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Aberrant B cell differentiation with reduced hypermutation limits humoral response maintenance and breadth in cancer patients

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Abstract

Patients with solid cancers exhibit impaired humoral immunity, yet the underlying B cell–intrinsic mechanisms remain incompletely characterized. Here, we leveraged longitudinal SARS-CoV-2 vaccination as a model of defined antigen exposure to dissect B cell responses in cancer patients. Despite preserved early expansion following booster vaccination, cancer patients failed to sustain long-term durability and variant breadth of antigen-specific B cell responses. This defect was associated with reduced resting-memory (CD21⁺CD27⁺) and germinal center–derived (CD27⁺IgG⁺) subsets, along with a shift toward double-negative 2/3 populations. Single-cell transcriptomic and B cell receptor (BCR) repertoire analyses revealed reduced somatic hypermutation across antigen-specific B cell subsets and enrichment of a transcriptionally distinct atypical T-bet⁺ B cell cluster in cancer patients, collectively indicating impaired germinal center-associated maturation as a central B cell–intrinsic defect. Notably, a fourth vaccine dose restored long-term B cell maintenance, supporting proactive booster vaccination strategies for cancer patients.

Introduction

Memory B cells play a central role in humoral immunity by enabling long-term protection, rapid recall responses, and adaptation to antigenic variation following antigen exposure. Although vaccination induces rapid B cell activation and expansion, durable protection depends on the establishment of memory B cells that can persist over time and respond to evolving pathogens¹. Disruption of these processes can compromise the maintenance of antigen-specific B cells and limit the breadth and effectiveness of recall responses, even when early expansion appears intact.

Patients with solid cancers represent a clinically important setting in which humoral immune responses are frequently impaired. Although reduced antibody responses following vaccination have been well documented in this population, including after SARS-CoV-2 vaccination²⁻⁹, and other vaccines such as influenza¹⁰, the underlying B cell-intrinsic perturbation remains incompletely understood. Tumor-associated immune dysregulation is characterized by chronic inflammation, altered cytokine milieu, and systemic immune remodeling¹¹, all of which may influence B cell activation and differentiation. In particular, it remains unclear how cancer-associated immune environments affect the differentiation trajectories of B cells and whether such alterations contribute to impaired durability of vaccine-induced immunity.

To address this question, we performed a comparative analysis of B cell and antibody responses in cancer patients and healthy controls following repeated antigen exposure through SARS-CoV-2 vaccination, using longitudinally collected peripheral blood samples. Notably, despite comparable expansion after booster vaccination, cancer patients exhibited a marked defect in the long-term maintenance and variant recognition of antigen-specific B cells. To investigate the underlying mechanisms, we

88 integrated phenotypic, functional, and single-cell transcriptomic analyses with clinical
89 data and revealed distinct alterations in B cell differentiation programs associated with
90 reduced durability. Together, these findings provide mechanistic insight into how
91 cancer-related immune remodeling constrains the persistence and breadth of vaccine-
92 induced humoral immunity in this population.

Materials and Methods

Study participants

We collected longitudinal peripheral blood samples following the second, third, or fourth vaccination (Supplementary Fig. 1). All cancer patients were recruited from Yonsei Cancer Center, Seoul, Korea. This cohort included male and female patients with solid malignancies undergoing active anti-cancer therapy, aged 19 or older, and with no prior SARS-CoV-2 infection. Exclusion criteria included hematologic malignancies, no anti-cancer therapy, neutrophil counts below 1,000/ μ L, life expectancy under three months, and lack of informed consent. Healthy controls in the vaccination cohort were healthcare workers from Severance Hospital, Samsung Medical Center, and Kyungpook National University Hospital, aged 19 or older, and with no prior SARS-CoV-2 infection. All samples were negative for anti-nucleocapsid (anti-N) antibodies using the Elecsys Anti-SARS-CoV-2 N assay (Roche Diagnostics, 9203079190 and 9216928190). Demographic details, including age, sex, tumor type, and treatment status, are provided in Supplementary Table 1.

This study was approved by the institutional review board of KAIST (KH2023-084), Severance Hospital (4-2020-1377, 4-2021-0478, 4-2023-0884), Samsung Medical Center (2021-01-165), and Kyungpook National University Hospital (2021-02-002) and conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all participants.

Flow cytometric analysis of SARS-CoV-2-specific B cells

To characterize the SARS-CoV-2 antigen-specific B cells, antigen probes

specific to WT spike, WT RBD, BA.1 RBD, BA.5 RBD, EG.5 RBD, and BA.2.86 RBD were produced. Samples were analyzed using FACSymphony A3 and A5 Cell Analyzers (BD Biosciences), and data were analyzed by FlowJo software (Supplementary Methods).

IgG ELISpot assay for antigen-specific memory B cells

PBMCs were stimulated with R848 and IL-2 for 72 hours, washed, and plated onto anti-IgG-coated ELISpot plates. After 16 hours of incubation, antigen-specific IgG-secreting cells were detected using biotinylated WT and BA.1 RBD antigens, followed by streptavidin-alkaline phosphatase and substrate development. Spots were enumerated using an automated ELISpot reader (Supplementary Methods).

Single-cell RNA sequencing and library preparation

Cryopreserved PBMCs from 11 healthy controls and 13 patients with solid cancers were thawed in RPMI supplemented with 10% fetal bovine serum and DNase I. Switched memory B cells were enriched using a human Memory B Cell Isolation Kit (Miltenyi Biotec, 130-093-617). Non-target cells obtained during enrichment were separately collected and subjected to whole-exome sequencing (WES) to enable genotype-based demultiplexing (Supplementary Fig. 4a). Detailed descriptions of the cell sorting strategy, library preparation, and downstream scRNA-seq and BCR-seq data processing are provided in the Supplementary Methods.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA was performed to quantify antibodies binding to various SARS-CoV-2 receptor-binding domain (RBD) proteins, including WT (Wuhan-Hu-1) RBD, Delta

(B.1.617.2) RBD, BA.1 (B.1.1.529) RBD, BA.5 (or BA.4) RBD, BA.2.86 RBD, and BQ.1 RBD (SPD-C5243) (Supplementary Methods).

SARS-CoV-2-pseudotyped reporter virus neutralization assay

Pseudoviruses expressing the spike protein of SARS-CoV-2 WT, Delta, Omicron BA.1, and BA.5 variants were generated through the transfection of Lenti-X 293T cell lines with lentiviral vectors. Neutralization capacity was measured by infecting hACE2 (human angiotensin-converting enzyme 2) 293T cells with a mixture of serially diluted plasma and the pseudovirus, and 50% neutralizing titer (NT₅₀) values were calculated (Supplementary Methods).

Statistical analysis

Statistical analyses were performed using GraphPad Prism V10.1.1. Our B cell and antibody data showed a non-normal distribution confirmed by the Shapiro-Wilk test, and thus, non-parametric methods were used. For comparison between different groups and between time points, the Mann-Whitney U test was used for unpaired analysis and proportions were compared using Fisher's exact test. Correlation analyses were performed using Spearman's correlation, and best-fit lines were drawn using simple linear regression. P-values <0.05 were considered significant.

Results

Durability and cross-reactivity of B cells induced by SARS-CoV-2 vaccination are impaired in cancer patients

We first investigated whether patients with solid cancers could mount a sufficient and sustained B cell response following two or three doses of SARS-CoV-2 vaccination compared to healthy controls (Fig. 1a and Supplementary Fig. 1). WT Spike⁺ and receptor-binding domain (RBD)⁺ B cells were identified by tetramer staining (Supplementary Fig. 2a). Demographic and clinical characteristics of the study participants are provided in Table 1 and Supplementary Table 1.

After two vaccine doses, WT Spike⁺ B cell counts were comparable between cancer patients and healthy controls (Fig. 1b). One month after the third dose, WT Spike⁺ B cells were robustly boosted in both groups to similar levels. However, while WT Spike⁺ B cell counts were maintained through six months in healthy controls, cancer patients exhibited a marked decline at three and six months, resulting in significantly lower WT Spike⁺ B cell counts at both time points. Importantly, despite the difference in the mean age between cohorts, the observed impairment in durability was not attributable to age. When analyses were focused on individuals aged 40-60 years, the reduced persistence of WT Spike⁺ B cells in cancer patients remained evident (Supplementary Fig. 2b). Consistently, multiple regression analysis adjusting for age and sex identified cancer status as the primary determinant of lower WT Spike⁺ B cell counts at three- and six-month post-vaccination (Supplementary Fig. 2c).

