BMJ Oncology

Predictive biomarkers and specific immune responses of COVID-19 mRNA vaccine in patients with cancer: prospective results from the CACOV-VAC trial

Laurie Spehner,[1,2] Emeline Orillard,[1,2] Antoine Falcoz,[1,3] Quentin Lepiller,[4] Adeline Bouard,[1,5] Hamadi Almotlak,[2] Stefano Kim,[2] Elsa Curtit,[1,2] Guillaume Meynard,[2] Marine Jary,[1,6] Charlee Nardin,[1,7] Kamal Asgarov,[1,5] Syrine Abdeljaoued,[1] Ugo Chartral,[1,2] Virginie Mougey,[1] Myriam Ben Khelil,[1] Morgane Lopez,[1,2] Romain Loyon ,[1] Dewi Vernerey,[1,3] Olivier Adotevi ,[1,2] Christophe Borg,[1,2,5] Laura Mansi,[1,2] Marie Kroemer [1,5,8]

To cite: Spehner L, Orillard E, Falcoz A, *et al*. Predictive biomarkers and specific immune responses of COVID-19 mRNA vaccine in patients with cancer: prospective results from the CACOV-VAC trial. *BMJ Oncology* 2023;**2**:e000054. doi:10.1136/bmjonc-2023-000054

Received 24 February 2023
Accepted 17 October 2023

For numbered affiliations see end of article.

Correspondence to
Dr Marie Kroemer;
mkroemer@chu-besancon.fr

ABSTRACT

Objective Vaccinated patients with cancer in follow-up studies showed a high seropositivity rate but impaired antibody titres and T cell responses following mRNA vaccine against COVID-19. Besides clinical characteristics and the type of anticancer treatment before vaccination, the identification of patients susceptible to non-response following vaccination using immunological markers is worth to be investigated.

Methods and analysis All patients (n=138, solid cancers) were included in the CACOV-VAC Study comprising three cohorts ((neo)-adjuvant, metastatic and surveillance). Immune responses were assessed using, respectively, anti-receptor-binding domain (RBD) SARS-CoV-S-IgG assay and interferon-γ ELISpot assay 3 months following the prime vaccination dose. Immunophenotyping of T cells and immunosuppressive cells from peripheral blood was performed before the prime dose. The serological threshold 3563 AU/mL was used to discriminate non-responders or suboptimal responders versus responders.

Results Most patients achieved seroconversion after receiving the two doses of vaccine (97.6%). The median serological level of anti-RBD SARS-CoV-S-IgG was equal to 3029 for patients at the metastatic stage. The patient's age was the main demographic characteristic that influenced vaccine efficacy. Among the immunological parameters measured at baseline, lower TIGIT (T cell immunoreceptor with Ig and ITIM domains) expression on CD8 T cells was associated with a better vaccine immunogenicity both in terms of humoral and cellular immune responses.

Conclusion Despite a high seroconversion rate, median serological levels of patients with cancer, particularly elderly patients, were below the threshold equal to 3563 AU/mL considered as a humoral correlate of protection against SARS-CoV-2. Our findings suggest that the inhibitory receptor TIGIT might be an interesting predictive biomarker of COVID-19 vaccine immunogenicity and beyond in an anticancer vaccine context.

Trial registration number
ClinicalTrials.gov Registry (NCT04836793).

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Most anticancer treatments weaken our adaptive immunity, foreshadowing a reduced efficacy of vaccine such as COVID-19 mRNA vaccine. Recent studies show that patients with solid tumours might have an altered or suboptimal serology following prophylactic mRNA vaccine against COVID-19. Anticancer treatment, cancer type and stage, the timing for vaccination as well as the patient's biological and immunological baseline characteristics are factors that might affect the vaccine response. Their impact in a real-world study on mRNA vaccine's humoral and cellular responses is worth to be investigated.

WHAT THIS STUDY ADDS

⇒ This study demonstrated that despite a high seroconversion rate following vaccine against COVID-19 among patients with cancer, most of them did not reach a protective serological threshold against SARS-CoV-2 and only half of them had specific T cell responses. Importantly, elderly patients were less protected than the young ones. Among immunological parameters investigated, baseline levels of immunosuppressive cells (myeloid-derived suppressor cells and regulatory T cells) did not influence anti-SARS-CoV-2 vaccine immunogenicity. Importantly, TIGIT (T cell immunoreceptor with Ig and ITIM domains) lower expression by CD8 T cells at baseline was associated with a better vaccine immunogenicity both in terms of humoral and cellular immune responses.

BACKGROUND

Patients with cancer are at higher risk of severe COVID-19.[1–4] Studies demonstrated the ability of patients with cancer to mount adaptive immune

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Most of patients had a suboptimal serology 3 months following the first vaccine dose, suggesting the value of routinely monitoring patients' serology to advocate or not an additional dose of vaccine, particularly for elderly ones. The interest of monitoring TIGIT receptor expression on CD8 T cells before the vaccination might have clinical implications that should be confirm in further clinical trials investigating curative anticancer mRNA vaccine.

responses following SARS-CoV-2 infection with some disparities in immunocompromised subgroups because of their anticancer treatment, corticosteroid or their cancer type and stage.[5–7] Since the beginning of the vaccination campaign, available evidence suggests that patients with cancer are a priority population for COVID-19 vaccine.[8 9] Given the limited and even the exclusion of patients with cancer from pivotal studies of mRNA vaccine, there is still insufficient awareness about vaccine efficacy relying on cancer type, anticancer treatment and the right timing for vaccination of those under treatment.[10–12] Vaccinated patients with cancer in follow-up studies showed a high seropositivity rate but impaired antibody titres and T cell responses following the vaccination against COVID-19.[13–15] Importantly, a major point remains the identification of a reliable immune correlate of protection, taking into consideration the emergence of novel variants of concern (VOCs). Interestingly, VOCs have been shown to escape humoral immunity, mostly due to mutations within the receptor-binding domain (RBD) of SARS-CoV-2, unlike cellular response.[16] Indeed, mutations known to impact T cell recognition are not localised within the RBD of the spike protein.[17]

Beyond clinical characteristics and the type of anticancer treatment before vaccination, identifying patients susceptible to non-response after vaccination using immunological markers is worth to be investigated. Currently, there is still a lack of literature on the relationship between regulatory T cell (Treg) and myeloid-derived suppressor cell (MDSC) rates with COVID-19 successful immunisation.[18 19] One study applying a high dimensional immune profiling on 92 healthy vaccinees quantifying 18 immune cell subsets identifies six vaccine-induced immune cell subsets that correlate with vaccine immunogenicity that do not include MDSCs.[20] Furthermore, the phenotype of T cells among patients with cancer just before SARS-CoV-2 mRNA vaccination has been poorly investigated. Inhibitory receptors like T cell immunoreceptor with Ig and ITIM domains (TIGIT) and programmed cell death 1 (PD-1) are largely expressed by circulating T cells of patients with cancer justifying to be mindful to their expression in a pre-vaccination context.[21–23]

In the present study, we analysed both humoral and cellular immune responses of 138 patients with solid tumours 3 months after the first mRNA COVID-19 vaccination dose and humoral responses 6 months after the first vaccination dose. Patients were separated according to their curative or palliative anticancer treatment. Then, we analysed clinical and biological parameters according to the serological threshold fixed at 3563 AU/mL. The existence of an optimal timing between anticancer treatment and vaccination was also investigated. Finally, we monitored immune parameters just before the vaccination phase to explore the existence of predictive factors for a successful immunisation against SARS-CoV-2.

MATERIAL AND METHODS

Patients and study design

We have started a prospective monocentric trial entitled CACOV-VAC (NCT04836793) in April 2021. All patients were enrolled after the signature of informed consent in accordance with the French regulation. Three cohorts of patients were enrolled: (1) patients in (neo)-adjuvant setting, (2) patients with metastatic solid tumours, (3) patients in surveillance (last treatment above 6 months). The analysed population was vaccinated against COVID-19 between January 2021 and November 2021. Patients with solid tumours were included if they were free of symptomatic COVID-19 infection 3 months before the vaccination date and if they signed the informed consent. The primary objective of the trial was to assess the ability of patients with cancer to mount efficient immune response following COVID-19 vaccination.

Vaccination phase and blood sample collection

All patients included in our trial received two doses of mRNA COVID-19 vaccine in accordance with the vaccination schedule recommended in early 2021. The mean delay between two doses was 28 days (95% CI 26.0 to 28.0). The patient's blood sample was collected just before the first vaccination dose (the same day, corresponding to day 0). The second blood sample was collected at 3 months after the first vaccination dose and the third blood sample was collected at 6 months.

Synthetic peptides

Peptides covering SARS-CoV-S protein were purchased from Miltenyi Biotec. Peptivator peptide pools consisting of 15-mer sequences with 11 amino acids overlap represent both CD4 and CD8 T cells, covering the N-terminal S1 domain sequence of the S protein (1–692 amino acid named SARS-CoV-Prot_S1), consisting of two functional domains: the S1 domain contains the surface binding site to the ACE2 receptor and the S2 subunit mediates membrane fusion.

