by Chi square test (CD4+ p = .033, CD8+ p = .001). (H) Percentage of spike-specific CD8+ T-cells (CD69+and/or CD137+ and IFN-γ+ and/or TNF-α+) of total CD8+ T-cells (y-axis) plotted against percentage of spike-specific CD4+ T-cells (CD137+and/or CD154+) of total CD4+ T-cells (x-axis). All patients at timepoint 2 weeks or 3 months, independent of cohort, are shown in the figure and dark triangles depict patients with an adequate anti-spike antibody response. Dotted lines either represent threshold for spike-specific CD8+ T-cell response on y-axis of 0.005% or spike-specific CD4+ T-cell response on x-axis of 0.05%. ns, not significant; *p < .05; **p < .01 or ***p < .001. PRE, before vaccination; 2W, 2 weeks after complete vaccination; 3M, 3 months after complete vaccination; 6M, 6 months after complete vaccination.

T-cell responses were measured in 49 patients lacking and 14 patients with adequate seroconversion (Table S2). Spike-specific CD4+ T-cell responses significantly increased in all disease cohorts 2 weeks after mRNA vaccination, whilst control CEFX-specific T-cells remained stable pre- and post-vaccination (Figures 1B and S3A). Spike-specific CD8+ T-cell responses based on reactivation with Spike peptides (Figure 1C) and spike/HLA-tetramer staining (Figure 1D) were increased in CLL and both NHL cohorts 2 weeks after vaccination. Most spike-specific CD4+ and CD8+ T-cell responses remained present over time with fluctuations (Figure 1B–D) and demonstrated to exhibit a type 1 cytokine profile (Figure S4). No clear effect of a third vaccination was seen at 6 months post-initial vaccination; however, only 62% of patients received a third vaccination and this was at various time points before the 6-month time point (median: 46 days, range: 1–70). Expression of PD-1, an early T-cell activation marker, was most prominent 2 weeks after vaccination (Figure S4D,K). In the subset of Spike-specific CD4+ T-cells, both T follicular helper cells and regulatory T-cells were present, though low in number (Figure S4G–H). Spike-specific CD8+ T-cells were mostly effector memory phenotype (Figure S4L). These results were in line with the phenotype observed in a healthy control cohort (n = 9) and previous studies in healthy cohorts (data not shown).[4]

Our in-depth T-cell analysis demonstrated induction of Spike-specific CD4+ responses in 75%, 92%, 81%, and 60% of CLL, aggressive NHL, indolent NHL, and MM, respectively (Figure 1A). Induction of Spike-specific CD8+ responses (presence of Spike-specific and/or Spike/HLA-tetramer+ CD8+ T-cells) were 77%, 82%, 71%, and 50%, respectively (Figure 1A). These data collectively indicate that induction of T-cell responses does not seem to be affected by disease. However, it is important to note that, though low in number of patients analyzed, T-cell responses seem to be hampered in MM. Possible explanation is the use of dexamethasone and/or anti-CD38 therapy, which are known to negatively affect T-cells and their function.

When comparing the Spike-specific T-cell responses between patients with and without adequate seroconversion, no significant differences were observed in the percentage of patients that generated a CD4+ T-cell response nor the percentage of Spike-specific CD4+ T-cells in individual patients (Figures 1E,F and S2B), indicating that the lack of humoral responses was not caused by lack of CD4+ T-cell help (Figure 1A). Furthermore, no difference was observed in the percentage of patients that generated a CD8+ T-cell response (Figures 1E and S2C); however, the frequency of

Spike-specific CD8+ T-cells was significantly higher in patients lacking serological response (Figure 1F) 2 weeks post-vaccination, which could represent an immune compensation mechanism that might contribute to a survival advantage in case of severe COVID-19.[3]

T-cell immunity is known to decline with advanced age. In this study, age was the only significant predictor of Spike-specific T-cell responses (CD4+ OR: 0.18, CD8+ OR: 0.05). (Figure S2B,C), both Spike-specific CD4+ and CD8+ T-cell responses decline significantly for patients aged over 68 years (Figure 1G).

Though cellular responses after SARS-COV-2 vaccination in patients with lymphoid malignancies have been suggested,5 we for the first time show with a highly specific and reproducible technique that nearly all lymphoid malignancy patients exhibit a good Spike-specific CD4+ and CD8+ T-cell response 2 weeks to 3 months after vaccination (Figure 1H).

Limitations of this study include a limited sample size, making it difficult to perform subgroup analysis especially within disease cohorts. Therefore, analyzing specific treatment-related effects is not possible. However, this cohort does represent a real-world situation for patients in two secondary-/tertiary-care centers in the Netherlands. In addition, the peptide pools used in the T-cell assays were based on the ancestral Wuhan strain sequence which is not the current circulating variant. However, it is important to note that it has been shown that T-cell responses induced by mRNA-1273 and BNT162b2 are minimally affected by the mutations found in omicron.[6]

In conclusion, this study demonstrates that the majority of patients lacking adequate seroconversion following SARS-CoV-2 mRNA vaccination were able to generate a cellular immune response. Moreover, a hampered humoral response is compensated by a stronger cellular response, which indicates that vaccination is of significance also in patients lacking seroconversion.