Consistent with the pattern observed in WT Spike⁺ B cells, the RBD⁺ compartment also exhibited impaired durability in cancer patients. After two vaccine

doses, WT RBD⁺ B cell counts were already significantly lower at one month in cancer patients compared to healthy controls (Fig. 1c), in agreement with previous reports⁵. This deficit was more pronounced for WT/BA.1 RBD⁺ and WT/BA.5 RBD⁺ cross-reactive B cells, where nearly half of the cancer patients exhibited undetectable B cell responses (Fig. 1d).

Following the third booster dose, WT RBD⁺ and WT/variant cross-reactive RBD⁺ B cells were robustly expanded at one month in both groups, reaching levels comparable to healthy controls (Fig. 1c). However, mirroring the kinetics of WT Spike⁺ B cells, RBD⁺ B cell counts declined significantly in cancer patients by three and six months, whereas WT RBD⁺ and WT/BA.1 RBD⁺ B cells were maintained in healthy controls over the same period, as previously reported^{12,13}. Only 20-26% of cancer patients retained detectable WT/BA.1⁺ and WT/BA.5 RBD⁺ B cells at six months (Fig. 1d), indicating that three doses were insufficient to sustain long-term variant-reactive B cell memory in cancer patients. A similar trend was observed in WT/EG.5 RBD⁺ and WT/BA.2.86 RBD⁺ B cells, although the difference was less evident due to low and variable B cell counts (Supplementary Fig. 2, d-e).

Reduced resting-memory and GC-derived B cells with skewing toward DN2/3 subsets contribute to impaired memory B cell durability in cancer patients

To elucidate the basis of reduced B cell durability in the cancer environment, we examined the phenotypic composition of vaccine-induced B cells, focusing on memory subsets known to support long-term persistence. WT Spike⁺ B cells were first classified according to CD21 and CD27 expression. At one month after the third vaccine dose, CD21⁻CD27⁺ activated memory and CD21⁻CD27⁻ double-negative B

cells comprised more than half of the Spike⁺ compartment in both groups (Fig. 2a and Supplementary Fig. 3a). However, the proportion of these subsets declined rapidly over time and contributed minimally to the long-term memory pool at six months. In contrast, CD21⁺CD27⁺ resting memory B cells, a subset associated with long-term maintenance¹⁴⁻¹⁷, were stably preserved through six months and constituted the majority of residual Spike⁺ B cells at later time points (Fig. 2a). Importantly, unlike total Spike⁺ B cells which were comparable at one month after the third vaccination (Fig. 1b), CD21⁺CD27⁺ resting memory B cells exhibited significantly lower counts as early as one month after the third vaccination in cancer patients, and this difference persisted at all subsequent time points (Fig. 2b). Given that CD21⁺CD27⁺ B cells constituted the predominant Spike⁺ memory subset at three and six months, insufficient early generation of this long-lived population may underlie the reduced durability observed in cancer patients. We next assessed CD27⁺IgG⁺ memory B cells, a subset predominantly derived from germinal center (GC) reactions¹⁸⁻²⁰. Similar to resting memory B cells, CD27⁺IgG⁺ B cell counts were consistently lower in cancer patients across all time points following the third vaccine dose (Fig. 2c), suggesting attenuated GC-derived memory formation in this population.

To directly test whether early subset composition influenced long-term persistence, we correlated B cell subset counts at one month with WT Spike⁺ B cell counts at six months. CD21⁺CD27⁺ and CD27⁺IgG⁺ Spike⁺ B cell counts at one month after the third vaccine dose showed significant positive correlations with Spike⁺ B cell counts at six-month (Fig. 2d), whereas CD21⁻CD27⁻ B cells showed no association (Supplementary Fig. 3b). Although CD21⁻CD27⁺ activated memory B cells also showed a significant correlation (Supplementary Fig. 3b), this subset declined rapidly

over time and therefore likely reflects a transient subset rather than a population supporting long-term persistence (Fig. 2a). These findings indicate that the magnitude of early generation of CD21⁺CD27⁺ resting and GC-derived memory subsets is a key determinant of long-term B cell maintenance.

To further resolve alterations in B cell differentiation, we analyzed heterogeneity within the total double-negative (DN; IgD⁻CD27⁻) B cell compartment (DN1: CD11c⁻CD21⁺; DN2: CD11c⁺CD21⁻; DN3: CD11c⁻CD21⁻). At one month after the third vaccination, DN subset composition differed between groups, with DN1 cells enriched in healthy controls, whereas DN2 and DN3 subsets were significantly increased in cancer patients (Fig. 2e). We next examined whether these early differences were associated with long-term B cell maintenance. The proportion of DN1 cells at one month positively correlated with Spike⁺ B cell counts at six months, whereas DN3 cells showed a significant negative correlation. DN2 exhibited a similar negative trend that did not reach statistical significance (Fig. 2f). A similar trend was observed for Spike⁺ B cell counts at three months (Supplementary Fig. 3c). These findings suggest that early skewing of the DN compartment away from DN1 and toward DN2/3 states is associated with impaired durability of antigen-specific B cell responses in cancer patients.

Single-cell transcriptomic and BCR profiling reveal reduced somatic hypermutation (SHM) and aberrant B cell differentiation

To gain mechanistic insight into the impaired durability and reduced generation of long-lived, GC-derived memory B cells observed by flow cytometry, we performed single-cell RNA sequencing with paired BCR sequencing of class-switched sorted B cells from 11 healthy controls and 13 cancer patients (Supplementary Fig. 4a).

Unsupervised clustering and UMAP projection identified seven transcriptionally distinct B cell clusters (Fig. 3a). Antigen-specific B cells were predominantly distributed within clusters C1, C3, and C6 (Fig. 3b). Cluster C1 was characterized by high expression of *CD27*, together with memory-associated genes including *HOPX*, *COCH*, *S100A10*, *CRIP1*, and *ITGB1* (Fig. 3c). *HOPX* has been implicated in the long-term maintenance and survival in T helper cell subsets^{21,22}, suggesting that C1 represents a long-lived memory population. In contrast, cluster C6 exhibited atypical B cell signatures, including *ITGAX*, *TBX21*, *ZEB2*, and *FCRL5*, indicative of a T-bet-associated atypical differentiation state²³. Cluster C3 represented a non-conventional *CD27*⁻ B cell population enriched for BCR signaling genes (*PRKCE*, *CAMK1D*, and *LYN*). Additional clusters comprised TNF-expressing memory B cells (C2), *CD24*⁺ memory B cells (C4), B cells enriched for canonical naïve markers (*TCL1A*, *IL4R*, and *FCER2*; C5), and *IgA*⁺ memory B cells (C7).

Analysis of B cell receptor (BCR) sequences further revealed qualitative differences in B cell response between cancer patients and healthy controls. Analysis of *V_H* gene usage revealed comparable patterns between groups (Supplementary Fig. 4b), with similar expansion of the top 14 *V_H* genes previously reported to be SARS-CoV-2-associated^{24,25}. In contrast, SHM analysis demonstrated significantly reduced SHM counts in cancer patients, both within antigen-specific B cells (Fig. 3d) and the total class-switched compartment (Supplementary Fig. 4c). Subset-resolved analysis further demonstrated that SHM counts remained significantly lower in cancer patients within both antigen-specific C1 and C6 populations (Fig. 3e), indicating that even within comparable differentiation states, B cells in cancer patients have undergone less somatic hypermutation. C3 was excluded from this analysis due to insufficient BCR coverage among antigen-specific cells.

Direct comparison between subsets showed that C6 atypical T-bet⁺ B cells harbored substantially fewer somatic hypermutations than C1 memory B cells (Fig. 3f), consistent with reduced GC experience in C6. Importantly, cancer patients exhibited a significantly higher proportion of atypical T-bet⁺ B cells (C6) on a per-donor basis (Fig. 3g), in agreement with the increased frequency of DN2 B cells observed by flow cytometry (Fig. 2e). Together, these findings indicate that the B cell compartment in cancer patients is skewed away from GC-experienced, long-lived memory states toward atypical T-bet⁺ B cell populations with limited somatic hypermutation.