Assessment of spontaneous T cell responses against SARS-CoV-S by interferon-γ ELISpot assay

Peripheral blood mononuclear cells (PBMCs) from patients with cancer and healthy donors were isolated by density centrifugation on Ficoll gradient (Eurobio). PBMCs were cryopreserved at a cell density of 8–12×10^6 cells/vial in CryoStor (CS10 and CS5) cell preservation media (Sigma-Aldrich) and were conserved at −196°C for flow cytometry and ELISpot assay analysis. Plasma from patients with cancer was isolated by centrifugation and

conserved at −80°C for ELISA analysis. PBMCs and plasma were analysed before, 3 months following the first mRNA COVID-19 vaccination dose and 6 months following the first vaccination dose.

The interferon-γ (IFNγ)-producing SARS-CoV-S-specific T cell responses were quantified by ELISpot assay. For that, 3×10^5 PBMCs/well were cultured in anti-human IFNγ monoclonal antibody in ELISpot plate with the SARS-CoV-Prot_S1 PepTivator (1 µg of peptide/mL) in X-Vivo 15 medium (Lonza) for 48 hours at 37°C. Cells cultured with medium alone or Phorbol-12-myristate-13-acetate/ionomycin (250 ng/mL; 10 µg/mL, Sigma-Aldrich) were used as negative and positive controls, respectively. All experiments were conducted in duplicates and each result presented is the mean of the duplicates. The IFNγ's spots were revealed following the manufacturer's instructions (Diaclone). Estimation of specific T cell number was expressed as spot-forming cells (SFCs)/3×10^5 PBMCs and calculated after subtracting negative control values (background). SFCs were counted using the CTL Immunospot system (Cellular Technology Limited) and assessed with Immunospot V.5.0 analyser software. Responses were considered as positive when IFNγ spot number was ≥10 and ratio twofold above background. Only the positive intensities of specific immune responses were indicated in this study.

Flow cytometry

For surface staining, PBMCs before vaccination were washed and stained for 30 min at 4°C in PBS (phosphate-buffered saline)/0.01% BSA (bovine serum albumin) and 2 mM EDTA with the following Fixable viability DyeeFluor 780 (eBioscience) and antibodies. Immune checkpoints and memory T cells were investigated performing surface staining with CD3-BV605 (clone HIT3a; BD Biosciences), CD4-BV786 (clone RPA-T4; BD Biosciences), PD-1-BV510 (clone EH12.1; BD Biosciences), CTLA-4-BB700 (clone BNI3; BD Biosciences), CD226-PercpCy5.5 (clone 11A8, Biolegend), TIGIT-APC (clone MBSA43, eBioscience), CD39-BV711 (clone A1; Sony), 4-1BB-A700 (clone 4B4-1; Sony), CCR7-FITC (clone 150503; Biotechne), CD95-BB700 (clone DX2; BD Biosciences) and CD45RA-APC (clone HI-100; BD Biosciences). Monocytic MDSCs (M-MDSCs) were characterised by surface staining using negative lineage Pacific blue (CD3, CD56 and CD19) (clone OKT3, HCD56 and SJ25C1; Biolegend), CD14-BV605 (clone M5E2; BD Biosciences), CD33-BV510 (clone WM53; BD Biosciences), CD11b-PeCy7 (clone ICRF44; BD Biosciences) and anti-HLA-DR-FITC (clone B-F1; Diaclone). For Treg analysis, T cells were first stained with surface antibodies CD3-BV605 (clone HTI3a; BD Biosciences), CD4-BV786 (clone RPA-T4; BD Biosciences), CD25-BV421 (clone MA-251; BD Biosciences), CD15s-A488 (clone LSLEX1; BD Biosciences), CD45RA-APC (clone HI-100; BD Biosciences), LAG-3-BV711 (clone 11C3C65; Sony), PD-1-BV510 (clone EH12.1; BD Biosciences) and CTLA-4-BB700 (clone BNI3; BD Biosciences). Intracellular staining was performed following the manufacturer's instructions (BD Biosciences). T cells were fixed and permeabilised with Human Foxp3 buffer set and then stained with Foxp3-PE (clone 259D/C7; BD Biosciences). Samples were directly acquired on a Facs Lyric (BD Biosciences) and analysed with DIVA software.

IgG ELISA assay

IgG antibodies were detected to SARS-CoV-S using the anti-RBD SARS-CoV-S IgG assay on Architect I2000SR (Abbott). Samples with a result ≥50 AU/mL (7.1 binding antibody units (BAU)/mL) were considered positive according to the manufacturer's instructions. Antibodies were considered to be at high levels above 3563 AU/mL (506 BAU/mL) for this anti-RBD assay in accordance with published data and with manufacturer's instructions.[24] Thus, patients with a serological threshold strictly inferior to 3563 AU/mL were considered non-responders or suboptimal responders, and patients with a serological threshold higher or equal to 3563 AU/mL were considered responders. Cytomegalovirus (CMV) status was determined using the Abbott Architect CMV IgG kit (Abbott) with a positive threshold value of 6.0 AU/mL.

Statistical analysis

Continuous parameters were summarised with median and IQR. Categorical variables were described using absolute and relative frequencies. Wilcoxon-Mann-Whitney test and X^2 (or Fisher's exact test, if appropriate) tests were used to compare median value of continuous parameters and frequencies of categorical parameters, respectively, between subgroups. Statistical analyses were performed using SAS V.9.4 (SAS Institute) and GraphPad Prism V.6 software (San Diego, California, USA). P values of <0.05 were considered statistically significant, and all tests were two sided. All p values are given on an exploratory purpose.

RESULTS

Demographic and clinical characteristics of patients with cancer

We prospectively enrolled 138 patients with cancer, from whom 33 were in the (neo)-adjuvant setting, 100 at metastatic stage and 5 in surveillance (table 1). Patients' median age at first vaccination was equal to 65.0 years (IQR: 54.8–75.2) and 37.0% (n=51) were older than 70 years. Beyond cancer, 28.3% (n=39) of patients had hypertension and 8.0% (n=11) had type 2 diabetes. Most common primary tumours were, respectively, located at the gastrointestinal tract 33.3% (n=46), the breast 31.2% (n=43), gynaecological organs 12.3% (n=17) and at the thoracic shaft 8.7% (n=12). Half of the patients had chemotherapy-based regimens during the vaccination phase. Regarding vaccination safety, 11.2% (n=12) of patients declared mild adverse events following the vaccination phase. Precision about anticancer treatment type ongoing during the vaccination phase is detailed in table 1. Briefly, 72 patients had chemotherapy-based

Table 1 Demographics, clinical and biological characteristics of patients with cancer

	All patients n=138		Patients with active treatment				Patients without active treatment n=5	
			(Neo)-adjuvant setting n=33		Metastatic setting n=100			
	n	%	n	%	n	%	n	%
Demographic data								
Age at first vaccination (years)								
Median	65.0 (54.8–75.2)		61.4 (52.4–74.0)		65.5 (55.8–75.9)		70.0 (52.1–75.5)	
≤70	87	63.0	23	69.7	62	62.0	2	40.0
>70	51	37.0	10	30.3	38	38.0	3	60.0
Sex								
Male	46	33.3	10	30.3	33	33.0	3	60.0
Female	92	66.7	23	69.7	67	67.0	2	40.0
BMI (kg/m^2)								
Median	24.2 (21.3–27.4)		25.8 (22.5–27.2)		23.9 (21.2–28.0)		20.4 (19.6–29.4)	
Missing	8	–	2	–	4	–	2	–
Comorbidity								
Hypertension	39	28.3	8	24.2	31	31.0	0	0.0
Diabetes	11	8.0	2	6.1	9	9.0	0	0.0
Autoimmune disease	4	2.9	1	3.0	2	2.0	1	20.0
Cancer-related information								
Cancer type								
Gastrointestinal	46	33.3	8	24.2	36	36.0	2	40.0
Breast	43	31.2	17	51.5	25	25.0	1	20.0
Gynaecological	17	12.3	4	12.1	13	13.0	0	0.0
Thoracic	12	8.7	2	6.1	9	9.0	1	20.0
Genitourinary	9	6.5	1	3.0	8	8.0	0	0.0
Melanoma or skin	7	5.1	1	3.0	5	5.0	1	20.0
Other	4	2.8	0	0.0	4	4.0	0	0.0
Treatment ongoing at vaccination								
Chemotherapy based	72	52.2	21	63.6	51	51.0	0	0.0
Immunotherapy alone or combined with targeted therapy	23	16.7	5	15.2	18	18.0	0	0.0
Targeted therapy and/or hormonotherapy	38	27.5	7	21.2	31	31.0	0	0.0
No treatment	5	3.6	0	0.0	0	0.0	5	100
Biological data before vaccine								
Complete blood count								
Leucocytes (10^9/L)	5.7 (4.3–7.1)		6.8 (4.7–8.7)		5.6 (4.2–6.5)		5.2 (4.5–6.0)	
1 Neutrophils (10^9/L)	3.6 (2.5–5.3)		4.6 (3.1–5.6)		3.5 (2.3–4.9)		3.7 (2.8–4.0)	

