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Letter

Immune response to three doses of mRNA SARS-CoV-2 vaccines in CD19-targeted chimeric antigen receptor T cell immunotherapy recipients

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Anti-SARS-CoV-2 vaccine response in patients with B cell lymphopenia is low compared to the general population. Here, we investigated humoral and cellular responses in 43 adult anti-CD19 chimeric antigen receptor (CAR) T cell recipients who received one (n = 11) or two (n = 22) booster doses of SARS-CoV-2 mRNA vaccine and 10 COVID-19 survivors in comparison to adult controls. The seroconversion rate was less than 20% in vaccinated recipients or COVID-19 survivors. T cell SARS-CoV-2-specific proliferation was detected in 42.4% and 57% of vaccinated recipients and COVID-19 survivors, respectively. Among the recipients tested consecutively after the first and second booster doses, no gain in specific humoral and cellular responses was observed. In responders, peripheral $CD4^+$ T cell and naive and central memory T cell counts were significantly higher. Cellular proliferative response was dependent on the timing of primary immunization; there were significantly more responders in the patients receiving primary immunization at least 6 months after CAR T cell infusion.

Severe cases of COVID-19 have been reported in recipients of autologous CAR T cells targeting CD19 (Busca et al., 2021; Hensley et al., 2021; Spanjaart et al., 2021) Not much is known about the immune response to mRNA vaccines against SARS-CoV-2 in recipients of CAR T cells. Small series have shown low seroconversion rates (<15%) after a single booster dose, particularly if vaccines were administrated within 6 months after CAR T cell infusion (Dhakal et al., 2021; Parvathaneni et al., 2022). Regarding the T cell repertoires, a study has reported that the frequency of Spike-specific memory $CD4^+$ T cell responses measured before and 1 month after the primary and booster vaccinations was not different in 12 CAR T cell-treated patients and eight healthy controls (Parvathaneni et al., 2022). French National Authority for Health has recommended the use of a second booster dose in immunosuppressed patients (Santé, 2022).

Here, we report the humoral and cellular responses after one or two booster doses of SARS-CoV-2 mRNA vaccine (n = 33) or after developing COVID-19 (n = 10) in a prospective observational single-center cohort of adults (≥18-year-old) who received commercial CAR T cells in comparison to control groups of mRNA-vaccinated adults (n = 5) or patients who developed mild to moderate ambulatory COVID-19 (n = 5). SARS-CoV-2-specific immunoglobulin (Ig) G was measured in sera harvested from CAR T cell recipients and control individuals (Supplemental information). Cellular response was assessed by $CD3^+$ T cell proliferation assay measured under antigenic peptide stimulation covering Spike, nucleocapsid, and the membrane of original SARS-CoV-2 ancestral strain.

In vaccinated CAR T cell recipients, 11 received one booster dose and 22 received two booster doses of either BNT162b2 (Pfizer/BioNTech) (n = 26) or mRNA-1273 (Moderna) (n = 7) (Figure S1A). The median age at primary dose or COVID-19 onset was 65 (interquartile range, IQR: 52–72) years, and sex ratio female/male was 0.48 (Table S1). The primary dose was administered either before (n = 8) or after (n = 25) CAR T cell infusion at a median time of 97 days (IQR: 68.5–141.2) and 257 days (IQR: 133–609), respectively. When primary dose was administered before CAR T cell infusion, the booster dose schedule was continued after infusion. The first booster dose was given 1 month apart from the primary dose, and the second booster dose was administered at a median time of 98.5 days (IQR: 61.2–146) after the first booster dose.

