Humoral and Cellular Immune Responses against SARS-CoV-2 after Third Dose BNT162b2 following Double-Dose Vaccination with BNT162b2 versus ChAdOx1 in Patients with Cancer

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ABSTRACT

Purpose: Patients with cancer display reduced humoral responses after double-dose COVID-19 vaccination, whereas their cellular response is more comparable with that in healthy individuals. Recent studies demonstrated that a third vaccination dose boosts these immune responses, both in healthy people and patients with cancer. Because of the availability of many different COVID-19 vaccines, many people have been boosted with a different vaccine from the one used for double-dose vaccination. Data on such alternative vaccination schedules are scarce. This prospective study compares a third dose of BNT162b2 after double-dose BNT162b2 (homologous) versus ChAdOx1 (heterologous) vaccination in patients with cancer.

Experimental Design: A total of 442 subjects (315 patients and 127 healthy) received a third dose of BNT162b2 (230 homologous vs. 212 heterologous). Vaccine-induced adverse events (AE) were captured up to 7 days after vaccination. Humoral immunity was assessed by SARS-CoV-2 anti-S1 IgG antibody levels and SARS-CoV-2 50% neutralization titers (NT50) against Wuhan and BA.1 Omicron strains. Cellular immunity was examined by analyzing $CD4^+$ and $CD8^+$ T-cell responses against SARS-CoV-2–specific S1 and S2 peptides.

Results: Local AEs were more common after heterologous boosting. SARS-CoV-2 anti-S1 IgG antibody levels did not differ significantly between homologous and heterologous boosted subjects [GMT 1,755.90 BAU/mL (95% CI, 1,276.95–2,414.48) vs. 1,495.82 BAU/mL (95% CI, 1,131.48–1,977.46)]. However, homologous-boosted subjects show significantly higher NT50 values against BA.1 Omicron. Subjects receiving heterologous boosting demonstrated increased spike-specific $CD8^+$ T cells, including higher IFNγ and TNFα levels.

Conclusions: In patients with cancer who received double-dose ChAdOx1, a third heterologous dose of BNT162b2 was able to close the gap in antibody response.

Introduction

Patients with cancer have increased risk for severe coronavirus disease (COVID-19) after SARS-CoV-2 infection (1, 2). As such, patients with cancer have been prioritized for COVID-19 vaccination (1). Because of immune incompetence, patients with cancer were excluded from pivotal vaccine approval trials. BNT162b2 and ChAdOx1 were the most widely administrated vaccines on the European continent, also for patients with cancer. The first studies evaluating the immunologic outcomes of vaccinated patients with cancer against COVID-19 demonstrated reduced humoral responses after double-dose BNT162b2 and even lower responses after double-dose ChAdOx1 vaccination, compared with healthy individuals (1–5). More recent data showed that a third vaccination dose further boosted immune responses for immunocompromised patients against COVID-19 (6–13). For double-dose vaccine schedules, it was observed that a heterologous double-dose elicited higher reactogenicity and higher levels of binding and neutralizing antibodies against SARS-CoV-2 compared with homologous double-dose vaccination (14–18). Recent studies took the first steps to gain knowledge about safety and immunologic outcomes of mixed schedules in a third dose setting (5). It was observed that a heterologous third dose led to higher increase in binding and neutralizing antibody titers compared with a homologous third dose (19, 20). In addition, lower infection rates were reported in people who received a heterologous third dose (19). Although these data provide valuable insights into mixing vaccines, they mainly address the immune response in healthy people. Moreover, no significant information on a third dose BNT162b2 after double-dose ChAdOx1 vaccination, or relevant comparison, are available. Currently, more data are needed on the use of homologous boosters (same vaccine as double-dose vaccination) versus heterologous boosters (different vaccine as double-dose vaccination) in patients with cancer. In addition to the production of antiviral antibodies, the cellular

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Clin Cancer Res 2023;29:635–46

doi: 10.1158/1078-0432.CCR-22-2185

Translational Relevance

Third vaccination doses against SARS-CoV-2 have been broadly administered, aiming to improve immunologic response and protection against COVID-19. The majority of the administered third doses were BNT162b2 and other mRNA vaccines due to their proven superior efficacy. Many individuals on the European continent, both healthy and immunocompromised, received double-dose mRNA or ChAdOx1 vaccination. Because of their impaired immunity, vaccine-induced protection against symptomatic COVID-19 is less efficient in patients with cancer. Currently, more data are needed on the use of homologous (same vaccine as double-dose vaccination) versus heterologous boosters (different vaccine as double-dose vaccination) in patients with cancer. As it is important to establish optimal vaccination schemes for these vulnerable patients, our study compared the immune response after homologous versus heterologous third dose in a large cohort of patients with cancer. Our study supports the recommendation of a third dose BNT162b2 in patients with cancer, irrespective of whether it constitutes a homologous or heterologous booster.

immune response—in particular T-cell-mediated immune response—has proven to be of significant importance in the defense against SARS-CoV-2 (21). Moreover, it has been described that T-cell responses are negatively correlated with COVID-19 severity (22). Recent data demonstrated the potential of T cells to protect against new viral variants. Hence, it is crucial that vaccines elicit both humoral and cellular immune responses (23, 24). Data about specific T-cell responses after different SARS-CoV-2 vaccination regimens are scarce and scattered, especially in cancer patients where T-cell immunity is often impaired (22, 25).

Therefore, it remains unclear whether there are differences regarding humoral and cellular immune responses in an onco-hematological population between a third dose BNT162b2 after a double-dose of BNT162b2 or ChAdOx1 vaccination. To address this knowledge gap, we prospectively investigated antibody responses, cellular responses, and safety of a third dose BNT162b2 after double-dose BNT162b2 or ChAdOx1 vaccination in a large cohort of patients with cancer.

Materials and Methods

Trial design and participants

In three parallel ongoing prospective COVID-19 vaccination studies, a third vaccination dose was given. A population of patients with cancer participating in the prospective B-VOICE study received a third dose BNT162b2 after double-dose BNT162b2 vaccination according to the amended protocol. A second population of patients with cancer was actively recruited to receive a third dose BNT162b2 after previous double-dose ChAdOx1 vaccination (Tri-VOICE plus; **Fig. 1**). The third study was conducted in a population of healthy staff members of the Antwerp University Hospital, without oncologic history (HEAL-V). All healthy individuals received a third dose BNT162b2 between 8 and 9 months after the administration of the first dose BNT162b2 or ChAdOx1 (**Fig. 1**). All participants signed informed consent and were ages 18 years or older with a life expectancy of at least 6 months. Pregnant or breastfeeding women and patients with an immune deficiency unrelated to cancer or cancer treatment were ineligible. Eligible patients were patients with cancer with a solid tumor or hematologic malignancies were divided into treatment cohorts (**Fig. 1**).

Trial oversight

The study was approved by the central ethics committee of the Antwerp University Hospital and the Federal Agency for Medicine and Health Products (EudraCT nos. 2021–000300–38 and 2021–003573–58 and EC nos. 2021–0543, 2021.0541, and 2021.0110) and was executed in accordance with Good Clinical Practice and the Declaration of Helsinki [ICH GCP E6(R2)].

Study procedures

Administration of third dose BNT162b2 and collection of blood samples

A group of patients with cancer and a group of healthy staff members of the Antwerp University Hospital all received 30 μg of the BNT162b2 vaccine intramuscularly after double-dose BNT162b2 or ChAdOx1 vaccination. Patients with cancer received a third vaccination dose 6 to 7 months after first dose administration, as described in the protocol. Healthy individuals received a third dose BNT162b2 8 to 9 months after administration of the first vaccination dose. From all study participants blood samples for analysis of the immune response, were collected on the day of third vaccination, prior to vaccine administration, and 28 days afterwards.

All blood samples were transferred to Biobank Antwerp for initial processing and storage until analysis.