Continued

Table 1 Continued

	All patients n=138		Patients with active treatment				Patients without active treatment n=5	
			(Neo)-adjuvant setting n=33		Metastatic setting n=100			
	n	%	n	%	n	%	n	%
Lymphocytes (10^9/L)	1.0 (0.8–1.5)		1.0 (0.7–1.6)		1.0 (0.8–1.4)		1.2 (0.9–1.6)	
<1	58	44.6	16	48.5	40	43.0	2	50.0
≥1	72	55.4	17	51.5	53	57.0	2	50.0
Missing	8	–	0	–	7	–	1	–
Serology SARS-CoV-2								
Median	3.9 (1.7–7.8)		3.8 (1.7–15.4)		3.9 (1.7–6.5)		8.0 (5.6–338.3)	
Missing	3	–	2	–	1	–	0	–
ELISpot IFNγ protein S1 spots								
Median	12 (11.5–23.0)		0 .0 (0.0–0.0)		12 (11.5–23.0)		0 .0 (0.0–0.0)	
Missing	45	–	13	–	29	–	3	–
Biological data 3 months after vaccine								
Complete blood count								
Leucocytes (10^9/L)	5.5 (3.8–7.1)		6.0 (4.6–8.1)		5.5 (3.8–6.7)		5.2 (3.8–7.3)	
Neutrophils (10^9/L)	3.5 (2.4–4.8)		3.8 (2.7–5.4)		3.2 (2.4–4.7)		3.3 (1.9–5.4)	
Lymphocytes (10^9/L)	1.0 (0.8–1.40)		1.0 (0.7–1.6)		1.0 (0.8–1.4)		1.2 (1.1–1.2)	
<1	51	42.5	13	44.8	38	43.2	0	0.0
≥1	69	57.5	16	55.2	50	56.8	3	100
Missing	18	–	4	–	12	–	2	–
Serology SARS-CoV-2								
Median	3 060 (1125–9118)		3 081 (653–11 561)		3 029 (1125–7878)		13 994 (3112–28 132)	
Frequency of responders (≥50 AU/mL)	123	97.6	27	93.1	92	98.9	4	100
Missing	12	–	4	–	7	–	1	–
ELISot IFNγ protein S1 spots								
Median	59.0 (34.0–123.5)		38.5 (13.5–86.5)		67.0 (36.0–144.0)		180.0 (180.0–180.0)	
Frequency of responders	48	49.5	12	57.1	35	47.3	1	50.0
Missing	41	–	12	–	26	–	3	–
Biological data 6 months after vaccine								
Complete blood count								
Leucocytes (10^9/L)	5.4 (3.9–7.1)*		5.5 (3.7–6.4)*		5.2 (4.1–7.2)		8.1 (8.1–8.1)	
Neutrophils (10^9/L)	3.5 (2.5–5.1)		3.2 (2.4–4.6)		3.6 (2.7–5.5)		6.2 (6.2–6.2)	

Continued

Table 1 Continued

	All patients n=138		Patients with active treatment				Patients without active treatment n=5	
			(Neo)-adjuvant setting n=33		Metastatic setting n=100			
	n	%	n	%	n	%	n	%
Lymphocytes (10^9/L)	0.9 (0.7–1.4)		0.9 (0.6–1.1)		0.90 (0.70–1.40)		1.2 (1.2–1.2)	
<1	31	58.5	5	62.5	26	59.1	0	0.0
≥1	22	41.5	3	37.5	18	40.9	1	100
Missing	84*/85	–	24*/25	–	56	–	4	–
Serology SARS-CoV-2								
Median	866.3 (329.5–1850)		1041 (63.2–1667)		774.6 (335.6–1772)		3969 (1143–7565)	
Frequency of responders (≥50 AU/mL)	104	95.9	17	80.9	83	97.6	4	100
Missing	28	–	12	–	15	–	1	–

BMI, body mass index; IFNγ, interferon-γ.

treatment, of which 29.2% were in the (neo)-adjuvant setting and 70.8% in palliative setting (metastatic stage). Other patients had immunotherapy alone or in combination with targeted therapy (16.7%), targeted therapy and/or hormonotherapy (27.5%).

Complete blood counts were performed for most of patients (n=130) before the vaccination phase (table 1 and figure 1A). Regardless of the disease stage at vaccination, more than half of the patients had a lymphocyte count superior to 1 g/L. Neutrophil count was significantly lower in patients at metastatic stage compared with patients in the (neo)-adjuvant setting (p=0.020). Moreover, 124 patients out of 138 (92.8%) had a negative SARS-CoV-2 serology, and 84 patients out of 93 (90.3%) had no T cell responses against the spike protein before the vaccination phase. We noticed that six patients had a positive SARS-CoV-2 serology at baseline suggesting either a past infection (beyond 3 months) or an asymptomatic infection in the last 3 months. All of them mounted a strong serological response following the vaccination scheduled at 3 months (superior to 20 000 BAU/mL). Three months following the first vaccination dose, the median serological level was equal to 3081.0 AU/mL (IQR: 653.0–11 561.0) for patients in the (neo)-adjuvant setting, 3029.0 AU/mL (IQR: 1125.0–7878.0) for patients at the metastatic stage and 13 994.0 AU/mL (IQR: 3112.0–28 132.0) for patients under surveillance (figure 1B). Median serological levels at 6 months were equal to 1041.0 AU/mL (IQR: 63.0–1581.0) for patients in the (neo)-adjuvant setting, 775.0 AU/mL (IQR: 337.0–1737.0) for patients at the metastatic stage and 3969.0 AU/mL (IQR: 1447.0–7004.0) for patients under surveillance. Additionally, we found that 30.3% of patients receiving chemotherapy at the time of vaccination had a serological threshold strictly inferior to 1000 AU/mL, while only 8.3% of patients free of chemotherapy had a threshold below 1000 AU/mL (p=0.0078). The rate of positive T cell responses 3 months following the first vaccination was equal to 49.5% (n=48) (figure 1C). Following a short stimulation, both CD4 and CD8 T cell elicited functional responses based on the secretion of IFNγ, interleukin 2 and tumour necrosis factor-α (data not shown). The detail of T cell responses according to patients' disease stage is presented in figure 1D. Collectively, these data demonstrated that COVID-19 mRNA vaccine promotes humoral and cellular immune responses 3 months following the first vaccination dose. Dramatically, most of the patients were below the protective threshold in the third months, suggesting the interest of routinely monitoring patients' serology to advocate or not an additional dose of vaccine.

Association of patients' baseline characteristics and anticancer therapy with vaccine immunogenicity

The next set of analyses was dedicated to investigate demographic and clinical features of patients with cancer according to patients' serological status. Feng *et al*[24] determined, using prospective data from a vaccination trial with 171 cases of SARS-CoV-2 infections and 1404 non-cases, that the antibody level which was associated with 80% vaccine efficacy against symptomatic COVID-19 was equal to 506 BAU/mL for anti-RBD IgG, corresponding to 3563 AU/mL and 264 BAU/mL for anti-spike IgG. Thus, patients with a serological threshold strictly inferior to 3563 AU/mL were considered non-responders or suboptimal responders, and patients with a serological threshold higher or equal to 3563 AU/mL were considered responders.

There was no difference between suboptimal responders and responders in terms of sex (p=0.890) and body mass index (p=0.806; table 2). However, patients' age at

