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

Humoral and cellular immune responses against SARS-CoV-2 after third dose BNT162b2 following double-dose vaccination with BNT162b2 versus ChAdOx1 in cancer patients

Running title: Heterologous vs homologous COVID-19 boosting in cancer patients

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Abstract

Cancer patients display reduced humoral responses after double-dose COVID-19 vaccination while their cellular response is more comparable to that in healthy individuals. Recent studies demonstrated that a third vaccination dose boosts these immune responses, both in healthy people and cancer patients. Due to 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 cancer patients. 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. 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 1755.90 BAU/mL [95% CI 1276.95-2414.48] vs 1495.82 BAU/mL (95% CI 1131.48-1977.46)). 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. In cancer patients who received double-dose ChAdOx1, a third heterologous dose of BNT162b2 was able to close the gap in antibody response.

Keywords

COVID-19 vaccination, BNT162b2, ChAdOx1, T cell, boosting dose, heterologous, homologous, oncological patients, cancer

Statement of translational relevance

Third vaccination doses against SARS-CoV-2 have been broadly administered, aiming to improve immunological 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. Due to their impaired immunity, vaccine-induced protection against symptomatic COVID-19 is less efficient in cancer patients. 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 cancer patients. 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 cancer patients. Our study supports the recommendation of a third dose BNT162b2 in cancer patients, irrespective of whether it constitutes a homologous or heterologous booster.

Background

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]. Due to immune incompetence, cancer patients were excluded from pivotal vaccine approval trials. BNT162b2 and ChAdOx1 were the most widely administrated vaccines on the European continent, also for cancer patients. The first studies evaluating the immunological outcomes of vaccinated cancer patients against COVID-19 demonstrated reduced humoral responses after double-dose BNT162b2 and even lower responses after double-dose ChAdOx1 vaccination, compared to 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 to homologous double-dose vaccination[14-18]. Recent studies took the first steps to gain knowledge about safety and immunological 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 to a homologous third dose[19,20]. Additionally, 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 cancer patients. In addition to the production of antiviral antibodies, the cellular 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 cancer patients.

Methods

Trial design and participants

In three parallel ongoing prospective COVID-19 vaccination studies, a third vaccination dose was given. A population of cancer patients 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 cancer patients was actively recruited to receive a third dose BNT162b2 after previous double-dose ChAdOx1 vaccination (Tri-VOICE plus) (Figure 1). The third study was conducted in a population of healthy staff members of the Antwerp University Hospital, without oncological history (HEAL-V). All healthy individuals received a third dose BNT162b2 between eight and nine months after the administration of the first dose BNT162b2 or ChAdOx1 (Figure 1). All participants signed informed consent and were aged 18 years or older with a life expectancy of at least six months. Pregnant or breastfeeding women and patients with an immune deficiency unrelated to cancer or cancer treatment were ineligible. Eligible patients were cancer patients with a solid tumor or hematological malignancies were divided into treatment cohorts (Figure 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 numbers 2021-000300-38 and 2021-003573-58 and EC numbers 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 cancer patients 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. Cancer patients received a third vaccination dose six to seven months after first dose administration, as described in the protocol. Healthy individuals received a third dose BNT162b2 eight to nine 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 cancer patients and healthy individuals, following the previously described protocol[3,28]. The subset of individuals was carefully selected in order 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 cancer patients 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 fetal bovine serum (FBS, Giboc, Thermofisher) 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 pre-warmed RPMI 1640 medium supplemented with 10% FBS, MEM Non-Essentiel Amino acid solution, L-Glutamine, penicillin and streptomycin (all from Gibco, Thermofisher). Next, 1 x 10^6 PBMCs were stimulated for six hours with 1µg/mL S1 and S2 spike specific peptide pools (JPT), 1µg/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 1µg/mL anti-CD28 beads to provide the required costimulatory signal. 1.5h post start of the incubation, 10µg/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 monoclonal antibodies: CD3-AF700, CD4-BV510, CD8-Pe-Cy7 as extracellular markers and CD137-BV605, CD154-BV421, IFNγ-AF488, TNFα-BV650, and IL-2-AF647 as intracellular markers (all from Biolegend). LIVE/DEAD™ Fixable Near-IR Dead Cell Stain Kit (Thermofisher) 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 10000.

Safety and breakthrough infections

An existing web-based electronic platform for toxicity telemonitoring, RemeCare Oncology, was used to assess patient-reported outcomes (PROs) about local vaccine reactions, systemic adverse events (AEs), 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 seven 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 cancer patients 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, this data were monitored up to four 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 post 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 patient-reported outcomes of local and systemic adverse events.

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 adverse events 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's 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.