Analysis of humoral immune response

Antibody levels were assessed in serum samples using the Siemens Healthineers Atellica IM SARS-CoV-2 IgG (sCOVG) assay for quantitative detection of anti-S1 IgG antibody levels against SARS-CoV-2 following the described protocol (26, 27). Quantitative anti-S1 IgG titers were converted to binding antibody units per mL (BAU/mL).

In vitro viral neutralizing antibody titers (NT50) against Wuhan-1 were assessed in a subset of patients with cancer and healthy individuals, following the previously described protocol (3, 28). The subset of individuals was carefully selected to have treatment cohorts equally represented. For each subset, the individuals mounting the highest SARS-CoV-2 IgG antibody titers (346.62–21800 BAU/mL) were selected for NT50 analysis. All samples with NT50-titres above 300 IU/mL against the Wuhan-1 strain were also tested against the BA.1 Omicron variant (B.1.1.529).

Analysis of cellular immune response

A subset of patients with cancer was selected via stratified sampling for the assessment of cellular immunity via flow cytometry. PBMCs were isolated from whole blood samples using density gradient centrifugation and stored in liquid nitrogen in FBS (Giboc, Thermo Fisher Scientific) with 10% DMSO (Sigma-Aldrich). For analysis of CD4 and CD8 T-cell responses against SARS-CoV-2-specific S1 and S2 spike peptides, flow cytometry was performed. Samples where thawed and washed on the day of analysis in prewarmed RPMI 640 medium supplemented with 10% FBS, MEM Non-Essential Amino acid solution, L-glutamine, penicillin, and streptomycin (all from Gibco, Thermo Fisher Scientific). Next, 1×10 (6) PBMCs were stimulated for 6 hours with 1 μg/mL S1 and S2 spike-specific peptide pools (JPT), 1 μg/mL Staphylococcus Enterotoxin B (Sigma-Aldrich) as a positive control or DMSO as a negative control. Negative control and S1 + S2 conditions were also supplemented with 1 μg/mL anti-CD28 beads to provide the required costimulatory signal. One and

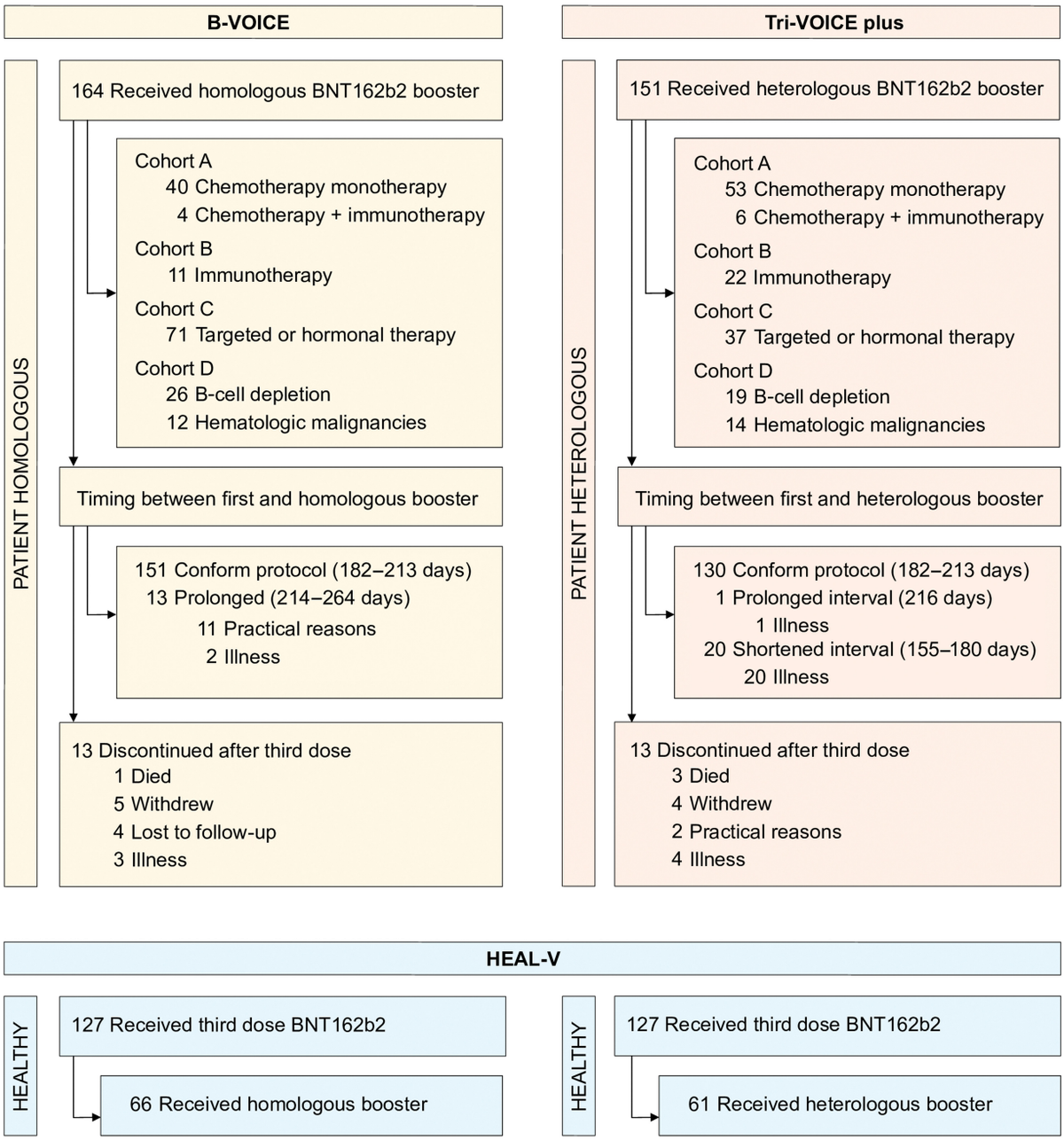

Figure 1.

Trial profile. Patients were assigned to a therapy cohort based on the type of anti-neoplastic treatment administered at the time of first vaccination dose. All patients received anti-neoplastic treatment when the first vaccination dose was administered. Patients with cancer with a solid tumor were divided into three treatment cohorts: receiving chemotherapy (cohort A), immunotherapy (cohort B), and targeted therapy or hormonal therapy (cohort C). Patients with hematologic malignancies were assigned to cohort D. In cohort D, a differentiation was made between patients receiving B-cell–depleting therapy and patients receiving other hematologic cancer treatments.

a half hours after start of the incubation, 10 μg/mL Brefeldin A (BD Biosciences) was added to stop cytokine release. After the incubation period, cells were stained for flow cytometry analysis for 30′ at 4°C with the following mAb: CD3-AF700, CD4-BV510, CD8-Pe-Cy7 as extracellular markers and CD137-BV605, CD154-BV421, IFNγ-AF488, TNFα-BV650, and IL2-AF647 as intracellular markers (all from BioLegend). LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Thermo Fisher Scientific) was used to discriminate between live and dead cells. For intracellular cytokine stainings, cells were fixed and permeabilized with cytofix/cytoperm solution (BD Biosciences). All samples were measured on a Novocyte Quanteon (Agilent) analyzer. Data analysis was performed using FlowJo v10.8.1 software package

(BD Biosciences). A quality threshold was set, where samples were excluded from data analysis when viability was below 50% or when the $CD4^+$ or $CD8^+$ T-cell count was below 10,000.

Safety and breakthrough infections

An existing web-based electronic platform for toxicity telemonitoring, RemeCare Oncology, was used to assess patient-reported outcomes (PRO) about local vaccine reactions, systemic adverse events (AE), and SARS-CoV-2 infections during the study period. Patients were educated for and equipped with this application. An alternative, via questionnaires on paper, was provided in case of restraint or difficulties using the application or in the case of healthy individuals not having access to the application. Participants registered local (pain, redness, swelling) and systemic reactions (nausea/vomiting, diarrhea, muscle/joint pain, fatigue, pain, fever) for 7 days after receiving the third vaccination dose. Local reactions were graded as mild, moderate, or severe. Subjects were actively asked about possible SARS-CoV-2 infections during their follow-up visit. In addition, all patients with cancer of the Antwerp University Hospital attending the oncology day care unit were screened biweekly for SARS-CoV-2 using PCR on mouth and oropharyngeal rinse samples before their treatment. Following the recommendations of the Belgian government, for persons that were in close contact with an infected person or with typical symptoms of SARS-CoV-2 infection (fever, cough, shortness of breath) testing for SARS-CoV-2 infection was highly recommended. For all patients, these data were monitored up to 4 months after third vaccination.