Humoral response in CAR T cell recipients showed that 6/33 (18%) and 1/7 (14%) tested positive for specific SARS-CoV-2 IgG in the vaccinated group and the COVID-19 survivor group, respectively, while 100% of controls were positive (Figure S1B). T cell spike-specific proliferation was observed in 14/33 (42.4%) of vaccinated patients (among whom 8 had received two booster doses) and in 100% of controls (Figure S1B). Among the COVID-19 survivor group, 4/7 (57%) had a positive T cell SARS-CoV-2 antigen-specific proliferation in response to at least one recall antigen (1/4 responded to all three, 1/2 responded to the Spike and nucleocapsid antigens, 1/4 responded to the Spike antigen, and 1/4 responded to the nucleocapsid antigen). Among the 16 patients tested consecutively after the first and second booster doses, no significant gain in humoral response or lymphocyte proliferation was observed ($p = 0.12$ and $p = 0.15$, respectively) (Figure S1C). Peripheral absolute $CD4^+$ T cell counts were significantly higher in responders than in non-responders, whether after one or two booster doses ($p = 0.021$ and $p = 0.016$, respectively) (Figure S1B). $CD8^+$ T cell, B cell, NK cell, and $CD19^+$ CAR T cell absolute counts were not different in responders and non-responders (Figure S1D).

When further investigating $CD4^+$ T cell subsets, responders had significantly more naive and central memory T cells and significantly less effector memory and terminally differentiated memory T cells than non-responders, whether after one or two booster doses ($p = 0.0160$ and $p < 0.0001$, respectively) (Figure S1B). When comparing responders and non-responders, receiving

the primary dose after CAR T cell infusion rather than before was the only factor significantly associated with a Spike-specific cellular response ($p = 0.049$) (Table S2). The number of responders was significantly lower if primary immunization was initiated within 6 months rather than beyond 6 months post-infusion ($p = 0.025$) (Table S2). Response was not different according to mRNA vaccine type ($p = 0.97$) (Table S2).

Overall, 82% of vaccinated CAR T cell recipients did not mount a humoral response, which is consistent with previous reports (Dhakal et al., 2021; Parvathaneni et al., 2022). In addition, we found that developing COVID-19 or receiving a second booster dose did not enhance specific IgG production in CAR T cell recipients. When considering the T cell response, studies conducted in healthy volunteers showed that both mRNA vaccines elicited systematically a Spike-specific $CD4^+$ and $CD8^+$ T cell response, although the responses varied inter-individually in magnitude and did not seem to be dose dependent (Jackson et al., 2020; Sahin et al., 2020). Here, specific T cell response was decreased by more than half (57.6%) in vaccinated recipients, indifferently of the number of booster dose, and by 43% in COVID-19 survivors who developed a response to at least one of the SARS-CoV-2 recall antigenic peptide. This suggests that developing COVID-19 generates a stronger and broader T cell response. Our results suggest a time-dependent efficacy of mRNA SARS-CoV-2 vaccines in inducing a specific cellular response after CAR T cell infusion. In addition, among peripheral $CD4^+$ lymphocyte subsets, naive $CD4^+$ lymphocyte count may be a predictive biomarker of appropriate cellular response. The present results are limited by the small sample size, and further studies are required. In the meantime, CAR T cell immunotherapy recipients must be advised to strictly maintain barrier measures, and vaccination of relatives must be strongly encouraged, particularly in the context of the new epidemiological landscape of Omicron takeover.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2022.01.010.

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AUTHOR CONTRIBUTIONS

F.A., P.S., F.V., and E.B. were involved in the design, implementation, and day-to-day management of the study. P.S., E.F., V.S., E.B., and F.A. included participants in the study. F.M.-S. was responsible for the virological analyses. M.G. and F.V. were responsible for the immunological analyses. P.S., F.A., and F.V. were involved in the statistical analyses. F.A. and F.V. wrote the original draft of the manuscript, which was reviewed and edited by P.S., E.F., V.S., M.G., F.M.-S., and E.B. All authors have read and approved the manuscript. The corresponding author had full access to all the data and had final responsibility for publication.

