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Article

B cell dynamics as predictive markers in triple-negative breast cancer treated with Pembrolizumab & radiation

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SUMMARY

By combining precise tumor targeting with immune system activation, radiation and immunotherapy have emerged as powerful approaches to extend cancer patient survival and enhance quality of life. While much of this progress has focused on T cell-mediated responses, our study focuses on the role of B cells in predicting response to combined pembrolizumab and radiation therapy in triple-negative breast cancer. We investigated how the combination of pembrolizumab, a programmed cell death 1 (PD-1) immune checkpoint inhibitor, and radiation therapy modulates immune cell dynamics in triple negative breast cancer patients who are responders versus non-responders. We hypothesized that responders would exhibit higher B cell proportions than non-responders throughout treatment, and that longitudinal changes in B cell abundance following PD-1 and radiation plus PD-1 (RT+PD-1) therapy would distinguish patients who benefit from combination therapy. We analyzed single cell RNA sequencing data from a publicly available 50-patient cohort that details a trial on RT+PD-1 therapy. Notably, our findings demonstrated that B cell proportions were higher in responders than non-responders at baseline and after PD-1 therapy, without evidence of a consistent within-responder increase. After RT+PD-1 therapy, this between-group difference was no longer significant. Non-responders exhibited consistently low B cell proportions at the onset and throughout their course of treatment. These results demonstrate a predictive role for B cells in PD-1 and RT+PD-1 therapy and their value as a biomarker for screening patients that could benefit from the combined therapy.

INTRODUCTION

Radiotherapy is a treatment provided to almost half of all cancer patients (1). It has long been regarded primarily as a local intervention that eliminates tumor cells by directly or indirectly damaging their DNA (2). Recent research, however, has revealed that its influence extends beyond direct tumor cell destruction by reshaping the surrounding tumor microenvironment through increased tumor antigen release, recruitment and activation of immune cells, and changes in vascular permeability and stromal composition (3). These insights have spurred interest in combining radiotherapy with other modalities to enhance overall treatment efficacy.

Preclinical and clinical data indicate that radiotherapy can work with immune checkpoint inhibitors to enhance antitumor immunity by inducing systemic responses such as the abscopal effect, where radiotherapy also affects distant tumors (4). Such observations have raised interest in combining radiotherapy with immunotherapies, specifically immune checkpoint inhibitors such as pembrolizumab. Pembrolizumab is a programmed cell death protein 1 (PD-1) inhibitor that works by blocking inhibitory signaling between PD-1 on T cells and its ligand, programmed cell death ligand 1 (PD-L1), which tumors use to suppress immune activation. By preventing this interaction, pembrolizumab restores T cell activity and enhances anti-tumor immune responses (5). However, while T cells have been at the forefront of such research, there is less exploration in the involvement of other immune cells, such as B cells.

Anti-tumor immune responses are shaped by the coordinated activity of multiple immune cell populations, including T cells, myeloid cells, mast cells, and B cells (6, 10). Each of these cell populations uniquely contributes to treatment response within the tumor microenvironment. T cells play a central role in mediating adaptive immune responses against tumors, while myeloid cells influence immune activation and suppression through antigen presentation, cytokine signaling, and tissue remodeling (9, 10). Mast cells, though less commonly studied in cancer immunotherapy, are increasingly recognized for their roles in inflammatory signaling and modulation of the tumor microenvironment (11). B cells are a vital component of the adaptive immune system and are traditionally associated with antigen recognition and antibody production (6). In the tumor microenvironment, B cells have also been shown to support anti-tumor immunity through antigen presentation, tertiary lymphoid structure formation, and the maintenance of long-term immunological memory (6). Although immune checkpoint blockade primarily targets T cell mediated inhibition, emerging evidence suggests that B cells can shape T cell responses and influence therapeutic outcomes, particularly through their organization within tertiary lymphoid structures (3, 6). However, few studies have quantitatively assessed how B cell proportion correlates with treatment response in patients who have received both radiation and immune checkpoint blockade (6).