Outcomes

The primary endpoint was the SARS-CoV-2 anti-S1 IgG antibody levels 28 days after administration of the third BNT162b2 vaccination dose. Secondary endpoints included neutralizing antibodies 28 days after third dose, differences in IgG antibody titers between homologous and heterologous vaccination schedules, $CD4^+$- and $CD8^+$-specific T-cell responses, breakthrough infections based on the incidence of PCR-confirmed SARS-CoV-2 infection, and vaccine safety based on PROs of local and systemic AEs.

Statistical analysis

All analyses were performed with the use of an intention-to-treat principle. The geometric mean titers (GMT) of the SARS-CoV-2 anti-S1 IgG titers 28 days post-third dose were compared between heterologous and homologous boosting. An analysis of variance was used between cohorts with pairwise comparison using Tukey's honestly significant difference (HSD) *post hoc* test. Exploratory analysis in treatment cohorts was performed using similar statistical techniques. The occurrence of AEs was compared between different boosting regimes with the use of a fisher exact test. GMT of NT50 values against Wuhan-1 and BA.1 Omicron were compared between both boosting types using an analysis of variance between treatment cohorts with pairwise comparison using Tukey HSD *post hoc* test. For T-cell analysis, differences between vaccination cohorts were assessed using Mann–Whitney *U* test. The correlation between SARS-CoV-2 anti-S1 IgG antibody levels and NT50 titers on one hand and the percentage $CD154^+$ $CD4^+$/$CD137^+$ $CD8^+$ T cells on the other hand, was analyzed 28 days after the third dose, with the use of the Spearman method. A two-sided P value <0.05 after Bonferroni–Holm correction for multiple testing was considered statistically significant.

Data availability

Data are available upon reasonable request by contacting T. Vandamme.

Results

Demographics of study groups

Of the 164 patients with cancer receiving a homologous booster, 92% received it 6 to 7 months after first dose and 8% received it between 7 and 9 months. In another group, 151 patients with cancer received a heterologous booster. In this group, 87% of the patients received the heterologous booster between 6 and 7 months and 13% 5 to 6 months after first dose. A total of 127 healthy controls received a third dose BNT162b2 between 8 and 9 months after administration of the first dose BNT162b2 or ChAdOx1. A group of 61 healthy subjects received heterologous booster vaccination and 66 received the homologous one. Demographic details of all enrolled subjects are available in **Table 1** and Supplementary Tables S1 and S2. A total of 287 patients with cancer and 125 healthy controls were evaluable 28 days after third dose administration. From the homologous group, 2 healthy individuals (3.0%) and 9 patients (5.5%) had a PCR-confirmed SARS-CoV-2 infection before administration of the third dose. From the heterologous group, 2 healthy individuals (3.3%) and 4 patients (2.6%) tested positive for SARS-CoV-2 before the third dose administration. SARS-CoV-2 anti-S1 IgG antibodies were detected in 284 subjects (68.9%) before administration of the third dose.

Safety and tolerability

Data about vaccine-induced AEs were collected up to 7 days after third dose in 212 homologous (151 patients vs. 61 healthy individuals) and 157 heterologous boosted subjects (96 patients vs. 61 healthy individuals; **Fig. 2**). The most frequently reported local AE was mild-to-moderate pain at the injection site. More than half of all subjects (52.7%) reported pain, either mild, moderate, or severe, within 7 days after third dose. Severe local reactogenicity after third dose was reported in 8.9% of the cancer patients (8.7% homologous vs. 9.4% heterologous boosted) and 9.0% of the healthy individuals (9.8% homologous vs. 8.2% heterologous boosted). The percentage of subjects reporting local pain and swelling was higher after heterologous compared with homologous boosting (60.5% vs. 46.9%; 19.1% vs. 10.9%). Subanalysis of the patients and the healthy controls revealed that this difference between homologous and heterologous boosting was only observed in the patient group (respectively 43.3% vs. 59.4% for local pain and 7.3% vs. 26.0% for swelling; **Fig. 2A**). Although the occurrence of local pain and swelling was significantly different between homologous and heterologous patients with cancer, the clinical relevance of this difference is unclear. The most frequently reported systemic AEs after third dose were fatigue (mean % of all groups; 27.2%), muscle/joint pain (26.6%) and pain (21.7%). No differences regarding systemic AEs were observed between the homologous and heterologous boosted subjects, for patients or healthy individuals (**Fig. 2B**). Patients with cancer did not report significantly more AEs than healthy individuals.

Serious adverse events (SAE) were only documented for the patients with cancer and all considered unrelated to the BNT162b2 vaccine. Eight SAEs (five hospitalizations and three deaths) were reported in the period between the administration of the third vaccination dose and 28 days after. Within this period, 3 patients were hospitalized because of disease progression, 1 patient because of hypotension and dehydration due to reduced intake, and another patient was hospitalized in the stroke unit neurology because of hypertension with vertigo and nausea. None of the hospitalizations were considered to be related to the administration of the BNT162b2 SARS-CoV-2 vaccine. Three patients died during the study period. These deaths were due to cancer-related disease progression and were considered unrelated to the

Table 1. Demographics of the enrolled subjects that were evaluable 28 days after third dose vaccination.

Cancer patients	Double-dose ChAdOx1 vaccination (*N* = 138)	Double-dose BNT162b2 vaccination (*N* = 149)	Overall (*N* = 287)
Gender			
Female	86 (62.3%)	103 (69.1%)	189 (65.9%)
Male	52 (37.7%)	46 (30.9%)	98 (34.1%)
Age at ICF			
Mean (SD)	60.5 (10.6)	61.7 (11.7)	61.2 (11.2)
Median [Min, Max]	62.0 [29.0, 89.0]	62.0 [27.0, 87.0]	62.0 [27.0, 89.0]
Missing	2 (1.4%)	0 (0%)	2 (0.7%)
Cohort			
B-cell depletion	19 (13.8%)	25 (16.8%)	44 (15.3%)
Chemotherapy	48 (34.8%)	33 (22.1%)	81 (28.2%)
Other hematologic cancer treatments	14 (10.1%)	11 (7.4%)	25 (8.7%)
Immunotherapy + chemotherapy	4 (2.9%)	4 (2.7%)	8 (2.8%)
Immunotherapy	21 (15.2%)	10 (6.7%)	31 (10.8%)
Targeted/hormone therapy	32 (23.2%)	66 (44.3%)	98 (34.1%)
ECOG performance status			
0	59 (42.6%)	132 (88.6%)	191 (66.5%)
1	64 (46.4%)	16 (10.7%)	80 (27.9%)
2	3 (2.2%)	0 (0%)	3 (1.0%)
3	3 (2.2%)	0 (0%)	3 (1.0%)
Missing	9 (6.5%)	1 (0%)	10 (3.5%)
Comorbidities			
Autoimmune disease	6 (4.3%)	7 (4.7%)	13 (4.5%)
Kidney disease	17 (12.3%)	6 (4.0%)	23 (8.0%)
Hypertension	38 (27.5%)	36 (24.2%)	74 (25.8%)
Diabetes	12 (8.7%)	12 (8.1%)	24 (8.4%)
Coronary disease	14 (10.1%)	16 (10.7%)	30 (10.5%)
Stage			
I	15 (10.9%)	22 (14.8%)	37 (12.9%)
II	21 (15.2%)	24 (16.1%)	46 (16.0%)
III	10 (7.2%)	8 (5.4%)	20 (7.0%)
IV	55 (39.9%)	57 (38.3%)	112 (39.0%)
Missing	3 (2.2%)	2 (1.3%)	5 (1.7%)
NA	34 (24.6%)	36 (24.2%)	70 (24.4%)

