53 EVALUATION OF TUMOR ANTIGEN-SPECIFIC ANTIBODY RESPONSES IN PATIENTS WITH METASTATIC TRIPLE NEGATIVE BREAST CANCER TREATED WITH CYCLOPHOSPHAMIDE AND PEMBROLIZUMAB

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Background Although T cells have been a central focus of cancer immunotherapy studies, there is a growing appreciation for the role of B cells in anti-tumor immunity. We recently reported that increased B cell gene signature expression and B cell receptor diversity in pretreatment samples were associated with clinical response to immune checkpoint blockade (ICB) in triple negative breast cancer (TNBC).[1] In murine models of TNBC, ICB response has been found to be dependent upon B cell responses.[2] Beyond TNBC, intratumoral presence of tertiary lymphoid structures (TLS; enriched with B cells, T cells and dendritic cells (DCs)) have been associated with ICB response in various cancer types.[3–7] Deeper understanding of B cell responses in the context of ICB is needed.

Methods We evaluated tumor antigen-specific antibody responses in patients with metastatic triple negative breast cancer treated with pembrolizumab following low dose cyclophosphamide therapy using custom peptide arrays (figure 1).

Results We found that a minority of predicted linear epitopes were associated with antibody signal, and antibody signal was associated with both mutated epitopes and self epitopes. No associations were observed between antibody signal and subcellular localization or RNA expression of parent proteins (figure 2). Patient-specific patterns of antibody signal boostability (e.g., antibody signal increase on-therapy) were observed and were independent of clinical response (figure 3). Intriguingly, measures of cumulative antibody signal intensity relative to immunotherapy treatment showed that the one complete responder in the trial had the greatest increase in total antibody signal (figure 3), which supports a putative association between ICB-dependent antibody boosting and clinical response. The antibody boost in the complete responder was largely driven by increased levels of IgG specific to a sequence of N-terminal residues in native Epidermal Growth Factor Receptor Pathway Substrate 8 (EPS8) protein (figure 4), a known oncogene in several cancer types including breast cancer. Structural protein prediction analysis showed that the antibody-targeted region of EPS8 was in a region of the protein with mixed linear/helical structure, and that this region was solvent-exposed and not predicted to bind to interacting macromolecules (figure 4).

Conclusions High-throughput peptide arrays can be used to map tumor antigen-specific antibody responses. This study highlights the potential importance of humoral immune responses recognizing neoantigen epitopes and unmutated self protein epitopes in anti-tumor immunity. Future studies will be needed to better elucidate the role of tumor antigen-specific antibodies in tumor growth inhibition and immunotherapy response.

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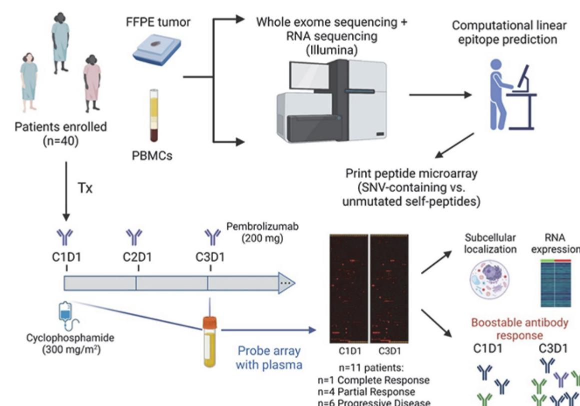
Ethics Approval This study was approved by UNC IRB #16–1025.



Abstract 53 Figure 1 Experimental approach to examine neoantigen-specific antibody responses. Patients with metastatic triple negative breast cancer underwent a clinical trial to examine the efficacy of regulatory T cell depletion with cyclophosphamide plus PD-1 inhibition with pembrolizumab. Eleven patients from this cohort were selected based on clinical response for analysis of tumor antigen-specific antibody responses via multiplex ELISA (peptide arrays). Downstream analyses included examination of protein subcellular localization and RNA expression, as well as antibody boostability relative to immunotherapy treatment. Antibody responses were seen at baseline, and in some patients, increased after therapy

