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Responses of patients with cancer to mRNA vaccines depend on the time interval between vaccination and last treatment

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ABSTRACT

Background Personalized mRNA vaccines are promising new therapeutic options for patients with cancer. Because mRNA vaccines are not yet approved for first-line therapy, the vaccines are presently applied to individuals that received prior therapies that can have immunocompromising effects. There is a need to address how prior treatments impact mRNA vaccine outcomes.

Method Therefore, we analyzed the response to BioNTech/Pfizer's anti-SARS-CoV-2 mRNA vaccine in 237 oncology outpatients, which cover a broad spectrum of hematologic malignancies and solid tumors and a variety of treatments. Patients were stratified by the time interval between the last treatment and first vaccination and by the presence or absence of florid tumors and IgG titers and T cell responses were analyzed 14 days after the second vaccination.

Results Regardless of the last treatment time point, our data indicate that vaccination responses in patients with checkpoint inhibition were comparable to healthy controls. In contrast, patients after chemotherapy or cortisone therapy did not develop an immune response until 6 months after the last systemic therapy and patients after Cht-immune checkpoint inhibitor and tyrosine kinase inhibitor therapy only after 12 months.

Conclusion Accordingly, our data support that timing of mRNA-based therapy is critical and we suggest that at least a 6-months or 12-months waiting interval should be observed before mRNA vaccination in systemically treated patients.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ It is well known that many oncological therapies impair the ability of the immune system to respond to vaccination. We provide direct evidence that the time interval between therapy and vaccination impacts the response to mRNA-based vaccines.

WHAT THIS STUDY ADDS

⇒ This study supports that in patients receiving certain systemic oncological treatments, a waiting interval of 6–12 months should be observed before mRNA vaccination to obtain effective vaccine response.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our data are important for planning the administration of personalized mRNA cancer vaccines in patients who have previously received other types of treatments and provide guidance for vaccination schedules in patients with different concomitant therapies.

INTRODUCTION

The COVID-19 pandemic revealed the power and the potential of mRNA-based vaccine strategies. The rapid development and clinical testing of COVID-19 mRNA vaccines were based on years of previous mRNA vaccine technology research in the field of oncology. However, the use of the COVID-19 vaccine provided the first data that mRNA-based vaccines are well tolerated in the general population including older people with complex comorbidities.[1–3] Moreover, the non-infectious molecules elicit robust humoral and cell-mediated immune responses in healthy individuals.[4] Currently, the development is refocusing on the utilization of mRNA vaccines for patients with cancer, following the observed vaccine efficacy to prevent COVID-19 infections. Several clinical trials are already in phase 2, such as the BNT122 vaccine in patients with stage II/II colorectal cancer after surgical resection of the tumor and completion of adjuvant chemotherapy. The study seeks to identify patients at high risk of recurrence using a blood test for circulating tumor DNA and investigate whether an individualized mRNA vaccine can prevent recurrence. As part of the trial, patients received adjuvant chemotherapy. Other clinical trials such as for melanoma

Table 1 Selected clinical trials of mRNA vaccines for cancer immunotherapies

	Trial phase	Targeting antigen	Cancer type	Cotherapy	Administration route
NCT03948763	1	mRNA-5671 (KRAS driver mutations)	Non-small-cell lung cancer, pancreatic cancer, colorectal neoplasms	Pembrolizumab	Intramuscular
NCT03313778	1	mRNA-4157 (personalized cancer vaccine)	Solid tumors	Pembrolizumab	Intramuscular
NCT03897881	2	mRNA-4157 (personalized cancer vaccine)	Melanoma	Pembrolizumab	Intramuscular
NCT04573140	1	Formulation with pp65 LAMP and tumor mRNA	Glioblastoma	Single agent	Intravenous

or glioblastoma are also using mRNA-based therapies in combination with additional immunomodulatory therapies (table 1). Because prior therapies often reduce the responsiveness of the immune system, it is important to choose the optimal timing for vaccination. Since no mRNA-based cancer vaccine has yet been approved by the US Food and Drug Administration (FDA) and none has reached phase 3 clinical trials, real world data on mRNA vaccine efficacy in patients receiving multiple combination therapies are missing.

The first FDA approved mRNA vaccine, BNT162b2 from BioNTech/Pfizer encoding the full-length SARS-CoV-2 spike protein, demonstrated up to 95% efficacy in preventing symptomatic SARS-CoV-2 infections.[1] This recent success proved the mRNA technology to be a powerful therapeutic tool. However, individuals with specific immunological deficits were excluded from the conducted phase II/III clinical trials,[5] resulting in a lack of data concerning the vaccine responses in immunocompromised populations. The various malignancies and their treatment options create a very diverse environment that poses special challenges, as the vaccine will probably have very different effects and lower efficacy in these patients. Given this uncertainty, the use of vaccines against endemic pathogens is usually postponed during and after oncology therapies or, if possible, provided before therapy. To date, several studies have examined the impact of various systemic cancer treatments on the BNT162b2 vaccine response.[6–11] Unsurprisingly, the vaccine response was lower in these groups compared with healthy controls. However, fewer studies addressed T cell responses, and more importantly there is a lack of data

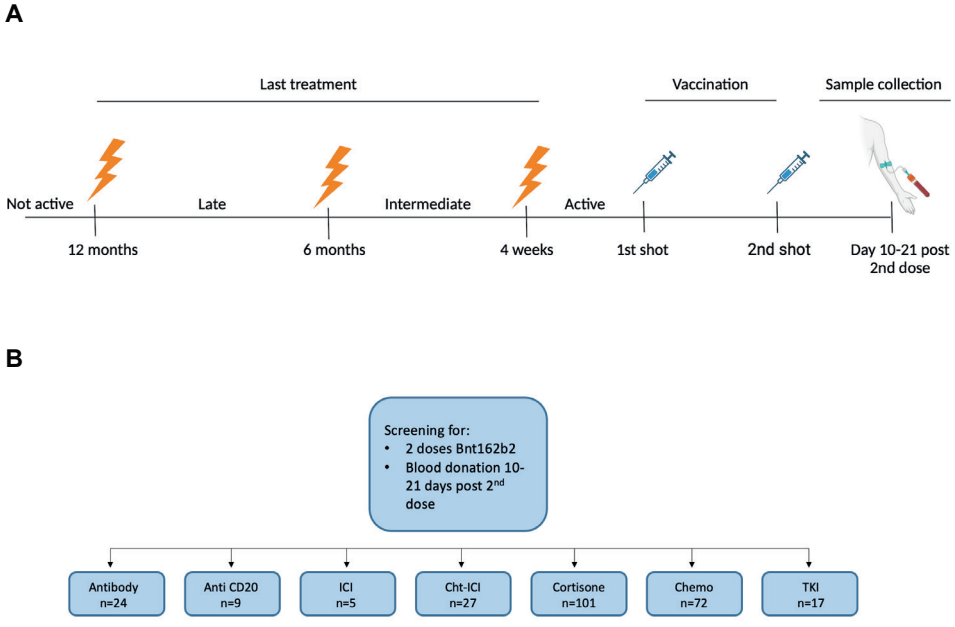

Figure 1 Schematic illustration of cohort formation. Outpatient data were collected from more than 900 patients with cancer of three oncology outpatient clinics. Those who received two doses of the BioNTech/Pfizer mRNA vaccine and donated blood 10–21 days post second dose, were included in this study. Patients were divided into different groups according to their treatment and additionally subgrouped considering the last time of therapy.

Table 2 Baseline demographics and clinical characteristics of the study population

	HD n=21	ICI n=5	TKI n=17	Anti-CD20 n=9	Antibody n=24	Cht-ICI n=27	Chemo n=72	Cortisone n=101
Age		59.6 (39–81)	68 (39–86)	69 (62–80)	66 (39–86)	66 (39–86)	63 (34–86)	64 (37–86)
Sex								
Male	7 (33%)	1 (20%)	9 (53%)	7 (78%)	14 (37%)	8 (30%)	23 (32%)	33 (33%)
Female	14 (67%)	4 (80%)	8 (47%)	2 (22%)	24 (63%)	19 (70%)	49 (68%)	68 (67%)
Therapy time point*								
Active		4	9	/	8	13	8	20
Intermediate		/	2	2	3	2	2	28
Late		/	1	2	4	2	1	10
Not active		1	5	5	9	10	14	41

Data are mean (SD) or n (%).
*Participants receiving systemic cancer therapy previous to vaccination were subdivided into active (last therapy up to 4 weeks before blood donation), intermediate (last therapy more than 4 weeks before blood donation but less than 6 months), late (last therapy 6–12 months before blood donation) and non-active therapies (last therapy more than 12 months before blood donation).
HD, healthy donor; ICI, immune checkpoint inhibitor; TKI, tyrosine kinase inhibitors.

correlating the vaccine response in patients with multiple concomitant therapies to the treatment regimen. These could help to provide recommendations for choosing optimal time points of vaccination adapted to the therapeutic regimen, allowing the best possible development of antibody titers and T cell responses. These time points are not only important for the application of prophylactic vaccines against infectious disease, but also for therapeutic tumor-specific vaccines.