Additionally, BCR isotype analysis revealed overall IgG and IgA predominance across clusters, whereas C5 naïve-like cells were enriched for IgM (Supplementary Fig. 4d). Notably, clusters C2, C3, and C6 in cancer patients displayed higher proportions of *IGHM* and *IGHD* compared to healthy controls, suggesting incomplete or impaired class-switch recombination in cancer patients. To further characterize transcriptional differences in B cells, we performed gene set enrichment analysis (GSEA) comparing cancer patients and healthy controls (Supplementary Fig. 4e). B cells in cancer patients were enriched for pathways related to B cell activation, including B cell receptor signaling and CD40-dependent T cell help, as well as humoral immune response and metabolic pathways such as oxidative phosphorylation. In contrast, gene sets associated with long-term memory were enriched in healthy controls, along with pathways related to B cell survival, including MYC and NF-κB signaling.

Collectively, these results demonstrate that B cell responses in cancer patients are characterized by reduced SHM and an increased representation of atypical T-bet⁺ B cell subsets, consistent with impaired GC-associated maturation.

Reduced functional responsiveness of memory B cells parallels impaired durability in cancer patients

To assess whether impaired durability and altered differentiation of memory B cells are functionally associated with reduced responsiveness, we next performed an IgG B cell ELISpot assay to directly assess the capacity of the memory B cell pool to differentiate into antigen-specific antibody-secreting cells (ASCs) upon stimulation (Supplementary Fig. 5a). PBMCs were stimulated with IL-2 and R848 for 72 hours, after which culture supernatants were collected for antibody quantification and cells were plated onto ELISpot membranes to quantify antigen-specific ASC formation.

Consistent with the flow cytometric findings, cancer patients exhibited comparable spot-forming unit (SFU) numbers to healthy controls at one month after the third vaccination (Supplementary Fig. 5b). However, by three months, SFU counts were significantly reduced in cancer patients for WT RBD, with a similar trend observed for BA.1 RBD. In parallel, antigen-specific IgG production following IL-2/R848 stimulation was markedly decreased in cancer patients at three and six months for both WT and BA.1 RBD, whereas responses at one month remained comparable between groups (Supplementary Fig. 5c).

These results indicate that, in addition to reduced long-term maintenance, the memory B cell pool in patients with solid cancers exhibits diminished functional capacity over time, characterized by impaired generation of antigen-specific ASCs and reduced antibody secretion upon stimulation.

B cell responses after SARS-CoV-2 vaccination in cancer patients are significantly correlated with the antibody responses

To determine the functional consequences of these quantitative and qualitative

alterations in the B cell compartment, we next examined the antibody responses following SARS-CoV-2 vaccination in cancer patients and healthy controls. Unlike healthy individuals, who elicited substantial antibody responses after two vaccine doses, cancer patients exhibited significantly lower responses compared to controls one month after the second vaccine dose (Fig. 4a). After the third booster dose, anti-WT RBD IgG titers markedly increased in both groups, resulting in comparable titers across all time points. However, antibody titers against the Omicron variants BA.1 and BA.5 remained significantly lower in cancer patients at one-month post-booster dose. This suggests that booster vaccines can enhance humoral immunity in cancer patients, but not as effectively for variants with greater antigenic distance from the original strain. Both groups showed similarly low responses to newer variants BQ.1 and BA.2.86 (Supplementary Fig. 6a). The neutralization titers were comparable between the two groups, although seropositive rates were consistently lower in cancer patients (Fig. 4b).

Furthermore, correlation analysis between B cell and antibody responses showed that WT RBD⁺ B cell counts strongly correlated with both anti-WT RBD IgG titers and WT neutralization titers at one month after the third vaccine dose (Fig. 4c). Similar positive correlations were observed for Omicron variants, with WT/BA.1 RBD⁺ B cell counts correlating with BA.1 neutralization titer, and WT/BA.5 RBD⁺ B cell counts correlating with BA.5 neutralization titers (Fig. 4d). These findings underscore the critical role of B cell expansion in driving antibody responses against both WT and VOCs.

Advanced age is associated with attenuated booster responses in B cells, while IV cytotoxic chemotherapy is related to impaired B cell durability in cancer

patients

We have demonstrated that B cell and antibody responses can be compromised in cancer patients. To identify key clinical factors influencing memory B cell responses, we classified the cancer patients based on epidemiological factors and medical conditions.

Initially, we investigated the impact of age by dividing cancer patients into two groups: those under 65 years (<65) and those 65 years and older (≥ 65). While patients aged <65 showed a peak in WT Spike⁺ B cells at one month post-third dose followed by a rapid decline, those aged ≥ 65 showed no clear increase in B cell counts after the third dose (Fig. 5a and Supplementary Fig. 7a). RBD⁺ B cells followed a similar pattern, with consistently lower B cell counts at one month in cancer patients aged ≥ 65 compared to those aged <65 (Fig. 5b and Supplementary Fig. 7b). Consistent with these findings, antibody responses one month after the third dose were comparable between healthy controls and cancer patients aged <65, but were significantly reduced in those aged ≥ 65 compared to healthy controls (Supplementary Fig. 7c), suggesting that advanced age impairs the magnitude of early humoral responses.

Next, we analyzed the effect of anti-cancer therapy, hypothesizing that cytotoxic agents may impair memory B cell formation. While diminished antibody responses to SARS-CoV-2 vaccination in cancer patients receiving cytotoxic therapy have been well documented^{8,9,26,27}, B cell responses in this population remain incompletely characterized. Cancer patients were divided into two groups: those who received intravenous (IV) cytotoxic chemotherapy within 28 days before vaccination ("IV cytotoxic" group) and those who did not ("no IV cytotoxic" group). After the third dose, WT Spike⁺ B cell counts were similar across all groups at one month (Fig. 5c and

Supplementary Fig. 8a). However, by three months, both cancer groups showed faster B cell decay than healthy controls, with a more pronounced decrease in the “IV cytotoxic” group, resulting in a statistically significant difference between the two cancer groups at the three-month timepoint (Fig. 5c and Supplementary Fig. 8a). Notably, WT/BA.1 RBD⁺ and WT/BA.5 RBD⁺ cross-reactive B cell positive rates showed a significant drop between 3-6 months in the “no IV cytotoxic” group, while the “IV cytotoxic” group experienced this drop between 1-3 months, indicating a faster decay (Fig. 5d and Supplementary Fig. 8b). For antibody responses, WT-specific titers at one month after the third dose were comparable regardless of treatment status, whereas BA.1 and BA.5 titers were significantly reduced only in the IV cytotoxic group compared to healthy controls (Supplementary Fig. 8c).

Together, these findings indicate that advanced age predominantly constrains the magnitude of memory B cell responses, while recent IV cytotoxic chemotherapy selectively compromises their durability in cancer patients.

Additional fourth-dose vaccination enhances the durability of B cell responses in cancer patients

To assess whether increased antigen exposure can modulate impaired memory B cell durability observed following the third vaccine dose in cancer patients, we analyzed samples collected at one, three, and over six months after a fourth vaccine dose (either monovalent or bivalent) (Fig. 6a). One month post-fourth dose, WT Spike⁺ and RBD⁺ B cell counts were similar to those observed after the third dose. However, unlike the rapid decline seen after the third dose, B cell counts remained stable after the fourth dose, leading to significantly higher counts at six months (Fig. 6b-c). The B cell response after four doses was not only superior to that after the third dose in

cancer patients but also comparable to healthy controls who received four doses (Fig. 6d-e). Moreover, while the fourth dose did not significantly improve antibody titers over the third dose (Supplementary Fig. 9a), it markedly enhanced neutralizing capacity against Delta and Omicron variants (Supplementary Fig. 9b). These findings suggest that the reduced B cell durability and neutralizing capacity observed after the third vaccine dose can be significantly improved by administering an additional fourth vaccine dose in cancer patients.

Discussion

In this study, we demonstrate that memory B cell responses in cancer patients are characterized by impaired durability and reduced variant breadth despite preserved early expansion following vaccination. This defect is accompanied by reduced generation of long-lived resting memory B cells (CD21⁺CD27⁺) and germinal center-derived B cells (CD27⁺IgG⁺), along with skewing of DN subsets from DN1 to DN2/3. Transcriptomic analyses further revealed diminished SHM and enriched atypical T-bet⁺ B cell subset, consistent with aberrant B cell maturation. Clinically, advanced age was associated with reduced magnitude of B cell responses while recent intravenous cytotoxic chemotherapy predominantly affected their durability. Finally, additional antigen exposure through a fourth vaccine dose restored long-term B cell maintenance. Collectively, these findings support a model in which cancer-associated immune environments reshape B cell differentiation by selectively impairing germinal center (GC)-dependent maturation while preserving early recall response, leading to reduced durability and limited breadth of B cell response.