Healthy individuals	Double-dose ChAdOx1 vaccination (*N* = 62)	Double-dose BNT162b2 vaccination (*N* = 63)	Overall (*N* = 125)
Gender			
Female	52 (83.9%)	52 (82.5%)	104 (83.2%)
Male	10 (16.1%)	11 (17.5%)	21 (16.8%)
Age at ICF			
Mean (SD)	38.7 (10.0)	40.5 (11.2)	39.6 (10.6)
Median [Min, Max]	40.0 [22.0, 58.0]	39.0 [23.0, 63.0]	40.0 [22.0, 63.0]

Notes: Demographics of individuals receiving a heterologous BNT162b2 booster after double-dose ChAdOx1 vaccination are shown in the first column. Demographics of individuals receiving a homologous BNT162b2 booster after double-dose BNT162b2 vaccination are shown in the second column. Patients with cancer were assigned to therapy cohorts based on type of treatment they were receiving when the first vaccination dose was administered. For patients with hematologic malignancies, a distinction was made between patients receiving B-cell–depleting therapy and all other treatments. Solid tumor staging was performed according to the TNM AJCC Cancer Staging Manual, eighth edition. Staging of hematologic malignancies was not performed and therefore is indicated as not applicable.
Abbreviations: ECOG, Eastern Cooperative Oncology Group; NA, not applicable.

BNT162b2 vaccine. Two hematological patients receiving B-cell depleting therapy died because of COVID-19, 1 and 4 months after receiving a third vaccination dose. Both patients had no detectable SARS-CoV-2 anti-S1 IgG antibodies.

Vaccine-induced antibody response per treatment cohort

No significant difference in GMT of SARS-CoV-2 anti-S1 IgG antibodies was observed between homologous and heterologous boosted subjects (cancer patients + healthy individuals), 28 days after third dose {GMT 1,755.90 BAU/mL [95% confidence interval (CI), 1,276.95–2,414.48] and 1,495.82 BAU/mL (95% CI, 1,131.48–1,977.46), respectively}. As expected, for both homologous and heterologous boosting, significantly lower anti-S1 IgG antibody levels were observed in patients with cancer [GMT 1,331.32 BAU/mL (95% CI, 857.41–2,067.29) and 1,163.44 BAU/mL (95% CI, 787.32–1,719.24), respectively] compared with the healthy control group [GMT 3,378.99 BAU/mL (95% CI, 2,789.50–4,093.05) and 2,616.93 BAU/mL (95% CI, 2,176.61–3,146.33), respectively; **Fig. 3A**]. The bimodal distribution of **Fig. 3A** was mainly driven by patients with cancer with hematologic malignancies receiving B-cell–depleting therapy.

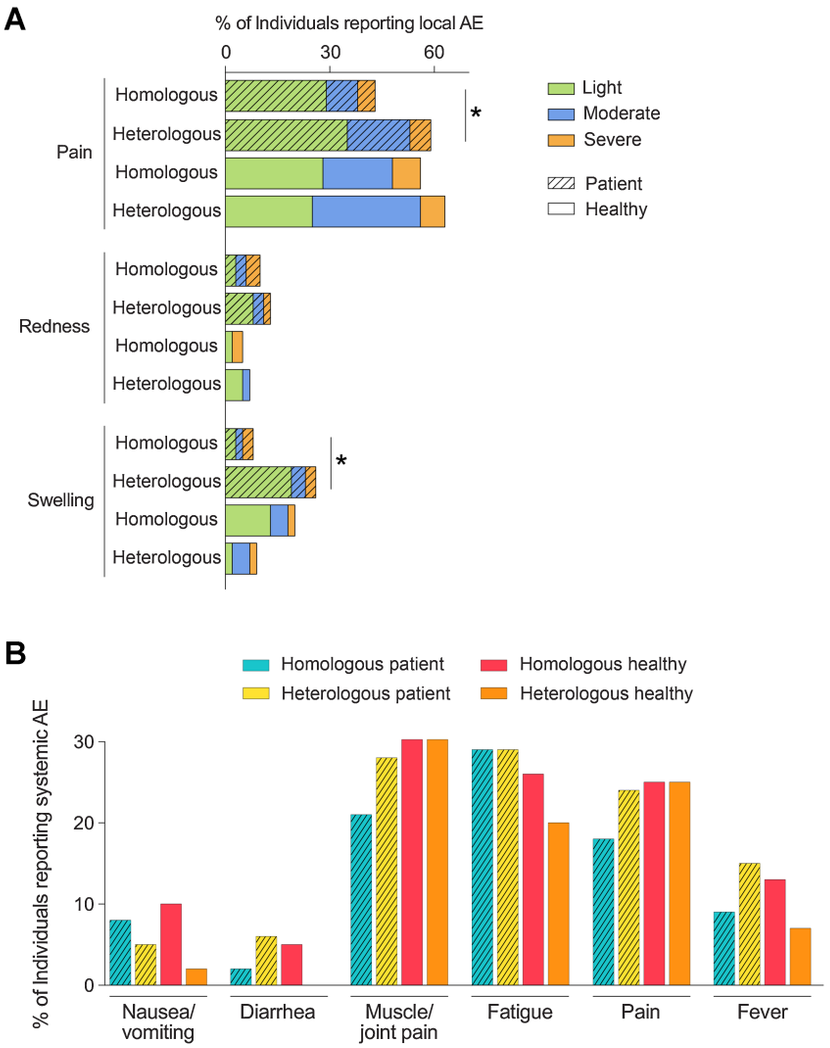

Figure 2.

Local and systemic AEs reported within 7 days after homologous and heterologous boosting in patients with cancer and healthy individuals. **A,** Local AEs were pooled from all study cohorts. Open bars represent the AEs reported by healthy individuals and dashed bars represent the AEs reported by patients with cancer. Different colors represent different grading of AEs. **B,** Systemic AEs were pooled from all study cohorts. Different colors represent different study cohorts. To show differences in the occurrence of AEs between cohorts, the proportion of subjects reporting AEs is represented as a percentage of the number of subjects in that cohort ($n = 151$ for homologous boosted cancer patients, $n = 96$ for heterologous boosted cancer patients, $n = 61$ for homologous boosted healthy individuals, $n = 61$ for heterologous boosted healthy individuals). Comparisons between boosting schedules were performed using a Fisher exact test with Bonferroni–Holm correction for the number of cohorts ($n = 4$) and the number of different local ($n = 3$) and systemic ($n = 6$) AEs. A two-sided P value <0.05 after Bonferroni–Holm correction for multiple testing was considered statistically significant: *, $P < 0.05$.

Neither in the heterologous, nor the homologous boosted cohort, significant differences could be observed in the SARS-CoV-2 anti-S1 IgG antibody levels between healthy individuals, solid patients with cancer receiving different treatment types (chemotherapy, immunotherapy, immunotherapy + chemotherapy, targeted or hormonal therapy), and hematologic patients not receiving B-cell–depleting therapy (**Fig. 3B**). On the contrary, hematologic patients receiving B-cell depletion therapy mount significantly lower binding antibody responses [GMT 25.86 BAU/mL (95% CI, 8.10–82.52) for heterologous and 8.25 BAU/mL (95% CI, 4.10–16.59) for homologous boosted subjects], compared with healthy individuals and all other patient cohorts.