Therefore, we initiated the COVID-19 Cancer Vision study, in which we analyzed data from 237 patients from 3 oncology outpatient clinics and correlated retrospectively the response rates to the vaccine with the type of treatment and tumor status. We thereby covered the entire spectrum of hematologic malignancies and solid tumors and subdivided patients according to their different therapies. We distinguished between chemotherapy, checkpoint inhibitors, monoclonal antibodies, tyrosine kinase inhibitors (TKIs), B cell depleting therapies and general corticosteroids, respectively. In contrast to previous studies, we subdivided these therapy groups further according to their treatment regimen, taking into account the last treatment administration and by the presence or absence of florid tumors. The outpatient centered data collected here provide useful guidelines on effective vaccination programs that are relevant to daily clinical practice.

METHODS

Human cohort

Venous blood and serum from 237 oncology patients of three clinical outpatient clinics and 21 healthy coworkers was collected approximately 14 days post 2nd dose or 3rd dose of the BNT162b2 (Biontech/Pfizer) vaccine. Patients with detectable tumors are classified as florid, while patients with undetectable tumors (adjuvant treatment) are classified as non-florid.

Serological assessment

Quantitative determination of spike-specific IgG antibodies was performed in sera using the Elecsys Anti-SARS-Cov-2 S Immunoassay (Roche Diagnostics, Switzerland). Antibodies above the limit of quantification of 0.4 U/mL were regarded as positive signal. Tests were performed on a cobas e801 (Roche) at the MVZ Freising laboratory, Freising, Germany.

Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood samples collected in either Lithium Heparin or Sodium-Heparin tubes. In brief, blood was diluted 1:1 in PBS (Phosphate-buffered saline) and layerd on Ficoll Paque Plus (GE Healthcare). Lymphocytes were recovered after centrifugation (40 min, 400rcf, RT, no brake), followed by two wash steps with PBS (10 min, 400rcf, 4°C). Cells were subsequently used for T cell stimulation or cryopreserved in heat-inactivated fetal

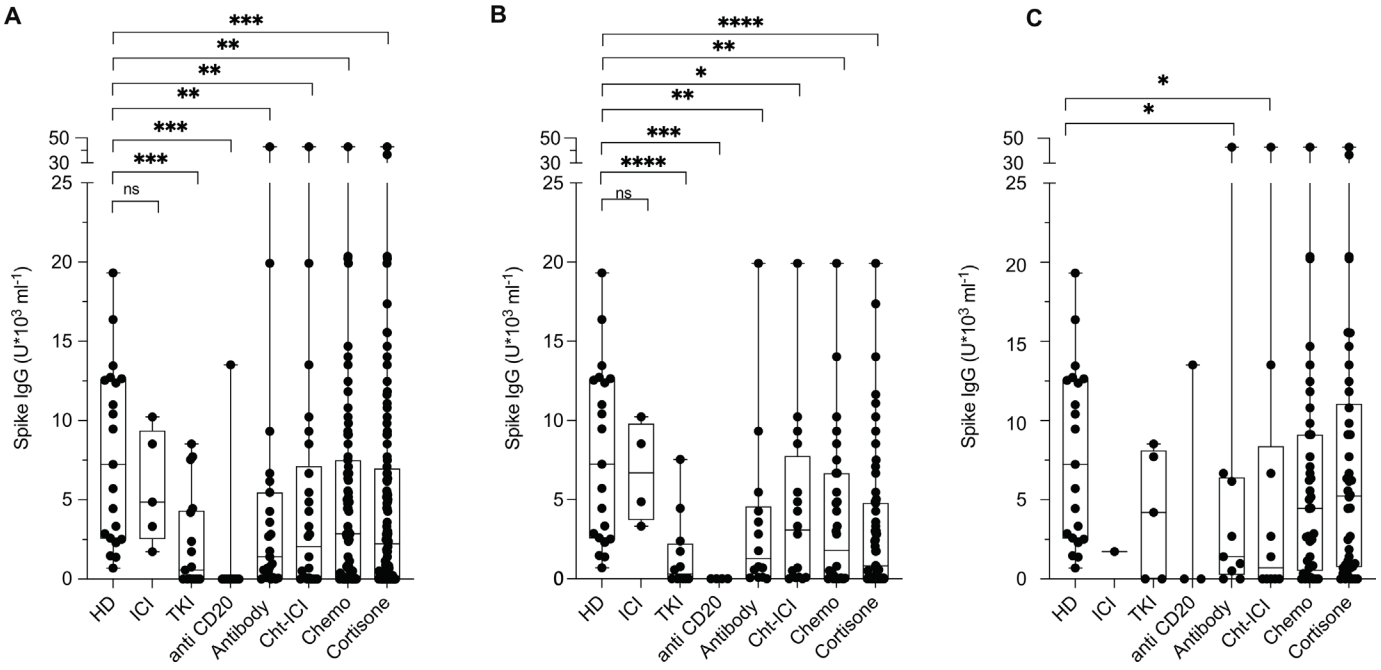

Figure 2 Antibody response to BNT162b2 mRNA vaccination in patients with cancer. Anti-SARS-CoV-2 spike-specific IgG titers (U/mL) compared between patients with cancer (n=237) and healthy donors (HD; n=20). Serum samples were obtained 14 days post second dose BNT162b2. (A) Patients were subdivided according to their type of therapy including checkpoint inhibition (checkpoint, n=5), tyrosine kinase inhibitors (TKI, n=17), B cell depleting therapies (anti-CD20, n=9), antibody therapy (n=24) immunotherapy with concomitant chemotherapy (Cht-ICI, n=27), chemotherapy (chemo, n=72) and corticosteroids (cortisone, n=101). Patients receiving various therapies are listed in multiple groups. (B) Exclusion of patients receiving their last therapy more than 12 months ago. (C) Patients receiving their last therapy more than 12 months ago. Symbols represent individual participants. Mann-Whitney U test was performed to calculate significance with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 and ns not significant. ICI, immune checkpoint inhibitor.

calf serum (FCS, Sigma-Aldrich) containing 10% DMSO (Dimethyl sulfoxide, Sigma).

T cell stimulation

Stimulations were predominantly performed on freshly isolated PBMCs. If needed, cryopreserved PBMCs were thawn briefly in a 37°C water bath and washed twice with RPMI 1640 supplemented with 10% FCS and Benzonase (50 U/mL). Overlapping peptide pools (PepMix, JPT) spanning the structural SARS-CoV-2 protein spike (spike vial 1 containing the receptor binding domain (RBD) and spike vial 2 containing fusion peptide, transmembrane domain and cytoplasmic peptide) were used for T cell stimulation in a final concentration of 1 µg/mL. T cells cultured in media with equivalent amounts of DMSO served as negative control. Stimulation was performed for 6 hours (37°C, 7% CO_2) and 10 µg/mL Brefeldin A (Sigma) was added after 2 hours.

Surface and intracellular antibody staining of human cells

Single cell suspensions were stained using in house labeled monoclonal anti-human antibodies: CD8 (clone OKT-8, BioXCell), CD4 (clone RPA-T4, BioXCell), CD3 (clone UCHT1, BioXCell) and IFNg (clone: B27, BioXCell). Cells were subsequently fixed for 30 min in PBS containing 2% formaldehyde. For permeabilization, cells were incubated in PBS containing 10% Saponin and 0.02% azide for 15 min prior to intracellular cytokine staining.

Statistics

Statistical analysis was performed in Prism (GraphPad). Unless stated otherwise, an unpaired, two-tailed Student's t-test was used to calculate significance between groups. Values of $p<0.05$ were considered significant with *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001. Values of $p>0.05$ were considered not significant (ns).