ACKNOWLEDGEMENTS

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SUPPLEMENTAL METHODS

Antibody measurement

For quantitative anti-SARS-CoV-2 Spike RBD antibody measurement the Roche Elecsys anti-SARS-CoV-2 S (Roche Diagnostics GmbH, Mannheim, Germany) was used. This test is an electrochemiluminescence assay (ECLIA) detecting total antibodies (IgG, IgA, and IgM) against the receptor binding protein of the SARS-CoV-2 Spike protein.

The test is standardized against an internal anti-RBD monoclonal antibody mixture and is expressed as units/mL (U/ml), with a measuring range of 0-250 U/ml). U/ml corresponds in a 1:1,0288 ratio with BAU/ml. The test was performed according to the manufacturer's instruction. Values higher than 0.8 BAU/ml were considered positive and values higher than 250 BAU/ml were considered as adequate response, as healthy individuals all have a value of 250 BAU/ml or higher. (1) Expected seroconversion rate using the Roche Elecsys assay after COVID-19 vaccination in comparable older individuals is 99% (prefrail category in Semelka et al.) (2).

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DETECTION OF SARS-COV-2 SPECIFIC T CELLS

PBMCs were isolated from fresh whole blood using Ficoll-Isopaque and cryo-preserved (in RPMI medium + 10% dimethyl sulfoxide (DMSO)) until further use. Upon thawing, the PBMCs were slowly diluted in culture medium consisting of Iscove Modified Dulbecco Medium (IMDM; Lonza) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Sigma-Aldrich), 2.7mM L-glutamine (Lonza), 100 U/mL penicillin (Lonza) and 100 µg/mL streptomycin (Lonza) (1% p/s). After thawing and washing, PBMCs were treated with 1.33 mg/ml DNAse to minimize cell clumping, counted, and used for T-cell stimulation assay as well as for peptide-HLA tetramer staining. For the T-cell stimulation assay, up to $2x10^6$ PBMCs were seeded in 100 µL culture medium and stimulated with 15-mer peptides with 11 amino acid overlap which cover the whole SARS-CoV-2 spike antigen (sb-PEPTIDE) in 96-well round bottomed plates. The peptides were dissolved in 20% DMSO in ddH_2O and added to the wells

in a 1 µg/mL final concentration. As a negative control, DMSO and ddH_2O in the same concentration was added. As a positive control, a pool of peptides from CMV, EBV, Flu etcetera (CEFX) was dissolved in 20% DMSO in ddH_2O and in a final concentration of 0.25 µg/mL (see **Table S3** for CEFX peptide pool details). After one hour incubation (37°C, 5% CO_2), 5 µg/mL Brefeldin A was added to the well and the plate was further incubated for 15 hours. The stimulation reaction was stopped by washing the cells in PBS followed by viability staining using Zombie-Red. After a PBS wash, cells were fixated and permeabilized using the FOXP3 buffer kit (Thermo Fisher). 20 µL antibody staining mix was added containing 0.8 mg/mL albumin, Brilliant Stain Buffer Plus and antibodies directed against CD3, CD4, CD8, CD154, CD137, CD69, IFN-γ, TNF-α, IL-2, IL-4, IL-17, PD-1, FOXP3 and CXCR5 (see **Table S4** for product details). After incubation for 30 minutes at room temperature (RT), the cells were washed in PBS containing 0.8 mg/mL albumin (FACS buffer) and dissolved in 100 µL FACS buffer for measurement on a 3-laser aurora (Cytek Biosciences).

For the tetramer staining, up to $2x10^6$ PBMCs were incubated for 16 hours in 100 µL culture medium. The cells were washed in FACS buffer and stained in two steps. First, 10 µL of an antibody cocktail directed against CD4, CCR7, CD45RA, PD-1 was added together with a tetramer pool in FACS buffer and incubated for 15 minutes at RT. Second, 10 µL of FACS buffer containing CD8 APC-H7 was incubated for an additional 15 minutes at RT (see **Table S3** for product details). The tetramer pool contained 23 in-house-made tetramers, consisting of spike peptides and HLAs (spike/HLA-tetramers), conjugated to PE as well as APC (see **Table S5** for tetramer details). Cells were washed and dissolved in 100 µL FACS buffer for measurement on a 3-laser aurora (Cytek Biosciences).

For flow cytometry analysis, OMIQ (http://www.omiq.ai) was used to set gates and retrieve percentages (see **Fig. S3** for gating strategy). Due to optimal reference controls used for unmixing on the 3-laser Cytek Aurora, no compensation was needed. The same gate was applied to all samples within one donor and as much as possible between donors. Percentage SARS-CoV-2 spike reactive $CD4^+$ T-cells was identified as $CD137^+$ and/or $CD154^+$ cells of total $CD4^+$ T-cells, corrected for background in DMSO. Percentage SARS-CoV-2-spike reactive $CD8^+$ T-cells was identified as $CD137^+$ and/or $CD69^+$ and IFN-γ^+ and/or TNF-α^+ of total $CD8^+$ T-cells. The same $CD137^+$ and/or $CD154^+$ or $CD137^+$ and/or $CD69^+$ was applied to all samples unless the background in DMSO was 0.1% or higher. Then the gate was adapted and applied to all samples of that donor.