DECLARATION OF INTEREST

P.S. declares Honoraria and Advisory/Consultancy from BMS, Novartis, and Kite/Gilead; E.F. declares Honoraria and Advisory/Consultancy from Kite/Gilead, Janssen, BMS, and Abbvie; V.S. declares Honoraria from Roche; E.B. declares Honoraria and Consultancy from Gilead, Novartis, Roche, Amgen, Janssen, Sanofi, and Abbvie. All other authors declare no conflicts of interest.

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Supplemental information

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Supplemental information

Supplemental Methods

This prospective observational single-center, cohort study was conducted in a French tertiary-care university hospital (Centre Hospitalier Lyon-Sud, Lyon) among adults (≥ 18-year-old) who received commercial $CD19^+$ CAR T cells. For these patients, the following characteristics were collected: demographics, baseline hematological malignancy, disease status at infusion (refractory or relapsed), prior autologous hematopoietic stem cell transplantation, CAR T cell construct (axicabtagene ciloleucel [axi-cel], lisocabtagene maraleucel [liso-cel], tisagenlecleucel [tisa-cel]), lymphodepletion regimen (fludarabine 30 mg/m^2 for axi-cel and liso-cel 25 mg/m^2 for tisa-cel and cyclophosphamide 500 mg/m^2 for axi-cel, 250 mg/m^2 for tisa-cel; 300 mg/m^2 for liso-cel), the occurrence of post-infusion cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) both according to the American Society for Transplantation and Cellular Therapy grading scale (Lee et al., 2019; Porter et al., 2018) the use of interleukin (IL)-6 inhibitor (tocilizumab) or corticosteroids (dexamethasone), current chemotherapy, immunotherapy or immunoglobulin replacement therapy. The severity of COVID-19 was graded according to hospital admission and ICU admission. Passive immunotherapy management strategy of COVID-19 by convalescent plasma or monoclonal antibody therapy was recorded.

Concomitantly, control individuals were recruited among donors to the Lyon (France) blood bank (*Etablissement Français du Sang*, EFS). According to the EFS standardized procedures for blood donation and to provisions of article R. 1243-49 (and those that follow) of the French public health code, written non-opposition to the use of donated blood for research purpose was obtained from these individuals. The age, sex, number of mRNA vaccines and history of proven COVID-19 of blood donors was transferred anonymously to the research laboratory. The study

was conducted in accordance with the Declaration of Helsinki and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline. The protocol was approved by the local Ethics Committees and the national authority for the protection of privacy and personal data in clinical research (*Commission Nationale Informatique et Libertés* [CNIL], approval no.18-076).

Reference values for the anti-SARS-CoV-2 specific T cell proliferation assay were obtained from a cohort of control donors. These donors were included after informed consent as part of the REA-IMMUNO-COVID (RICO) clinical study, which was approved by ethics committee (*Comité de Protection des Personnes Ile de France 1 - N°IRB / IORG #: IORG0009918*) under agreement number 2020-A01079-30 and registered at ClinicalTrials.gov (NCT04392401).

S1-RBD specific IgG were measured using the Atellica IM SARS-CoV-2 IgG (sCOVG) (Siemens) diagnosis kits according to manufacturer's recommendations. For standardization of this assay according to the first WHO international standard, the concentrations were transformed in BAU/mL using the conversion factor provided by the manufacturer.

T $CD4^+$ and $CD8^+$ absolute counting was performed on an automated volumetric flow cytometer from Beckman Coulter (Aquios CL) as previously described (Gossez et al., 2017) . T, B and NK lymphocyte subpopulation immunophenotyping was performed using lyophilized antibody panels from Beckman Coulter (Duraclone kit). Data were acquired on a Navios flow cytometer (Beckman Coulter, Hialeah, FL) and flow data were analyzed using Navios software (Beckman Coulter).