The study cohort comprised patients with solid cancers undergoing active anti-

cancer therapy, representing a setting in which cancer-associated and therapy-induced immune perturbations are superimposed and are therefore expected to result in profound systemic immune dysregulation. Despite this immune impairment, short-term antigen-specific B cell responses following the third-dose vaccination were largely preserved, with magnitudes comparable to those in healthy controls. This is consistent with our antibody data demonstrating robust boosting of anti-wild-type (WT) responses, also in line with prior studies in patients with solid cancers^{28,29}, and other immunocompromised groups³⁰⁻³³. Given that antigen-specific B cell expansion early after boosting is driven by rapid proliferation of pre-existing memory B cells which can occur without GC involvement, these findings suggest that GC-independent B cell responses remain largely intact in this setting.

Despite comparable B cell expansion shortly after the third booster dose, clear qualitative differences were evident in cancer patients. Specifically, the B cell compartment showed reduced CD21⁺CD27⁺ resting memory B cells, a subset known to support long-term persistence of humoral immunity¹⁴⁻¹⁷, as well as CD27⁺IgG⁺ memory B cells, which are primarily derived from germinal centers¹⁸⁻²⁰. Notably, both subsets were significantly correlated with long-term antigen-specific B cell persistence. Consistent with these alterations, early antibody responses against antigenically distant variants such as Omicron were reduced, in contrast to the similar boosting of anti-WT responses, collectively suggesting a relative deficiency in GC-associated maturation.

In addition to the changes in CD27⁺ conventional memory subsets, the composition of non-classical double-negative (DN; IgD⁻CD27⁻) B cells were also altered, with a shift from DN1 toward DN2/3 populations. DN1 cells, together with CD27⁺ switched B cells, have been described as a durable memory subset in

vaccination settings³⁴, consistent with our data showing that DN1 proportion was positively correlated with long-term Spike⁺ B cell responses. In contrast, DN2 and DN3 subsets are characterized as a more transient, extrafollicular differentiation states³⁴. DN2 cells have also been reported to represent a transient population displaying a pre-antibody-secreting cell phenotype³⁵, suggesting a bias toward effector differentiation rather than durable memory maintenance. DN3 cells, although less well characterized, have recently been described as an extrafollicular subset that accumulates in inflammatory settings and infiltrates inflamed tissues³⁶. Increased DN3 frequencies have also been reported in cancer patients following COVID-19 vaccination, where they were associated with impaired antibody responses³⁷. This is consistent with our finding that DN3 frequency was negatively associated with long-term Spike⁺ B cell responses, further supporting an association between DN3 enrichment and impaired B cell durability. Together, these findings indicate that, although the magnitude of the early B cell response is maintained, its qualitative composition is substantially altered across both CD27⁺ and CD27⁻ B cell compartments in cancer patients, which may contribute to reduced durability and limited variant breadth.

Single-cell transcriptomic and paired BCR analyses provided deeper insight into the qualitative defects underlying impaired long-term memory formation in patients with solid cancers. The distribution of V_H gene usage among antigen-specific B cells was broadly comparable between cancer patients and healthy controls, and gene set enrichment analysis indicated preserved or even enhanced activation signals including B cell receptor and CD40-mediated pathways, suggesting that initial antigen recognition, B cell activation, and clonal selection were largely preserved. In contrast, B cell responses in cancer patients were characterized by markedly reduced SHM.

Given that SHM accumulation reflects cumulative GC experience³⁸, these findings suggest an impaired GC response in cancer patients, in accordance with our flow-cytometry results demonstrating reduced GC-derived B cell subset. Similar reductions in SHM and antigen-specific memory B-cell generation have also been reported in anti-TNF-treated patients with inflammatory bowel disease, raising the possibility that impaired GC output may represent a common mechanism underlying impaired vaccine-induced humoral immunity across diverse immunocompromised states³⁹.

Moreover, we identified an increased proportion of atypical T-bet⁺ B cells (cluster C6) in cancer patients, which has not been previously described in the context of solid cancers. This suggests that the cancer immune environment extends beyond disrupting GC processes, fundamentally reshaping the B cell differentiation fate, redirecting cells toward non-durable memory states such as cluster C6. Notably, reduced SHM was consistently observed not only in total antigen-specific B cells but also within individual B cell subsets, including C1. This indicates that the reduction in SHM is not solely attributable to an increased proportion of low-mutated subsets, but reflects impaired GC activity even within the same B cell subsets. Taken together, these findings indicate that B cell responses in cancer patients exhibit preserved activation, but are characterized by inefficient GC-associated maturation and altered differentiation trajectories.

Multiple, non-mutually exclusive mechanisms may underlie this shift. In cancer, profound lymphopenia is common, driven by skewed immune cell composition and an increased myeloid-to-lymphoid ratio¹¹. Given that sustained GC responses require continuous input from the naïve B cell pool⁴⁰, such lymphopenic conditions may limit effective GC maintenance. In addition, cytokine networks are broadly dysregulated in cancer, with elevated levels of both immunosuppressive and stimulatory cytokines

including MIF, TNF α , IFN- γ , IL-6, IL-8, IL-10, IL-18, and TGF- β ^{37,41}. Notably, excessive inflammatory cytokines such as TNF and IFN- γ have been shown to impair T follicular helper cell differentiation and GC formation in experimental models⁴², highlighting their potential to disrupt GC responses. Also, as IFN- γ has been shown to be a key signal for inducing T-bet⁺ B-cell differentiation^{43,44}, elevated IFN- γ in cancer may have contributed to altered B-cell fate decisions, favoring atypical T-bet⁺ B-cell differentiation. Furthermore, the tumor microenvironment is enriched with immunosuppressive cell populations, including regulatory T cells, myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages¹¹. These cells can interfere with B cell development and function, as exemplified by MDSCs, which have been shown to impair B cell development both in the bone marrow and peripheral compartments⁴⁵.

Cytotoxic chemotherapy may further exacerbate this defect by disrupting GC dynamics. During the GC response, rapid proliferation of centroblasts and subsequent SHM in the dark zone are essential for generating diverse, high-affinity B cell repertoires³⁸. As actively dividing cells are particularly susceptible to cytotoxic agents⁴⁶, such therapies are likely to impair these processes, thereby limiting the generation of memory B cells with a diverse repertoire. Therefore, our data support the current clinical recommendations for cancer patients, to administer vaccination prior to the initiation of anticancer therapy when clinically feasible⁴⁷, as vaccination during periods of maximal immune suppression may compromise the effective GC activity. Although GC responses were not directly assessed in this study, collective evidence of phenotypic, transcriptomic, and functional alterations observed here supports impaired GC-associated maturation as a central mechanism underlying defective B cell durability in cancer patients.

Importantly, our findings extend beyond mechanistic insight to reveal clinically

actionable opportunities to improve B cell immunity in cancer patients. We show that administration of a fourth vaccine dose effectively mitigates the rapid decline in memory B cells observed after the third dose. While the precise mechanisms governing the transition from initial expansion to long-term B cell memory are not fully understood⁴⁸, prior evidence suggests that repeated antigenic stimulation reinforces GC activity and promotes clonal maturation³⁸. Accordingly, additional vaccination may expand and reshape the antigen-specific memory pool in ways that could support more sustained B cell responses over time. Collectively, these findings support a more proactive and strategically timed booster approach in patients with solid cancers, particularly those receiving cytotoxic chemotherapy, who are at heightened risk for impaired durability of B cell immunity.

A limitation of this study is the exclusive use of peripheral blood samples, preventing the direct evaluation of germinal center responses. To fully understand the mechanisms underlying B cell impairment in cancer patients, aspiration of the lymph nodes and analysis of GC B cells and follicular helper T cells will be necessary. Additionally, the cancer cohort was older than the healthy group, which could be a confounding factor. However, multiple regression showed that cancer was the most significant factor affecting B cell responses, with the trend of reduced B cell durability remaining consistent even in individuals aged 40-60. Finally, since two-thirds of the patients enrolled had gastric cancer, we could not assess the impact of different cancer types on B cell responses. Further studies involving diverse patient populations are needed to generalize our findings across various types of solid cancers.