Triple-negative breast cancer (TNBC) accounts for approximately 10 to 15% of breast cancer cases and is associated with poor clinical outcomes, including a U.S. 5-year survival rate of approximately 78% overall and only 15% for distant-stage disease (12). Because TNBC lacks established targeted therapies and exhibits heterogeneous responses to treatment, identifying biomarkers that predict response to combined immunotherapy and radiotherapy remains an important clinical challenge.

In this study, we examined treatment-associated changes across immune cell populations, with a particular focus on

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the relationship between B cell proportion and response to combined radiation therapy and pembrolizumab (RT+PD-1). Building on the work of Shiao et al., who analyzed treatment-induced immune modulation at the group level in TNBC patients receiving RT+PD-1, we conducted our analysis due to TNBC's aggressive clinical course and the established use of immunotherapy-based treatment strategies (7). We hypothesized responders would exhibit higher B cell proportions than non-responders throughout treatment, and that longitudinal changes in B cell proportion following PD-1 and RT+PD-1 therapy would distinguish responders from non-responders and serve as predictive biomarkers of therapeutic response. To test this hypothesis, we analyzed longitudinal single-cell RNA sequencing (scRNA-seq) data from patients receiving RT+PD-1 therapy, comparing immune-cell proportions between responders and non-responders across multiple treatment time points (7). Our findings demonstrate that higher B cell proportions at baseline and following PD-1 blockade are associated with treatment response, supporting the potential of B cells as predictive biomarkers for selecting patients most likely to benefit from combined RT+PD-1 therapy.

RESULTS

To evaluate the immunological impact of RT+PD-1 therapy, we analyzed a scRNA-seq dataset from a published clinical trial involving 50 patients with operable TNBC (7). The authors collected biopsies at three key timepoints from the same group of patients: prior to treatment, after one dose of PD-1 therapy, and after the initial administration of RT+PD-1 therapy. Surgery was performed after completion of preoperative treatment, and treatment response was determined at surgery using pathological complete response and residual cancer burden (RCB) scoring; thus, tumors were not surgically removed prior to or during the biopsy timepoints analyzed in this study. We focused on changes in B cells and T cells across these timepoints, given their central roles in adaptive anti-tumor immunity, while also examining myeloid and mast cell populations to capture broader immune remodeling within the tumor microenvironment that may influence treatment response. Immune cell proportions were compared between responders and non-responders. In this study, responders were defined as patients with RCB of 0 or 1 following the combination treatment while non-responders exhibited a higher RCB of 2 or 3.

Cell type identities were assigned using the original authors' gene expression-based clustering annotations (7). Immune cell proportion was defined as the fraction of a given immune cell type relative to the total immune cell population within each sample. Using this definition, we examined longitudinal changes in B cell, T cell, myeloid cell, and mast cell proportions across treatment stages in responders and non-responders. B cell proportion was significantly higher at baseline in responders compared to non-responders (p=0.0133, **Figure 1A**). After PD-1 inhibition, responders continued to show higher B cell proportions than non-responders (p=0.0247, **Figure 1B**); this difference was no longer significant following RT+PD-1 therapy (p=0.3285, **Figure 1C**). T cell proportion showed no significant difference at baseline between responders and non-responders (p=0.6099, **Figure 1D**). However, following PD-1 inhibition, T cell proportions were significantly lower in non-responders