Percentage SARS-CoV-2-spike $CD4^+$ T-cells was removed from analysis if there were less than 10.000 events in CD4 gate and percentage SARS-CoV-2-spike $CD8^+$ T-cells was removed from analysis if there were less than 10.000 events in CD8 gate. For CLL this was diminished to 5.000 events within the $CD4^+$ T-cell gate and the $CD8^+$ T-cell gate, because of the large B-cell population. A threshold was set on 0.05% for frequency $CD154^+$ and/or $CD137^+$ of total $CD4^+$ T-cells, 0.005% for frequency $CD137^+$ and/or $CD69^+$ AND IFN-γ^+ and/or TNF-α^+ of total CD8 T-cells and 0.005% for frequency of spike/HLA-tetramer$^+$ of total $CD8^+$ T-cells. Further gating to calculate percentage of spike-specific cells that are IFN-γ, TNF-α, IL-2, IL-4, IL-17, Tfh (T follicular helper cells; $CXCR5^+PD\text{-}1^+$), PD-1 ($CXCR5^-PD\text{-}1^+$) or Treg ($FOXP3^+$IFN-γ^- TNF-α^-) positive was only done if frequency threshold was met and if there were more than 25 events in the $CD154^+$ and/or $CD137^+$ gate for $CD4^+$ T-cells and $CD137^+$ and/or $CD69^+$ for the $CD8^+$ T-cells. Percentage of naïve (CCR7+CD45RA+), central memory (CM; CCR7+CD45RA-), effector memory (EM; CCR7-CD45RA-) or terminal effector memory (TEMRA; CCR7-CD45RA+) was measured of spike/HLA-tetramer$^+$ $CD8^+$ T-cells and only calculated if the frequency of spike/HLA-tetramer$^+$ $CD8^+$ T-cells was above threshold and contained at least 10 events. The percentages were exported and further analyzed in Graphpad Prism 9.0.1. All timepoints of the same patient were measured simultaneously to minimize technical variance within one patient. Patients were measured and analyzed in random order to minimize technical variance and bias between cohorts. In the last analysis step the data was separated for the different cohorts to prevent bias during analysis.