Results

Demographics of study groups

Of the 164 cancer patients receiving a homologous booster, 92% received it six to seven months after first dose and 8% received it between seven and nine months. In another group, 151 cancer patients received a heterologous booster. In this group, 87% of the patients received the heterologous booster between six and seven months and 13% five to six months after first dose. A total of 127 healthy controls received a third dose BNT162b2 between eight and nine 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 Suppl. Tables 1 and 2. A total of 287 cancer patients and 125 healthy controls were evaluable 28 days after third dose administration. From the homologous group, two healthy individuals (3.0%) and nine patients (5.5%) had a PCR-confirmed SARS-CoV-2 infection before administration of the third dose. From the heterologous group, two healthy individuals (3.3%) and four 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.

Table 1: Demographics of the enrolled subjects that were evaluable 28 days after 3rd dose vaccination

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. Cancer patients were assigned to therapy cohorts based on type of treatment receiving when the first vaccination dose was administered. For patients with hematological 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 8th edition. Staging of hematological malignancies was not performed and therefore indicated as not applicable.

ECOG; Eastern Cooperative Oncology Group, NA; not applicable, SD; standard deviation.

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 hematological 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			

Cancer patients	Double-dose ChAdOx1 vaccination (N=138)	Double-dose BNT162b2 vaccination (N=149)	Overall (N=287)
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			
Auto immune 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]

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) (Figure 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 to homologous boosting (60.5% vs 46.9%; 19.1% vs 10.9%). Sub analysis 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) (Figure 2A). Although the occurrence of local pain and swelling was significantly different between homologous and heterologous cancer patients, 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 (Figure 2B). Cancer patients did not report significantly more AEs than healthy individuals.

Serious adverse events (SAEs) were only documented for the cancer patients 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, three patients were hospitalized because of disease progression, one 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 BNT162b2 vaccine. Two hematological patients receiving B-cell depleting therapy died because of COVID-19, one and four 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 geometric mean titer (GMT) of SARS-CoV-2 anti-S1 IgG antibodies was observed between homologous and heterologous boosted subjects (cancer patients + healthy individuals), 28 days post third dose {GMT 1755.90 BAU/mL [95% confidence interval (CI) 1276.95-2414.48] and 1495.82 BAU/mL (95% CI 1131.48-1977.46), respectively}. As expected, for both homologous and heterologous boosting, significantly lower anti-S1 IgG antibody levels were observed in cancer patients {GMT 1331.32 BAU/mL [95% CI 857.41-2067.29] and 1163.44 BAU/mL (95% CI 787.32-1719.24), respectively} compared to the healthy control group {GMT 3378.99 BAU/mL [95% CI 2789.50-4093.05] and 2616.93 BAU/mL (95% CI 2176.61-3146.33), respectively} (Figure 3A). The bimodal distribution of Figure 3A was mainly driven by cancer patients with hematological malignancies receiving B-cell depleting therapy.

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 cancer patients receiving different treatment types (chemotherapy, immunotherapy, immunotherapy + chemotherapy, targeted or hormonal therapy) and hematological patients not receiving B-cell depleting therapy (Figure 3B). On the contrary, hematological 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 to healthy individuals and all other patient cohorts.

Neutralizing antibodies: Wuhan vs 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 cancer patients and 20 healthy individuals. The subset of individuals was carefully selected in order 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 3813.41 IU/mL [95% CI 2960.54-4911.97] and 2586.43 IU/mL (95% CI 1950.54-3429.63), respectively}. Sub-analysis revealed that NT50 values against BA.1 were significantly lower after heterologous boosting compared to homologous boosting in cancer patients {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 cancer patients {GMT 2191.26 IU/mL [95% CI 1580.48-3038.07] vs 3297.73 IU/mL (95% CI 2450.44-4438.00)} and healthy individuals {GMT 5020.31 IU/mL [95% CI 3554.1-7091.39] vs 6818.76 IU/mL (95% CI 5352.46-8686.75)} (Figure 4). It was also observed that NT50 values against BA.1 Omicron were significantly lower in heterologous boosted cancer patients compared to 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 (ρ=0.74) and BA.1 Omicron (ρ=0.88) strains. NT50 values against Wuhan and BA.1 Omicron showed a strong correlation (ρ=0.79) (Suppl. Figure 1). Out of 80 tested subjects, 1 heterologous boosted healthy individual and 24 cancer patients (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 haematological malignancies, either receiving B-cell depleting therapy or other types of hematological cancer treatments. A detailed overview of the NT50 values per cohort can be found in Suppl. Figure 2.