RESULTS

The focus of our study was to analyze differences in the immune response depending on the time interval between vaccination and the last oncological treatment administration. For this purpose, we used the approved mRNA vaccine from Biontech/Pfizer BNT162b2 as a model. Between February and September 2021, we collected blood samples from patients that were vaccinated with BNT162b2 in three oncological outpatient clinics. Of this cohort, we analyzed data from 237 patients with cancer and 21 healthy individuals who matched our study criteria (figure 1A). Participants received two doses of the mRNA vaccine BNT162b2. Blood was donated approximately 14 days post second dose (range >10 and <21 days). Patients received either chemotherapy (n=72),

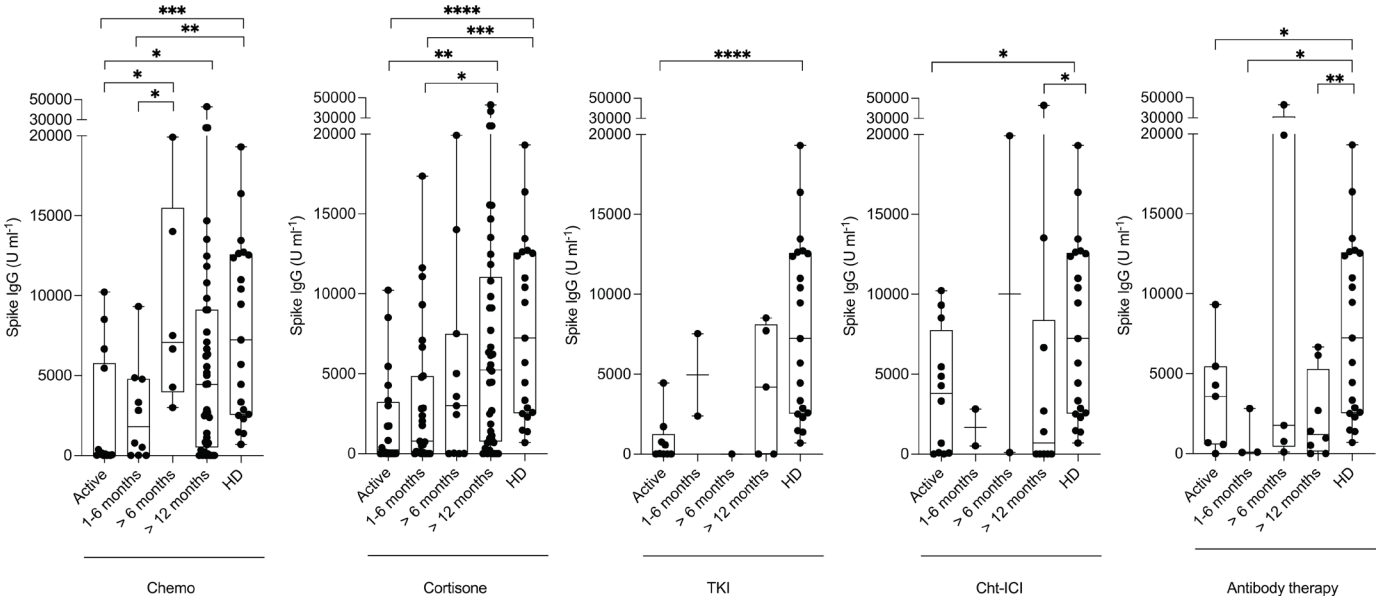

Figure 3 Time point of last therapy impacts antibody formation post vaccination in patients with cancer. Anti-SARS-CoV-2 spike-specific IgG titers (U/mL) compared between patients with cancer during different stages of chemotherapy, cortisone treatment, TKI's, combined immune and chemotherapy and antibody therapy. Serum samples were obtained 14 days post second dose BNT162b2. Each treatment was subdivided into active (last therapy received up to 4 weeks prior to blood donation), intermediate (last therapy more than 4 weeks before blood donation but less than 6 months), late (last therapy 6–12 months before blood donation) and not active therapy (last therapy more than 12 months before blood donation). Patients receiving various therapies are listed in multiple groups. Symbols represent individual participants. Mann-Whitney U test was performed to calculate significance with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. HD, healthy donors; TKIs, tyrosine kinase inhibitor.

immune checkpoint inhibitors (ICI) (n=5), monoclonal antibodies (n=38), TKI (n=17), B cell depleting therapies (n=9), high-dose corticosteroids (n=101) or chemo-immunotherapy (Cht-ICI; n=27). Patients receiving various therapies are thereby listed in multiple groups (figure 1B). The healthy control group included volunteers who were vaccinated during the early phase of the SARS-CoV-2 pandemic. All patient and baseline characteristics are shown in table 2 and specific medications are listed in online supplemental table S1. Blood and serum samples were collected, respectively, to compare cellular and serological immune responses.

The timing of mRNA vaccine administration impacts the antibody response in oncology patients

Testing for antibody titers is generally accepted as a correlate to vaccine efficacy. Hence, our first objective was to determine SARS-CoV-2 spike protein RBD IgG titers approximately 14 days after the second dose BNT162b2 in our cohorts using the Elecsys Anti-SARS-Cov-2 S Immunoassay (Roche Diagnostics, Switzerland).

Robust circulating anti-Spike IgGs were detectable in all healthy participants (100%) with a median of 7236 U/mL (figure 2A), which is in concordance

Table 3 HD and patient T cell response to stimulation with SARS-CoV-2 Spike S1 and S2 peptide pool stimulations

	CD8 T cell response S1	CD8 T cell response S2	CD4 T cell response S1	CD4 T cell response S2
HD n=8	7 (87.5%)	7 (87.5%)	8 (100%)	8 (100%)
ICI n=4	3 (75%)	3 (75%)	4 (100%)	4 (100%)
TKI n=9	7 (77.7%)	4 (44.4%)	7 (77.7%)	9 (100%)
Anti-CD20 n=5	5 (100%)	1 (20%)	5 (100%)	5 (100%)
Antibody n=17	13 (76.4%)	8 (47%)	16 (94.1%)	17 (100%)
Cht-ICI n=12	9 (75%)	6 (50%)	12 (100%)	12 (100%)
Chemo n=25	16 (64%)	12 (48%)	23 (92%)	24 (96%)
Cortisone n=31	21 (67.7%)	15 (48.3%)	29 (93%)	30 (96.7%)

Data are n (%)
HD, healthy donor; ICI, immune checkpoint inhibitor; TKI, tyrosine kinase inhibitors.