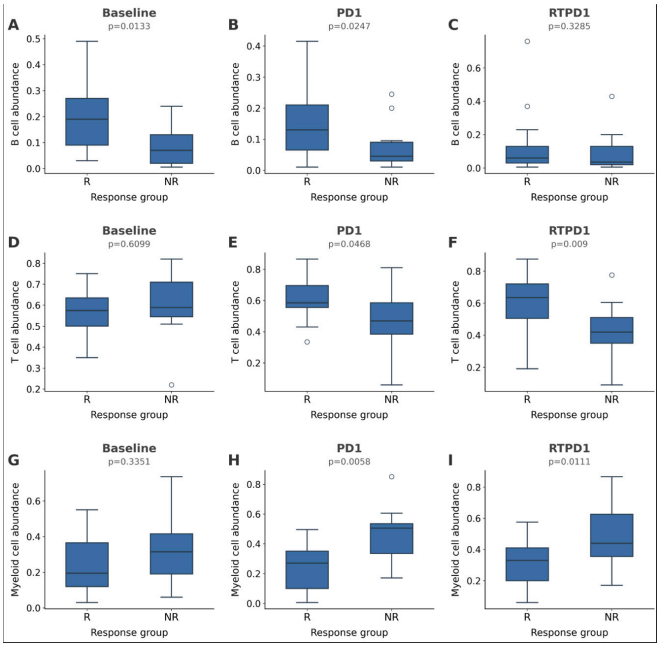

Figure 1: Changes to immune cell proportion after PD-1 and RT+PD-1 in responders versus non-responders. Boxplots comparing immune cell proportions between responders (R) and non-responders (NR) across treatment timepoints. (A–C) B cell proportions at baseline (A), after PD-1 therapy (B), and after combined PD-1 and radiation therapy (RT+PD-1) (C). (D–F) T cell proportions at baseline (D), after PD-1 therapy (E), and after RT+PD-1 (F). (G–I) Myeloid cell proportions at baseline (G), after PD-1 therapy (H), and after RT+PD-1 (I). Immune cell proportions are defined as the fraction of each immune cell type within the total immune cell population. Outliers (defined as values outside 1.5× the interquartile range) are shown as open circles. Statistical comparisons between responders and non-responders at each timepoint were performed using the Mann–Whitney U test, with exact p-values reported.

compared to responders (p=0.0468, Figure 1E). This gap between groups was more pronounced following RT+PD-1 therapy (p=0.009, Figure 1F). T cell responder proportions had no significant change across timepoints. At baseline, myeloid cell proportions followed a similar pattern to T cells, with no significant difference between responders and non-responders (p=0.3351). Following PD-1 inhibition (p=0.0058) and RT+PD-1 (p=0.0111), myeloid cell proportions increased significantly in non-responders compared to responders (**Figure 1G-I**), in contrast to the post-treatment T cell pattern. Within the myeloid population, mast cells showed no significant changes in proportion across treatment groups (p > 0.2 in all conditions), which indicates a minimal role of mast cells in modulating an immune response in this context (**Figure 2**). No further data was available on remaining myeloid subtypes. The patient-level line plot illustrates substantial heterogeneity in individual B cell proportion trajectories across timepoints, while responders generally maintained higher B cell proportions than non-responders at baseline and after PD-1 (**Figure 3**). These findings indicate that changes in B cell proportions relative to total immune cell number do not mirror trends observed in the other analyzed immune cell types, indicating distinct B cell dynamics over the course of treatment. Total immune cell counts were not directly compared between responders and non-responders in this

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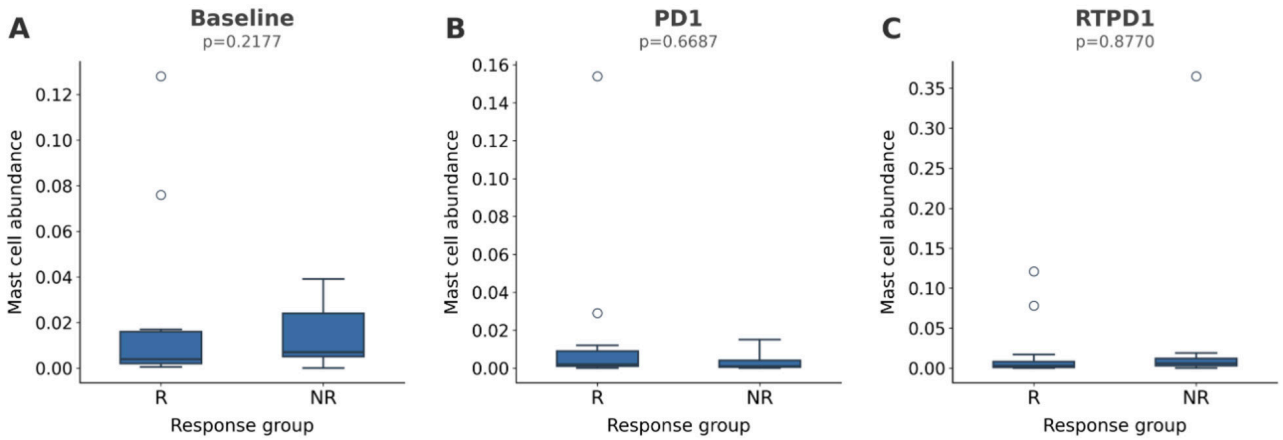