Comparable occurrence of breakthrough infections

Data from the occurrence of breakthrough infections were collected in 412 out of 442 vaccinated individuals. Within four months after the third vaccination dose, 32 breakthrough infections (13 patients vs 19 healthy), were observed in homologous boosted subjects, while 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 to 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 cancer patients. 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, while 41 and 42 patients were included for the heterologous cohort. Activation-induced markers were quantified for spike-specific CD4+ (CD154 or CD40L, IFNγ, IL-2 and TNFα) and CD8+ (CD137 or 4-1BB, IFNγ, IL-2 and TNFα) T cells (Suppl. Figure 3). No significant differences were observed for any activation marker of CD4+ T cells between patients that received homologous and heterologous booster vaccination (Figure 5A). Responses were always of the same magnitude with the median being 0.05 vs 0.032 for CD154, 0.033 vs 0.03 for IFNγ, 0.052 vs 0.042 for IL-2 and 0.036 vs 0.03 for TNFα for homologous vs heterologous booster vaccination groups, respectively. Zooming in on the different sub-cohorts (Suppl. Figure 4), only a significant lower response was observed in the hematology sub-cohort 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 cancer patients 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 cancer patients 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 (Figure 5B). No difference was observed for the IL-2 response (median 0.016 vs 0.026). Analysis of the sub-cohorts 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 sub-cohort and for CD137 in the hematology sub-cohort (median 0.001 vs 0.019) (Suppl. Figure 5). 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 (Suppl. Figure 6). 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 (ρ=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

Cancer patients 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 cancer patients[7-9,12,13,29].

We are the first to compare the immune response after a BNT162b2 booster following different double-dose vaccination schedules in a cancer patient 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 to BNT162b2, for the same cancer populations[4]. Other studies observed that individuals receiving double-dose ChAdOx1 vaccination showed even lower antibody responses compared to double-dose BNT162b2 or heterologous double-dose vaccination[4,17,30]. 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 et al.[31] and Ehmsen et al.[32] for cancer patients. We also confirm that both homologous and heterologous boosting elicited lower neutralizing antibodies against Omicron BA.1 compared to Wuhan. Other studies also showed that the current vaccines mount lower or even undetectable NT50 values against Omicron BA.1 compared to other viral variants[31,33,34]. 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[33,35,36]. Previously, higher NT50 values against different SARS-CoV-2 variants were observed after heterologous (mRNA) compared to 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 to homologous boosting[20,37]. 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 to 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. Since a strong correlation between neutralizing antibodies against Wuhan and BA.1 Omicron was observed for each treatment cohort, the 10 homologous and heterologous boosted cancer patients 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 cancer patients compared to that of the heterologous boosted. Since it is known that binding antibody titers strongly correlate with neutralizing antibody titers[3,31,33,34], this could be a possible explanation for our observation.

Our prospective study demonstrated an acceptable safety profile of a third dose BNT162b2 in cancer patients. 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[5,20,31,38]. Homologous and heterologous boosting have a similar safety profile, but local pain and swelling were more frequently reported after heterologous boosting in cancer patients. It has already been reported that reactogenicity is higher after heterologous compared to 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 to 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[39]. Since 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 10-30 times lower compared to 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[19,40].

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[41]. 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[42]. 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 cancer patients is even more scarce, especially concerning responses to third dose vaccination, where to our knowledge, only Rouhani et al. and Oostling et al. described this with solely mRNA-based vaccine schemes[39,43]. 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 et al., although their more limited dataset concerns healthy individuals[44]. 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 versus 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[20,45]. When looking at the number of responders across those studies, it is clear that –

independent of the vaccination regimen – cancer patients have a higher proportion of individuals displaying no CD4+ T cell response, even after third dose[20,44,46]. This might be because of the often immunosuppressed status of cancer patients or their treatment which affects responses to vaccination, something also observed by others after double-dose vaccination[47]. When looking at the CD8+ T cells, a significantly higher spike-specific response was observed after heterologous boosting compared to homologous, which is in accordance with findings observed in healthy individuals and mice[48-51]. Remarkably, both magnitude as well as the number of responders were rather equal when compared to healthy individuals[20,44,46]. 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[52]. 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[43,53]. 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 et al. demonstrated, although their samples originated from healthy individuals and different vaccination schemes were not included[54]. This indicates 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 immunological 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, since to the best of our knowledge, no data is available. As it is more and more recognised 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 cancer patients. However it also underscores that a significant portion of cancer patients with no cellular response, could remain at high risk for SARS-CoV-2 infection. Identifying these patients could thus be highly clinically relevant[52].

The strength of this study is that we analysed SARS-CoV-2 binding and neutralising antibodies in a large and well-defined population that included both cancer patients and healthy individuals, thus providing representation of the humoral immune response against SARS-CoV-2. Moreover, this study provides new insight into the neutralising capacity against the Omicron BA.1 variant, which has not been monitored frequently[39]. Furthermore, we compared cellular immunity for two third dose vaccination schedules in a large cohort of cancer patients. 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 cancer patients. A third dose BNT162b2 can mount a good antibody response in

cancer patients irrespective whether the double-dose vaccination was BNT162b2 or ChAdOx1. Even despite three vaccination doses, some cancer patients still can't 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 serological 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 to double-dose BNT162b2 vaccination in cancer patients.