Taken together, this study provides the first in-depth characterization of vaccine-induced B cell responses in patients with solid cancers and reveals previously unrecognized alterations in the memory B cell compartment, including reduced

557 durability, variant breadth, and remodeling of memory B cell subsets. These findings
558 provide a mechanistic framework for understanding impaired humoral immunity in
559 cancer patients and may also offer valuable insights for optimizing vaccine strategies
560 against potential pathogens in the future pandemics.

Author contributions: K.S.L., E.L., E.-C.S., J.-H.K., M.J., and J.E.O. conceived and designed the study. J.Y.A., M.K.J., J.Y.C., S.-W.K., J.P., J.-Y.L., J.-H.K., and M.J. enrolled the study participants and collected the clinical specimens and information. K.S.L., E.L., J.Y.A., S.J.H., and S.K. performed the experiments. K.S.L., E.L., J.Y.A., and J.E.O. analyzed and interpreted the data. S.J.H. performed the single-cell transcriptomic and BCR repertoire analyses. K.S.L., E.L., S.-H.P., and J.E.O. prepared figures and performed visualization. K.S.L., E.L., J.Y.A., and J.E.O. wrote the manuscript. All authors provided critical feedback and review of the manuscript.

Data and materials availability: Detailed materials and methods are provided in the Supplementary Methods. Human scRNA-seq data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) under accession number GSE330235. All other data are available from the corresponding author (J.E.O.) upon reasonable request.

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Figure legends

Fig. 1 | Durability and cross-reactivity of B cells following SARS-CoV-2 vaccination in cancer patients and healthy controls. **a** Schematic overview of the study design and sample collection. Peripheral blood was collected from healthy controls and patients with solid cancers after two or three doses of SARS-CoV-2 vaccination. Samples were obtained approximately 1 and 3 months after the second dose (2-VAC) and 1, 3, and 6 months after the third dose (3-VAC). The timeline indicates mean days post-vaccination (ranges in parentheses). **b** Total WT Spike⁺ B cell counts per 10⁶ lymphocytes at the indicated time points. **c** B cell counts per 10⁶ lymphocytes for WT RBD⁺ and cross-reactive WT/BA.1 RBD⁺ and WT/BA.5 RBD⁺ B cells. **d** Proportion of samples with detectable WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ B cells. Each dot represents an individual sample; lines indicate medians. Sample sizes are shown above the x-axes. Statistical comparisons were performed using the Mann–Whitney U test, and detection rates were compared using Fisher’s exact test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. PBMC, peripheral blood mononuclear cells; VAC, vaccination; M, months; d, days.

Fig. 2 | Impaired generation of resting and germinal center–derived memory B cells with altered DN subset distribution in cancer patients following SARS-CoV-2 vaccination. **a** Representative gating of WT Spike⁺ B cell subsets based on CD21 and CD27 expression (CD21⁺CD27⁺, CD21⁺CD27[−], CD21[−]CD27[−]) and their proportional composition in healthy controls and cancer patients at the indicated time points after vaccination. **b** CD21⁺CD27⁺ resting memory WT Spike⁺ B cell counts per 10⁶ lymphocytes. **c** CD27⁺IgG⁺ germinal center-derived WT Spike⁺ B cell counts

per 10^6 lymphocytes. **d** Correlation of CD21⁺CD27⁺ and CD27⁺IgG⁺ WT Spike⁺ B cell counts at one month after the third vaccination with total WT Spike⁺ B cell counts at six months (n = 15). **e** Distribution of DN subsets (DN1, DN2, and DN3) within DN B cells in healthy controls and cancer patients at one month after third vaccination. **f** Correlation of DN subset proportions at one month with WT Spike⁺ B cell counts at six months (n = 15). Each dot represents an individual sample; lines indicate medians. Sample sizes are shown above the x-axes. Statistical comparisons were performed using the Mann-Whitney U test. Correlations were analyzed using Spearman's correlation and best-fit lines were generated by simple linear regression. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. VAC, vaccination; M, months.

Fig. 3 | Single-cell transcriptomic and BCR analyses reveal reduced somatic hypermutation and enrichment of atypical T-bet⁺ B cells in cancer patients. **a** UMAP visualization of class-switched B cells from healthy controls and cancer patients showing seven transcriptionally distinct clusters (C1–C7). **b** Distribution of Spike-specific B cells across clusters. **c** Dot plots showing expression of representative marker genes across clusters. **d, e** Somatic hypermutation (SHM) counts in total antigen-specific B cells (**d**) and within C1 and C6 antigen-specific subsets (**e**). **f** Comparison of SHM counts between C1 and C6 B cells. **g** Proportion of cluster C6 in healthy controls and cancer patients. Statistical comparisons were performed using the Mann–Whitney U test.

Fig. 4 | Antibody responses and their correlation with B cell responses following SARS-CoV-2 vaccination in cancer patients and healthy controls. **a** Anti-RBD IgG titers (endpoint titers) against WT, Delta, Omicron BA.1, and BA.5 after two or three

vaccine doses. **b** Neutralization titers (NT_{50}) against WT, Delta, Omicron BA.1, and BA.5 after the third vaccination. Percentages indicate the proportion of samples with detectable neutralization. **c, d** Correlation between antigen-specific B cell counts and antibody responses at one month after the third vaccination in cancer patients ($n = 34$). Correlations were assessed between WT RBD⁺ B cell counts and anti-WT antibody and neutralization titers (**c**), and between variant cross-reactive RBD⁺ B cell counts and corresponding neutralization titers (**d**). Sample sizes are shown above or below the x-axes. Data are presented as median and interquartile range where applicable. Correlations were analyzed using Spearman's correlation, and best-fit lines were generated by simple linear regression. Statistical comparisons were performed using the Mann–Whitney U test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. LOD, limit of detection; M, months; VAC, vaccination.

Fig. 5 | Impact of age and recent IV cytotoxic chemotherapy on B cell responses following SARS-CoV-2 vaccination in cancer patients. a WT Spike⁺ B cell counts per 10^6 lymphocytes after the third vaccination in healthy controls, cancer patients aged <65 years, and cancer patients aged ≥ 65 years. **b** WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ cross-reactive B cell counts at one month after the third vaccination in the indicated groups. **c** WT Spike⁺ B cell counts per 10^6 lymphocytes after the third vaccination in healthy controls, cancer patients without intravenous cytotoxic chemotherapy within 28 days before vaccination (“no IV cytotoxic”), and those who received intravenous cytotoxic chemotherapy within 28 days before vaccination (“IV cytotoxic”). **d** Proportion of samples with detectable WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ B cells after the third vaccination in the indicated

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Fig. 6 | Additional fourth SARS-CoV-2 vaccine dose improves B cell durability in cancer patients. **a** Schematic overview of sample collection after the third or fourth SARS-CoV-2 vaccination. Numbers indicate mean days post-vaccination (ranges in parentheses). **b, c** WT Spike⁺ (**b**) and WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ (**c**) B cell counts per 10⁶ lymphocytes after three or four vaccine doses in cancer patients. **d, e** WT Spike⁺ (**d**) and WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ (**e**) B cell counts after four vaccine doses in healthy controls and cancer patients. Healthy control samples at three months after the fourth vaccination were not available. Sample sizes are shown above the x-axes; lines indicate medians where applicable. Statistical comparisons were performed using the Mann–Whitney U test. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$. d, days; M, months; VAC, vaccination.

Figures

Figure 1.