Figure 2. Changes to mast cell proportion after PD-1 and RT+PD-1 in responders versus non-responders. Boxplots comparing mast cell proportion in responders (R) and non-responders (NR) across three treatment stages. Mast cell proportions were assessed at baseline (A), following PD-1 (B), and after RT+PD-1 (C). Statistical testing was performed using the Mann-Whitney U-test; outliers are shown as open circles.

analysis, and because these analyses were based primarily on relative proportions, potential differences in total immune cell proportion between responders and non-responders may contribute to the observed patterns and were not directly assessed. This reinforces the need for further investigation into the role of B cells in modulating or predicting responses to combination immunotherapy. Given the repeated-measures structure across only three ordinal timepoints, patient-level B cell trajectories were visualized descriptively (**Figure 3**) rather than formally tested for within-patient change. Responders maintained higher B cell proportions than non-responders at baseline and after PD-1, but responder proportions declined toward non-responder levels by RT+PD-1 (**Figure 3**).

DISCUSSION

We analyzed the immune cell populations of responders versus non-responders treated with the combination RT+PD-1 therapy and found proportions in T cells remained the same in responders. Importantly however, we also discovered higher B cell proportions and lower myeloid cell proportions associated with treatment success.

Our analysis shows that combination RT+PD-1 therapy is associated with shifts in immune cell composition in patients with TNBC. These findings reinforce the rationale for combination therapy. However, patient-level visualization revealed that responders exhibited higher B cell proportions than non-responders at baseline and after PD-1 therapy, while B cell proportions in responders appeared more variable and often lower by the RT+PD-1 timepoint.

Among the various immune cells, non-responders showed significantly lower T cell proportions than responders at both post-treatment timepoints, with the largest between-group gap observed after RT+PD-1. The retention of T cells with responders is consistent with known mechanisms of this combination therapy such as immunogenic cell death and enhanced antigen presentation (7). In non-responders, reduced T cell proportions may be due to a persistently immunosuppressive tumor microenvironment that limits antigen presentation and effective T cell recruitment during therapy (8). Myeloid cell responder proportion decreased following combination therapy, while myeloid non-responder proportion increased. This suggests that myeloid cells such as macrophages may serve an immunosuppressive role (10). Still, their functional role, either immunosuppressive or stimulatory, requires further investigation. Finally, mast cell proportion remained unchanged across treatments, which suggests they are likely not involved in modulating immune response in this context.

The divergent pattern of B cells as opposed to other immune cells is particularly notable. Despite evidence that B cells contribute to long-term immune memory, antigen presentation, and tertiary lymphoid structure formation within tumors, they remain under-investigated in clinical trials of checkpoint blockade and radiation therapy (3). Our findings suggest that the proportions of specific immune cells exhibit patterns that may be predictive of immunotherapy responses for the treatment of triple negative breast cancer. These observations raise the hypothesis that radiotherapy-associated microenvironmental changes may influence B cell persistence or localization, although this study did not directly test underlying mechanisms.