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Figure Legends

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. Cancer patients with a solid tumor were divided in to three treatment cohorts: receiving chemotherapy (cohort A), immunotherapy (cohort B) and targeted therapy or hormonal therapy (cohort C). Patients with hematological malignancies were assigned to cohort D. In cohort D, a differentiation was made between patients receiving B-cell depleting therapy and patients receiving other hematological cancer treatments.

Figure 2: Local and systemic adverse events (AEs) reported within seven days after homologous and heterologous boosting in cancer patients 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 cancer patients. 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 were 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's 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*

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 cancer patients.

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 geometric mean titer (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 10.90-16350.00 BAU/mL. Values below the detection were imputed half of it (5.45 BAU/mL), values above the measuring interval were imputed 33% above the upper limit of detection (21800 BAU/m)L with dotted line indicating LLQ and ULQ, respectively. *p < 0.05 with homologous boosted cancer patients receiving B-cell depleting therapy. , #p < 0.05 with heterologous boosted cancer patients receiving B-cell depleting therapy,##p < 0.05 with heterologous boosted cancer patients receiving other hematological cancer treatments.*

Figure 4: Virus neutralization test in healthy individuals (A) and cancer patients (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 towards the SARS-CoV-2 Wuhan and BA.1 Omicron strains 28 days after homologous or heterologous boosting in healthy individuals (A) and cancer patients (B). Geometric mean titers (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. I 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*

Figure 5: Spike-specific CD4+ and CD8+ T cell responses post homologous or heterologous booster vaccination.

*A percentage of CD154 (CD40L), IFNγ, IL-2, 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, IL-2 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 non-responder (zero) threshold. Each dot represents a single patient. Numbers indicate the number of responders/total patients. Each dot represents a single patient. 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. indicates not significant.*