SUPPLEMENTARY FIGURES

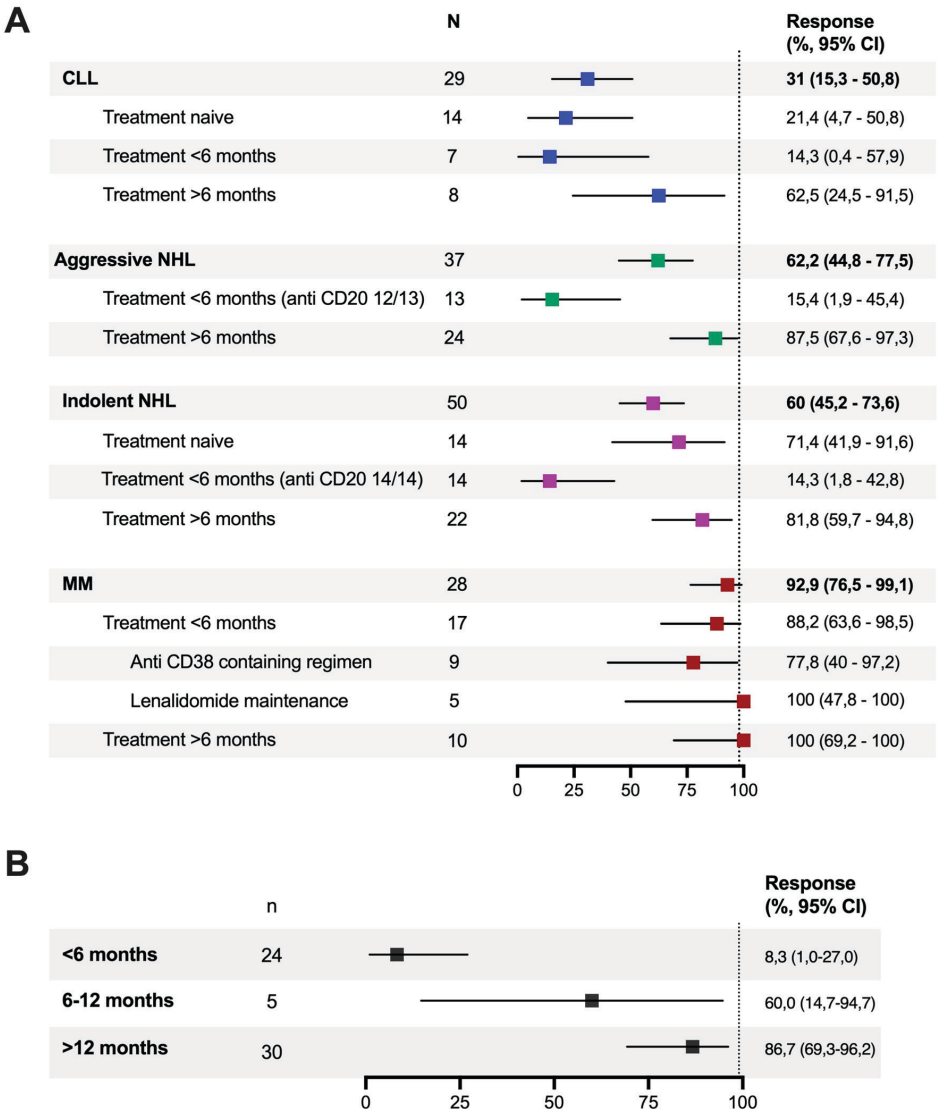

Figure S1 Serological response rates 2 weeks after SARS-CoV-2 mRNA vaccination.
Serological response rate was based on the percentage of patients with adequate anti-SARS-CoV-2 Spike antibodies (>250 BAU/ml). All proportions were compared to the expected population proportion of 99%, using one sample binomial testing. Clopper-Pearson method was used to estimate 95% confidence intervals. A) Data stratified to hematological disorder, time since treatment and if applicable type of treatment. Subgroups with n<5 were excluded from this figure. B) Data stratified to time since anti-CD20 containing regimen treatment.

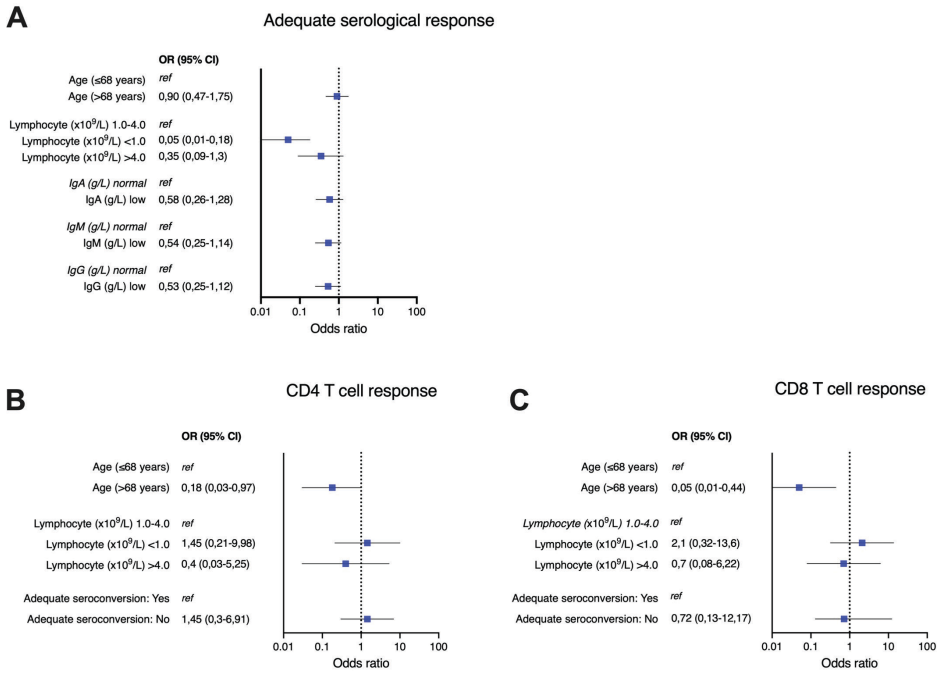

Figure S2 Potential predictive factors for serological CD4⁺ or CD8⁺ T-cell response.
Univariable logistic regression for possible outcome predictors of adequate serological (A), CD4⁺ (B) and CD8⁺ T-cell response (C). Showing odds ratios (OR) including 95% confidence interval. An OR <1 indicates a negative impact on the outcome and an OR >1 indicates a positive impact on the outcome. CD4⁺ T-cell response was defined as >0.05% of SARS-CoV-2 Spike-specific CD4⁺ T-cells, CD8⁺ T-cell response was defined as <0.005% of SARS-CoV-2 Spike-specific or spike/HLA-tetramer binding CD8⁺ T-cells.