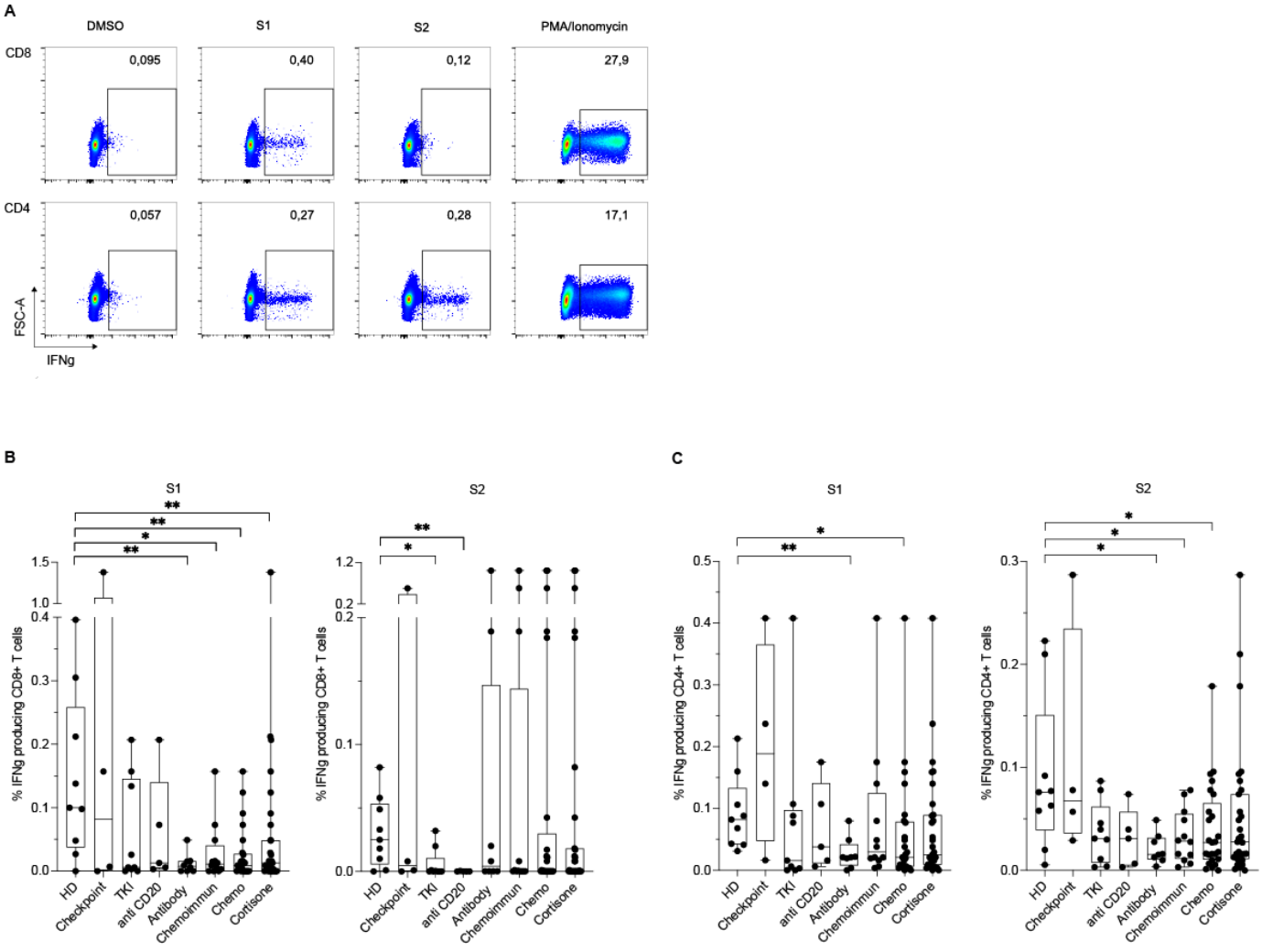

Figure 4 T cell response to BNT162b2 mRNA vaccination in patients with cancer. (A) Representative flow cytometry plots showing IFNg expression of $CD8^+$ (upper row) and $CD4^+$ (lower row) T cells in a healthy individual after stimulation with DMSO (negative control), spike pool 1 (S1), spike pool 2 (S2) and PMA and Ionomycin (positive control), respectively. Percentage of IFNg expressing $CD8^+$(B) and $CD4^+$(C) T cells in patients with cancer receiving checkpoint inhibition (Checkpoint, n=4), tyrosine kinase inhibitors (TKI, n=9), B cell depleting therapies (anti-CD20, n=5), antibody therapy (n=8), immunotherapy with concomitant chemotherapy (Cht-ICI, n=12), chemotherapy (chemo, n=24) and corticosteroids (cortisone, n=31) and healthy individuals (HD, n=9) after stimulation with S1 and S2, respectively. Each dot represents one donor and was calculated by background subtraction. Mann-Whitney U test was performed to calculate significance with *p<0.05, **p<0.01.

with previous studies on the efficacy of BNT162b2 in healthy individuals.[4] Compared with that, antibodies above the limit of quantification of 0.4 U/mL were detected in 62 (86%, median 2860 U/mL) patients receiving chemotherapy, 6 patients receiving checkpoint inhibitors (100%, median 4861 U/mL), 22 (91%, median 1405 U/mL) with monoclonal antibody therapy, 11 (64%, median 571 U/mL) receiving TKIs, 2 (22%, median 0.4 U/mL) on B cell depleting therapies, 84 (83%, median 2218 U/mL) on corticosteroids and in 21 (77%, median 2051 U/mL) individuals with Cht-ICI therapy. Thus, a significantly lower antibody response was observed in each treatment group compared with healthy individuals, with the exception of patients receiving checkpoint inhibition.

Interestingly, substantial variability was observed in patients receiving the same therapy. Some patients showed antibody responses comparable to or even stronger than those of the control group, while other patients showed no antibody response at all. For example, one patient under B cell depletion developed IgG levels comparable to those of the control group, while the remaining patients in the anti-CD20 group showed no antibody response. These observations reveal that not only the type of therapy is critical for the response rate to mRNA vaccination, but also the timing of administration. We also observed that individuals with a florid tumor (M1 classification) and with therapy length over 6 months had a significantly reduced immune response, compared with individuals with a non-florid tumor (M0) and shorter than 6 months therapy (online supplemental figure S1), in groups with n>3. In addition to a possible suppressive

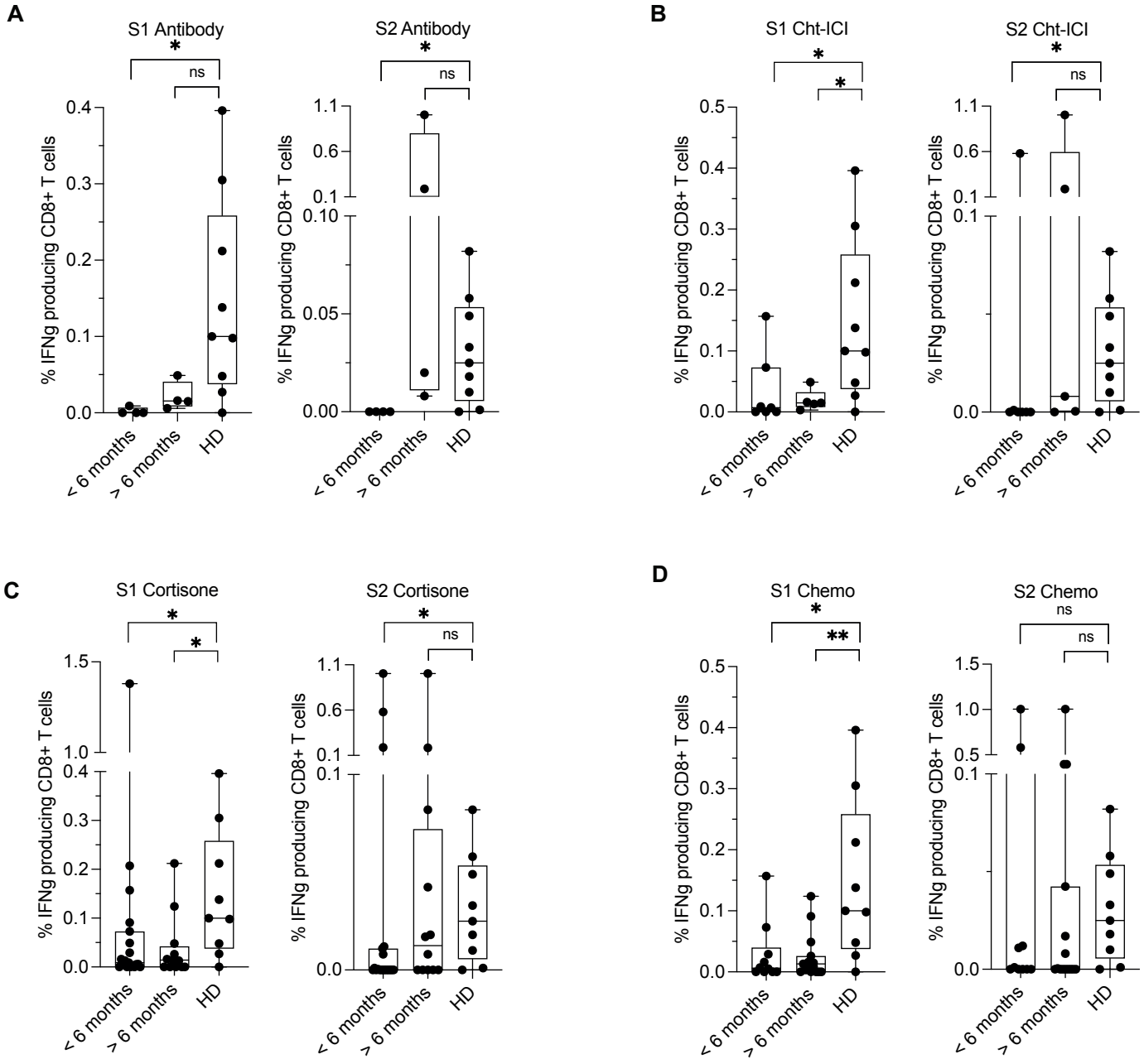

Figure 5 Impact of treatment regimen on spike-specific CD8⁺ T cell response post vaccination in patients with cancer. Percentage of IFNg producing CD8⁺ T cells in patients with cancer and healthy donors (HD, n=9)) after stimulation with spike pool 1 (S1) or spike pool 2 (S2) 14 days post second dose BNT162b2. Patients receiving antibody therapy (n=8), immunotherapy with concomitant chemotherapy (Cht-ICI, n=12), corticosteroids (cortisone, n=31) or chemotherapy (chemo, n=24) were distinguished by whether their last treatment more or less then 6 months prior to the first dose of the BNT162b2 vaccine. Each dot represents one donor and was calculated by background subtraction. Mann-Whitney U test was performed to calculate significance with *p<0.05, **p<0.01 and ns not significant. ICI, immune checkpoint inhibitor.

