Cancer Cell, Volume *39*

Supplemental information

Humoral and cellular immune responses in SARS-CoV-2 mRNA-vaccinated patients with cancer

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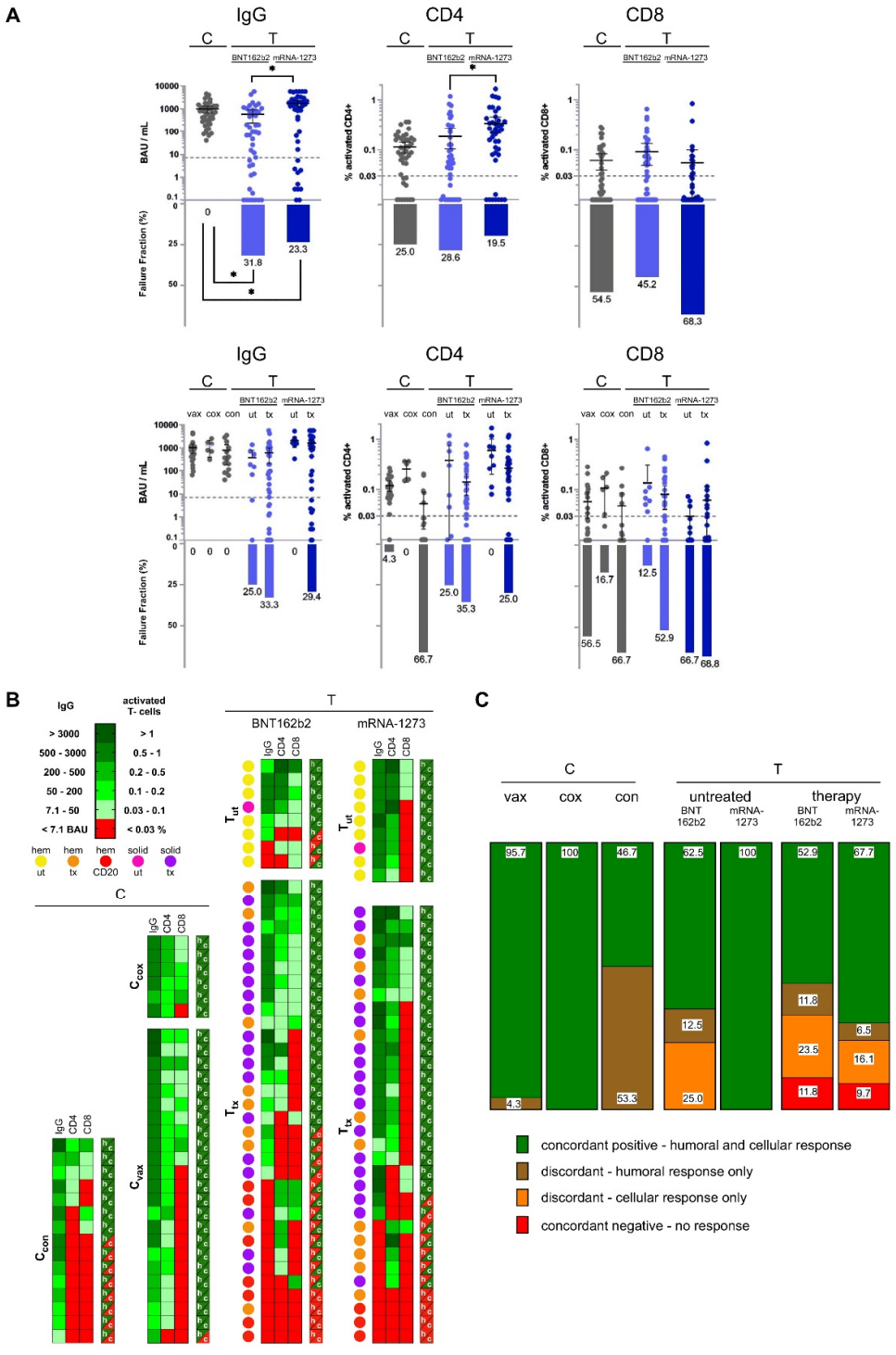

Figure S1. Individual humoral and cellular SARS-CoV-2 spike protein-specific responses to mRNA vaccines in patients with cancer