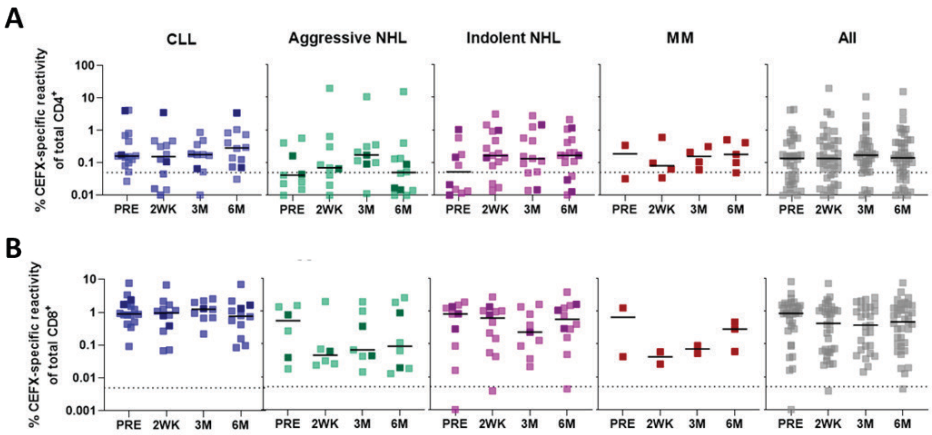

Figure S3 CD4⁺ and CD8⁺ T-cell responses after stimulation with CEFX peptide pool.
PBMCs from patients with CLL (blue), aggressive NHL (green), indolent NHL (purple) and MM (red) were isolated and stimulated with a CEFX peptide pool, after which flowcytometric analysis was performed to investigate CEFX-specific T-cell responses before vaccination (PRE), 2 weeks after vaccination (2WK), 3 months after vaccination (3M) and 6 months after vaccination (6M). Patients with adequate seroconversion following SARS-CoV-2 vaccination are depicted as dark squares while patients lacking adequate seroconversion are depicted as lightly colored squares. CD4⁺ T-cell response was defined as the percentage of CD4⁺ T-cells that express CD137⁺ and/or CD154⁺ phenotype after stimulation with CEFX peptide pool. CD8⁺ T-cell response was defined as the percentage of CD8⁺ T-cells that express CD69⁺ and/or CD137⁺ and IFNγ⁺ and/or TNFα⁺ phenotype after stimulation with the CEFX peptide pool. Values were corrected for background measured in DMSO. Each dot represents a patient. Horizontal black line shows the median. A) Percentage of CEFX-specific CD4⁺ T-cells of total CD4⁺ T-cells measured before vaccination and after vaccination. Dotted line represents a threshold of 0.05%. B) Percentage of CEFX-specific CD8⁺ T-cells of total CD8⁺ T-cells measured before vaccination and after vaccination. Dotted line represents a threshold of 0.005%.

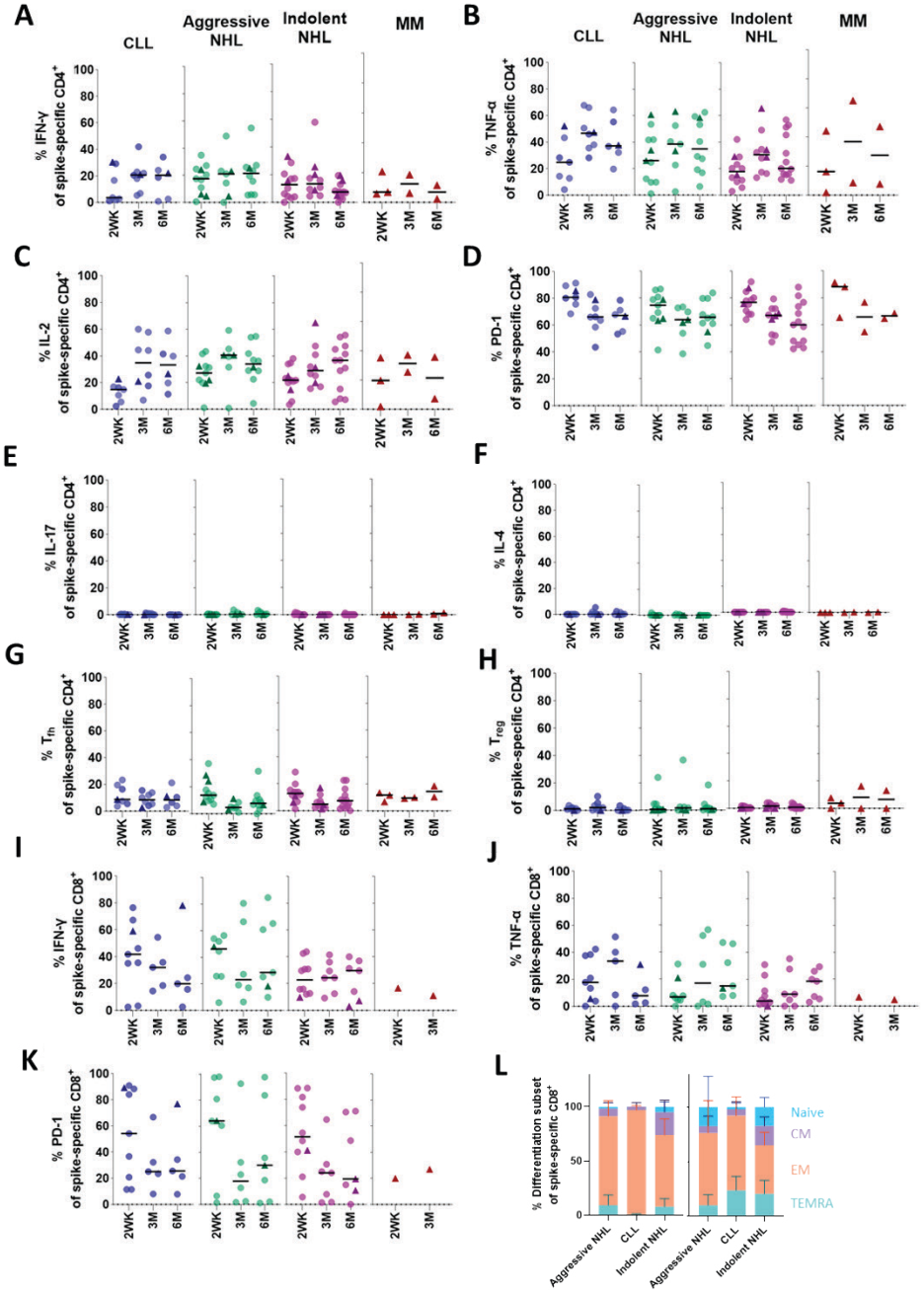
A
CLL
Aggressive NHL
Indolent NHL
MM
% IFN-γ of spike-specific CD4+
B
% TNF-α of spike-specific CD4+
C
% IL-2 of spike-specific CD4+
D
% PD-1 of spike-specific CD4+
E
% IL-17 of spike-specific CD4+
F
% IL-4 of spike-specific CD4+
G
% Tfh of spike-specific CD4+
H
% Treg of spike-specific CD4+
I
% IFN-γ of spike-specific CD8+
J
% TNF-α of spike-specific CD8+
K
% PD-1 of spike-specific CD8+
L
% Differentiation subset of spike-specific CD8+
Naive
CM
EM
TEMRA
2WK
3M
6M
Aggressive NHL
CLL
Indolent NHL