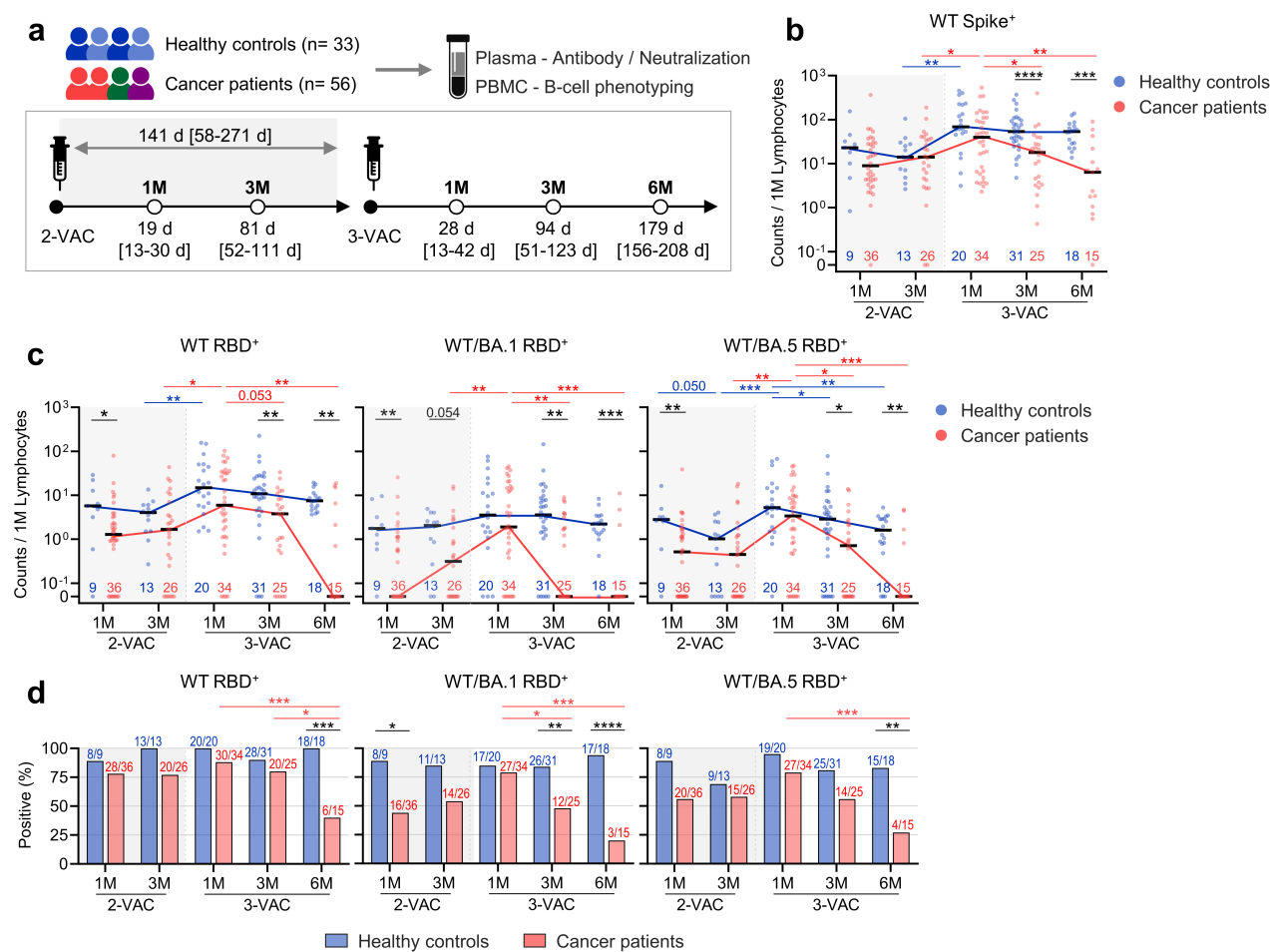


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Figure 2.