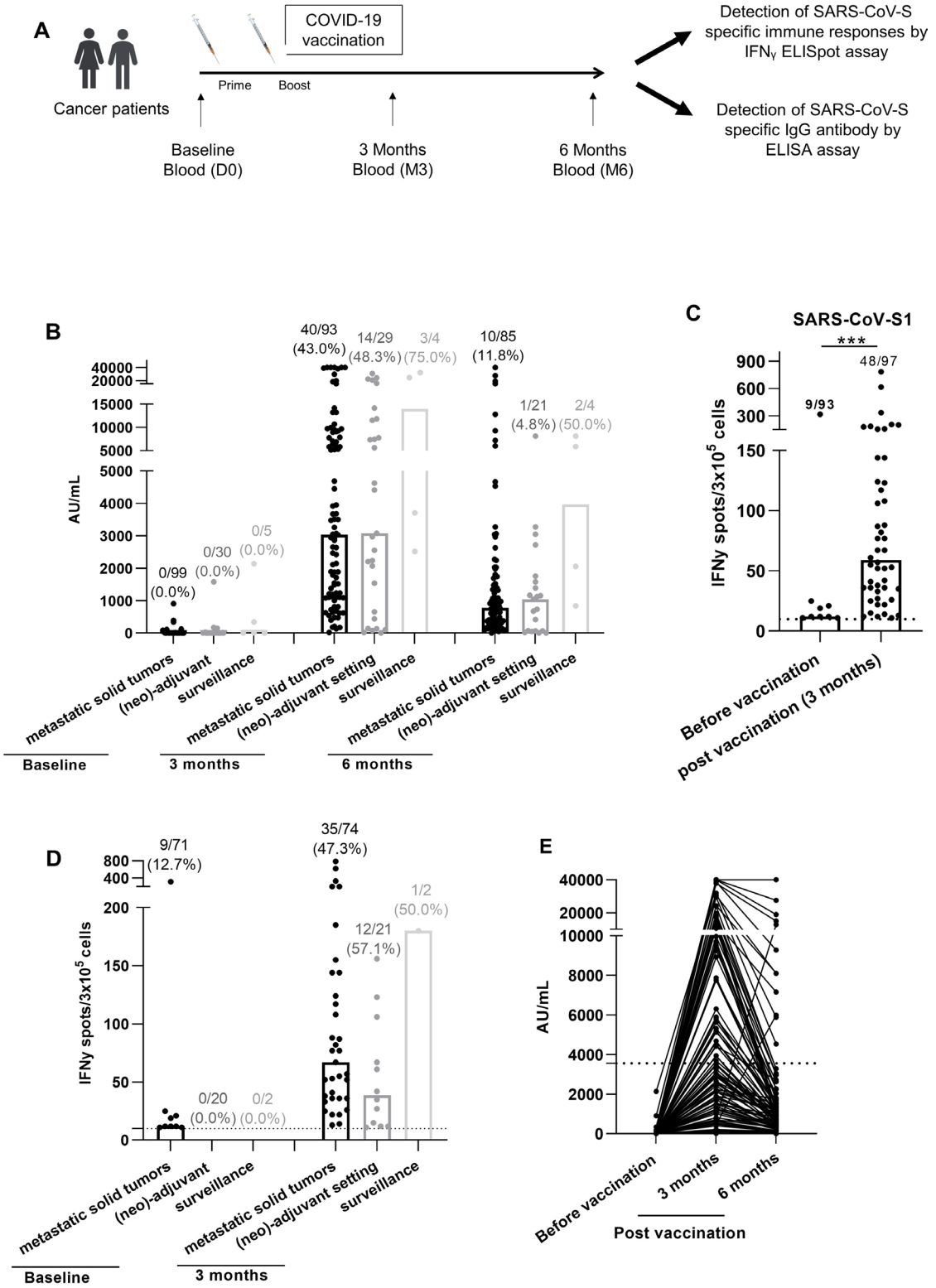

Figure 1 SARS-CoV-2-specific B and T cell responses were increased after COVID-19 vaccination in patients with cancer. (A) SARS-CoV-2-specific humoral and cellular immune responses analysed by ELISA and IFN$_\gamma$ ELISpot assay. (B) Serology (BAU/mL) of patients with cancer according to their disease stage before and after COVID-19 vaccination. (C) SARS-CoV-S-specific cellular responses analysed by ex vivo IFN$_\gamma$ ELISpot assay before (n=93) and 3 months (n=97) after COVID-19 vaccination. Missing data were associated with uninterpretable results because of excessive background. Intensity of positive SARS-CoV-Prot_S1-specific immune responses in patients with cancer. Mann-Whitney test, ***p<0.001. Medians were indicated on graphs. Responses were considered positive when IFN$_\gamma$ spot number was ≥10 and ratio twofold above background. Only the positive intensities of specific immune responses are indicated. (D) Cellular responses in patients with cancer according to their disease stage before and after COVID-19 vaccination. (E) Serology (BAU/mL) of patients with cancer analysed by ELISA assay before (n=134), 3 months (n=126) and 6 months (n=110) after COVID-19 vaccination. BAU, binding antibody units; IFN$_\gamma$, interferon-γ.

Table 2 Characteristics of patients with cancer according to the serological threshold fixed at 3563 AU/mL

	Serological threshold <3563 AU/mL at M3		Serological threshold ≥3563 AU/mL at M3		
	n=69		n=57		
	n	%	n	%	P value
Demographic data					
Age at vaccination (years)					
Median	69.1 (59.8–76.4)		58.6 (51.5–68.4)		0.0010
≤70	36	52.2	45	79.0	0.0018
>70	33	47.8	12	21.0	
Sex					
Male	21	30.4	18	31.6	0.8900
Female	48	69.6	39	68.4	
BMI (kg/m^2)					
Mean (SD)	25.3 (4.7)		25.4 (5.1)		0.8061
Missing	4	–	4	–	
Cancer-related information					
Disease stage at vaccination					
(Neo)-adjuvant	15	21.8	14	24.5	0.6321
Metastatic	53	76.8	40	70.2	
Surveillance	1	1.5	3	5.3	
Treatment ongoing at vaccination					
Chemotherapy based	39	56.5	27	47.4	0.4744
Immunotherapy alone or combined with targeted therapy	11	15.9	8	14.0	
Targeted therapy and/or hormonotherapy	18	26.1	19	33.3	
No treatment	1	1.5	3	5.3	
Biological data before vaccine					
Complete blood count					
Leucocytes (g/L)	5.8 (4.3–7.4)		5.8 (4.4–7.4)		1.0000
Neutrophils (g/L)	3.5 (2.6–5.5)		3.7 (2.4–5.0)		0.4786
Lymphocytes (g/L)	0.9 (0.6–1.5)		1.1 (0.9–1.7)		0.0927
<1	32	50.8	21	37.5	
≥1	31	49.2	35	62.5	
Missing	6	–	1	–	
Protein S1 spots					
Median	12.0 (11.0–12.0)		20.0 (12.0–25.0)		0.2237
Frequency of responders	3	5.9	6	14.3	
Missing	18	–	15	–	
Biological data 3 months after vaccine					
Complete blood count					
Leucocytes (10^9/L)	5.2 (3.8–6.6)		6.2 (4.1–7.6)		0.0470
Neutrophils (10^9/L)	3.2 (2.3–4.3)		3.8 (2.6–5.3)		0.1012
Lymphocytes (10^9/L)	1.0 (0.7–1.4)		1.1 (0.9–1.6)		0.1741
<1	32	49.2	18	33.3	0.0803
≥1	33	50.8	36	66.7	
Missing	4	–	3	–	
Protein S1 spots					
Median	72.0 (41.0–123.0)		52.5 (33.0–124.0)		0.6510
Frequency of responders	22	40.7	26	60.5	

Continued

Table 2 Continued

	Serological threshold <3563 AU/mL at M3		Serological threshold ≥3563 AU/mL at M3		
	n=69		n=57		
	n	%	n	%	P value
Missing	15	–	14	–	

BMI, body mass index; M3, month 3.

vaccination was significantly higher in non-responders or low responders comparatively with responders both in the whole cohort (p=0.001) and in the chemotherapy-treated patients' cohort (p=0.019) (online supplemental table 1). No differences in terms of disease stage (p=0.632) and ongoing anticancer treatment during the vaccination phase (p=0.474) were observed. Before the vaccination phase, the level of lymphocytes tended to be higher among protected patients (p=0.093). This result was similar when focusing on patients treated with chemotherapy-based protocols (online supplemental table 1).

Importantly, 54.8% and 88.2% of patients had a serological level below the threshold of 3563 AU/mL, respectively, 3 months and 6 months following the first vaccination dose (figure 1E). Of note, two patients with a negative serology at 3 months had a third dose of vaccine that promotes a serology superior to 3563 AU/mL.

Then T cell responses were analysed 3 months following the first dose of vaccine. Interestingly, 60.5% of responders had positive SARS-CoV-2-specific T cell responses vs 40.7% of non-responders or suboptimal responders (p=0.054). In the chemotherapy-treated patients' cohort, there was no difference in their ability to mount specific T cell responses between responders (52.4%) and non-responders or suboptimal responders (46.4%) (p=0.680) (online supplemental table 1).

Altogether, these results suggest that the patient's age was the main demographic characteristic that influenced vaccine efficacy. Likewise, protected patients tended to have more T cell responses than unprotected ones.

Timing of vaccination in relation to anticancer treatment did not affect SARS-CoV-2-specific humoral responses

A consensus on the best timing to administrate vaccines to treated patients with cancer has still not been reached. Thus, we next investigated if the timing of vaccination before and after anticancer treatment, with a focus on chemotherapy, might influence SARS-CoV-2-specific humoral immune responses. To this end, we analysed the serological index of each patient at 3 months in regard to the time between the last anticancer treatment and the first vaccination dose. Median time was equal to 7.0 days (IQR: 3.0–13.5) among non-responders or suboptimal responders and 6.0 days (IQR: 0.0–11.0) (p=0.180) among responders (table 3). When focusing on patients treated by chemotherapy, the median time was equal to 8.0 days (IQR: 3.0–14.0) among non-responders or suboptimal responders and 7.0 days (IQR: 2.0–14.0) (p=0.594) among responders (table 3). Interestingly, neither a timing threshold of 7 days (p=0.513) nor 10 days (p=0.542) predicted a successful immunisation for the whole cohort of patients with cancer. Similar results were obtained when focusing on chemotherapy-treated patients.

Whatever the anticancer treatment, no optimal timing for a successful vaccine immunisation was demonstrated, both in the whole cohort and in the chemotherapy-treated patients' cohort.

Monitoring of biological and immunological parameters at baseline

We next explored biological and immunological parameters associated with SARS-CoV-2 vaccine immunogenicity among patients with cancer. We first analysed these parameters according to the serological threshold. Absolute lymphocyte count (p=0.090) and neutrophil-to-lymphocyte ratio (NLR) (p=0.054) tend to be higher among responders (table 4, left part). Likewise, the CD4/CD8 ratio tended to be higher among responders (p=0.060). Among immunological parameters, CTLA-4 expression on CD4 T cells tended to be higher among non-responders or suboptimal responders (p=0.097), whereas TIGIT was less expressed by CD8 T cells among responders (36.3, IQR: 19.5–50.5 vs 44.8, IQR: 35.9–55.1, p=0.036). Memory phenotypes were similar regardless of the serological threshold with the exception of central memory (higher among non-responders or suboptimal responders) (p=0.007) and naïve phenotype (almost lower among non-responders or suboptimal responders) (p=0.052).