Figure S4 Cytokine production and marker expression of spike-specific $CD4^+$ and $CD8^+$ T-cells.

PBMCs from patients with CLL (blue), aggressive NHL (green), indolent NHL (purple) and MM (red) were isolated and stimulated with SARS-CoV-2 Spike peptides followed by flowcytometry. Percentage of spike-specific T-cells that are cytokine or marker positive 2 weeks (2WK), 3 months (3M) and 6 months (6M) after vaccination was analyzed for each disease group.Patients with adequate antibody responses following SARS-CoV-2 vaccination are depicted as dark triangles while patients lacking an adequate antibody response are depicted as circles. Each dot represents a patient. Horizontal black line shows the median. A) Percentage of IFNγ production by spike-specific CD4+ T-cells, shown per disease group. B) Percentage of TNFα production by spike-specific CD4+ T-cells, shown per disease group. C) Percentage of IL-2 production by spike-specific CD4+ T-cells, shown per disease group. D) Percentage of PD-1 expression by spike-specific CD4+ T-cells, shown per disease group. E) Percentage of IL-17 production by spike-specific CD4+ T-cells, shown per disease group. F) Percentage of IL-4 production by spike-specific CD4+ T-cells, shown per disease group. G) Percentage of Tfh cells of total spike-specific CD4+ T-cells, shown per disease group. H) Percentage of Treg cells of total spike-specific CD4+ T-cells, shown per disease group. I) Percentage of IFNγ production by spike-specific CD8+ T-cells, shown per disease group. J) Percentage of TNFα production by spike-specific CD8+ T-cells, shown per disease group. K) Percentage of PD-1 expression by spike-specific CD4+ T-cells, shown per disease group. L) Percentage of spike/HLA-specific CD8+ T-cells which are naïve (blue; CCR7+CD45RA+), central memory (CM; purple; CCR7+CD45RA-), effector memory (EM; orange; CCR7-CD45RA-) or terminal effector memory (TEMRA; green; CCR7-CD45RA+).