An *ex vivo* functional assays for the determination of SARS-CoV-2-specific T cell response was used. Peripheral blood mononuclear cells (PBMCs) from patients and donors were isolated from fresh whole blood by Ficoll-Paque PREMIUM (GE Healthcare) density gradient centrifugation and immediately processed for cell culture. The number of PBMCs per well was adjusted to 1.10^6 cells/ml re-suspended in complete culture media (RPMI 1640 medium with

HEPES, L-glutamine, 10% human AB plasma, 20 mg/ml streptomycin and 2.5 mg/ml fungizone). Cells were then stimulated during 7 days at 37°C, 5% CO_2 with pools of SARS-CoV-2 peptide pools (JPT technology) or tuberculin PPD (AJVaccines) as a control for antigen-specific stimulation. The peptide pools consisted of 15 amino acid long peptides with 11 amino acid overlap. The following SARS-CoV-2 peptide pools were evaluated: pool of 315 peptides covering the spike (15 mers with 11-aa overlap, delivered in two sub-pools of 158 [S1 pool] and 157 [S2 pool] peptides), pool of 102 peptides covering the nucleoprotein, pool of 53 peptides covering the membrane from the original SARS-CoV-2 strain. Peptides were used at a final concentration of 1.25 µg/ml. Tuberculin PPD was used at a final concentration of 10 µg/mL. All samples were performed in duplicates. EdU click-it reaction was performed using EdU Click-it kit (Thermo Fisher) as previously described (Poujol et al., 2014). $CD3^+$ T cell proliferation was analyzed by monitoring EdU-AF488 incorporation into cells. Results were expressed as percentages of cells incorporating EdU.

Statistical analyses were conducted using Prism (version 7.0e, Graphpad Software). Categorical variables were presented using frequencies and percentages, while continuous variables by median, interquartile range (IQR). For immunological data, a Mann-Whitney test was performed as a nonparametric *t* test and a Kruskal-Wallis test with Dunns test was performed for multiple group comparisons. CAR T cell recipients characteristics were compared by cellular vaccine response (responders *versus* non responders) using the x^2 test for categorical variables. Effect of second booster dose on humoral and cellular responses was evaluated through a non-parametric Wilcoxon paired test in the subcohort of patients with paired samples. A p value ≤ 0.05 was considered statistically significant.

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Table S1. Demographics and characteristics of CAR T cell recipients
Data are represented as absolute count (n, percent total) or median [IQR, interquartile range].

	n = 43
Age (years)	65 [52; 72]
Sex F/M (ratio)	14/29 (0.48)
Hematological characteristics	
Baseline hematological malignancy	
DLBCL	27 (62.8)
FL	13 (30.2)
Other NHL[a]	3 (7)
Refractory/relapsed disease	28/15 (65.1/34.9)
Prior autologous HSCT	11 (25.6)
Lymphodepletion regimen	
Flu-Cyc	43 (100)
CAR T cell product	
Axicabtagene ciloleucel	23 (53.5)
Tisagenlecleucel	13 (30.2)
Lisocabtagene maraleucel	7 (16.3)
Post-infusion complications and related treatments	
CRS ≥ grade 2	12 (27.9)
Anti-IL-6R (tocilizumab)	25 (58.1)
ICANS ≥ grade 2	3 (7)
Corticosteroids (dexamethasone)	17 (39.5)
Current chemotherapy and/or immunotherapy[b]	2 (4.6)
Current Ig replacement therapy	3 (7)
Vaccine procedure	
mRNA-vaccinated CAR T cell recipients	33
Primary dose administrated after infusion	25 (75.7)
Time from infusion to primary dose (days)	257 [133;609]
Primary dose administrated before infusion	8 (24.3)
Time from primary dose to infusion (days)	97 [68.5;141.2]
Number of booster dose	
One	11 (33.3)
Two	22 (66.7)
Time interval between first and second booster dose	98.5 [61.2; 146]
Anti-SARS-CoV-2 mRNA vaccine	
BNT162b2 (Pfizer-BioNTech)	26 (78.8)
mRNA-1273 (Moderna)	7 (21.2)
COVID-19 cases	10 (23.2)
Time from infusion to COVID-19	536.5 [491; 622.5]
Hospital admission	7 (70)
ICU admission	4 (40)
Monoclonal antibody or convalescent plasma therapy	4 (40)
COVID-19-related death	2 (20)