Some of the limitations of this analysis include that the data is from scRNA-seq immune profiling, which reflects relative proportions, not absolute counts or spatial context (7). Additionally, this study is based on a single-arm clinical trial in which all patients received the same RT+PD-1 therapy, limiting the ability to disentangle treatment-specific effects from temporal changes. As a result, it is not possible to determine whether observed differences in immune cell proportions are driven by PD-1 blockade, radiation, or the passage of time itself, and these findings should be interpreted as hypothesis-generating rather than confirmatory. Finally, B cell function, such as antibody production, antigen presentation, and tertiary lymphoid structure formation, was not directly measured. Because multiple immune cell types were compared across several timepoints, some marginal *p*-values, such as the T cell and myeloid comparisons following PD-1, should be interpreted with caution, as testing multiple hypotheses increases the likelihood of observing statistically significant results by chance.

Future research should further explore the potential use of B cells as biomarkers for treatment response. Responders

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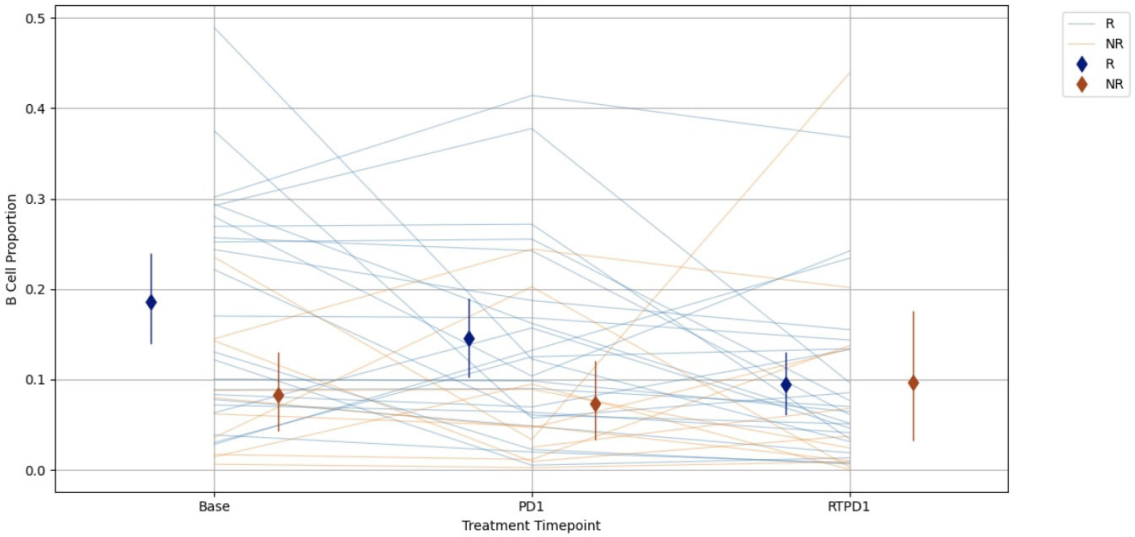

Figure 3: Patient-level B cell proportions across treatment timepoints. Line plot showing patient-level B cell proportions across treatment timepoints for responders (R) and non-responders (NR). Each line represents an individual patient's B cell proportion across three timepoints: baseline (Base), following PD-1, and after RT+PD-1. Diamond markers represent the means while the bars represent standard errors. Each line represents an individual patient, with lines colored blue for responders and orange for non-responders. Mean B cell proportions for responders and non-responders are shown at each treatment timepoint to enable visual comparison across baseline, PD-1, and RT+PD-1 therapy.

maintained higher B cell proportions at baseline and after PD-1 blockade, while the between-group difference was no longer observed following RT+PD-1 therapy, which suggests that B cell profiling could be used to serve as a screening tool to identify patients who are most likely to benefit from RT+PD-1 therapy. In addition to this, the increase in myeloid cell proportion observed in non-responders following both PD-1 and RT+PD-1 therapy suggests that elevated myeloid proportion could serve as a negative prognostic indicator. Together, these patterns support an approach in which both myeloid and B cell profile assessments could provide information on which patients are most likely to benefit. The use of myeloid cell screening, though not as definitive as B cell differences, could be used in ambiguous cases where B cell data solely is not enough.