Figure 1

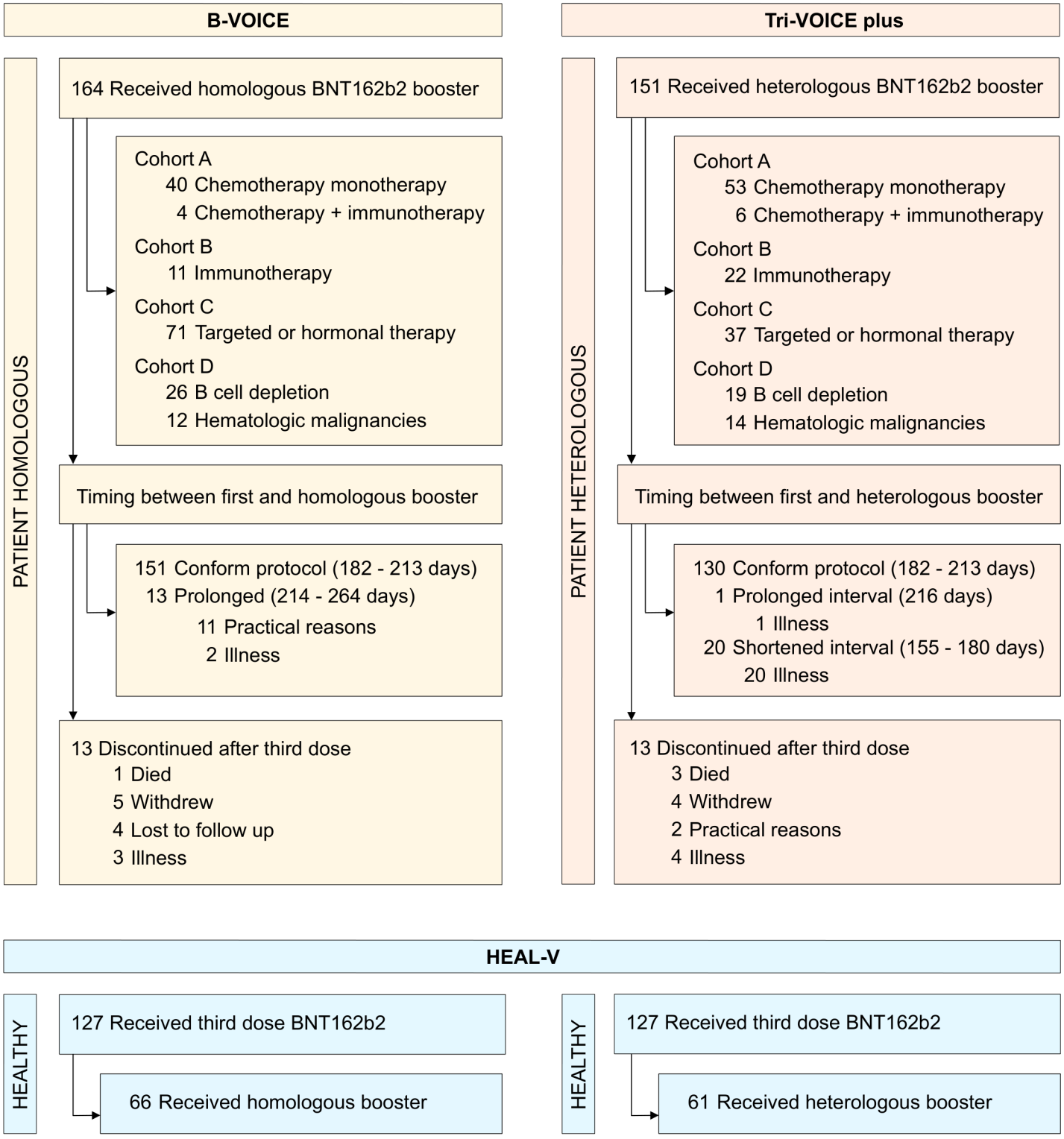
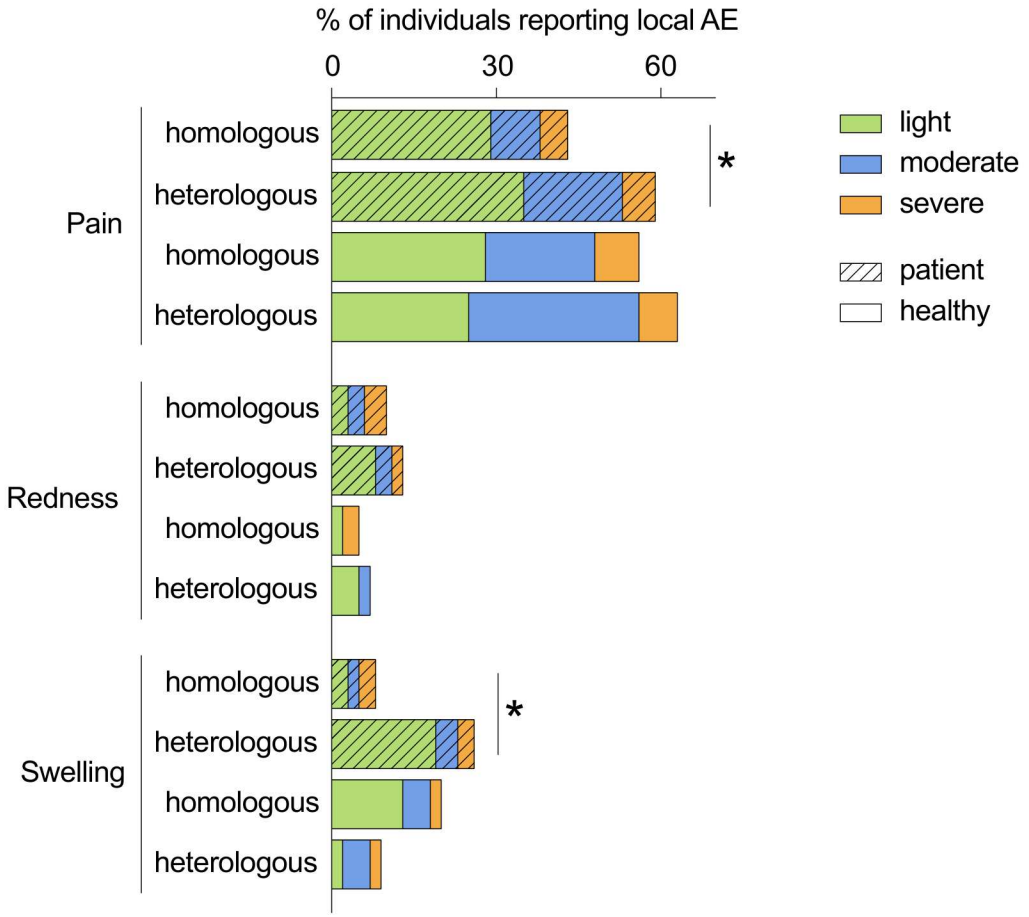
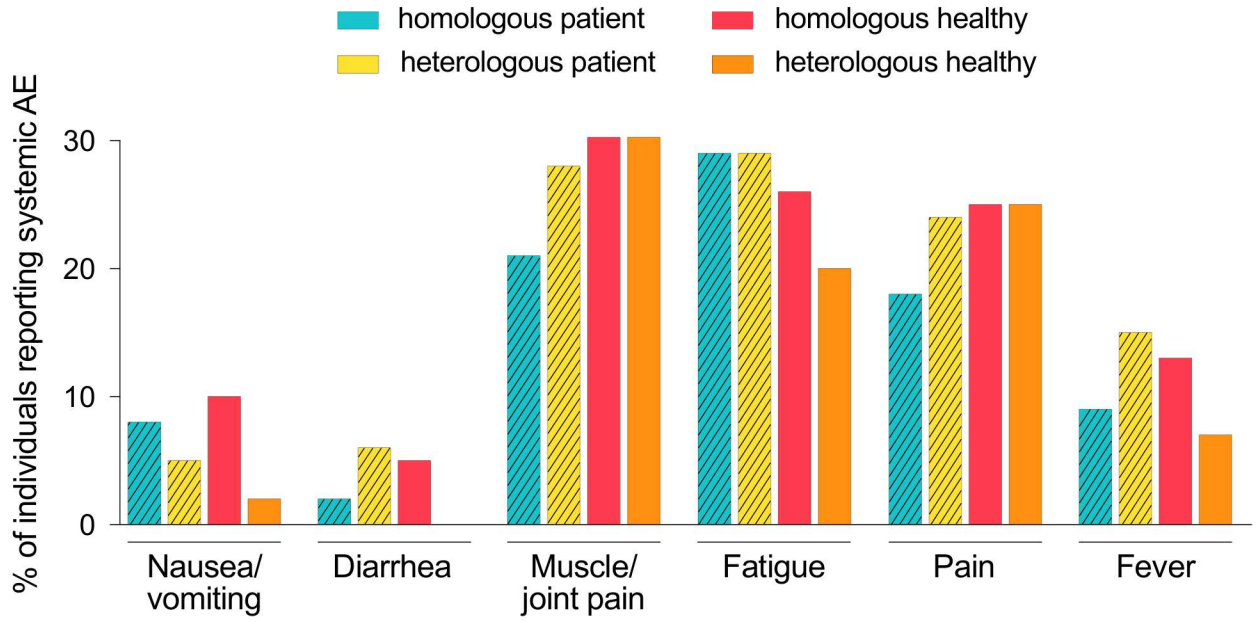