[a]Mantle cell lymphoma (n=2), and primary mediastinal B cell lymphoma (n=1)
[b]Treatments were administered within 6 months before vaccination or during the vaccine study period.
CAR, chimeric antigen receptor; **COVID-19**, coronavirus disease 2019; **CRS**, cytokine release syndrome; **Cyc**, cyclophosphamide; **DLBCL**, diffuse large B cell lymphoma; **F**, female; **FL**, follicular lymphoma; **Flu**, fludarabine; **HSCT**, hematopoietic stem cell transplantation; **ICANS**, immune effector cell-associated neurotoxicity syndrome; **ICU**, intensive care unit; **IL-6R**, interleukin 6 Receptor; **IQR**, interquartile range; **M**, male; **mRNA**, messenger RNA; **NHL**, non-Hodgkin lymphoma; **SARS-CoV-2**, severe acute respiratory syndrome coronavirus 2.

Table S2. Comparison of variables in responders and non-responders to spike-specific T cell proliferation assay among vaccinated CAR T cell recipients

	Responders (n= 14)	**Non-responders (n= 19)**	***P***
Sex ratio (F/M)	0.75	0.35	0.32
Primary immunization started after CAR T-cell infusion	13	12	***0.049***
[a]Timing of vaccination < 6 months	2	7	***0.025***
[b]Timing of vaccination ≥ 6 months	11	5	
Prior autologous HSCT	4	4	0.62
Refractory	11	12	0.34
CRS ≥ grade 2	5	5	0.56
Anti-IL-6R (tocilizumab)	8	12	0.73
ICANS ≥ grade 2	0	3	0.12
Corticosteroids (dexamethasone)	7	8	0.65
Type of vaccine (BNT162b2)	11	3	0.97

CAR, chimeric antigen receptor; **CRS**, cytokine release syndrome; **HSCT**, hematopoietic stem cell transplantation; **ICANS**, immune effector cell-associated neurotoxicity syndrome; **IL-6R**, interleukin 6 Receptor.

[a]Sample size was based on the number of recipients vaccinated after CAR T cell infusion (n=25). Primary immunization administrated before 6 months after CAR T cell infusion.

[b]Primary immunization administrated after 6 months after CAR T cell infusion.