effect of the tumor, the reduced immune response could also be due to the duration of the ongoing therapy. To investigate the effects of precise timing on the success of the humoral immune response in more detail, patient cohorts were stratified according to the interval between the last systemic oncological therapy and mRNA vaccination. We started by distinguishing between patients in whom the last therapy was administered less (figure 2B) or more (figure 2C) than 12 months before vaccination. As expected, IgG levels appeared less heterogeneous in the patient group receiving their last therapy treatment within the last 12 months. Moreover, IgG titers were less or not at all affected in individuals who had received their last therapy more than 12 months ago, especially for those receiving cortisone or chemotherapy (figure 2C).

Based on these findings, we divided the therapy groups further into active (last therapy up to 4 weeks before blood donation), intermediate (last therapy more than 4 weeks before blood donation but less than 6 months), late (last therapy 6–12 months before blood donation) and

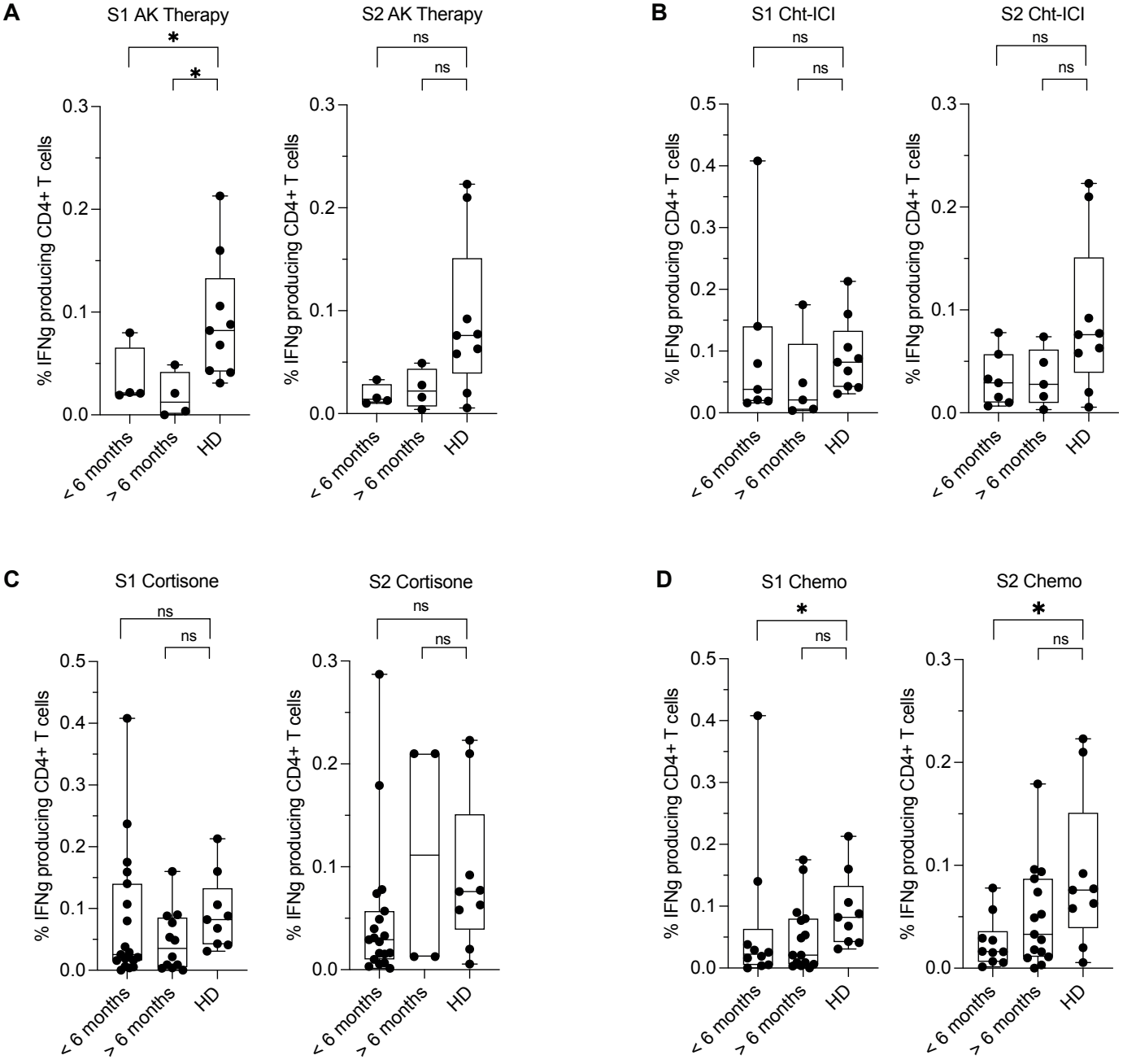

Figure 6 Impact of treatment regimen on spike-specific CD4⁺ T cell response postvaccination in patients with cancer. Percentage of IFNg producing CD4⁺ T cells in patients with cancer and healthy donors (HD, n=9)) after stimulation with spike pool 1 (S1) or spike pool 2 (S2) 14 days post second dose BNT162b2. Patients receiving antibody therapy (n=8), immunotherapy with concomitant chemotherapy (Cht-ICI, n=12), corticosteroids (cortisone, n=31) or chemotherapy (chemo, n=24) were distinguished by whether their last treatment more or less then 6 month prior to the first dose of the BNT162b2 vaccine. Each dot represents one donor and was calculated by background subtraction. Mann-Whitney U test was performed to calculate significance with *p<0.05 and ns not significant. ICI, immune checkpoint inhibitor.

non-active therapies (last therapy more than 12 months before blood donation). Since patients on checkpoint inhibition did not show any impact on the IgG response, and IgG titers were generally not detectable for patients after anti-CD20 treatment, further subdivision was not applied for those two groups. For individuals on chemotherapy and cortisone therapy, we observed that anti-spike IgG levels developed to a level comparable to the control group only 6 months after the end of therapy (figure 3). For antibody therapy, as well as the combination of chemotherapy and immunotherapy, anti-spike IgG did not develop to levels comparable to those in the control group even 12 months after their last therapy administration. A similar tendency was observed for patients with TKI treatment, but due to the rare number of subjects in the intermediate and late therapy group, we cannot make any conclusions about the vaccine efficacy when applied 1–6 months post-therapy. These data clearly show