B-VOICE
Tri-VOICE plus
PATIENT HOMOLOGOUS
164 Received homologous BNT162b2 booster
Cohort A
40 Chemotherapy monotherapy
4 Chemotherapy + immunotherapy
Cohort B
11 Immunotherapy
Cohort C
71 Targeted or hormonal therapy
Cohort D
26 B cell depletion
12 Hematologic malignancies
Timing between first and homologous booster
151 Conform protocol (182 - 213 days)
13 Prolonged (214 - 264 days)
11 Practical reasons
2 Illness
13 Discontinued after third dose
1 Died
5 Withdrew
4 Lost to follow up
3 Illness
PATIENT HETEROLOGOUS
151 Received heterologous BNT162b2 booster
Cohort A
53 Chemotherapy monotherapy
6 Chemotherapy + immunotherapy
Cohort B
22 Immunotherapy
Cohort C
37 Targeted or hormonal therapy
Cohort D
19 B cell depletion
14 Hematologic malignancies
Timing between first and heterologous booster
130 Conform protocol (182 - 213 days)
1 Prolonged interval (216 days)
1 Illness
20 Shortened interval (155 - 180 days)
20 Illness
13 Discontinued after third dose
3 Died
4 Withdrew
2 Practical reasons
4 Illness
HEAL-V
HEALTHY
127 Received third dose BNT162b2
66 Received homologous booster
HEALTHY
127 Received third dose BNT162b2
61 Received heterologous booster