Neutralizing antibodies: Wuhan versus BA.1 Omicron

The humoral response was further investigated by analyzing the *in vitro* neutralizing capacity (NT50) against the wild-type Wuhan strain and the BA.1 Omicron variant in a subset of 80 patients with cancer and 20 healthy individuals. The subset of individuals was carefully selected to have treatment cohorts equally represented in 40 heterologous and 40 homologous boosted patients. Among the healthy subjects, this was performed for 10 homologous and 10 heterologous boosted individuals. In both homologous and heterologous boosting regimes, NT50 values against the BA.1 strain [GMT 233.26 IU/mL (95% CI, 176.18–308.83) and 102.30 IU/mL (95% CI, 77.45–135.13), respectively] were significantly lower than against the wild-type Wuhan strain [GMT 3,813.41 IU/mL (95% CI, 2,960.54–4,911.97) and 2,586.43 IU/mL (95% CI, 1,950.54–3,429.63), respectively]. Subanalysis revealed that NT50 values against BA.1 were significantly lower after heterologous boosting compared with homologous boosting in patients with cancer [GMT 84.33 IU/mL (95% CI, 61.90–114.89) vs. 221.71 IU/mL (95% CI, 157.35–312.40)]. NT50 values against the Wuhan strain were comparable between heterologous and homologous boosting in both patients with cancer [GMT 2,191.26 IU/mL (95% CI, 1,580.48–3,038.07) vs. 3,297.73 IU/mL (95% CI, 2,450.44–4,438.00)] and healthy individuals [GMT 5,020.31 IU/mL (95% CI, 3,554.1–7,091.39) vs. 6,818.76 IU/mL (95% CI, 5,352.46–8,686.75); **Fig. 4**]. It was also observed that NT50 values against BA.1 Omicron were significantly lower in heterologous boosted patients with cancer compared with healthy individuals [GMT 84.33 IU/mL (95% CI, 61.90–114.89) vs. 209.08 IU/mL (95% CI, 130.77–334.29), respectively]. There was a statistically significant correlation between SARS-CoV-2 anti-S1 IgG antibody titers and NT50 values against both Wuhan ($\rho = 0.74$) and BA.1 Omicron ($\rho = 0.88$) strains.

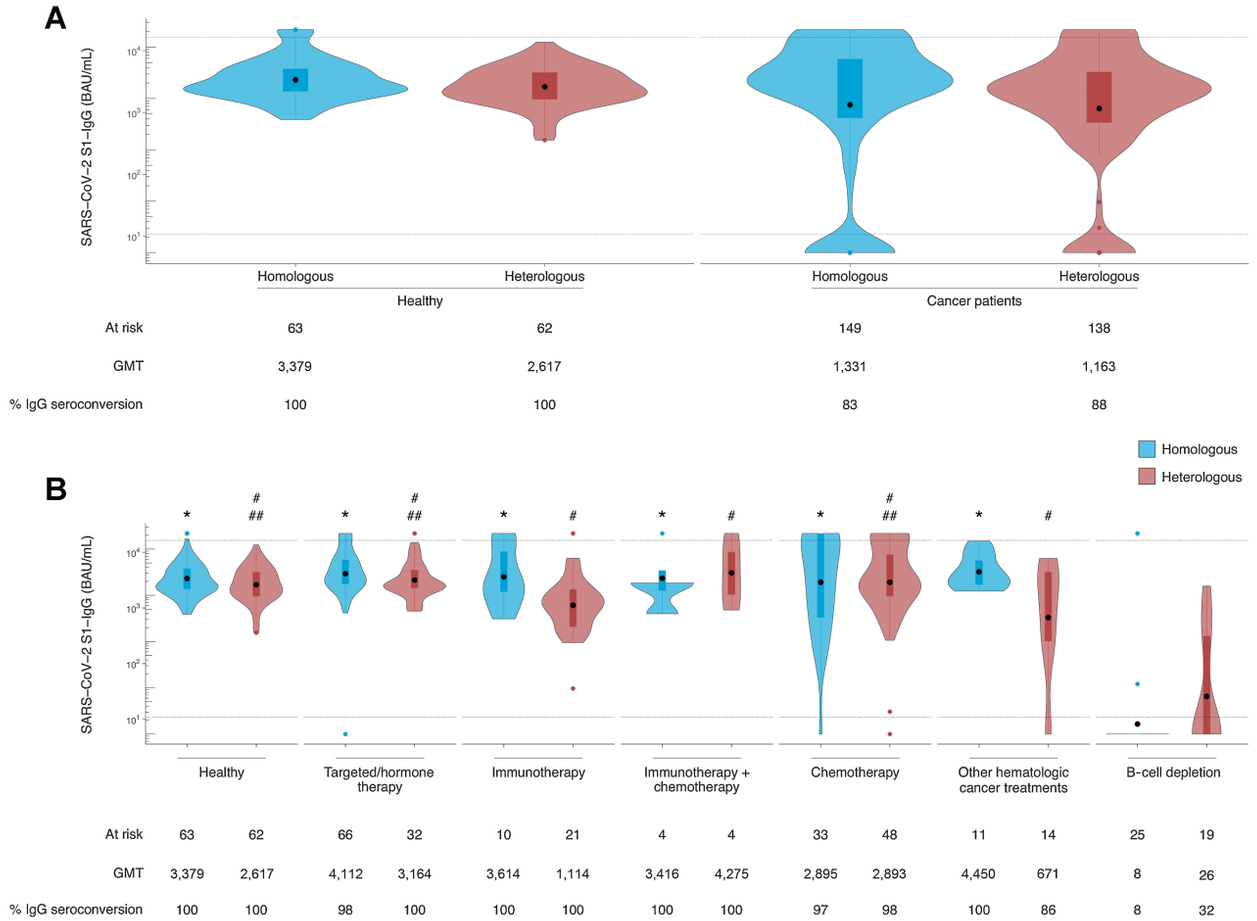

Figure 3.

SARS-CoV-2 S1-IgG antibody levels 28 days after homologous or heterologous boosting in healthy individuals and cancer patients. **A,** Violin plots of log-transformed SARS-COV-2 anti-S1 IgG antibody titers 28 days after homologous or heterologous boosting with BNT162b2 in healthy individuals and patients with cancer. **B,** Subanalysis of log-transformed SARS-CoV-2 anti-S1 IgG antibody titers 28 days after homologous or heterologous boosting with BNT162b2 in different treatment cohorts. Inside each violin plot, the GMT is depicted as a black point and outliers are depicted as colored dots. Anti-S1 IgG-class antibody titers were quantified using a SARS-CoV-2 immunoassay, Siemens Healthineers Atellica IM SARS-CoV-2 IgG (sCOVG) assay, for the detection of antibodies (BAU/mL). The measuring interval was 10.90 to 16,350.00 BAU/mL. Values below the detection were imputed to half of it (5.45 BAU/mL), values above the measuring interval were imputed to 33% above the upper limit of detection (21,800 BAU/m)L with dotted line indicating LLQ and ULQ, respectively. *, $P < 0.05$ with homologous boosted patients with cancer receiving B-cell–depleting therapy. #, $P < 0.05$ with heterologous boosted patients with cancer receiving B-cell–depleting therapy. ##, $P < 0.05$ with heterologous boosted patients with cancer receiving other hematologic cancer treatments.

NT50 values against Wuhan and BA.1 Omicron showed a strong correlation ($\rho = 0.79$; Supplementary Fig. S1). Of 80 tested subjects, 1 heterologous boosted healthy individual and 24 patients with cancer (7 homologous and 17 heterologous boosted) had undetectable levels of neutralizing antibodies against BA.1 Omicron. The majority of patients that were unable to mount neutralizing antibodies against the BA.1 Omicron strain were patients with hematologic malignancies, either receiving B-cell depleting therapy or other types of hematologic cancer treatments. A detailed overview of the NT50 values per cohort can be found in Supplementary Fig. S2.

Comparable occurrence of breakthrough infections

Data from the occurrence of breakthrough infections were collected in 412 of 442 vaccinated individuals. Within 4 months after the third vaccination dose, 32 breakthrough infections (13 patients vs. 19 healthy) were observed in homologous boosted subjects, whereas 30 heterologous boosted subjects (9 patients vs. 21 healthy) tested positive for SARS-CoV-2 infection within the same period. SARS-CoV-2 breakthrough infections were significantly more reported in healthy individuals compared with cancer patients (32.8% vs. 8.9%). No significant difference in the occurrence of breakthrough infections was observed between homologous and heterologous boosted subjects.