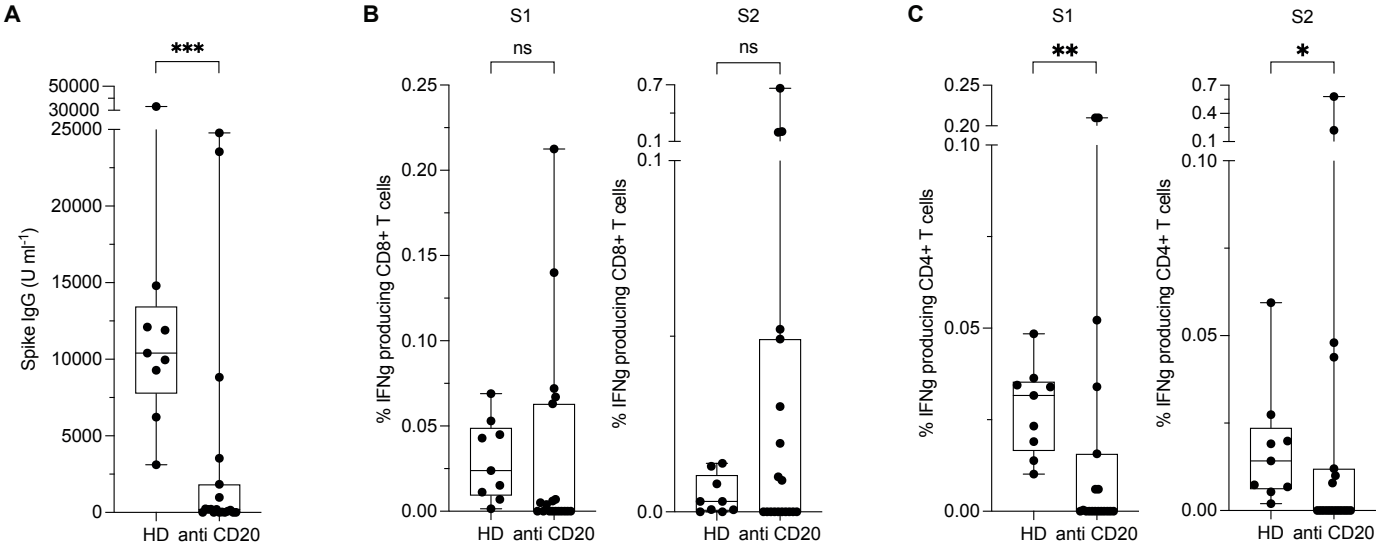

Figure 7 Third BNT162b2 boost vaccination in patients with ongoing B cell depleting therapies. Blood and serum samples were obtained one to 5 months post third vaccination with BNT162b2. (A) Anti-SARS-CoV-2 spike-specific IgG titers (U/mL) compared between patients receiving anti-CD20 therapy (n=9) and healthy donors (HD; n=9). Percentage of IFNg producing $CD8^+$ (B) and $CD4^+$ (C) T cells in after stimulation with spike pool 1 (S1) or spike pool 2 (S2) compared between patients receiving anti-CD20 therapy (n=19) and (HD; n=9). Mann-Whitney U test was performed to calculate significance with *p<0.05, **p<0.01, ***p<0.001 and ns not significant.

that the timing of mRNA-based vaccines is critical for the humoral immune response in oncological and hematological patients.

Long-term reduced mRNA vaccine induced T cell responses post cancer therapies

Personalized cancer vaccines on a mRNA base are designed to induce or boost anti-tumor immunity by activating individual neoantigen-specific T cells.[12] To study the impact of different cancer treatments on the antigen-specific T cell response post vaccination with BNT162b2, we used the approach of PBMC stimulation with overlapping SARS-CoV-2 spike peptide pools covering both the RBD (S1) and the transmembrane and cytoplasmic domains (S2). PBMCs were stimulated for 6 hours with 15-mer peptides with 11-mer overlap, containing in total 150 peptides per pool. We focused on screening Interferon-gamma (IFNg) cytokine secretion within $CD4^+$ and $CD8^+$ T cells. The full gating strategy is provided in online supplemental figure S1.

As expected, we observed detectable IFNg $CD8^+$ and $CD4^+$ T cells against at least one of the SARS-CoV-2 peptide pools in each individual of our healthy cohort (table 3). Compared with that, IFNg producing T cells were less frequently detected in the majority of the different treatment groups (figure 4, online supplemental figure S3,4). Similar to our observation of RBD IgG development, each treatment except for checkpoint inhibition shows a significant reduction in the frequency of $IFNg^+$ $CD8^+$ T cells either against the S1 or S2 peptide pool. Additionally, reduced CD4 T cell responses were observed for all individuals except those receiving checkpoint, TKI or anti-CD20 treatment.

When comparing the T cell response of individuals with a florid or non-florid tumor to healthy individuals, there we noted a tendency toward a lower frequency of IFNg producing T cells for individuals with a florid tumor compared with tumor-free patients (online supplemental figure S5). Again, this could be due to an immunosuppressive effect of the tumor micorenvironment or it could be a result of different treatment regimens applied to patients with florid tumors. Also, similar to the analysis of the antibody response, we observed a lot of heterogeneity in the T cell response within each treatment group and therefore further divided T cell responses according to the treatment regimen (figures 5 and 6).

Since T cell analysis goes along with higher efforts compared with IgG detections, we limited T cell stimulations to a proportion of patients. Adjusted to the small sample size, patients were divided into two groups, taking into account whether the last treatment was more or less than 6 months before vaccination. We observed that $CD8^+$ T cell responses were significantly reduced in each therapy group when treatment was given within 6 months prior to vaccination. In contrast to our antibody analysis, patients receiving Cht-ICI, chemo or cortisone therapy more than 6 months ago, still showed a significantly reduced $CD8^+$ T cell response. Moreover, $CD4^+$ T cell responses were only impacted in individuals receiving antibody and chemotherapy. Since $CD8^+$ T cells are those that are specifically targeted with mRNA vaccines for cancer immunotherapy, the vaccine administration should be carefully adapted between treatment regimens to allow the induction of optimal T cell responses.

Impaired vaccine response after third boost vaccination in anti-CD20 treated patients

Given the observation, that patients receiving B cell depleting therapies did not mount proper humoral vaccine responses after two doses, we surveilled the third boost response in this therapy group. Samples were obtained 1–5 months post third vaccination for both, control and treated group. With a median of 198 U/mL, patients receiving anti-CD20 therapy showed significantly reduced anti-spike IgG titers compared with the healthy control group with a median of 10 400 U/mL (figure 7A). Interestingly, $CD8^+$ T cell responses were not impaired (figure 7B), whereas significantly lower frequencies of IFNg producing $CD4^+$ T cells were observed (figure 7C).

DISCUSSION

Cancer vaccines such as BioNTech/Pfizer's mRNA vaccines, which encode up to 20 patient-specific neoantigens, are a promising new technology for eliciting an antitumor immune response. Many of the vaccines have already been approved for clinical trials. Currently, the mRNA-4157 vaccine is already in phase II, assessing whether postoperative adjuvant therapy with the mRNA vaccine in combination with pembrolizumab improves recurrence-free survival compared with pembrolizumab alone in participants with complete resection of cutaneous melanoma and a high risk of recurrence. To our knowledge, there are no data yet on the effects of immunomodulatory and immunosuppressive treatments and the relationship of the time interval with the humoral and cellular immune response. In our study, we investigated how different systemic oncological or hematological treatment regimens affect the efficacy of BioNTech/Pfizer's first approved mRNA vaccine BNT162b2. We have, therefore, recruited patients with a variety of different therapies and stratified them based on their last systemic treatment administration into active, intermediate, late and non-active therapy subgroups.

We found that T-cell and anti-spike IgG responses in individuals receiving ICI were similar to those in healthy individuals as described elsewhere,[13 14] which has also been shown for the COVID-19 mRNA-1273 vaccine from Moderna.[9] Our data showed that there is no impairment of the humoral and cellular immune response, which is important considering that mRNA-based cancer vaccines might often be used in combination with ICI therapy.

Strong impairments of both humoral and cellular immune responses were expected for patients under immunosuppressive and immunocompromising therapy, as demonstrated in previous reports.[8 15–17] Unsurprisingly, we observed low seropositivity and limited T-cell responses in patients receiving chemotherapy or cortisone therapy. Regarding timing of vaccination we observed that anti-spike IgGs did not develop until 6 months after the end of therapy for both chemo and cortisone therapy, whereas T cells continued to show an impaired response for longer than 6 months after therapy administration. During Cht-ICI as well as antibody therapy, we observed an impaired IgG development even more than 12 months after administration of the last therapy. The T cell response showed to be more impacted when treatments were administered 6 months prior to vaccination. The observed delayed immune response after these therapies could have a critical impact on the efficiency of mRNA-based cancer vaccines and could be clinically relevant for other mRNA-based therapies. Based on our results we presently recommend that there should be at least 6 months between last systemic therapy and vaccination in order to have a sufficient immune response.

B cell depleting therapies result in a prevention of seroconversion, thus drastically reducing the humoral immune response.[18] It is therefore not surprising that we did not observe any significant antibody response during ongoing therapy (within the 12-month interval). This has been reported previously in other studies and, moreover, a direct correlation of anti-spike IgG titers with absolute B cell counts has been shown.[19–24] With regard to the cellular response, we observed both IFNg producing $CD8^+$ and $CD4^+$ T cells, which is consistent with previous reports demonstrating less affected T cell responses in seronegative patients during ongoing B cell depleting therapies.[7 24 25] Thus, in the event of a breakthrough infection, at least the cellular adaptive immune response would be available to these patients. However, the lack of spike-specific IgG antibodies makes them more susceptible to infections in the first place, so they should be considered for passive immunization for the time period of and up to 12 months after therapy.