Figure 2

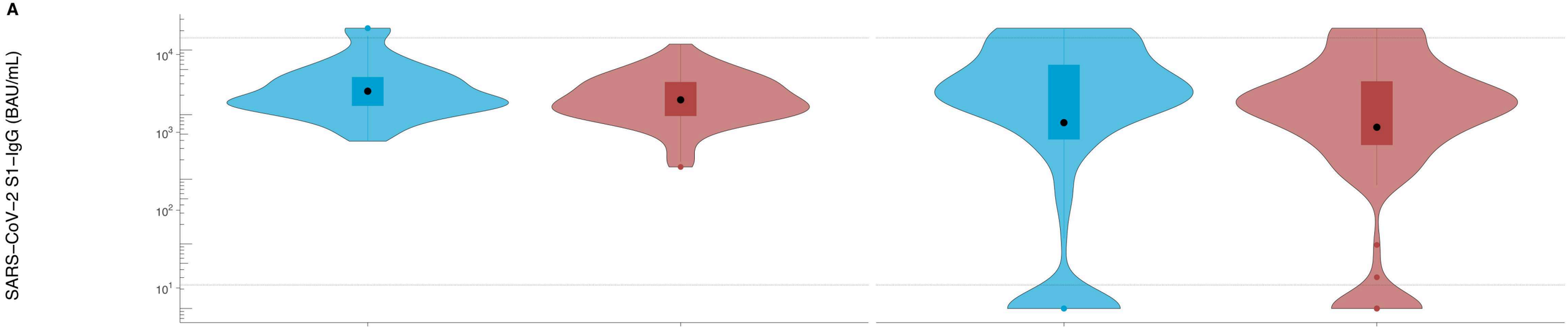
A


% of individuals reporting local AE
0
30
60
Pain
Redness
Swelling
homologous
heterologous
homologous
heterologous
homologous
heterologous
homologous
heterologous
homologous
heterologous
homologous
heterologous
*
*
light
moderate
severe
patient
healthy

B


homologous patient
heterologous patient
homologous healthy
heterologous healthy
% of individuals reporting systemic AE
0
10
20
30
Nausea/ vomiting
Diarrhea
Muscle/ joint pain
Fatigue
Pain
Fever