Figure S1

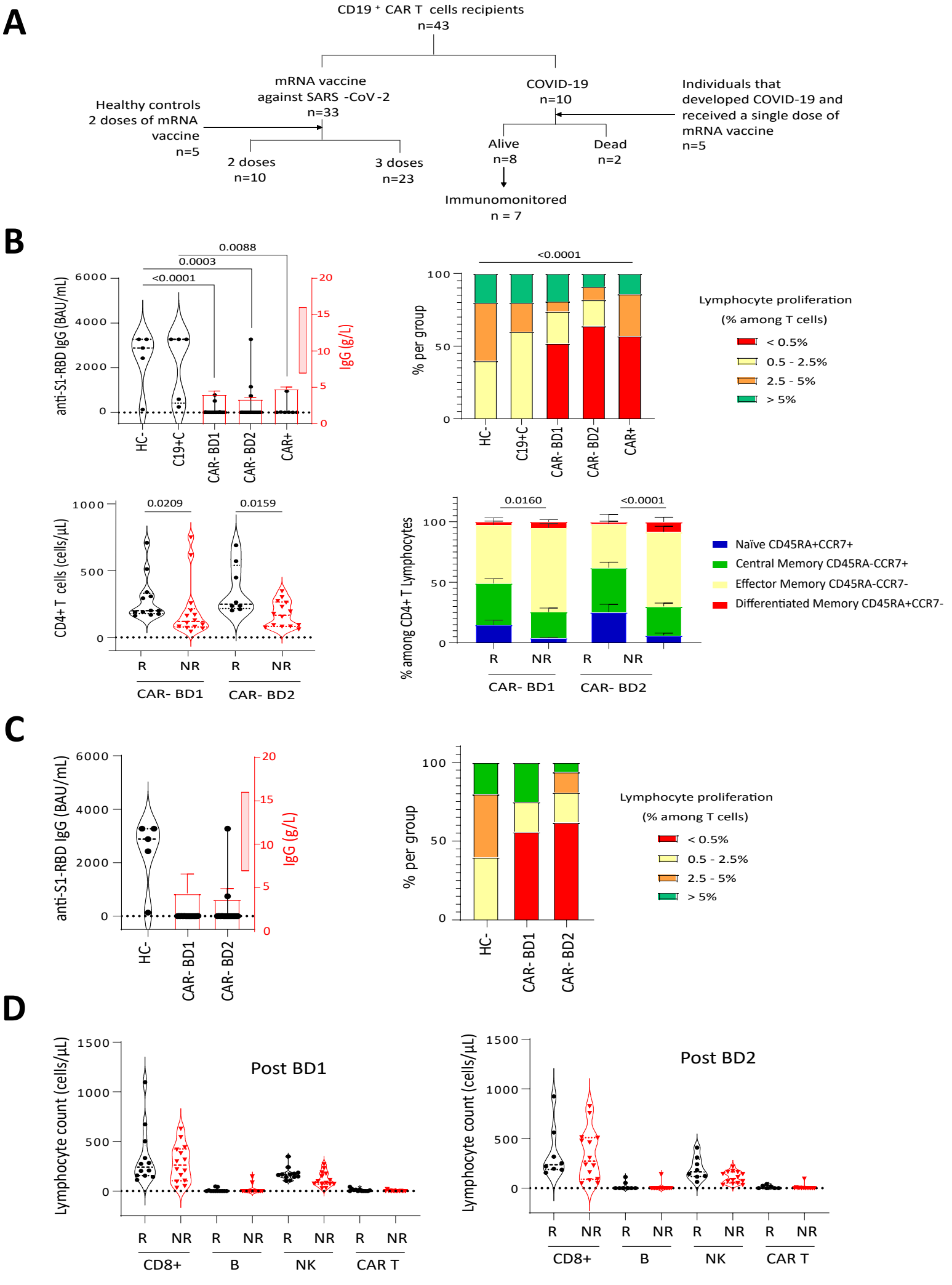
A
CD19 + CAR T cells recipients
n=43
mRNA vaccine
against SARS -CoV-2
n=33
Healthy controls
2 doses of mRNA
vaccine
n=5
2 doses
n=10
3 doses
n=23
COVID-19
n=10
Individuals that
developed COVID-19 and
received a single dose of
mRNA vaccine
n=5
Alive
n=8
Dead
n=2
Immunomonitored
n = 7
B
anti-S1-RBD IgG (BAU/mL)
IgG (g/L)
0.0088
0.0003
<0.0001
HC-
C19+C
CAR- BD1
CAR- BD2
CAR+
% per group
<0.0001
Lymphocyte proliferation
(% among T cells)
< 0.5%
0.5 - 2.5%
2.5 - 5%
> 5%
CD4+ T cells (cells/µL)
0.0209
0.0159
R
NR
CAR- BD1
CAR- BD2
% among CD4+ T Lymphocytes
0.0160
<0.0001
Naïve CD45RA+CCR7+
Central Memory CD45RA-CCR7+
Effector Memory CD45RA-CCR7-
Differentiated Memory CD45RA+CCR7-
C
anti-S1-RBD IgG (BAU/mL)
IgG (g/L)
HC-
CAR- BD1
CAR- BD2
% per group
Lymphocyte proliferation
(% among T cells)
< 0.5%
0.5 - 2.5%
2.5 - 5%
> 5%
D
Lymphocyte count (cells/µL)
Post BD1
Post BD2
CD8+
B
NK
CAR T