Notably, similar observations were made for a large fraction of patients on TKI therapy. This was surprising, because earlier studies on TKI patients showed robust anti-spike IgG titers comparable to those in the control group against COVID-19 vaccines.[26 27] The lack of robust IgG titers in a proportion of our TKI group may be attributed to the administration of specific TKIs as well as ongoing cotherapies. Patients who showed lower IgG than the control group received Bruton TKIs (BTKIs), which are often used in combination with B cell depleting antibodies for the treatment of B cell lymphoma. Thus, patients on BTKIs should also be considered for passive immunization.

Our study shows that the success of mRNA-based therapies for oncology and hematology patients strongly depends on the time between the last systemic therapy and the time of vaccination. This could be especially important for study designs and trials using mRNA vaccines, which are also based on lipid nanoparticle technology and administered intramuscularly. Since these vaccines are currently being tested in combination with checkpoint inhibitors,[28] our study provides important data that could be relevant for future studies. It needs to be investigated which patients will benefit from mRNA-based therapies if they have already received one or more systemic therapies. Altogether, our data imply that individuals receiving chemo, cortisone, Cht-ICI

or antibody therapy would mostly benefit from vaccinations if applied more than 6 months post-treatment administration. Nevertheless, it needs to be considered that although our study included a very large number of patients in whom we assessed T-cell responses, the number of patients within our individual subgroups is much smaller. Therefore, much larger patient cohorts are needed to confirm and refine this interval but our data already provides clear evidence that a longer waiting period is beneficial.

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Contributors DH, LH and DZ initiated and conceptualized the study. DZ and DH acquired funding. CS measured IgG titers. LVD performed T cell based experiments, analyzed all data and performed statistical analysis. JVdO provided demographic patient informations. LH provided important scientific input. The manuscript was written by LVD, LH and DZ. DZ is responsible for the overall content as guarantor.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Human blood samples were collected in in accordance with the ethical standard of the responsible committee of the Bavarian Chamber Of Physician (BLÄK) with the ethic committee's approval No. 20037 and with the Declaration of Helsinki of 1964 and its later amendments, as well as the Technical University of Munich (TUM; 736/20S).

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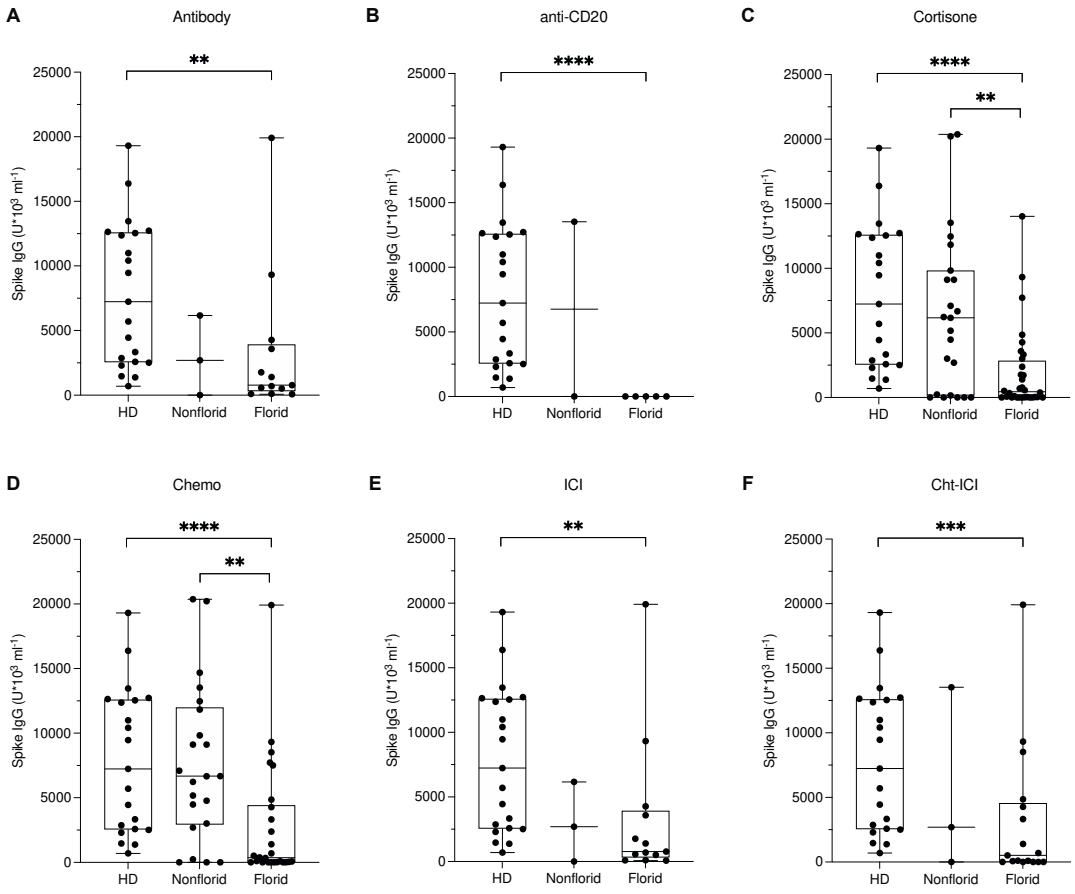

Fig. S1: Correlation of tumor control and antibody titers. Anti SARS-CoV-2 Spike specific IgG titers (U ml^{-1}) compared between and healthy donors (HD; n = 20) and cancer patients receiving antibody therapy (A), B cell depleting therapies (B), corticosteroids (C), chemotherapy (D), checkpoint inhibition (ICI; E) or immunotherapy with concomitant chemotherapy (Cht-ICI; F). Serum samples were obtained 14 days post 2nd dose BNT162b2. Patients were distinushied by their tumor control, with (florid) and without (nonflorid) detectable tumor mass. Symbols represent individual participants. Mann-Whitney test was performed to calculate significance with *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 and ns not significant.

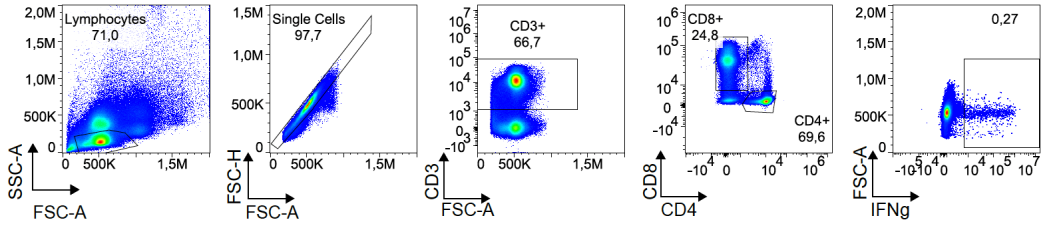

Fig. S2: Gating strategy

Flow cytometric gating strategy of IFNg producing $CD4^+$ and $CD8^+$ T cells.

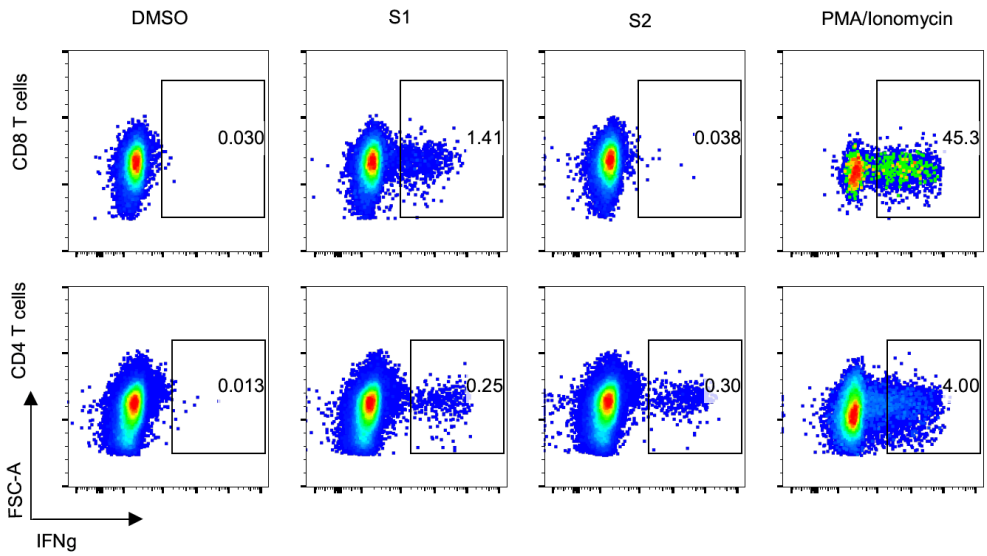

Fig. S3 T cell response to BNT162b2 mRNA vaccination in cancer patients receiving checkpoint inhibition.