Then, biological and immunological parameters in relation to the SARS-CoV-S-restricted T cell responses were investigated (table 4, right part). Interestingly, neither the absolute lymphocyte count nor the NLR influenced the specific T cell responses. PD-1 expression on both CD4 and CD8 T cells tended to be higher among patients without specific T cell responses (p=0.096 and p=0.091), while CTLA-4 expression on CD8 T cells was higher on patients with specific T cell responses (p=0.044). Both responders and patients with specific T cell responses had a lower level of CD8 T cell expressing TIGIT than patients without T cell responses (37.4, IQR: 22.0–44.8 vs 46.2, IQR: 36.1–58.5, p=0.330). The expression of TIGIT by CD8 T cells at 3 months was similar to the one before vaccination (data not shown). At last, memory phenotypes

Table 3 Delay between the last anticancer treatment and the first vaccination dose

	Serological threshold <3563 AU/mL at M3		Serological threshold ≥3563 AU/mL at M3		
	n	%	n	%	P value
Delay between the last anticancer treatment and the first vaccination dose (all patients)—days	n=69		n=57		
Mean (SD)	10.9 (18.5)		8.2 (12.9)		0.1802
Median (IQR)	7.0 (3.0–13.5)		6.0 (0.0–11.0)		
≤7	35	51.5	31	57.4	0.5134
>7	33	48.5	23	42.6	
≤10	43	63.2	37	68.5	0.5418
>10	25	36.8	17	31.5	
Delay between the last anticancer treatment and the first vaccination dose (chemotherapy-treated patients)—days	n=39		n=27		
Mean (SD)	12.6 (23.0)		11.3 (16.2)		0.5940
Median (IQR)	8.0 (3.0–14.0)		7.0 (2.0–14.0)		
≤7	19	48.7	15	55.6	0.5847
>7	20	51.3	12	44.4	
≤10	25	64.1	19	70.4	0.5954
>10	14	35.9	8	29.6	

M3, month 3.

were similar regardless of the serology index except for the naïve phenotype that tends to be higher in patients with specific T cell responses (p=0.078).

We finally analysed biological and immune parameters according to patients' cancer treatment, grouped as follows: chemotherapy-based regimen versus other (figure 2 and online supplemental table 2). Neither the absolute lymphocyte count nor the NLR and CD4/CD8 ratio were dependent of anticancer treatments (figure 2A). CTLA-4 and PD-1 expression on CD4 T cells were similar whatever the anticancer treatment (figure 2B). Regarding CD8 T cells, CTLA-4 and PD-1 expression were similar whatever the anticancer treatment, whereas TIGIT expression tends to be lower in the group of patients treated by chemotherapy (figure 2C).

These data suggest that among immunological parameters measured at baseline, TIGIT lower expression by CD8 T cells was associated with better vaccine immunogenicity both in terms of humoral and cellular immune responses.

Presence of immunosuppressive cells in patients with cancer before COVID-19 vaccination did not impair specific immune responses

Finally, we wondered whether the baseline presence of immunosuppressive cells was altering vaccine immunogenicity. We first analysed the level of Tregs and M-MDSC as well as their immune feature according to the serological threshold. The level of naïve Tregs (nTregs) was higher among protected patients (1.1%, IQR: 0.9–1.8 vs 0.8%, IQR: 0.5–1.3; p=0.012), while the level of effector Tregs (eTregs) tended to be higher among non-responders or suboptimal responders (table 4). Considering immune feature of Tregs, there were no differences between responders and non-responders or suboptimal responders except for PD-1 expression by nTregs that was higher among unprotected patients (p=0.048). The level of M-MDSC at baseline was similar between both groups (1.5%, IQR: 0.6–2.9 vs 1.4%, IQR: 0.6–3.8).

Given these observations, we wondered about the level of Tregs and M-MDSC in relation to the SARS-CoV-S-restricted T cell response (table 4). In line with the results obtained regarding the humoral response, there were no differences depending on the T cell response except for PD-1 expression by nTregs that tended to be higher among patients without specific T cell response (p=0.063). The level of M-MDSC at baseline was similar between both groups (1.4%, IQR: 0.7–2.5 vs 1.5%, IQR: 0.6–3.8).

We finally investigated the level of immunosuppressive cells according to patients' cancer treatment as described above. Neither the level of Tregs nor the level of M-MDSC was associated with anticancer treatments (figure 2D). Only the expression of LAG-3 by nTregs was higher among patients treated by chemotherapy, other immune parameters were similar between both groups.

Table 4 Biological and immunological parameters of SARS-CoV-2 vaccinated patients with cancer according to their humoral and cellular responses

	Serological threshold ≥3563 AU/mL at M3	Serological threshold <3563 AU/mL at M3		SARS-CoV-S-restricted T cell responses at M3	Without SARS-CoV-S T cell responses at M3	
	n=57	n=69	P value	n=49	n=48	P value
Biological data before vaccine						
CMV serology level (AU/mL)	1.3 (0.4–239.6)	58.1 (0.5–196.2)	0.779	102.6 (0.5–241.3)	59.6 (0.45–243.4)	0.598
Missing	1	2		0	1	
Absolute lymphocyte count (10^9/L)	1.1 (0.9–1.7)	0.9 (0.6–1.5)	0.090	1.1 (0.7–1.4)	1.0 (0.7–1.6)	0.966
Monocytes (10^9/L)	0.4 (0.3–0.6)	0.4 (0.3–0.5)	0.988	0.4 (0.2–0.6)	0.5 (0.3–0.6)	0.226
Missing	1	6		3	5	
NLR	3.0 (1.8–4.4)	3.7 (2.5–5.9)	0.054	3.4 (2.3–4.5)	3.1 (1.7–6.1)	0.933
Missing	2	6		3	5	
Immunological data before vaccine						
CD4 (%)	37.5 (29.3–51.1)	34.9 (24.0–42.1)	0.143	32.9 (29.3–44.0)	36.3 (22.1–44.2)	0.559
CD8 (%)	18.6 (11.8–30.2)	25.9 (16.1–31.2)	0.175	23.1 (14.4–32.0)	23.1 (13.3–30.7)	0.765
CD4/CD8 (%)	1.7 (1.2–3.8)	1.5 (0.9–1.9)	0.060	1.6 (0.9–2.7)	1.6 (0.7–2.1)	0.721
Missing	22	24		11	13	
CTLA-4 on CD4 T cells (%)	2.2 (1.5–3.5)	3.0 (1.9–5.0)	0.097	3.3 (1.7–5.0)	2.2 (1.6–3.3)	0.154
PD-1 on CD4 T cells (%)	11.1 (6.3–16.8)	13.0 (5.3–18.1)	0.493	10.0 (4.2–16.3)	14.1 (8.8–18.7)	0.096
LAG-3 on CD4 T cells (%)	1.0 (0.4–2.2)	1.2 (0.5–2.8)	0.689	2.0 (0.3–2.8)	1.4 (0.4–2.4)	0.601
Missing	24	27		13	16	
TIGIT on CD4 T cells (%)	19.5 (16.2–24.7)	21.6 (18.5–26.2)	0.147	19.8 (17.9–25.7)	21.5 (18.8–27.0)	0.528
CD226 on CD4 T cells (%)	55.4 [43.5–65.4)	64.6 (48.7–69.3)	0.165	50.3 (37.7–65.4)	64.4 (50.8–75.9)	0.051
Missing	30	36		22	22	
CD39 on CD4 T cells (%)	6.6 (2.3–9.2)	6.5 (4.8–11.4)	0.207	5.6 (2.8–8.6)	8.6 (5.0–11.7)	0.113
Missing	30	36		13	16	
CTLA-4 on CD8 T cells (%)	0.4 (0.2–0.8)	0.7 (0.4–1.0)	0.086	0.7 (0.3–1.1)	0.5 (0.2–0.7)	0.122
PD-1 on CD8 T cells (%)	11.4 (6.1–16.8)	11.9 (6.4–11.9)	0.516	10.1 (4.8–17.5)	13.3 (7.4–20.4)	0.124
Missing	24	26		13	16	
TIGIT on CD8 T cells (%)	36.3 (19.5–50.5)	44.8 (35.9–55.1)	**0.036**	37.4 (22.0–44.8)	46.2 (36.1–58.5)	**0.033**
CD226 on CD8 T cells (%)	66.1 (43.6–76.6)	66.0 (55.9–78.5)	0.624	66.1 (45.1–78.6)	68.6 (52.5–77.9)	0.940
4-1BB on CD8 T cells (%)	0.3 (0.2–0.4)	0.2 (0.1–0.5)	0.424	0.2 (0.1–0.4)	0.2 (0.1–0.4)	0.961
CD39 on CD8 T cells (%)	2.7 (1.5–4.7)	2.6 (1.6–5.2)	0.950	2.6 (1.3–4.6)	2.7 (1.7–5.1)	0.774
Missing	30	36		22	22	
Naïve T cell	45.1 (34.4–59.7)	35.1 (22.2–49.8)	0.052	39.3 (31.3–59.3)	31.0 (21.3–47.3)	0.078