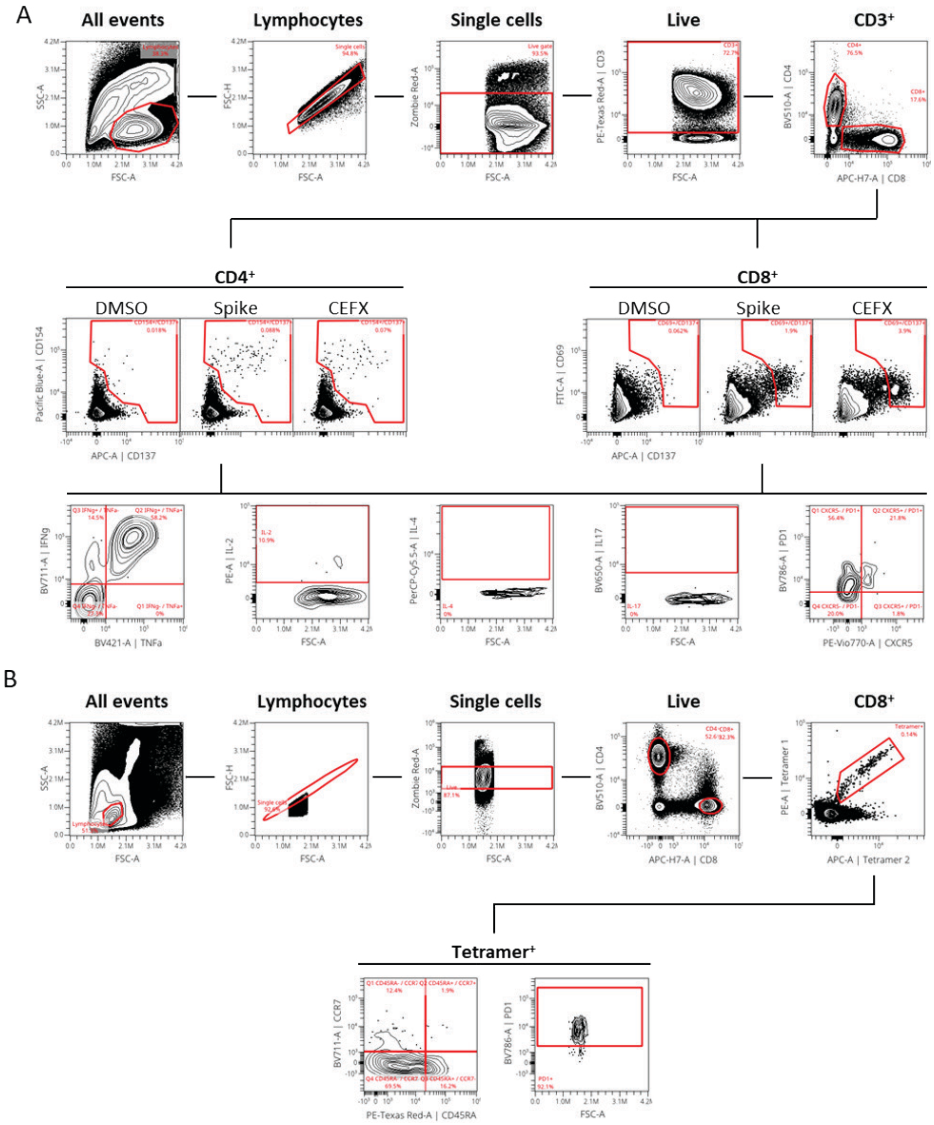

Figure S5 Flow cytometry gating example for peptide stimulation assays and tetramer staining.

A) Representative example of flow cytometry gating strategy for peptide-specific CD4+ and CD8+ T-cells. All events were gated on lymphocytes, single cells, viable cells, CD3 positive and subsequently either CD4 or CD8 positive. For CD4+ T-cells, activated cells were gated on CD137 and/or CD154 positive whilst for CD8+ T-cells CD137 and/or CD69 and IFN-γ and/or TNF-α positive cells were gated. DMSO functioned as a negative control and CEFX functioned as a positive control. From the activated cell gate onwards, cytokine production and marker expression were calculated. B) Representative example of flow cytometry gating strategy for spike/HLA-tetramer positive CD8+ T-cells. All events were gated on lymphocytes, single cells, viable cells, CD8 positive and spike/HLA-tetramer positive. Tetramer positive events were subsequently gated on CCR7, CD45RA or PD-1.

Table S1 Baseline characteristics

Characteristic	N (%) / median [IQR]
Sex	
Male	93 (58)
Female	67 (42)
Age (years)	68 [60-73]
Haematological diagnosis	
CLL	31 (19)
Treatment naïve	16 (52)
Treatment <6M	7 (23)
• Anti CD20 containing regimen	1 (14)
• Ibrutinib	4 (57)
• Venetoclax	2 (29)
Treatment >6M	8 (26)
Aggressive NHL	39 (24)
Treatment <6M*	13 (33), 2/13 (15)
• Anti CD20 containing regimen	12 (92)
• Chemotherapy	1 (8)
Treatment >6M	26 (67)
Indolent NHL	57 (36)
Treatment naïve	15 (26)
Treatment <6M**	19 (33)
• Anti CD20 containing regimen	14 (74)
• Ibrutinib	2 (11)
• Other	3 (16)
Treatment >6M	22 (39)
MM	30 (19)
Treatment naïve	2 (7)
Treatment <6M***	18 (60)
• Anti CD38 containing regimen	10 (56)
• Lenalidomide maintenance	5 (28)
• Other	3 (17)
Treatment >6M	10 (33)
Other	3 (2)
Presence of anti SARS-CoV-2 RBD prevaccination	8 (5)
Vaccine type (initial vaccination)	127 (80)
• mRNA-1273	25 (16)
• BNT162b	8 (5)
• Unknown	

Table S1 *Continued*

Characteristic	N (%) / median [IQR]
Booster vaccination**	110 (69)
• Received	29 (18)
• Not received	21 (13)
• Unknown	
Leukocytes (*10^9/L)	5,9 [4,6-8,7]
Leukocytes (*10^9/L)	
Total IgA (g/L)	1,08 [0,56-1,87]
Total IgG (g/L)	7,65 [5,26-10,50]
Total IgM (g/L)	0,38 [0,21-0,67]

* 2 patients have also received an autologous stem cell transplant <6 months ago
** 1 patient has also received an autologous stem cell transplant <6 months ago
*** 3 patients have also received an autologous stem cell transplant <6 months ago
**** Patients that have received a third/booster vaccination before the 6 months timepoint

Table S2 Baseline characteristics T-cell subset

Characteristic	N (%) / median [IQR]	Anti SARS-CoV-2 Ig pos/ neg (n)
Age (years)	69 [62-74]	
CLL	20 (32)	18/2
Treatment naïve	10 (16)	
Treatment <6M	6 (10)	
• Anti CD20 containing regimen	1 (2)	
• Ibrutinib	4 (6)	
• Venetoclax	1 (2)	
Treatment >6M	4 (6)	
Aggressive NHL	16 (25)	13/3
Treatment <6M	12 (19)	
• Anti CD20 containing regimen	12 (19)	
Treatment >6M	4 (6)	
Indolent NHL	19 (30)	15/4
Treatment naïve	6 (10)	
Treatment <6M	13 (21)	
• Anti CD20 containing regimen	12 (19)	
• Ibrutinib	1 (2)	
MM	5 (8)	0/5
Treatment <6M	5 (8)	
• Anti CD38 containing regimen	5 (8)	
Other	3 (5)	3/0