Other immune cells, such as $CD8^+$ T cells and myeloid cells, have also been explored as screening tools, especially in TNBC, with the former correlating with better response to combination therapy and the latter with worse response (8). These findings demonstrate the growing influence of immune profiling in building personalized treatment strategies (9,10). Overall, our findings showcase the complexity of immune cell dynamics in combination therapies. Furthermore, they encourage the development of screening frameworks that can integrate both positive and negative immune cell indicators to better assess patient benefits. B cell-based screening could help identify those most likely to respond to therapy, while myeloid profiling has the potential to help in inconclusive cases. Such strategies can thus help in guiding future clinical decisions.

MATERIALS AND METHODS

Collecting the data

Publicly available processed scRNA-seq data (GSE246613) generated from a phase Ib/II single-arm clinical trial evaluating preoperative RT+PD-1 therapy in patients with operable triple-negative breast cancer were obtained from a publicly available dataset published by Shiao et al. (7). Fifty patients aged 18 years or older were enrolled between November 2017 and June 2021. Eligible patients had histologically confirmed TNBC, defined by the absence of estrogen and progesterone receptor expression and lack of HER2 overexpression, with tumors greater than 2cm, and no evidence of distant metastatic disease at enrollment. All patients received pembrolizumab (200 mg intravenously) followed by focal radiotherapy prior to initiation of standard-of-care chemotherapy and surgery.

Ultrasound-guided tumor biopsies were collected at three timepoints: prior to treatment (Baseline), after a single dose of PD-1 therapy, and following RT+PD-1 therapy. The dataset included immune cell annotations and clinical response classifications (responder vs. non-responder), allowing for analysis of immune dynamics.

All tumor biopsies were collected from patients upon informed consent, and the clinical trial was approved by the Cedars-Sinai Institutional Review Board and the Department of Defense Human Research Protection Office, as described in the original study (7).

Clinical response classification

Clinical response was determined at the time of surgery using RCB scoring performed by a breast pathologist in the original study. Patients with an RCB score of 0 (pathologic complete response) or 1 (minimal residual disease) were classified as responders. Among the 34 patients with complete evaluable longitudinal biopsies included in this analysis, 23 patients (67.6%) were classified as responders and 11 patients (32.4%) were classified as non-responders (**Table 1**).

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Characteristic	Responders (RCB 0-1)	Non-responders (RCB 2-3)
Number of patients	23	11
Median age (range)	53 (33-79)	55 (36-94)
Sex	Female (100%)	Female (100%)
Tumor stage	Stage II: 21 (91%); Stage III: 2 (9%)	Stage I: 1 (9%); Stage II: 9 (82%); Stage III 1 (9%)
Nodal involvement at baseline	10 (43%)	4 (36%)
Prior cancer treatment	None	None

Table 1: Baseline clinical characteristics of the patient cohort stratified by response status. Age, tumor stage, and nodal status for each patient were obtained from Table S1 in Shiao et al. (7), and independently re-stratified by response group (RCB 0–1 vs. RCB 2–3) for this analysis.

Analyzing the data

Cell type identities were assigned using the original authors' definitions based on gene expression and clustering. We focused on four primary immune compartments: T cells, B cells, myeloid cells, and mast cells. For each sample, we calculated the fraction of each immune cell type within the total immune cell population (which we refer to as immune cell proportion), enabling comparison of immune composition across treatments and between response groups.

Patients were stratified into responders and non-responders based on the clinical response data provided in the original publication. For each immune compartment, we analyzed longitudinal changes in relative proportion across the three treatment stages. Boxplots were generated to visualize the distribution of immune cell fractions across response groups and timepoints. In addition to group-level boxplots, patient-level line plots were generated to show longitudinal changes in immune cell proportions for each individual, highlighting heterogeneity in response trajectories.

Statistical testing

To assess differences in immune cell proportions between responders and non-responders at each treatment stage, we used the Mann-Whitney U test.

Software

We used Python 3.12.2 with libraries (NumPy 1.26.4, Pandas 2.2.2, Scanpy 1.10.4) for analysis. For statistical testing, we used SciPy 1.13.1, and for data visualization we used Matplotlib 3.9.2.

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