Continued

Table 4 Continued

	Serological threshold ≥3563 AU/mL at M3	Serological threshold <3563 AU/mL at M3		SARS-CoV-S-restricted T cell responses at M3	Without SARS-CoV-S T cell responses at M3	
	n=57	n=69	P value	n=49	n=48	P value
T stem cell memory (%)	7.6 (5.2–9.6)	7.0 (4.0–10.5)	0.834	7.9 (5.2–10.1)	7.3 (3.1–9.6)	0.411
T central memory (%)	19.1 (14.0–25.3)	26.4 (20.6–31.0)	**0.007**	23.9 (13.7–27.8)	24.8 (19.0–34.2)	0.242
T effector memory (%)	18.6 (10.9–24.8)	18.4 (12.6–29.3)	0.391	18.2 (10.9–24.9)	20.0 (12.6–29.9)	0.437
T terminal effector cell (%)	3.6 (2.8–6.2)	5.1 (3.0–9.4)	0.233	4.2 (3.0–6.4)	5.2 (3.2–11.3)	0.318
Missing	22	24		11	13	
nTregs	1.1 (0.9–1.8)	0.8 (0.5–1.3)	**0.012**	0.9 (0.7–1.6)	1.0 (0.4–1.2)	0.325
CTLA-4 on nTregs (%)	3.2 (0.9–7.0)	5.7 (2.5–10.1)	0.053	5.8 (1.2–8.6)	4.5 (2.0–8.7)	0.781
PD-1 on nTregs (%)	2.4 (0.5–4.9)	3.1 (0.7–8.4)	0.219	2.6 (0.3–4.9)	5.2 (1.0–10.2)	**0.033**
LAG-3 on nTregs (%)	1.0 (0.0–2.8)	0.6 (0.0–2.6)	0.668	0.6 (0.0–3.3)	0.9 (0.0–2.4)	0.928
Missing	24	27		13	16	
eTregs	1.2 (0.6–1.6)	1.4 (0.9–2.2)	0.062	1.3 (0.7–1.6)	1.6 (0.8–2.1)	0.166
CTLA-4 on eTregs (%)	35.2 (29.1–47.9)	35.8 (25.0–46.8)	0.764	44.0 (27.2–52.6)	32.4 (23.8–44.3)	0.208
PD-1 on eTregs (%)	19.4 (10.6–31.0)	20.8 (13.2–32.9)	0.744	16.8 (10.4–32.5)	22.9 (15.3–33.4)	0.380
LAG-3 on eTregs (%)	0.8 (0.2–2.0)	1.5 (0.4–2.8)	0.333	1.0 (0.4–3.0)	1.5 (0.3–2.4)	0.895
Missing	24	27		13	16	
M-MDSC (%)	1.5 (0.6–2.9)	1.4 (0.6–3.8)	0.893	1.4 (0.7–2.5)	1.5 (0.6–3.8)	0.672
Missing	22	27		13	14	

Bold values represents statistically significant values.
CMV, cytomegalovirus; eTreg, effector Treg; M3, month 3; M-MDSC, monocytic myeloid-derived suppressor cell; NLR, neutrophil-to-lymphocyte ratio; nTreg, naïve Treg; PD-1, programmed cell death 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; Treg, regulatory T cell.

To conclude, these results demonstrate that immunosuppressive cells did not affect anti-SARS-CoV-2 vaccine immunogenicity among patients with cancer at baseline apart from the level of nTregs, which positively influenced the humoral response.

DISCUSSION/CONCLUSION

Most anticancer treatments weaken our immunity, foreshadowing a reduced efficacy of vaccine whom COVID-19 mRNA vaccine. Recent studies show that patients with solid tumours might fail or suboptimally respond to such vaccines.[25–27] Few real-world studies have been conducted to determine the impact of biological and immunological baseline characteristics of patients with cancer for vaccine response. The goal of this prospective study was to evaluate mRNA vaccine immune responses among patients with solid tumours 3 months following the first dose of vaccine. We observed that most of patients achieved seroconversion after receiving two doses of vaccine (97.6%). However, among them, less than a half were responders using the cut-off level of 3563 AU/mL (46.3%) suggesting the interest of routinely monitoring patients' serology to advocate or not an additional dose of vaccine. The patient's age was the main demographic characteristic that influenced the vaccine efficacy. As expected, responders tended to have more T cell responses than non-responders or suboptimal responders. From an immunological point of view, a low expression of the TIGIT immune checkpoint on CD8 T cells was associated with a better vaccine immunogenicity. At last, baseline levels of immunosuppressive cells (MDSCs and Tregs) did not influence anti-SARS-CoV-2 vaccine immunogenicity among patients with cancer at the exception of nTregs.

At the time of the first vaccination campaign, in early 2021, a correlate of protection was not yet known, since the prediction of vaccine effectiveness against SARS-CoV-2 infection has been demonstrated in both prospective and retrospective studies.[26 28–31] Evidence that the level of

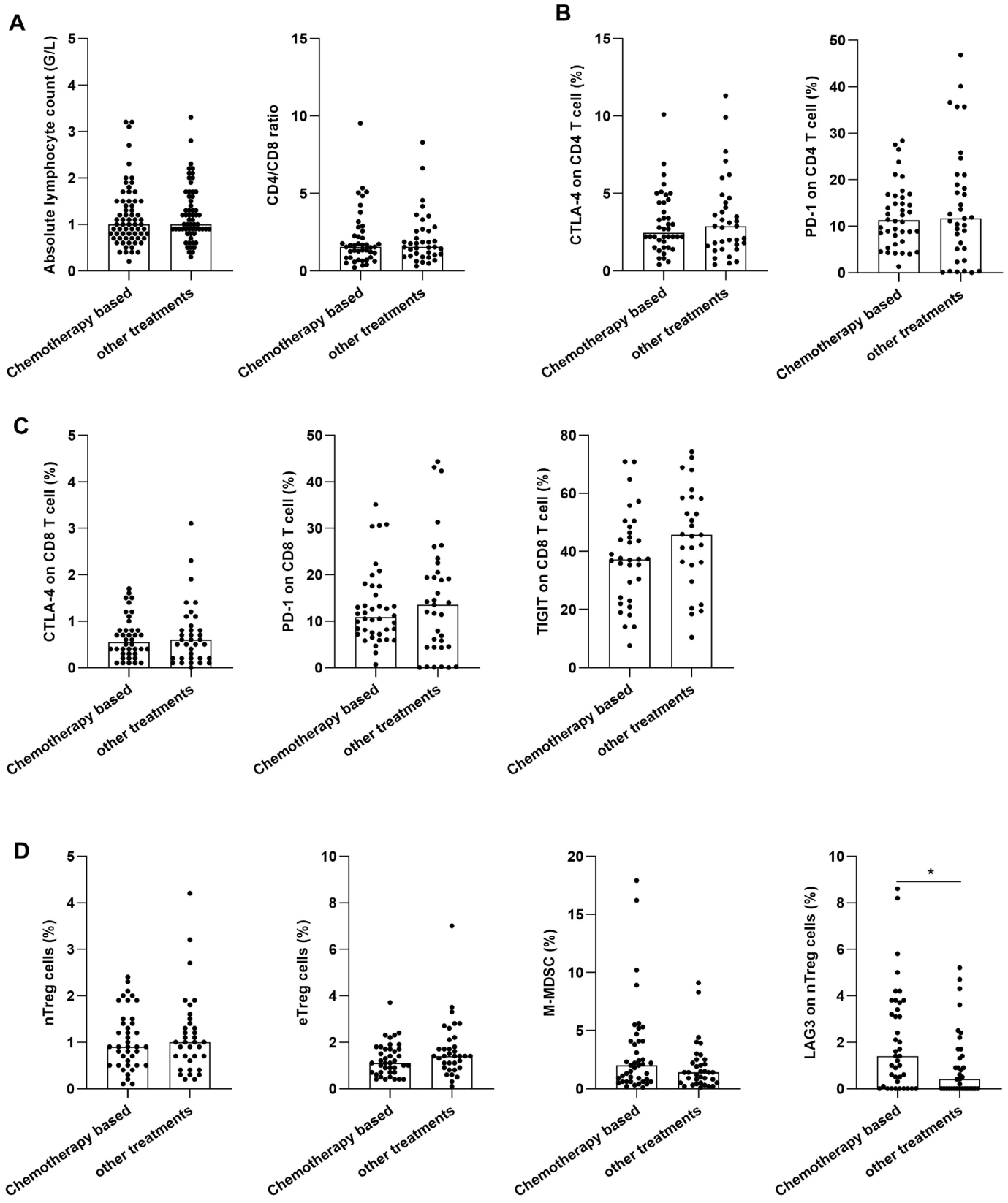

Figure 2 Immunological parameters according to patients' cancer treatment before vaccination. Immune checkpoint expression on T cells and immunosuppressive cell rates were analysed before mRNA vaccination by flow cytometry. (A) Median of absolute lymphocyte count and CD4/CD8 ratio. (B) Median expression of CTLA-4 and PD-1 expression on CD4 T cells. (C) Median expression of CTLA-4, PD-1 and TIGIT expression on CD8 T cells. (D) Frequencies of nTreg, eTreg, M-MDSC and LAG-3 expression on nTreg in patients with cancer. Mann-Whitney test, *p<0.05. Medians were indicated on graphs. eTreg, effector Treg; M-MDSC, monocytic myeloid-derived suppressor cell; nTreg; naïve Treg; PD-1, programmed cell death 1; TIGIT, T cell immunoreceptor with Ig and ITIM domains; Treg, regulatory T cell.