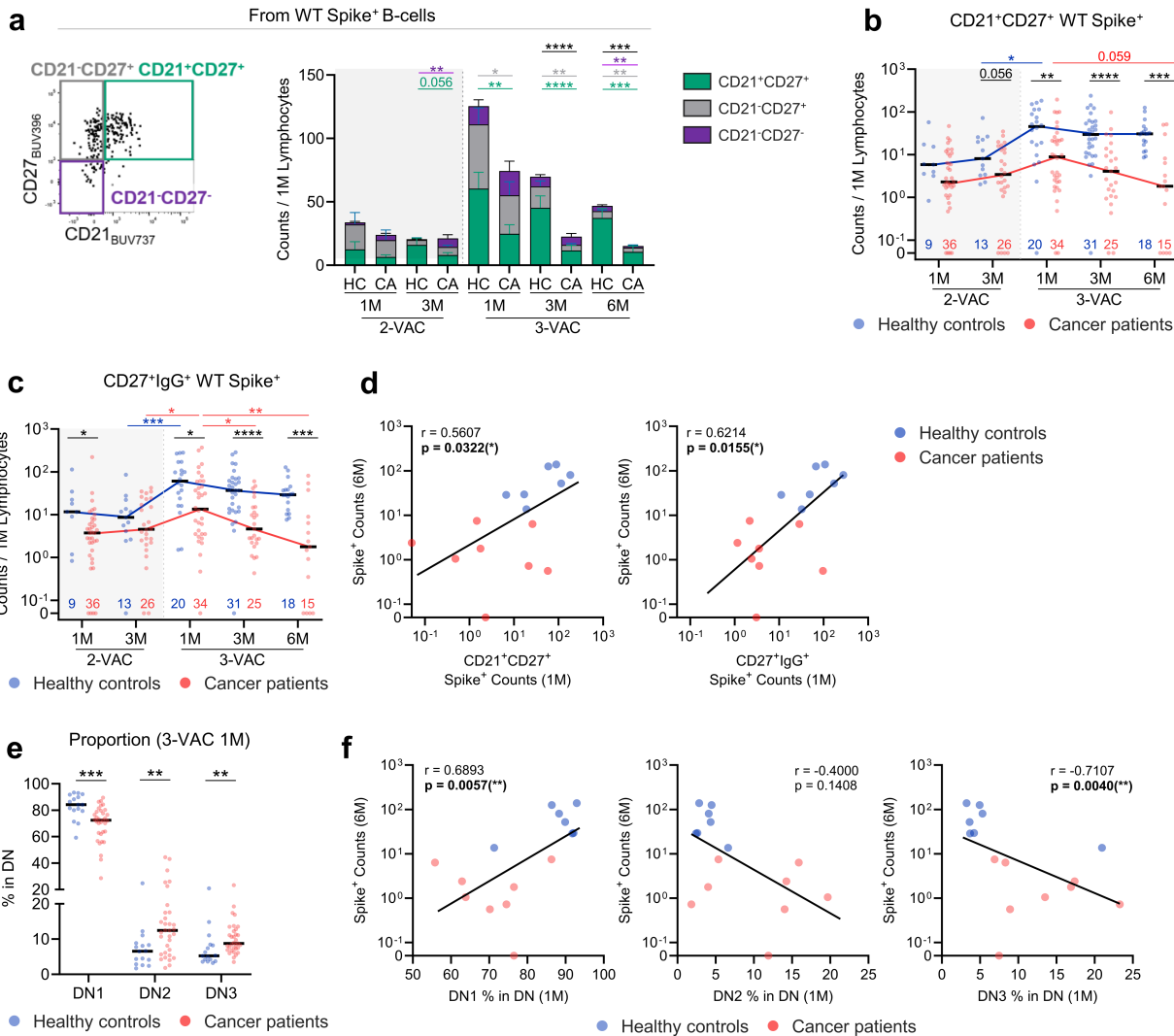


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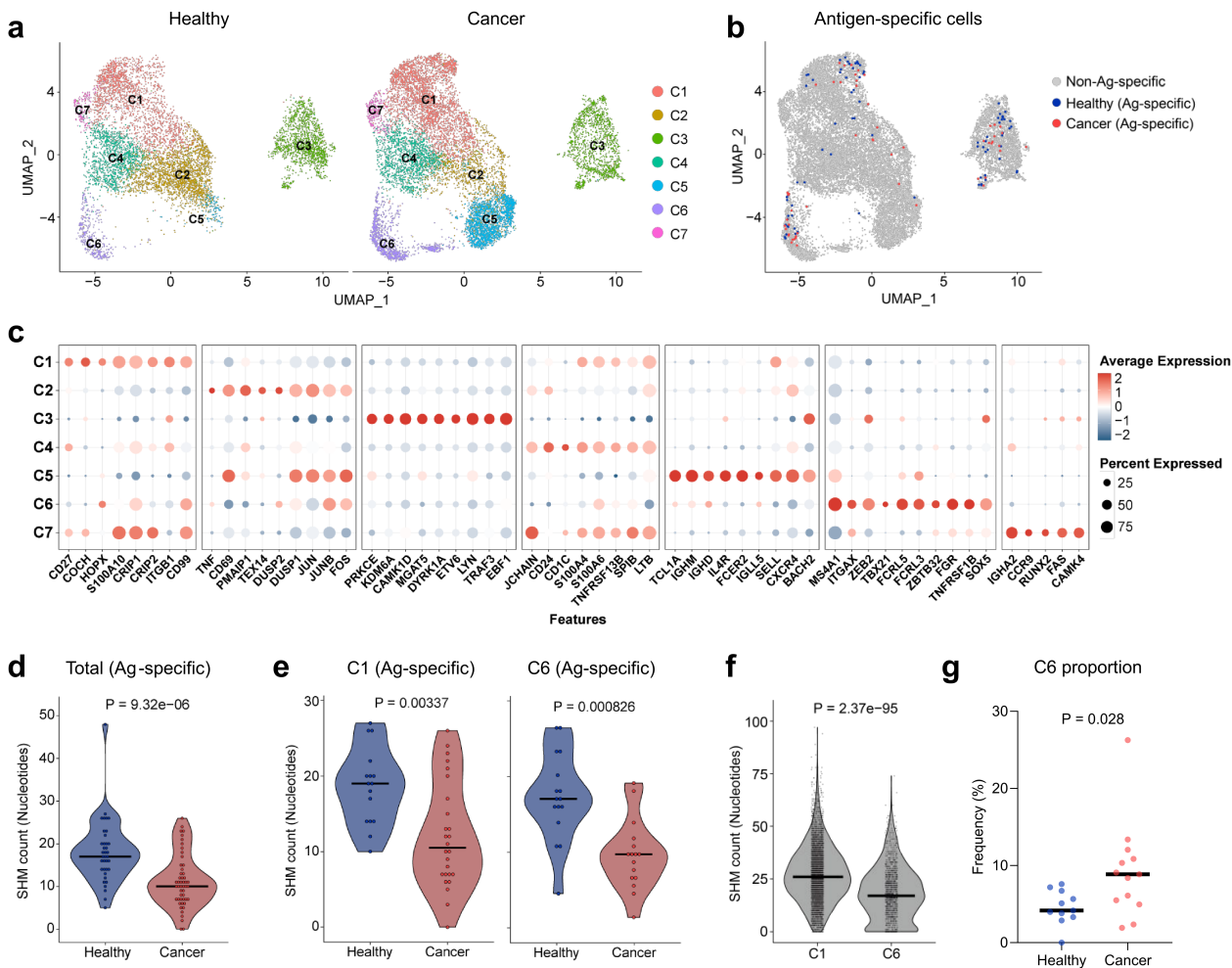


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Figure 4.

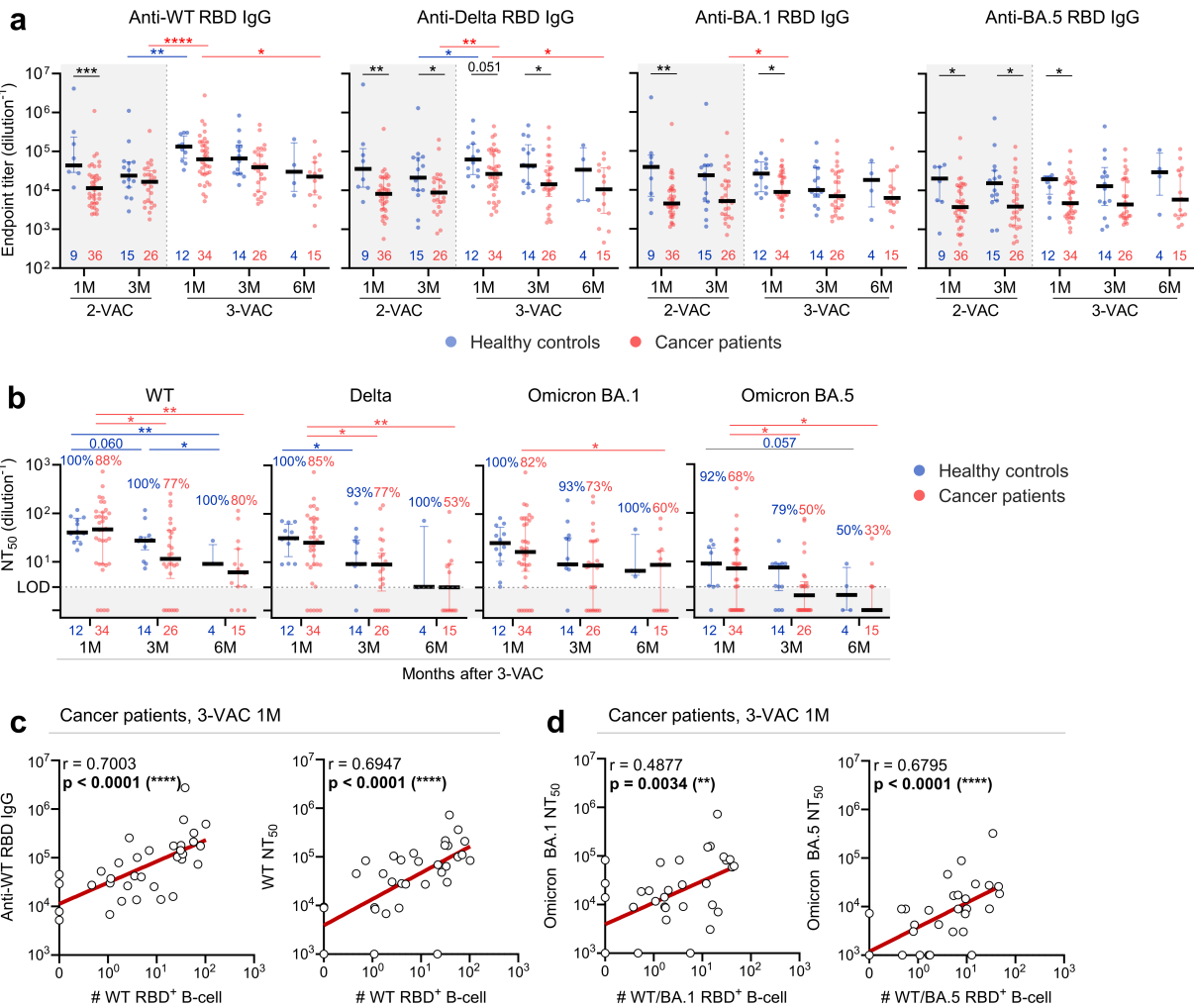


Figure 4

Fig. 4 | Antibody responses and their correlation with B cell responses following SARS-CoV-2 vaccination in cancer patients and healthy controls. **a** Anti-RBD IgG titers (endpoint titers) against WT, Delta, Omicron BA.1, and BA.5 after two or three vaccine doses. **b** Neutralization titers (NT₅₀) against WT, Delta, Omicron BA.1, and BA.5 after the third vaccination. Percentages indicate the proportion of samples with detectable neutralization. **c, d** Correlation between antigen-specific B cell counts and antibody responses at one month after the third vaccination in cancer patients (n = 34). Correlations were assessed between WT RBD⁺ B cell counts and anti-WT antibody and neutralization titers (**c**), and between variant cross-reactive RBD⁺ B cell counts and corresponding neutralization titers (**d**). Sample sizes are shown above or below the x-axes. Data are presented as median and interquartile range where applicable. Correlations were analyzed using Spearman's correlation, and best-fit lines were generated by simple linear regression. Statistical comparisons were performed using the Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. LOD, limit of detection; M, months; VAC, vaccination.

Figure 5.

