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Supplemental information

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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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.

Supplemental References

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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

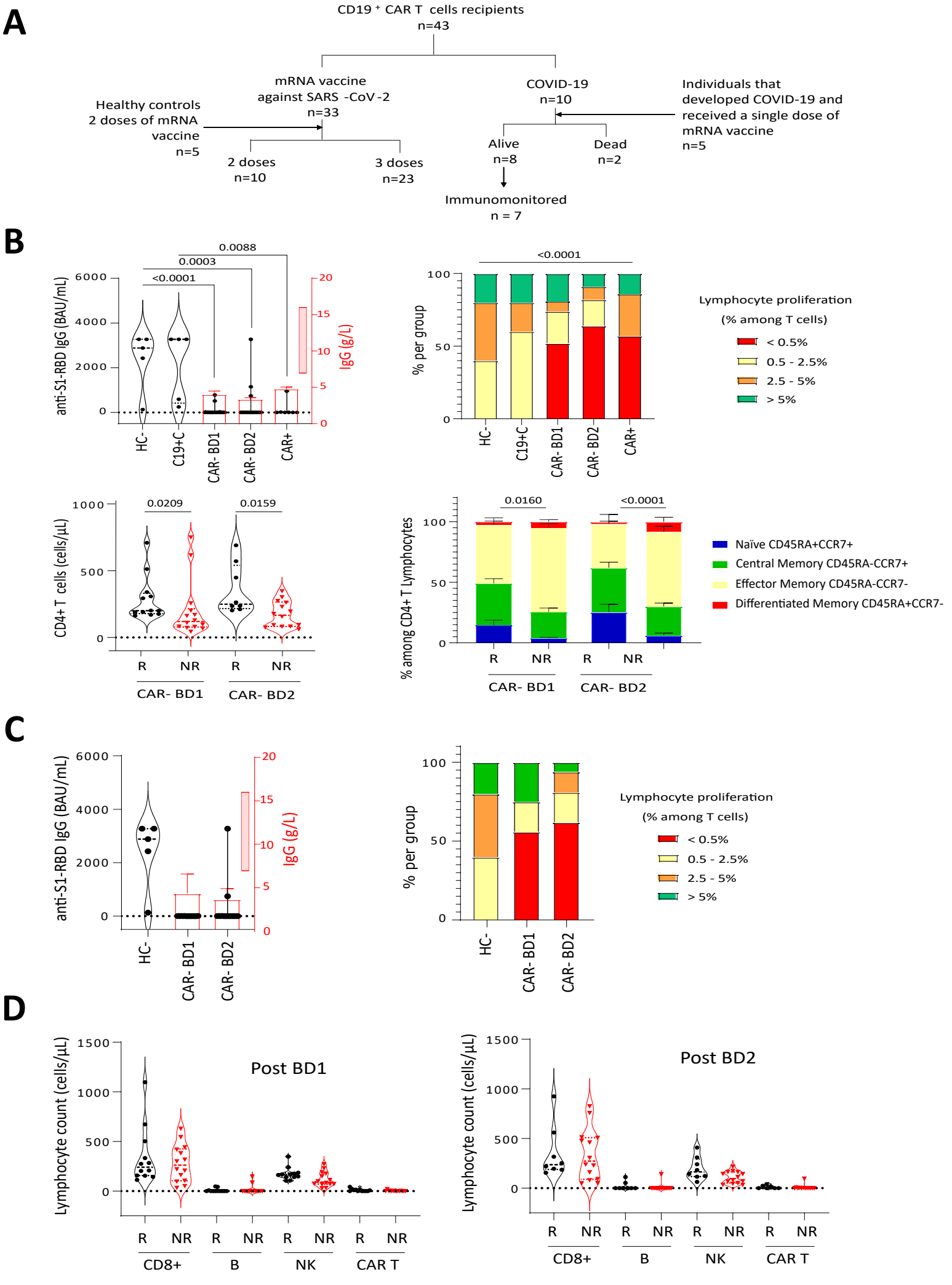
A
CD19 + CAR T cells recipients
n=43
mRNA vaccine
against SARS -CoV-2
n=33
Healthy controls
2 doses of mRNA
vaccine
n=5
2 doses
n=10
3 doses
n=23
COVID-19
n=10
Individuals that
developed COVID-19 and
received a single dose of
mRNA vaccine
n=5
Alive
n=8
Dead
n=2
Immunomonitored
n = 7
B
anti-S1-RBD IgG (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.