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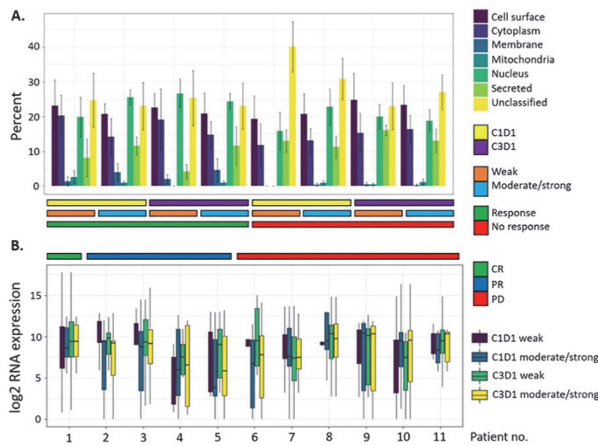
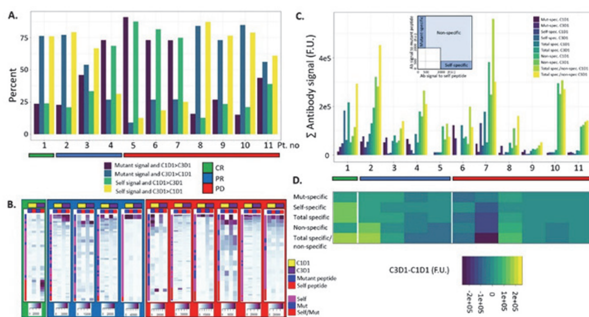
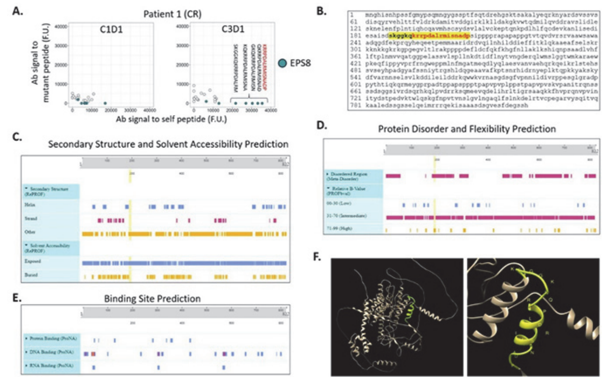


Abstract 53 Figure 2 Subcellular protein localization and RNA expression do not associate with antibody signal or response group. (A) Subcellular localization of parent proteins of self/mutant peptide pairs subset based on associated antibody signal level (weak vs. moderate/strong), and further categorized by treatment time point and response. Peptide pairs were considered to have moderate/strong signal if either peptide of the pair (self or mutant) had >2,000 F.U. signal intensity. Peptide pairs were considered to have weak signal if both peptides of the pair (self and mutant) had signal intensity >500 F.U. and <2000 F.U. Error bars represent standard error (n=5 for responders; n=6 for non-responders). (B) Distribution of RNA expression of parent proteins categorized according to antibody signal level, treatment time point and response



Abstract 53 Figure 3 Boostability of antibody response relative to ICB treatment. (A) Percentage of mutant or self-peptides that had associated antibody signal (>500 F.U. at either C1D1 or C3D1) and greater signal relative to the other time point. (B) Heatmap depiction of antibody signal relative to treatment time point. Signal was ranked according to intensity of signal to C3D1 mutant peptide. A peptide pair was included if there was any signal >2,000 F.U. for either self or mutant peptide at either C1D1 or C3D1 time point. Colored sidebar denotes whether antibody signal was associated with mutant peptide, self peptide or both. (C) Absolute antibody signal (summed) categorized by signal specificity, treatment time point and response. Inlay depicts signal thresholds that were used to denote specificity classes. (D) ICB-associated boostability difference in antibody signal, which is derived by subtracting C1D1 antibody signal from C3D1 signal for respective specificity classes



Abstract 53 Figure 4 Complete responder displayed ICB-dependent boostability of both mutant and self-peptides, with particularly strong boost of IgG specific to native EPS8 peptides. (A) Relative antibody signal to self- versus mutant peptides at C1D1 and C3D1. Annotation shows ICB-dependent boost of antibody signal to self-EPS8 peptides. Peptides with greatest boost corresponding to S187-P107 residues are shown. (B) Primary protein structure of EPS8, with S187-P107 highlighted. The primary structure for EPS8 protein was input into PredictProtein (https://predictprotein.org) to query secondary structure and solvent accessibility (C), protein disorder and flexibility (D), and macromolecular binding site predictions (E). (F) Tertiary structure of EPS8 as predicted by Alphafold Protein Structure Database (https://alphafold.com), with S187-P107 highlighted in yellow. 3D structure was visualized and annotated using Chimera v1.16 software (https://www.cgl.ucsf.edu/chimera/)

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