Heterologous BNT162b2 booster vaccination induces higher S1+S2 spike-specific $CD8^+$ T-cell reactivity

T-cell activity was assessed on PBMCs collected prior to and 28 days after third dose BNT162b2 vaccination for 142 patients with cancer. After quality control of sample viability and flow cytometry acquisition data, 56 and 54 patients were included in the homologous cohort for $CD4^+$ and $CD8^+$ T cells, respectively, whereas 41 and 42 patients were included for the heterologous cohort. Activation-induced markers were quantified for spike-specific $CD4^+$ (CD154 or CD40L, IFNγ,

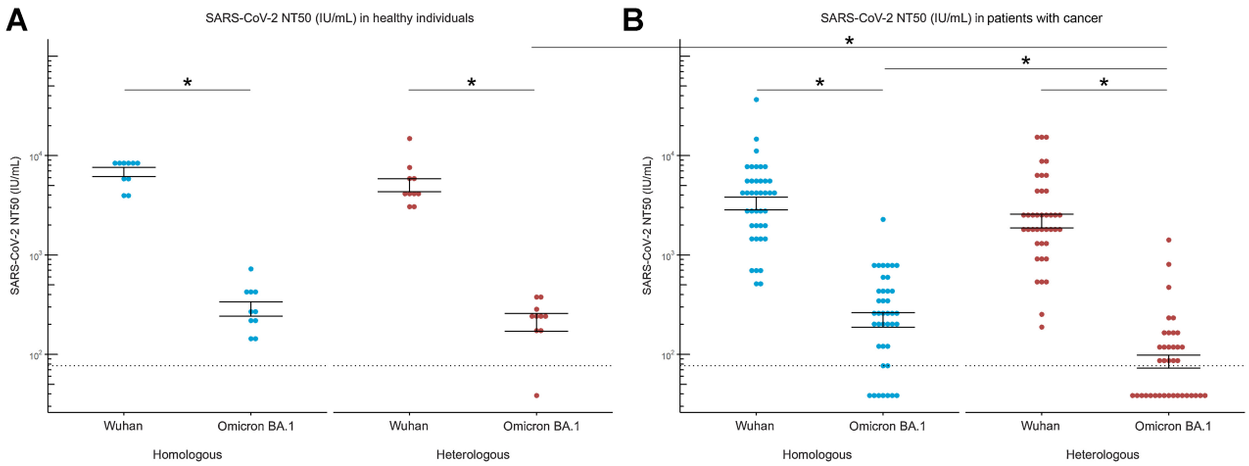

Figure 4.
Virus neutralization test in healthy individuals (**A**) and patients with cancer (**B**) with 50% neutralization titers (NT50), defined as the sample dilution (reciprocal titer) conveying 50% neutralization in SARS-CoV-2 (strains 2019-nCoV-Italy-INMI1 and VLD20211207) infected wells. *In vitro* virus neutralization test toward the SARS-CoV-2 Wuhan and BA.1 Omicron strains 28 days after homologous or heterologous boosting in healthy individuals (**A**) and patients with cancer (**B**). GMTs of the NT50 values of each cohort are depicted by a black point. The lower limit of detection (LLQ) is 77 IU/mL and is indicated with a dotted line. Values below the lower limit of detection (LLQ) are imputed to 38.5 IU/mL. Error bars indicate standard errors. A two-sided *P* value <0.05 after Bonferroni–Holm correction for multiple testing was considered statistically significant: *, $P < 0.05$.

IL2, and TNFα) and $CD8^+$ (CD137 or 4–1BB, IFNγ, IL2, and TNFα) T cells (Supplementary Fig. S3). No significant differences were observed for any activation marker of $CD4^+$ T cells between patients that received homologous and heterologous booster vaccination (**Fig. 5A**). Responses were always of the same magnitude with the median being 0.05 versus 0.032 for CD154, 0.033 versus 0.03 for IFNγ, 0.052 versus 0.042 for IL2, and 0.036 versus 0.03 for TNFα for homologous versus heterologous booster vaccination groups, respectively. Zooming in on the different subcohorts (Supplementary Fig. S4), only a significant lower response was observed in the hematology subcohort

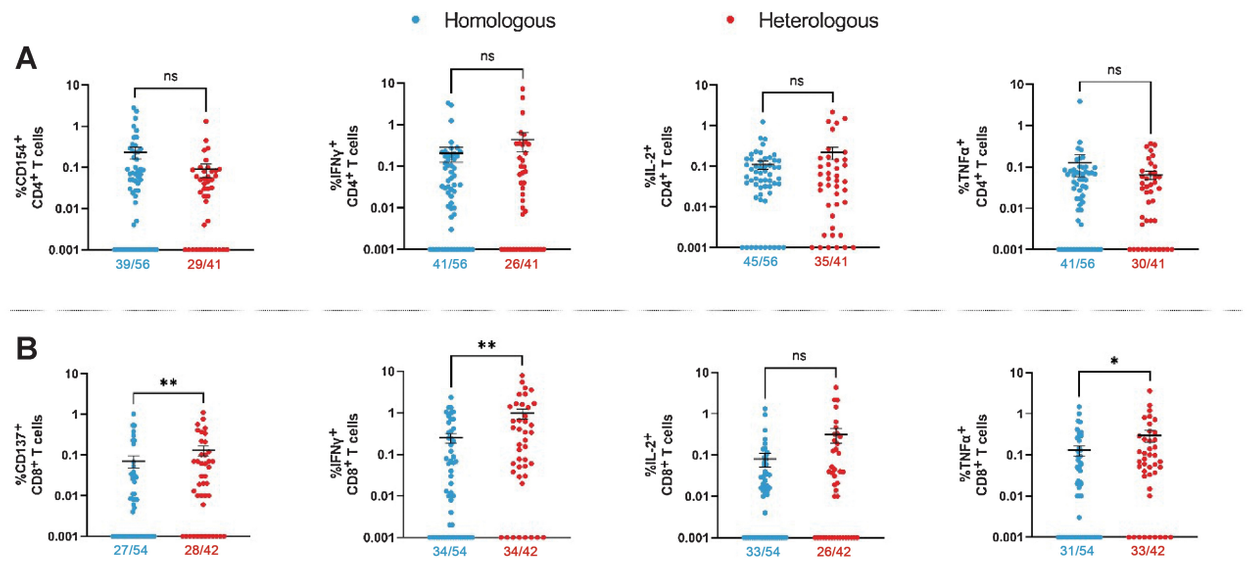

Figure 5.
Spike-specific $CD4^+$ and $CD8^+$ T-cell responses after homologous or heterologous booster vaccination. **A,** Percentage of CD154 (CD40L), IFNγ, IL2, and TNFα activation-induced markers of S1+S2 spike-specific $CD4^+$ T cells after deduction of negative (unstimulated) control. **B,** Percentage of CD137 (4–1BB), IFNγ, IL2, and TNFα activation-induced markers of S1+S2 spike-specific $CD8^+$ T cells after deduction of negative (unstimulated) control. Values below 0.001 are equaled to 0.001 as nonresponder (zero) threshold. Each dot represents a single patient. Numbers indicate the number of responders/total patients. Medians are compared using the Mann–Whitney *U* test after checking for normal distribution using the Shapiro–Wilk test (*, $P < 0.05$; **, $P < 0.01$; and n.s., not significant).

where the CD154 response to heterologous boosting was lower (median 0.130 vs. 0.038). Importantly, it was observed that 30% of patients in either vaccination scheme did not mount a $CD4^+$ T-cell response at all. Responses were detected in the majority of patients with cancer receiving B-cell depleting therapy, which did not show an antibody response.