(A) Quantitative SARS-CoV-2-specific IgG, and spike-activated $CD4^+$ and $CD8^+$ responses (top) in the indicated groups; BNT162b2 and mRNA-1273 referring to two cancer patient cohorts fully vaccinated to these respective mRNA vaccines. Dashed lines indicate thresholds for positive results; thus, values below are considered negative and contribute to the percentages of failures in the indicated response categories (bottom). In the top panel, IgG analyses are based on 44 control participants and 44 (BNT162b2) and 43 (mRNA-1273) patients with cancer; $CD4^+$ and $CD8^+$ analyses are based on 44, 42, and 41 participants, respectively. In the bottom panel, IgG analyses are based on 23 C_{vax}, 6 C_{cox} and 15 C_{con} control participants, and 8 untreated (ut) and 36 treated (tx) BNT162b2- as well as 9 ut and 34 tx mRNA-1273-vaccinated patients with cancer; $CD4^+$ and $CD8^+$ analyses are based on the same numbers in control subgroups, and included 8 untreated (ut) and 34 treated (tx) BNT162b2- as well as 9 ut and 32 tx mRNA-1273-vaccinated patients with cancer. Error bars indicate mean ± 95% confidence intervall, and "*" indicates significance based on $p < 0.05$.

(**B)** Control and patient with cancer groups are color-coded to represent the quantification and tumor type/treatment designation. On the right of each column, green and red indicate positive and negative humoral (h) and cellular (c) responses, respectively. Concordant results are h/c green or red; discordant results are color-mixed. The indicated groups consist of 15 (C_{con}), 6 (C_{cox}), 23 (C_{vax}) control participants, and 8 ut and 34 tx BNT162b2- as well as 9 ut and 32 tx mRNA-1273-vaccinated patients with cancer.

(**C**) Fractions of participants with concordant or discordant humoral (IgG) and cellular (either $CD4^+$ or $CD8^+$ or both) responses. The percentages are based on individual data triads as shown in B.

Table S1. Characteristics of study participants, and assessment of odds ratios for concordant/discordant vaccination responses in patients with cancer

A. Characteristics of study participants. B. Odds ratio assessment for the indicated parameters regarding concordant *vs.* discordant responses to mRNA vaccination in patients with cancer.

Supplemental materials and methods

Patients with cancer and control participants in the vaccination and post-COVID-19 observation program

Beginning in March 2021 with the first dose and a booster dose three to four weeks later, the Kepler University Medical Center Linz was able to offer patients with cancer 18 years of age or older as part of a particularly vulnerable population at risk of more severe COVID-19 courses in a first series BNT162b2-based, in a second series mRNA-1273-based SARS-CoV-2 vaccination. This tumor patient cohort (termed group T) had no evidence of preceding COVID-19 and included patients with solid and hematologic malignancies (with the exception of non-melanoma skin cancers) without systemic anticancer therapy in the last three months and without anti-CD20 therapy in the last six months (subgroup T_{ut}) or with recent or current anticancer therapy (subgroup T_{tx}). Vaccination controls consisted of staff working at the Kepler University Medical Center with no record of solid or hematologic malignancies during the last three years who received two doses of BNT162b2 starting January 2021. Subgroup C_{vax} comprised subjects with no evidence of preceding SARS-CoV-2 infection, whereas subgroup C_{cox} reflected those with previous COVID-19. An additional subgroup (termed C_{con}) was formed by post-COVID-19 SARS-CoV-2-negative, non-

vaccinated convalescents who were taken care of at Kepler University Medical Center from April 2020 onwards.

Blood samples

Sample collection from patients with cancer, non-cancer controls and convalescent COVID-19 patients was approved by the ethic committee of Upper Austria, the institutional review board of the Medical Faculty of the Johannes Kepler University Linz, with permit number 1070/2020 and its subsequent amendment in 2021. For detailed patient characteristics, see Table S1A. After obtaining informed consent, venous blood was collected from all vaccine program-enrolled participants for antibody quantification prior to the first vaccination dose and three weeks after the second vaccination, the time, when also blood was taken to harvest peripheral blood mononuclear cells (PBMC). In the non-vaccinated convalescent group, serum and PMBC samples were taken around two weeks after nasopharyngeal swabs turned SARS-CoV-2 PCR-negative again. Serum aliquots were stored at -80°C until analysis.

PBMC isolation:

For PBMC isolation, blood was drawn into Vacutainer NH-CPT tubes (BD Biosciences) and centrifuged at 1500x g for 20 minutes at room temperature. The buffy coat was collected from the top of the gel and diluted 1:3 with PBS. PBMC were pelleted (300x g, 10 min), residual red blood cells were removed by resuspension of the pellet in 3 ml NH_4Cl lysis buffer and incubated for six minutes at room temperature. Tubes were filled with PBS to 13 ml, centrifuged and washed again in 5 ml PBS. An aliquot was removed for viable cell count, cells were pelleted (300x g, 5 min), resuspended in freezing medium (fetal bovine serum with 10% dimethyl sulfoxide [DMSO]), aliquoted in cryotubes and frozen down by using polystyrene foam containers that provided a

constant cooling rate of -1°C/minute. Samples were stored at -80°C until T-cell measurements were carried out.

