Background Resistance to immune checkpoint inhibition (ICI) in patients with triple-negative breast cancer (TNBC) remains a common problem, with the underlying mechanisms not well understood. Tumor antigens bound to major histocompatibility complex-I (MHC-I) are required for CD8-mediated tumoricidal activity, and thus, anti-PD-1/L1 targeted ICI. However, many breast tumors downregulate, or heterogeneously express, MHC-I, potentially making them less susceptible to ICIs. This heterogeneity in MHC-I expression is not examined in most preclinical studies, limiting our understanding of how to overcome ICI resistance in the context of heterogeneous MHC-I expression, as is often observed clinically.

Methods We evaluated tumor specific MHC-I expression with clinical outcome via multiplexed immunofluorescence (mIF) staining on pre-treatment biopsies from metastatic TNBC patients (n=84) in a randomized Phase II clinical trial evaluating carboplatin ± atezolizumab (NCT03206203). Further, we evaluated MHC-I heterogenous expression across breast cancer subtypes at a single-cell level (n=314). To model patterns of MHC-I heterogeneity and how this impacts tumor immune infiltration and response to immunotherapy, we examined murine mammary cancer models of enforced MHC-I heterogeneity via scRNA sequencing, flow cytometry, and RNA gene expression profiling. Lastly, we applied spatial technologies on TNBC tumors (ROI=154) to characterize the immune cell infiltration patterns around regions of high, low, and heterogeneous MHC-I expression.

Results The current study demonstrates that TNBC patients show remarkable intratumor heterogeneity in MHC-I expression patterns. In preclinical mouse models, complete loss of MHC-I negates anti-tumor immunity and ICI response, whereas intratumor MHC-I heterogeneity leads to increased local infiltration of natural killer (NK) cells. These findings are replicated in human breast cancers using spatial technologies where MHC-I heterogeneity is associated with clinical resistance to anti-PD-L1 therapy and increased NK:T cell ratios in breast tumors. MHC-I heterogeneous tumors require NKG2A to suppress NK cell function and to eliminate MHC-I negative tumor cells. Combining anti-NKG2A and anti-PD-L1 therapies restores complete response in heterogeneous MHC-I murine models, and it is dependent on both activated, tumor-infiltrating NK and CD8+ T cells.

Conclusions These data reinforce the growing interest on how tumor-specific antigen presentation via MHC-I plays a role in modifying anti-tumor immunity and ICI response. Together, they endorse the unmet translational and clinical need to address heterogeneity in MHC-I expression as a variable in understanding breast cancer anti-tumor immunity and response to immunotherapy. Moreover, these data showcase the potential to harness NK cell function in advancing cancer immunotherapy combinations.

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