neutralising antibodies is highly predictive of immune protection from symptomatic SARS-CoV-2 infection comes from the statistical model developed by Khoury *et al*.[28] That study estimated the neutralising level required for 50% protection from COVID-19 to be equal to 20% of the mean titre for persons in the convalescent phase. A potential correlation of protection using prospective data from a vaccination trial with 171 cases of SARS-CoV-2 infections and 1404 non-cases was established by Feng *et al*.[24] In this study, the antibody level associated with 80% vaccine efficacy against symptomatic COVID-19 was equal to 506 BAU/mL for anti-RBD IgG and 264 BAU/mL for anti-spike IgG. In our study, the Abbot technique detecting anti-RBD IgG was used. The correlation between BAU/mL and AU/mL corresponds to the formula 1 BAU=0142×AU, justifying the serological threshold used equal to 3563 AU/mL to discriminate responders and non-responders or suboptimal responders. In the trial published by Oosting *et al*,[26] a cut-off level at 300 BAU/mL (2113 AU/mL) was used to discriminate between suboptimal and adequate responders among patients receiving anticancer treatment. This was consistent with our threshold since the authors used an anti-spike IgG detection assay, approaching the 264 BAU/mL threshold determined by Feng *et al*, for antibodies targeting the spike protein. Finally, in another prospective multicentre study conducted among 1551 patients with haematological disorders, a serological titre cut-off of 250 BAU/mL was predictive of breakthrough infection and its severity.[32] In this study, results were reported as SARS-CoV-2-reactive IgG BAU/mL. This was consistent with our threshold and the one of Feng *et al*, equal to 264 BAU/mL for anti-spike IgG.

In our study, most of patients had a SARS-CoV-2 antibody response 3 months following the beginning of the vaccination phase (consisting two doses of vaccine). This finding is consistent with previous reports evaluating mRNA-based vaccine efficacy among patients with cancer.[13 14 26 33 34] However, we observed that more than half of the patients had a suboptimal response according to the cut-off level dichotomising responders from non-responders or suboptimal responders. Oosting *et al* previously suggested the interest of a third dose of vaccine that could potentially turn suboptimal responders into responders.[26] To go further, in a context of recurrent seasonal epidemic of COVID-19, our study and others demonstrated the interest to monitor SARS-CoV-2-binding antibodies among patients with cancer to adapt the vaccination strategy to the antibody level.

We also analysed T cell responses against the spike protein among 97 patients with cancer. On the whole, 48 patients had a positive T cell response. As previously described, a cellular response was not systematically associated with a humoral response among patients with cancer.[14 25 26] The rate of spike-specific T cell responses was lower in our study compared with that of Oosting *et al* (63.5%), while it was similar to that described by Cortés *et al*[14] (50.9% for CD4 T cell responses; 45.5% for CD8 T cell responses). Surprisingly, in relation to the serological threshold, rates of T cell responses were similar in our study and that of Oosting *et al*, with, respectively, 47% and 40.7% of T cell responses for non-responders or suboptimal responders and, respectively, 70% and 60.5% of T cell responses for responders. Currently, it remains unknown the extent of the magnitude of T cell responses and their correlation with COVID-19 protection. Nevertheless, unlike serological responses, several studies demonstrated that T cell responses against the spike protein were not affected by circulating variants.[25 35 36]

The right timing of vaccination with cancer treatment is key to achieving better seroconversion and vaccine protection, in a population already weakened by their illness and treatment. In the CACOV-VAC Study, local guidelines were most of the time followed. Indeed, immunosuppressive treatments (chemotherapy or corticoid) were discontinued, if possible, 10 days following each vaccination dose. Our results did not establish an optimal timing of vaccination according to humoral responses. In a recent study including 63 patients with cancer with solid tumour treated by chemotherapy, the timing of vaccination did not affect the vaccine-induced humoral responses.[37] Additionally, our results are in line with other studies analysing other vaccine immunogenicity among patients with cancer with solid tumour such as influenza and pneumococcal vaccines.[38 39]

From an immunological point of view, M-MDSC levels did not impact vaccination immunogenicity both in terms of humoral and cellular response. This result was in line with the study of Takano *et al* on healthy vaccinees in which M-MDSC dynamics did not correlate with neutralising antibody response at the time of the first two doses of mRNA vaccine.[20] However, the rate of nTregs ($CD25^+$ $FoxP3^+$ CD15s–) was significantly higher in patients with a serological threshold superior to 3563 AU/mL and the one of eTreg ($CD25^+$ $FoxP3^+$ $CD15s^+$) tended to be lower in this category of patients. These observations support previous results demonstrating that eTregs are suppressive cells that might alter immune responses following TCR stimulation, unlike nTreg.[40] A high and sustained expression of inhibitory receptors is a hallmark of exhausted T cells.[41] Our results show that low TIGIT level on CD8 T cells was significantly associated with higher cellular and humoral responses following COVID-19 vaccination. In cancer context, TIGIT expression by CD8 T cells limits their effector function and expansion capacities.[42] Why among all inhibitory receptors only TIGIT was associated with vaccine immunogenicity is worth to be further investigated in vaccination context. Here, we demonstrated that low expression of the TIGIT receptor might be an interesting predictive biomarker of vaccine efficacy.

This study had the inherent limitation that it was not powered to conclude for specific subgroups, such as patients' tumour types or anticancer treatment regimens. Further analyses on memory T cell persistence and T cell correlates of immunity are still hardly needed especially in the context of VOC emergence and reduced humoral

efficacy. Furthermore, the information about COVID-19 infection in the past 3 months was declarative and only patients with symptomatic COVID-19 during this period were excluded, in accordance with the vaccination recommendations at the time of the patients' recruitment. Because cross-reactive peptides are mainly localised in the S2 subunit of the S protein, spontaneous T cell responses against the S1 subunit have been presented in this study. We noticed spontaneous T cell responses against the S2 subunit that were weaker compared with the one against the S1 subunit (data not shown). Collectively, these results demonstrated that despite a high seroconversion rate following vaccine against COVID-19 among patients with cancer, their median serological levels were below the threshold equal to 3563 AU/mL considered as a humoral correlate of protection against SARS-CoV-2. Importantly, elderly patients appeared to be less protected than the young ones. Among immunological parameters investigated, the inhibitory receptor TIGIT might be considered as an interesting predictive biomarker of COVID-19 vaccine immunogenicity and beyond in an anticancer vaccine context.

Author affiliations
[1]Université Franche-Comté, INSERM, EFS BFC, UMR1098 RIGHT Interactions Greffon-Hôte-Tumeur/Ingénierie Cellulaire et Génique, Besançon, France
[2]Service d'oncologie médicale, CHU Besançon, Besançon, France
[3]Methodology and Quality of Life Unit in Oncology, CHU Besançon, Besançon, France
[4]Department of Virology, CHU Besançon, Besançon, France
[5]ITAC Platform, University of Franche-Comté, Besançon, France
[6]Medical Oncology, Hôpital Jean Minjoz, Besançon, France
[7]Dermatology, CHU Besançon, Besançon, France
[8]Department of Pharmacy, University Hospital Centre Besançon, Besançon, France

Acknowledgements The authors are grateful to the patients included in this study, the clinical research team (DB, AM, AS), the medical teams from the Department of Oncology, Infectious Disease, Pharmacy and Biology of the CHU of Besançon (TN, OH, AF, BF, CL, CL, CE, DP, JE, KG, MG, MC, NU, SA, T-V, CV, VW, KB, CC, TR), the UMR1098 research team (MP) and the biomonitoring platform (CL, EG, AR, FS, ES, CC, RA).

Contributors Conception, design and supervision—MK, LM and CB. Development of methodology—MK, LM, CB, AF and DV. Statistical analysis—AF, DV and LS. Acquisition of data—LS, AB, KA, SA, UC, VM, MBK and ML. Analysis and interpretation of data—LS, AB, CB, MK, LM, QL, AF and EO. Writing, reviewing and/or revision of the manuscript—MK, LM, CB, OA, LS, EO and CB. Administrative, technical or material support—HA, SK, EC, GM, MJ, CN, RL and QL. Guarantor, MK.

Funding This work was supported by a grant from the Fondation ARC (CANCER EMERGENCY 11 & COVID-19, December 2020) and from the regional health agency Bourgogne Franche-Comté (ARS BFC) to CB (Evaluation of efficacy and predictors of response to COVID-19 vaccine in cancer patients in hospitals of Regional Institute of Franche-Comté (IRFC), January 2021).