Quantification of SARS-CoV-2 spike-specific antibody titers

IgG antibodies recognizing the receptor binding domain (RBD) of the spike protein were quantified using a CE-IVD-certified chemiluminescence-microparticle-based assay (Abbott) according to the manufacturer's instructions. Chemiluminescence measurements were performed using the proprietary ARCHITECT system (Abbott). Raw measurements were converted into binding antibody units (BAU)/mL according to WHO standards. The cut-off for sample positivity was set by the manufacturer to 7.1 BAU/mL, reflecting a 50% inhibitory dose (ID50) at a dilution of 1:20 in a plaque reduction neutralization test with 95% probability, and the upper detection limit of the assay was 5.680 BAU/mL.

Flow cytometric quantification of SARS-CoV-2 spike-specific activated $CD4^+$ and $CD8^+$ T-cells

Spike-specific activated T-cells were quantified using the SARS-CoV-2 Prot_S human T Cell Analysis Kit (Miltenyi Biotech). To maximize spike-specific responses, S1 and S+ Peptivator pools were admixed to the Prot_S Peptivator pool included in the kit. Peptides were added to each participant's individual PBMC population, of which the antigen-presenting cells therein take up the peptides, process and present them *via* major histocompatibility class I or II molecules to autologous $CD4^+$ or $CD8^+$ T-cells. Cryopreserved PBMC were thawed and allowed to recover over-night in TexMACS medium (Miltenyi Biotech). Viable PBMC were quantified, and approximately 1 x 10^6 cells were used for T-cell stimulation and staining according to the manufacturer's instructions. In brief, 100 µl of each PBMC sample were dispensed into 96-well plates,

and 2 µl of the spike peptide pools S, S1 and S+ were added. For each sample, cells supplemented with 6 µl of 10% DMSO in milliQ water were included as a negative control. After two hours of stimulation at 37°C and 5% CO_2, 2 µl of Brefeldin A to inhibit intracellular protein transport were added, and incubation was continued for another four hours. Then, cells were incubated with a viability dye for 30 minutes, fixed, permeabilized and stained with the eight antibodies contained in the kit. After a final washing step, cell pellets were resuspended in 200 µl of PEB buffer (*i.e.* PBS, pH 7.4, supplemented with 2 mM EDTA and 0.5% bovine serum albumin). T-cell analysis was performed using a CytoFlex flow cytometer (Beckman-Coulter). 10^5 events were collected for each sample whenever possible. Below 5×10^4 total events, measurements were considered inconclusive. Data analysis was performed using the Kaluza software package (Beckman Coulter). Compensation was determined using OneComp beads (Thermo). Gates were set utilizing fluorescence-minus-one controls and negative control samples. Gating was performed as follows: In a forward scatter/side scatter dot plot, a cell gate was set. These cells were further gated into viable $CD3^+$ cells, which were then differentiated on a CD4/CD8 dot plot. CD4-positive cells were analyzed for tumor necrosis factor-alpha (TNF-α) and CD154 expression as activation markers, with the fraction of double-positive cells constituting the spike-activated $CD4^+$ T-cells. CD8-positive cells were analogously analyzed for interferon-gamma (IFN-γ) and TNF-α expression, with the fraction of double-positive cells constituting the spike-activated $CD8^+$ T-cells. Measurements were baseline-corrected by subtracting the fraction of double-positive cells observed in the negative controls. The threshold for activated $CD4^+$ and $CD8^+$ T-cells was established at 0.03%, considering a baseline level below 0.01%.

Statistical analyses

Statistical differences between two experimental groups were calculated using the unpaired two-tailed Student's *t*-test. If the groups did not follow a Gaussian distribution, the Mann-Whitney U-test was used where indicated. Statistical differences between three or more groups were calculated using the two-way ANOVA test. For non-Gaussian distributions, the Kruskal-Wallis test was used where indicated. A significance level of $p < 0.05$ was used throughout the study. Odds ratios were calculated according to Altman-DG (1991), Practical statistics for medical research, using the MedCalc webtool (https://www.medcalc.org/calc/odds_ratio.php).