Figure S1. Humoral and cellular responses to mRNA SARS-CoV-2 vaccines in a prospective cohort of CD19-targeted chimeric antigen receptor T cell immunotherapy recipients

A. Flow chart of recipients of autologous chimeric antigen receptor (CAR)-genetically engineered T cells targeting CD19 included in the study. B. Immune response to mRNA vaccines in CAR T cell recipients. Top left, humoral immune response. Anti-S1 RBD IgG concentrations were measured in control groups of mRNA-vaccinated adults (Healthy controls: HC, n=5), patients that developed mild-to-moderate ambulatory COVID-19 (C19+C, n=5), $CD19^+$ CAR T cells recipients who received either one SARS-CoV-2 mRNA vaccine booster dose (CAR-BD1, n=11) or two SARS-CoV-2 mRNA vaccine booster dose (CAR-BD2, n=22), or $CD19^+$ CAR T cells recipients after developing COVID-19 (CAR+, n=10). Results are shown as individual values and violin plots. Threshold for positivity is shown as dotted line. Total IgG concentrations in patients are shown as means and SEM in red boxes. Normal values are illustrated as a red box. **Top right, cellular immune response.** Percentages of T cells proliferating in response to Spike antigens are shown as mean proportions of patients in each group of proliferative response. **Bottom left, $CD4^+$ T lymphocyte absolute count.** Numbers of circulating $CD4^+$ T lymphocytes were measured in $CD19^+$ CAR T cells recipients who received either one SARS-CoV-2 mRNA vaccine booster dose (CAR-BD1, n=11) or two SARS-CoV-2 mRNA vaccine booster dose (CAR-BD2, n=22). Patients were categorized in 2 groups depending on their proliferative response to Spike antigens (proliferation > 0.5%: responders = R, n = 1, black plots; proliferation <0.5%: non-responders = NR, n = 19, red plots). **Bottom right, $CD4^+$ T cell subpopulations.** Percentages of $CD4^+$ T cell subpopulations are shown in responders and non-responders as means and SEM. Differences in continuous values were evaluated through Mann Whitney test. Differences in proportions were evaluated through Chi-square test. **C. SARS-CoV-2-specific immune response in 16 CAR T cell recipients consecutively sampled post-booster dose 1 and 2. Left, humoral immune response.** Anti-

S1 RBD IgG concentrations were measured in control groups of mRNA-vaccinated adults (HC, n=5), 16 $CD19^+$ CAR T cells recipients sampled both after one SARS-CoV-2 mRNA vaccine booster dose (CAR-BD1, n=11) and two SARS-CoV-2 mRNA vaccine booster dose (CAR-BD2, n=22). Results are shown as individual values and violin plots. Threshold for positivity is shown as dotted line. Total IgG concentrations in patients are shown as means and SEM in red boxes. Normal values are illustrated as a red box. **Right, cellular immune response.** Percentages of T cells proliferating in response to Spike antigens are shown as mean proportions of patients in each group of proliferative response. **D. Lymphocyte counts in CAR T cells recipients who received either one or two SARS-CoV-2 mRNA vaccine booster doses.** Numbers of circulating $CD8^+$ T lymphocytes, B lymphocytes, NK lymphocytes and $CD19^+$ CAR T lymphocytes were measured in $CD19^+$ CAR T cells recipients who received either one SARS-CoV-2 mRNA vaccine booster dose (CAR-BD1, n=11, left) or two SARS-CoV-2 mRNA vaccine booster dose (CAR-BD2, n=22, right). Patients were categorized in two groups depending on their proliferative response to Spike antigens (proliferation > 0.5%: responders = R, n = 14 [black plots]; proliferation <0.5%: non-responders = NR, n = 19 [red plots]). For panels B, C and D, a p value ≤ 0.05 was considered statistically significant.