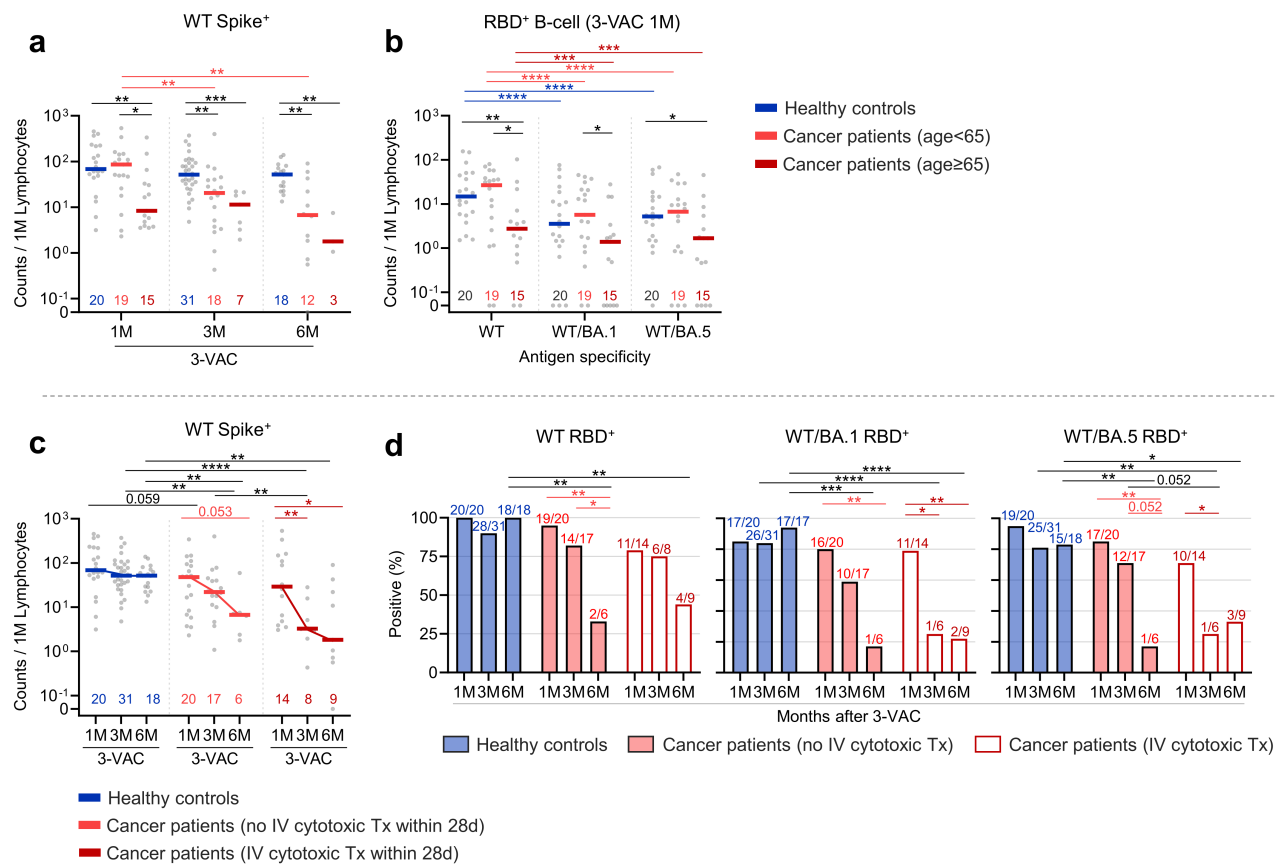


Figure 5

Fig. 5 | Impact of age and recent IV cytotoxic chemotherapy on B cell responses following SARS-CoV-2 vaccination in cancer patients. **a** WT Spike⁺ B cell counts per 10⁶ lymphocytes after the third vaccination in healthy controls, cancer patients aged <65 years, and cancer patients aged ≥65 years. **b** WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ cross-reactive B cell counts at one month after the third vaccination in the indicated groups. **c** WT Spike⁺ B cell counts per 10⁶ lymphocytes after the third vaccination in

healthy controls, cancer patients without intravenous cytotoxic chemotherapy within 28 days before vaccination (“no IV cytotoxic”), and those who received intravenous cytotoxic chemotherapy within 28 days before vaccination (“IV cytotoxic”). **d** Proportion of samples with detectable WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ B cells after the third vaccination in the indicated groups. Sample sizes are shown above the x-axes; lines indicate medians where applicable. Statistical comparisons were performed using the Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. M, months; VAC, vaccination; IV, intravenous; Tx, therapy.

Figure 6.

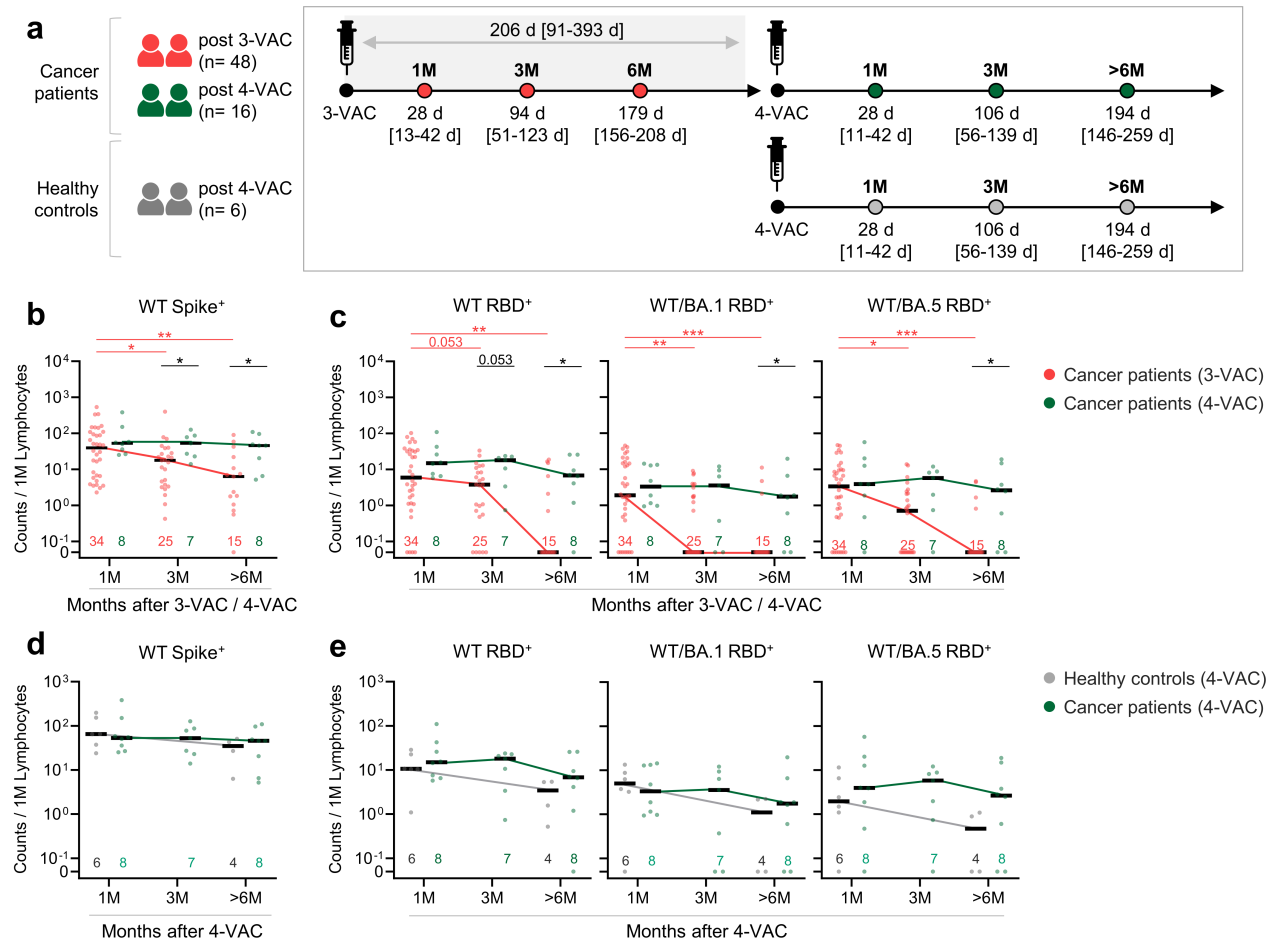


Figure 6

Fig. 6 | Additional fourth SARS-CoV-2 vaccine dose improves B cell durability in cancer patients. a Schematic overview of sample collection after the third or fourth SARS-CoV-2 vaccination. Numbers indicate mean days post-vaccination (ranges in parentheses). **b, c** WT Spike⁺ (**b**) and WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ (**c**) B cell counts per 10⁶ lymphocytes after three or four vaccine doses in cancer patients. **d, e** WT Spike⁺ (**d**) and WT RBD⁺, WT/BA.1 RBD⁺, and WT/BA.5 RBD⁺ (**e**) B cell counts after four vaccine doses in healthy controls and cancer patients. Healthy control samples at three months after the fourth vaccination were not available. Sample sizes are shown above the x-axes; lines indicate medians where applicable. Statistical comparisons were performed using the Mann–Whitney U test. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, and *****P* < 0.0001. d, days; M, months; VAC, vaccination.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [EMMTable1Leeetal.xlsx](#)
- [EMMSupplementarymaterialsLeeetal.pdf](#)