Disclaimer The sponsors had no role in the study design, data collection and analysis, in the preparation of the manuscript or in the decision to publish.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants. The CACOV-VAC Study was approved by the ethical review board on 25 March 2021 (no. 2021-24 A00166-35). Written informed consent was obtained from all patients in accordance with the French regulation and after approval by the local and national ethics committee.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

ORCID iDs
Romain Loyon http://orcid.org/0000-0001-8039-7893
Olivier Adotevi http://orcid.org/0000-0002-7742-136X
Marie Kroemer http://orcid.org/0000-0001-9274-619X

REFERENCES

1 Dai M, Liu D, Liu M, *et al*. Patients with cancer appear more vulnerable to SARS-Cov-2: a multicenter study during the COVID-19 outbreak. *Cancer Discov* 2020;10:783–91.
2 Robilotti EV, Babady NE, Mead PA, *et al*. Determinants of COVID-19 disease severity in patients with cancer. *Nat Med* 2020;26:1218–23.
3 Kuderer NM, Choueiri TK, Shah DP, *et al*. Clinical impact of COVID-19 on patients with cancer (Ccc19): a cohort study. *Lancet* 2020;395:1907–18.
4 Elkrief A, Desilets A, Papneja N, *et al*. High mortality among hospital-acquired COVID-19 infection in patients with cancer: a multicentre observational cohort study. *Eur J Cancer* 2020;139:181–7.
5 Marra A, Generali D, Zagami P, *et al*. Seroconversion in patients with cancer and oncology health care workers infected by SARS-Cov-2. *Ann Oncol* 2021;32:113–9.
6 Mansi L, Spehner L, Daguindau E, *et al*. Study of the SARS-Cov-2-specific immune T-cell responses in COVID-19-positive cancer patients. *Eur J Cancer* 2021;150:1–9.
7 Lièvre A, Turpin A, Ray-Coquard I, *et al*. Risk factors for Coronavirus disease 2019 (COVID-19) severity and mortality among solid cancer patients and impact of the disease on anticancer treatment: a French nationwide cohort study (GCO-002 CACOVID-19). *Eur J Cancer* 2020;141:62–81.
8 Ribas A, Sengupta R, Locke T, *et al*. Priority COVID-19 vaccination for patients with cancer while vaccine supply is limited. *Cancer Discov* 2021;11:233–6.
9 van der Veldt AAM, Oosting SF, Dingemans A-MC, *et al*. COVID-19 vaccination: the VOICE for patients with cancer. *Nat Med* 2021;27:568–9.
10 Corti C, Curigliano G. Commentary: SARS-Cov-2 vaccines and cancer patients. *Ann Oncol* 2021;32:569–71.
11 Baden LR, El Sahly HM, Essink B, *et al*. Efficacy and safety of the mRNA-1273 SARS-Cov-2 vaccine. *N Engl J Med* 2021;384:403–16.
12 Polack FP, Thomas SJ, Kitchin N, *et al*. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020;383:2603–15.
13 Addeo A, Shah PK, Bordry N, *et al*. Immunogenicity of SARS-Cov-2 messenger RNA vaccines in patients with cancer. *Cancer Cell* 2021;39:1091–8.
14 Cortés A, Casado JL, Longo F, *et al*. Limited T cell response to SARS-Cov-2 mRNA vaccine among patients with cancer receiving different cancer treatments. *Eur J Cancer* 2022;166:229–39.
15 Barrière J, Chamorey E, Adjtoutah Z, *et al*. Impaired Immunogenicity of BNT162b2 anti-SARS-Cov-2 vaccine in patients treated for solid tumors. *Ann Oncol* 2021;32:1053–5.
16 Skelly DT, Harding AC, Gilbert-Jaramillo J, *et al*. Two doses of SARS-Cov-2 vaccination induce robust immune responses to emerging SARS-Cov-2 variants of concern. *Nat Commun* 2021;12:5061.
17 Moss P. The T cell immune response against SARS-Cov-2. *Nat Immunol* 2022;23:186–93.
18 Grassi G, Notari S, Gili S, *et al*. Myeloid-derived Suppressor cells in COVID-19: the paradox of good. *Front Immunol* 2022;13:842949.

19 Terao T, Naduka T, Ikeda D, *et al*. Depletion of Cd38-positive regulatory T cells by anti-Cd38 Monoclonal antibodies induces a durable response to SARS-Cov-2 vaccination in patients with plasma cell Dyscrasia. *Br J Haematol* 2022;197:417–21.
20 Takano T, Morikawa M, Adachi Y, *et al*. Distinct immune cell Dynamics correlate with the Immunogenicity and Reactogenicity of SARS-Cov-2 mRNA vaccine. *Cell Rep Med* 2022;3:100631.
21 Chauvin J-M, Pagliano O, Fourcade J, *et al*. TIGIT and PD-1 impair tumor antigen-specific Cd8 T cells in Melanoma patients. *J Clin Invest* 2015;125:80445:2046–58.:.
22 He W, Zhang H, Han F, *et al*. Cd155T/TIGIT signaling regulates $Cd8^+$ T-cell metabolism and promotes tumor progression in human gastric cancer. *Cancer Res* 2017;77:6375–88.
23 Simon S, Voillet V, Vignard V, *et al*. PD-1 and TIGIT Coexpression identifies a circulating Cd8 T cell subset predictive of response to anti-PD-1 therapy. *J Immunother Cancer* 2020;8:e001631.
24 Feng S, Phillips DJ, White T, *et al*. Correlates of protection against symptomatic and asymptomatic SARS-Cov-2 infection. *Nat Med* 2021;27:2032–40.
25 Rouhani SJ, Yu J, Olson D, *et al*. Antibody and T cell responses to COVID-19 vaccination in patients receiving anticancer therapies. *J Immunother Cancer* 2022;10:e004766.
26 Oosting SF, van der Veldt AAM, GeurtsvanKessel CH, *et al*. COVID-19 vaccination in patients receiving chemotherapy, Immunotherapy, or Chemoimmunotherapy for solid tumours: a prospective, Multicentre, non-inferiority trial. *Lancet Oncol* 2021;22:1681–91.
27 Massarweh A, Eliakim-Raz N, Stemmer A, *et al*. Evaluation of Seropositivity following BNT162b2 messenger RNA vaccination for SARS-Cov-2 in patients undergoing treatment for cancer. *JAMA Oncol* 2021;7:1133–40.
28 Khoury DS, Cromer D, Reynaldi A, *et al*. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-Cov-2 infection. *Nat Med* 2021;27:1205–11.
29 Cromer D, Steain M, Reynaldi A, *et al*. Predicting vaccine effectiveness against severe COVID-19 over time and against variants: a meta-analysis. *Nat Commun* 2023;14:1633.
30 Khoury DS, Schlub TE, Cromer D, *et al*. Correlates of protection, thresholds of protection, and Immunobridging among persons with SARS-Cov-2 infection. *Emerg Infect Dis* 2023;29:381–8.
31 Gilbert PB, Montefiori DC, McDermott AB, *et al*. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. *Science* 2022;375:43–50.
32 Piñana JL, Vazquez L, Calabuig M, *et al*. One-year breakthrough SARS-Cov-2 infection and correlates of protection in fully vaccinated hematological patients. *Blood Cancer J* 2023;13:8.
33 Thakkar A, Gonzalez-Lugo JD, Goradia N, *et al*. Seroconversion rates following COVID-19 vaccination among patients with cancer. *Cancer Cell* 2021;39:1081–90.
34 Monin L, Laing AG, Muñoz-Ruiz M, *et al*. Safety and Immunogenicity of one versus two doses of the COVID-19 vaccine BNT162b2 for patients with cancer: interim analysis of a prospective observational study. *Lancet Oncol* 2021;22:765–78.
35 Edara V-V, Pinsky BA, Suthar MS, *et al*. Infection and vaccine-induced neutralizing-antibody responses to the SARS-Cov-2 B.1.617 variants. *N Engl J Med* 2021;385:664–6.
36 Planas D, Veyer D, Baidaliuk A, *et al*. Reduced sensitivity of SARS-Cov-2 variant Delta to antibody neutralization. *Nature* 2021;596:276–80.
37 Peeters M, Verbruggen L, Teuwen L, *et al*. Reduced humoral immune response after BNT162b2 Coronavirus disease 2019 messenger RNA vaccination in cancer patients under antineoplastic treatment. *ESMO Open* 2021;6:100274.
38 Keam B, Kim M-K, Choi Y, *et al*. Optimal timing of influenza vaccination during 3-week cytotoxic chemotherapy cycles. *Cancer* 2017;123:841–8.
39 Choi W, Kim JG, Beom S-H, *et al*. Immunogenicity and optimal timing of 13-Valent Pneumococcal conjugate vaccination during adjuvant chemotherapy in gastric and colorectal cancer: a randomized controlled trial. *Cancer Res Treat* 2020;52:246–53.
40 Miyara M, Chader D, Sage E, *et al*. Sialyl Lewis X (Cd15S) identifies highly differentiated and most suppressive Foxp3High regulatory T cells in humans. *Proc Natl Acad Sci U S A* 2015;112:7225–30.
41 Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. *Nat Rev Immunol* 2015;15:486–99.
42 Dougall WC, Kurtulus S, Smyth MJ, *et al*. TIGIT and Cd96: new Checkpoint receptor targets for cancer Immunotherapy. *Immunol Rev* 2017;276:112–20.