In contrast to $CD4^+$ T-cell responses, a significant difference in CD8+ T cell response was observed in patients with cancer that received heterologous boosting, evidenced by a higher response of CD137 (median 0.003 vs. 0.025), IFNγ (median 0.017 vs. 0.197), and TNFα (median 0.018 vs. 0.069) activation markers (**Fig. 5B**). No difference was observed for the IL2 response (median 0.016 vs. 0.026). Analysis of the subcohorts revealed a significantly higher frequency after heterologous vaccination for IFNγ (median 0.001 vs. 0.833) and TNFα (median 0.020 vs. 0.234) response in the chemotherapy subcohort and for CD137 in the hematology subcohort (median 0.001 vs. 0.019; Supplementary Fig. S5). As for the $CD4^+$ T-cell responses, an even bigger proportion of patients (i.e., 50% and 33% for homologous and heterologous booster vaccination, respectively) showed no $CD8^+$ T-cell response at all, but the majority of patients receiving B-cell-depleting therapy also mounted a $CD8^+$ T-cell response.

To investigate a possible link between $CD4^+$ and $CD8^+$ T-cell responses with the SARS-CoV-2 IgG binding antibodies, correlations were investigated (Supplementary Fig. S6). Here we observed no correlation between $CD4^+$ and $CD8^+$ T cells responses, an no correlation between $CD8^+$ T cells and the amount of binding antibodies in the blood. However, a weak but significant correlation ($\rho = 0.23$) was observed between $CD4^+$ T cells and anti-S1 antibody titers. These findings persisted after exclusion of hematologic patients receiving B-cell–depleting therapy.

Discussion

Patients with cancer display reduced antibody responses after SARS-CoV-2 infection or double-dose BNT162b2 vaccination (1, 3). Emerging evidence from various studies shows that a third vaccination dose efficiently boosts immune responses and provides better protection against SARS-CoV-2 infection in patients with cancer (7–9, 10, 12, 13).

We are the first to compare the immune response after a BNT162b2 booster following different double-dose vaccination schedules in a patient with cancer population. Our study showed no significant difference for SARS-CoV-2 anti-S1 IgG antibody titers after BNT162b2 booster between ChAdOx1 and BNT162b2 primed individuals. This is an interesting observation since we previously reported significantly lower antibody responses after double-dose ChAdOx1 compared with BNT162b2, for the same cancer populations (4). Other studies observed that individuals receiving double-dose ChAdOx1 vaccination showed even lower antibody responses compared with double-dose BNT162b2 or heterologous double-dose vaccination (4, 17, 29). Hence, it seems that boosting with BNT162b2 resulted in an increased response for patients who first received ChAdOx1, reaching the same antibody levels as homologous boosted patients. Although, this increased response could be due to to lower antibody levels at start and/or the reaching of a plateau after three doses of BNT162b2, these findings support the idea that a third dose BNT162b2 has excellent boosting capabilities regardless of the type of double-dose vaccination.

The antibody levels after a third dose BNT162b2 observed in our study are similar to observations of Lasagna and colleagues (30) and Ehmsen and colleagues (31) for patients with cancer. We also confirm that both homologous and heterologous boosting elicited lower neutralizing antibodies against Omicron BA.1 compared with Wuhan. Other studies also showed that the current vaccines mount lower or even undetectable NT50 values against Omicron BA.1 compared with other viral variants (30, 32, 33). This can be explained by the highly mutated spike protein of the BA.1 Omicron variant which is related to antibody evasion and decreased protection by vaccination (32, 34, 35). Previously, higher NT50 values against different SARS-CoV-2 variants were observed after heterologous (mRNA) compared with homologous double-dose vaccination with a viral vector vaccine (14, 15). Other studies also observed lower NT50 values against BA.1 Omicron after heterologous boosting compared with homologous boosting (20, 36). Although no significant difference in NT50 values against the Wuhan strain was observed between both boosting regimes, NT50 values against Omicron were significantly lower after heterologous boosting compared with homologous boosting. Despite hinting towards a higher immunogenicity of mRNA vaccines against BA.1 Omicron, this observation could be a consequence of the sample selection. Because a strong correlation between neutralizing antibodies against Wuhan and BA.1 Omicron was observed for each treatment cohort, the 10 homologous and heterologous boosted patients with cancer that mounted the highest SARS-CoV-2 anti-S1 IgG antibody titers were selected for neutralizing antibody analysis to provide comparable cohorts and assure neutralizing antibody detection. Although the same selection principle was used, SARS-CoV-2 anti-S1 IgG antibody titers were significantly higher in the homologous boosted selection of patients with cancer compared with that of the heterologous boosted. Because it is known that binding antibody titers strongly correlate with neutralizing antibody titers (3, 30, 32, 33), this could be a possible explanation for our observation.

Our prospective study demonstrated an acceptable safety profile of a third dose BNT162b2 in patients with cancer. The most frequently reported AE after the administration of a third dose BNT162b2 was pain at the injection site. Other studies also described local pain as a frequently, but temporary, occurring AE (5, 20, 30, 37). Homologous and heterologous boosting have a similar safety profile, but local pain and swelling were more frequently reported after heterologous boosting in patients with cancer. It has already been reported that reactogenicity is higher after heterologous compared with homologous double-dose COVID-19 vaccination (16–18). Although these findings were only reported in studies that investigated homologous versus heterologous vaccination in a regime of two doses, increased reactogenicity after heterologous compared with homologous boosting in a three doses vaccination is therefore not unexpected.

No difference in the amount of breakthrough infections between both boosting regimes was observed (38). Because the study was not powered to differentiate breakthrough infections, it cannot be stated whether heterologous or homologous boosting provides better protection against SARS-CoV-2 infections. Not surprisingly, the number of breakthrough infections increased when the Omicron BA.1 variant became dominant. In the entire study population, NT50 values against BA.1 Omicron were 10 to 30 times lower compared with the Wuhan strain, resulting in decreased protection against this specific viral strain. Other studies reporting on breakthrough infections after different boosting regimens did not observe significant differences between homologous and heterologous boosting (19, 39).

T-cell responses play an important role in antiviral immunity with, upon activation, $CD8^+$ T cells that produce cytokines which limit viral reproduction and kill infected cells directly. Also $CD4^+$ T cells, which become T helper 1 (Th1) cells upon viral peptide recognition, produce cytokines which limit viral reproduction and

support CD8$^+$ T-cell and B-cell responses (40). Therefore, eliciting adequate T-cell responses is crucial for protection against viral infections. Nevertheless, the exact role of the importance of T-cell responses in the context of SARS-CoV-2 still has multiple outstanding questions (41). Data on T-cell responses after different SARS-CoV-2 vaccination regimens has been gathered but is rather scattered and often includes only a small number of subjects. Moreover, data focusing on the immune response of patients with cancer is even more scarce, especially concerning responses to third dose vaccination, where to our knowledge, only Rouhani and colleagues and Oostling and colleagues described this with solely mRNA-based vaccine schemes (38, 42). Therefore, spike-specific CD4$^+$ and CD8$^+$ T cells responses were investigated after the two different vaccination schemes most widely used on the European continent. Our results indicate no difference between both vaccination regimens concerning CD4$^+$ T-cell responses, which is in full accordance with data from Vogel and colleagues, although their more limited dataset concerns healthy individuals (43). Regarding the magnitude of the response, it is hard to make direct comparisons since different assays are often used (i.e., ELISPOT versus IFNγ ELISA vs. flow cytometry). Despite this limitation, the responses observed in our homologous boosted cancer population seem to be slightly lower than in healthy individuals with equally assessed T-cell activity. Few studies evaluated T-cell activity following mRNA-1273, Ad26.COV2.S, and BNT162b2 vaccination but did not include double-dose ChadOx1 vaccinated individuals (20, 44). When looking at the number of responders across those studies, it is clear that—independent of the vaccination regimen—patients with cancer have a higher proportion of individuals displaying no CD4$^+$ T-cell response, even after third dose (20, 43, 45). This might be because of the often immunosuppressed status of patients with cancer or their treatment which affects responses to vaccination, something also observed by others after double-dose vaccination (46). When looking at the CD8$^+$ T cells, a significantly higher spike-specific response was observed after heterologous boosting compared with homologous, which is in accordance with findings observed in healthy individuals and mice (47–50). Remarkably, both magnitude as well as the number of responders were rather equal when compared with healthy individuals (20, 43, 45). Whether this higher CD8$^+$ T-cell response was also of clinical significance, is a conclusion that cannot be drawn from our study. Larger, ongoing studies might shed a new light on this topic (51). Another important observation is that patients who received anti-CD20 therapy and thus displayed no antibody response, did show a good CD4$^+$ and/or CD8$^+$ T-cell response in the majority of cases, as also reported by others (42, 52). This is an important feature for these specific sets of patients, which still might have a layer of protection against SARS-CoV-2 despite their treatment.