Representative flow cytometry plots showing IFNg expression of $CD8^+$ (upper row) and $CD4^+$ (lower row) T cells after stimulation with DMSO (negative control), spike pool 1 (S1), spike pool 2 (S2) and PMA and Ionomycin (positive control), respectively.

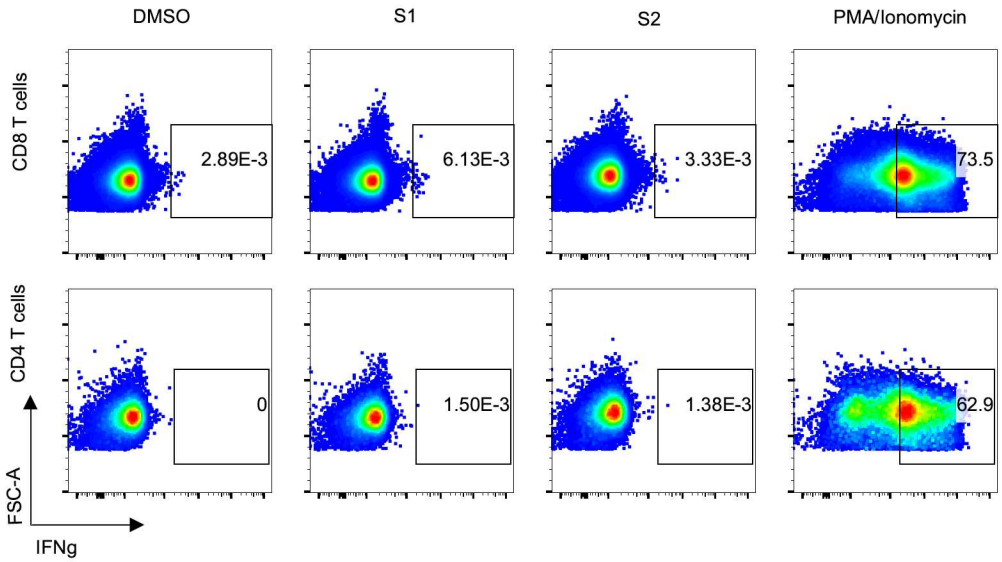

Fig. S4 T cell response to BNT162b2 mRNA vaccination in cancer patients receiving B cell depleting therapy.

Representative flow cytometry plots showing IFNg expression of $CD8^+$ (upper row) and $CD4^+$ (lower row) T cells after stimulation with DMSO (negative control), spike pool 1 (S1), spike pool 2 (S2) and PMA and Ionomycin (positive control), respectively.

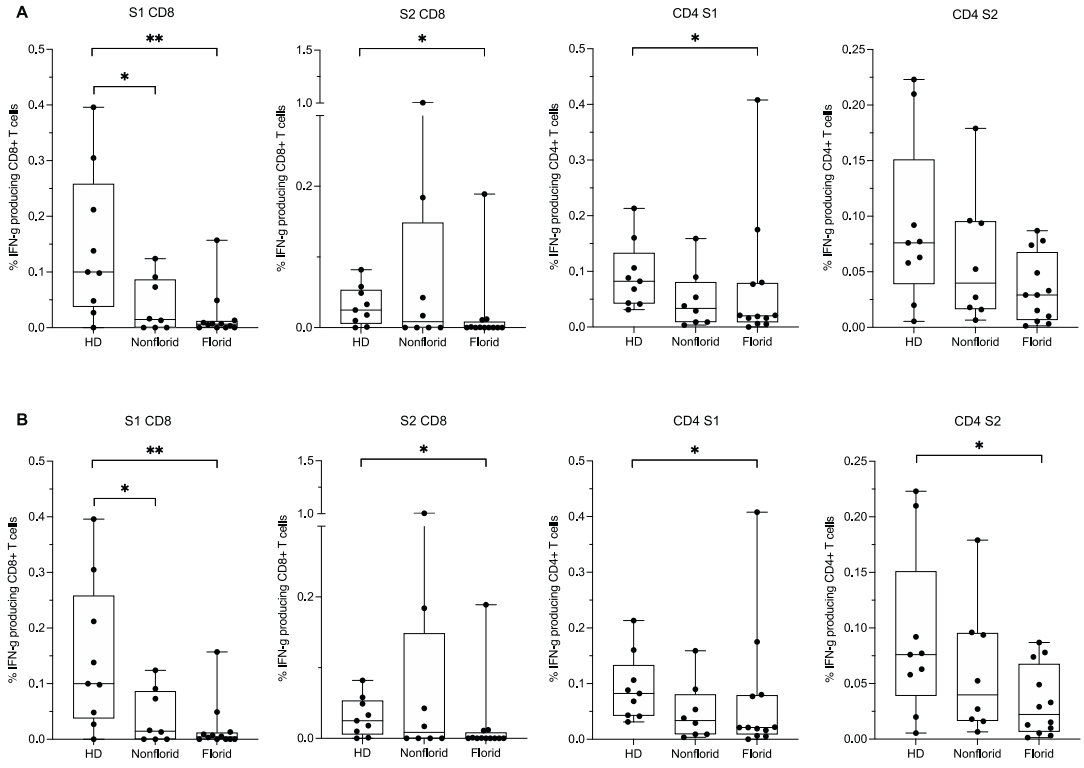

Fig. S5: Correlation of tumor control and T cell response. Percentage of IFNg producing $CD8^+$ T cells $CD4^+$ T cells in healthy donors (HD, n = 9)) and cancer patients receiving chemo (A) or cortisone (B) therapy after stimulation with spike pool 1 (S1) or spike pool 2 (S2) 14 days post 2^{nd} dose BNT162b2. Patients were distinushied by their tumor control, with (florid) and without (nonflorid) detectable tumor mass. Each dot represents one donor and was calculated by background subtraction. Mann-Whitney test was performed to calculate significance with *p<0.05, **p<0.01 and ns not significant.

38 **Table S1 List of administered drugs in each patient group**

	All patients (n = 237)
Antibody	
Atezolizumab	3 (7%)
Bevacizumab	9 (23%)
Brentuximabvedotin	2 (5%)
Cetuximab	2 (5%)
Caratumumab	2 (5%)
Cenosumab	4 (10%)
Obinutuzumab	2 (5%)
Ofatumumab	1 (2%)
Panitumumab	1 (2%)
Pembrolizumab	2 (5%)
Pertuzumab	7 (18%)
Ramucirumab	1 (2%)
Rituximab	8 (21%)
Trastuzumab	7 (18%)
Anti CD20	
Obinutuzumab	2 (22%)
Ofatumumab	1 (11%)
Rituximab	8 (88%)
Immune checkpoint inhibitors	
Atezolizumab	3 (60%)
Pembrolizumab	2 (40%)
Cytostatics	
Bortezomib	3 (4%)
Brentuximabvedotin	1 (1%)
Capecitabin	3 (4%)
Carboplatin	13 (18%)
Cisplatin	3 (4%)
Cyclophosphamid	28 (40%)
Docetaxel	3 (4%)
Doxorubicin	12 (17%)
Epirubicin	16 (22%)
Etoposid	2 (2%)
Fludarabin	1 (1%)
Fluorouracil	10 (14%)

Gemcitabin	5 (7%)
Irinotecan	7 (10%)
Oxaliplatin	10 (14%)
Paclitaxel	17 (24%)
Procarbazin	1 (1%)
Temozolomid	3 (4%)
Vinorelbin	2 (2%)
Tyrosine kinase inhibitors	
Axitinib	1 (5%)
Dasatinib	2 (11%)
Ibrutinib	7 (41%)
Imatinib	2 (11%)
Lapatinib	1 (5%)
Lenvatinib	1 (5%)
Nilotinib	2 (11%)
Pazopanib	2 (11%)
Ponatinib	1 (5%)
Regorafenib	1 (5%)
Ruxolitinib	2 (11%)
Sunitinib	1 (5%)