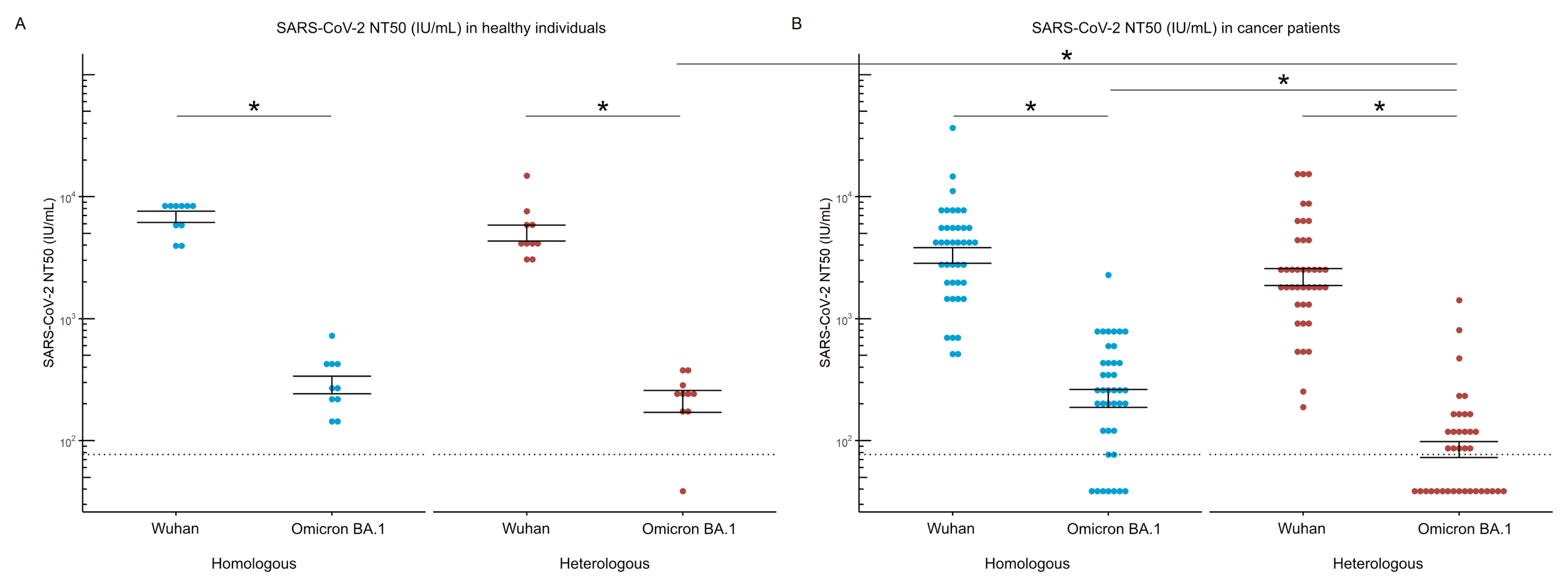
A

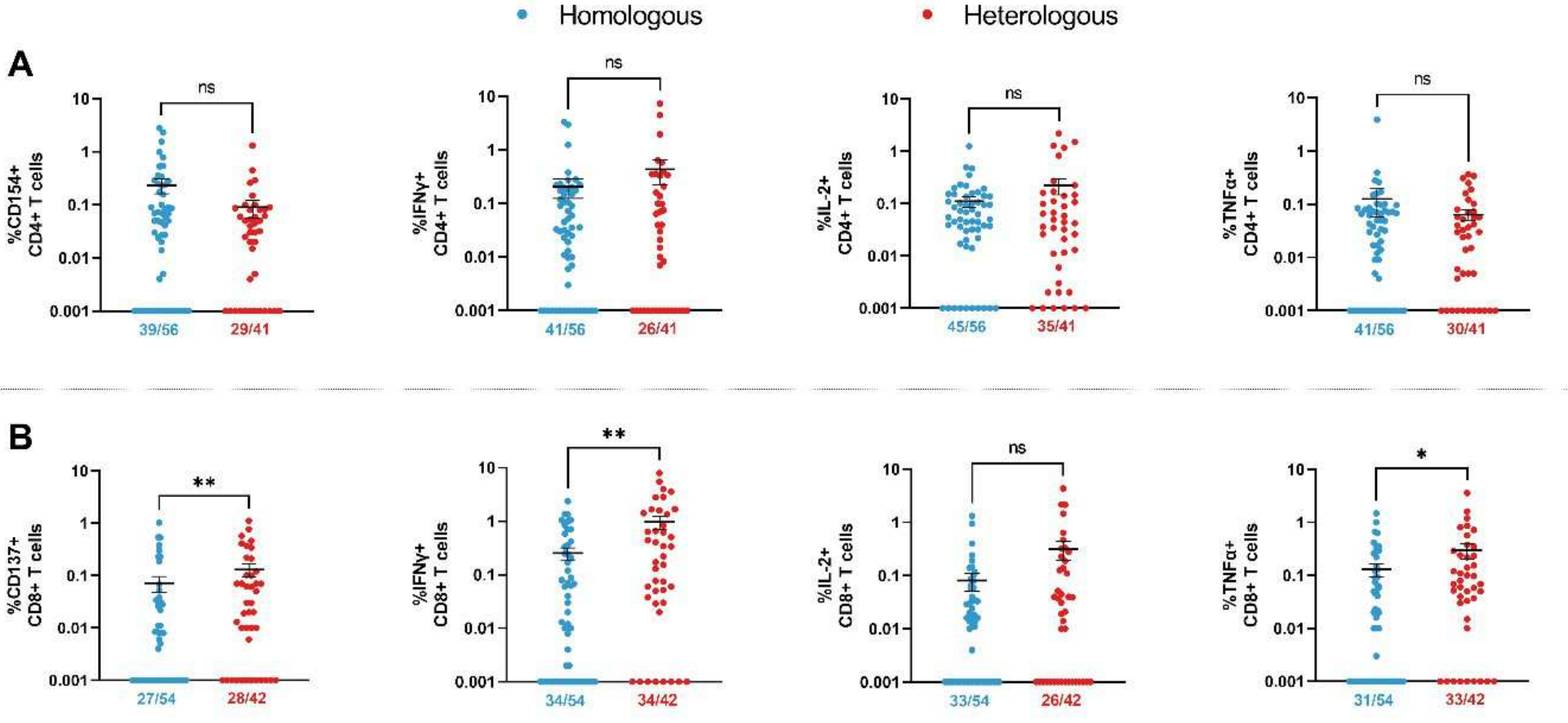


	Healthy		Cancer patients	
At risk	63	62	149	138
GMT	3379	2617	1331	1163
% IgG seroconversion	100	100	83	88

B

	Healthy		Targeted/Hormone therapy		Immunotherapy		Immunotherapy + chemotherapy		Chemotherapy		Other hematologic cancer treatments		B cell depletion	
At risk	63	62	66	32	10	21	4	4	33	48	11	14	25	19
GMT	3379	2617	4112	3164	3614	1114	3416	4275	2895	2893	4450	671	8	26
% IgG seroconversion	100	100	98	100	100	100	100	100	97	98	100	86	8	32


A
SARS-CoV-2 NT50 (IU/mL) in healthy individuals
SARS-CoV-2 NT50 (IU/mL)
10^4
10^3
10^2
*
*
Wuhan
Omicron BA.1
Wuhan
Omicron BA.1
Homologous
Heterologous
B
SARS-CoV-2 NT50 (IU/mL) in cancer patients
SARS-CoV-2 NT50 (IU/mL)
10^4
10^3
10^2
*
*
*
*
Wuhan
Omicron BA.1
Wuhan
Omicron BA.1
Homologous
Heterologous


Homologous
Heterologous
A
%CD154+ CD4+ T cells
%IFNγ+ CD4+ T cells
%IL-2+ CD4+ T cells
%TNFα+ CD4+ T cells
ns
39/56
29/41
41/56
26/41
45/56
35/41
41/56
30/41
B
%CD137+ CD8+ T cells
%IFNγ+ CD8+ T cells
%IL-2+ CD8+ T cells
%TNFα+ CD8+ T cells
**
*
27/54
28/42
34/54
34/42
33/54
26/42
31/54
33/42