Finally, we observed no correlation between the level of CD4$^+$ or CD8$^+$ T-cell responses and the SARS-CoV-2 IgG binding antibodies, similar to what Kared and colleagues demonstrated, although their samples originated from healthy individuals and different vaccination schemes were not included (53). These indicate that having no or low antibody response does not mean that a T-cell response is also lacking. Given the importance of having more than one layer of immunologic protection against SARS-CoV-2 (i.e., humoral and cellular protection), this finding underscores the importance to also monitor T-cell responses in often immune-compromised patient populations as we did. Our finding, that there is no link between the amount of spike-specific CD4$^+$ and CD8$^+$ T cells, also points into the direction that often at least one of both spike-specific T-cell subsets is present, which each might pose a layer of protection against the virus. It is not certain whether this is also the case in other patient populations and healthy individuals, because to the best of our knowledge, no data are available. As it is more and more recognized that cellular immunity plays an important role in the protection against and control of a SARS-CoV-2 infection, including new viral variants, this is a reassuring observation for the majority of patients with cancer. However, it also underscores that a significant portion of patients with cancer with no cellular response, could remain at high risk for SARS-CoV-2 infection. Identifying these patients could thus be highly clinically relevant (51).

The strength of this study is that we analyzed SARS-CoV-2 binding and neutralizing antibodies in a large and well-defined population, which included both patients with cancer and healthy individuals, thus providing representation of the humoral immune response against SARS-CoV-2. Moreover, this study provides new insight into the neutralizing capacity against the Omicron BA.1 variant, which has not been monitored frequently (38). Furthermore, we compared cellular immunity for two third dose vaccination schedules in a large cohort of patients with cancer. In-depth longitudinal T-cell analysis is warranted to obtain better insight in SARS-CoV-2-specific T-cell responses and possible correlations between CD4$^+$/CD8$^+$ T cells and humoral immunity, induced by different vaccination schedules in both healthy and diseased individuals. We acknowledge that more control groups are needed for direct comparisons between homologous and heterologous boosting. A trial where different types of booster vaccines are administered would provide additional insights on the possible advantage of heterologous boosting. In this study, humoral immunity, including neutralizing antibodies, and cellular immunity were compared for the first time after homologous and heterologous boosting in a large cancer population, providing data to support COVID-19 vaccination strategies in cancer and other immunocompromised patients.

Conclusions

A third dose BNT162b2 after double-dose BNT162b2 or ChAdOx1 vaccination has an acceptable safety profile in patients with cancer. A third dose BNT162b2 can mount a good antibody response in patients with cancer irrespective whether the double-dose vaccination was BNT162b2 or ChAdOx1. Even despite three vaccination doses, some cancer patients still cannot mount a detectable antibody, CD4$^+$ or CD8$^+$ T-cell response, leaving these patients at a possibly higher risk for SARS-CoV-2 infection. Although a true serologic correlate of protection is not yet established, a heterologous BNT162b2 booster is seems to mitigate the impaired humoral immune response that was mounted after double-dose ChAdOx1 compared with double-dose BNT162b2 vaccination in patients with cancer.

Authors' Disclosures

Y. Debie reports grants from Sciensano and Kom op tegen Kanker during the conduct of the study. J.R.M. Van Audenaerde reports grants from Kom op tegen Kanker and Sciensano during the conduct of the study. T. Vandamme reports nonfinancial support from AstraZeneca outside the submitted work. L. Verbruggen reports grants from Kom op tegen Kanker and Sciensano during the conduct of the study. D. Le Blon reports grants from Sciensano and Kom op tegen Kanker during the conduct of the study. M.E. Goossens reports grants from Belgian Government during the conduct of the study. E.L.J. Smits reports grants from Kom op tegen Kanker and Sciensano during the conduct of the study. C. Vulsteke reports personal fees from Janssen-Cilag, Roche, GSK, MSD, BMS, Pfizer, AstraZeneca, and Bayer outside the submitted work. M. Peeters reports grants from Sciensano during the conduct of the study. No disclosures were reported by the other authors.

Authors' Contributions

Y. Debie: Data curation, formal analysis, investigation, visualization, writing–original draft, project administration, writing–review and editing. **J.R.M. Van Audenaerde:** Data curation, formal analysis, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **T. Vandamme:** Conceptualization, resources, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **L. Croes:** Data curation, investigation, writing–review and editing. **L.-A. Teuwen:** Investigation, visualization, writing–review and editing. **L. Verbruggen:** Conceptualization, funding acquisition, methodology, project administration. **G. Vanhoutte:** Conceptualization, funding acquisition, methodology, writing–review and editing. **E. Marcq:** Investigation, writing–review and editing. **L. Verheggen:** Data curation, project administration, writing–review and editing. **D. Le Blon:** Investigation, writing–review and editing. **B. Peeters:** Formal analysis, investigation, writing–review and editing. **M.E. Goossens:** Resources, funding acquisition, writing–review and editing. **P. Pannus:** Investigation, writing–review and editing. **K.K. Ariën:** Formal analysis, investigation, writing–review and editing. **S. Anguille:** Supervision, investigation, writing–review and editing. **A. Janssens:** Investigation, writing–review and editing. **H. Prenen:** Supervision, investigation, writing–review and editing. **E.L.J. Smits:** Supervision, investigation, writing–review and editing. **C. Vulsteke:** Supervision, investigation, writing–review and editing. **E. Lion:** Resources, supervision, investigation, project administration, writing–review and editing. **M. Peeters:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing–review and editing. **P.A. van Dam:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, writing–review and editing.

Acknowledgments

We kindly thank the B-VOICE and Tri-VOICE plus patients and HEAL-V participants for participation; the nursing staff members at the Day Care Unit of the Antwerp University Hospital and AZ Maria Middelares; and the staff members of the Biobank Antwerp and all recruiting physicians: Sevilay Altintas, Zwi Berneman, Sarah Debussche, Charlotte De Bondt, Ximena Elzo-Kraemer, Veerle Galle, Félix Gremonprez, Konstantinos Papadimitriou, Jo Raskin, Marika Rasschaert, Kirsten Saevels, Wiebren Tjalma, Xuan Bich Trinh, Christophe Van Berckelaer, Jan Van den Brande, Ann Van de Velde and Anke Verlinden. We are grateful to Leo Heyndrickx, Johan Michiels, and Betty Willems for neutralising antibody testing, to Hans De Reu, Stefanie Peeters, Carole Faghel, Céline Merlin, and Ho Wa Lau for T-cell analysis, and to the clinical biology study team for performing serologic analysis. We are thankful to Silke Raats, Isolde Van der Massen, Sanne Wouters, and Sven De Keersmaecker for logistic support and coordination and to Abraham Lin for proofreading of the manuscript. In addition, we thank the B-VOICE, Tri-VOICE plus, and HEAL-V study teams for patient inclusion and sample collection. This work was supported by the Belgian Government through Sciensano (grant nos. COVID-19_SC004, COVID-19_SC059, COVID-19_SC061), Kom op tegen Kanker (KOTK_UZA/2020/12604/1), Methusalem (FFB150082, FFB200035), and UA core facility funding (KF120000). T. Vandamme is holder of Senior Clinical Investigator grant 1803723N of the Research Foundation - Flanders (Belgium) (FWO).

The publication costs of this article were defrayed in part by the payment of publication fees. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Received July 19, 2022; revised September 14, 2022; accepted November 2, 2022; published first November 3, 2022.

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