Characteristic	N (%) / median [IQR]	Anti SARS-CoV-2 Ig pos/ neg (n)
Booster vaccination*	39 (62)	
• Received	12 (19)	
• Not received	12 (19)	
• Unknown		
Leukocytes (*10^9/L)	5.6 [4.1-10.2]	
Lymphocytes (*10^9/L)	1.4 [0.8-5.8]	

*Patients that have received a third/booster vaccination before the 6 months timepoint

Table S3 List of peptides used in this study

Peptide pools				
Pathogen	**Antigen**	**Supplier**	**Catalogous #**	**Peptide characteristics**
CMV	PP65	JPT	Custom-made	15-mer, 11aa overlapping
Pool	Pool	JPT	PM-CEFX-3	15-mer, 11aa overlapping
EBV	BZLF1	JPT	PM-EBV-BZLF1	15-mer, 11aa overlapping
EBV class I	Mix	LUMC	Custom-made	9-mer, known epitopes (see below)
Influenza A	NP1	JPT	N/A	15-mer, 11aa overlapping, NCBI: ABB79814

EBV class I peptide pool				
Antigen	**Sequence (aa)**	**HLA restriction**	**Supplier**	**Peptide characteristics**
LMP2	ESEERPPTPY	A*01:01	LUMC	9-mer
BMLF1	GLCTLVAML	A*02:01	LUMC	9-mer
LMP2	CLGGLLTMV	A*02:01	LUMC	9-mer
LMP2	FLYALALLL	A*02:01	LUMC	9-mer
BRLF1	RVRAYTYSK	A*03:01	LUMC	9-mer
EBNA3A	RLRAEAQVK	A*03:01	LUMC	9-mer
EBNA3B	IVTDFSVIK	A*11:01	LUMC	9-mer
EBNA3B	AVFDRKSDAK	A*11:01	LUMC	9-mer
BRLF1	DYCNVLNKEF	A*24:02	LUMC	9-mer
EBNA3A	RPPIFIRRL	B*07:02	LUMC	9-mer
BZLF1	RAKFKQLL	B*08:01	LUMC	9-mer
EBNA3A	QAKWRLQTL	B*08:01	LUMC	9-mer
EBNA3A	FLRGRAYGL	B*08:01	LUMC	9-mer
EBNA3A	YPLHEQHGM	B*35:01	LUMC	9-mer

Table S4 List of antibodies and reagents used for flow cytometry

Antibodies				
Antigen	**Format**	**Clone ID**	**Supplier**	**Catalogous #**
CD4	BV510	SK3	BD Biosciences	562970
CD8	APC-H7	SK1	BD Biosciences	560179
CD3	Pe-Texas-Red	7D6	Invitrogen	MHCD0317
CD69	FITC	Clone L78	BD Biosciences	347823
CD137	APC	4B4-1	BD Biosciences	550890
CD154	Pacific blue	24-31	Biolegend	310820
IFNγ	BV711	B27	BD Biosciences	564039
TNFα	BV421	MAb11	BD Biosciences	562783
IL2	PE	SCPL1362	BD Biosciences	560436
IL4	PERCPCY5.5	MP4-25D2	Biolegend	500822
FOXP3	AF700	PCH101	Thermo Fisher	56-4776-41
CXCR5	PE-Vio770	REA103	Miltenyi	130-117-358
PD1	BV786	EH12.1	BD Biosciences	563789
IL17	BV650	N49-653	BD Biosciences	563746
CCR7	BV711	3D12	BD Biosciences	563712
CD45RA	Pe-Texas-Red	MEM-56	Invitrogen	MHCD45RA17

Other reagents		
Product	**Supplier**	**Catalogous #**
Brilliant Violet Stain Buffer Plus	BD Biosciences	566385
FOXP3 buffer kit	Thermo fisher	00-5521-00
Zombie-red	Biolegend	423110

Table S5 List of peptide-HLA tetramers used for flow cytometry

Tetramers		
Peptide sequence (aa)	**HLA allele**	**Supplier**
LTDEMIAQY	A*01:01	LUMC
FLPFFSNV	A*02:01	LUMC
RLNEVAKNL	A*02:01	LUMC
RLQSLQTYV	A*02:01	LUMC
VLNDILSRL	A*02:01	LUMC
YLQPRTFLL	A*02:01	LUMC
GTHWFVTQR	A*03:01/A*11:01	LUMC
KCYGVSPTK	A*03:01/A*11:01	LUMC
IYKTPPIKDF	A*24:02	LUMC
QYIKWPWYI	A*24:02	LUMC
RFDNPVLPF	A*24:02	LUMC
TQDLFLPFF	A*24:02	LUMC
TYVPAQEKNFT	A*24:02	LUMC
LPQGFSAL	B*07:02	LUMC
MIAQYTSAL	B*07:02	LUMC
SPRRARSVA	B*07:02	LUMC
CVADYSVLY	B*15:01	LUMC
LVKNKCVNF	B*15:01	LUMC
VASQSIIAY	B*15:01	LUMC
IYKTPPIKDF	B*35:01	LUMC
QPTESIVRF	B*35:01	LUMC