Analysis of immune infiltrates (assessed via multiplex fluorescence immunohistochemistry) and immune gene expression as predictors of response to the checkpoint inhibitor pembrolizumab in the neoadjuvant I-SPY 2 TRIAL

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BACKGROUND

Pembrolizumab (Pembro), an anti-PD-1 immune checkpoint inhibitor, has been approved for the treatment of a variety of cancers including melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, and urothelial carcinoma. Pembro was recently evaluated in HER2 breast cancer patients in the neoadjuvant I-SPY 2 trial conducted at the UCSF NCI (TN), HRPR2, and HER2 signatures. HER2 positive patients were randomized to receive Pembro+paclitaxel followed by doxorubicin/cyclophosphamide (P+AC) vs. T-AC. We and others have shown that TN breast cancers tend to have high numbers of immune infiltrates, including T cells and tumor associated macrophages (TAMs). We hypothesize that characterizing the tumor microenvironment in these cases via multiplex fluorescence IHC (IHC) and immune expression signatures will identify biomarkers that predict response to Pembro.

METHODS

Gene Expression: Data from 248 patients (Pembro: 69 controls: 179) were available. Pre-treatment biopsies were assessed by Agilent gene expression arrays. Signature scores were calculated by averaging cell type specific genes. All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess biomarker performance. A biomarker is considered a specific predictor of Pembro response if it associates with response in the Pembro arm but not the control arm, and if the biomarker’s treatment interaction is significant (likelihood ratio test, p<0.05). This analysis is performed adjusting for HR status as covariates, and within receptor subtypes. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

Multiplex fluorescence Immunohistochemistry (IFC): Pre-treatment FFPE samples were immunostained using 20-lane reagent kit (Perkin Elmer) on a fully automated Ventana Discovery platform, imaged with a Vectra® 3.0 automated Cell Phenotype Map software (Perkin Elmer). The 7-plex panel included CD3, CD68, PD-L1, PD-L2, IL6, K67, and cytokeratin. An algorithm for tumor/stroma segmentation developed in I-SPY was used to randomly select 7-10 high power fields (hps) for imaging that contained at least 40% tumor. Cell phenotype maps were generated for each of these hps for each sample. Cell densities were determined per area of stroma, tumor, or total tissue and averaged across all hps for a given case.

RESULTS

Association of Immune Cell Infiltrates with Response

Total CD3+ T cells, CD68+ cells, and macrophages, as well as PD-L1+ Tumor cells, are not significantly associated with pCR. Similar results were obtained when immune cell infiltrates were analyzed by location (tumor vs. stroma).

Heatmap of Marker Genes Defining Immune Cell Populations

Correlations of Immune Cell Gene Signatures with IHC Results

SUMMARY

• None of the immune cell types identified by IHC were significantly associated with response (pCR) to pembrolizumab + chemotherapy.
• T cell gene signatures correlated with T cell infiltrates by IHC, whereas the macrophage signature did not correlate with CD68+ macrophage infiltrates.
• Several immune cell gene signatures, as well as PD-L1 expression, were associated with response (pCR) to pembrolizumab + chemotherapy.
• In particular, the CD4, CD8, and dendritic cell signatures were significantly associated with pCR when adjusted for response in the control arm (chemotherapy only) and for HR status.
• Interestingly, a mast cell signature was negatively associated with response, particularly in the HR- subgroup.

ACKNOWLEDGEMENTS:

I-SPY operates as a prospective consortia, with study sponsors FNIH (2010-2012) and Quintaunt Health/Quintiles (2012-present).


This work was also funded in part by a grant from the Breast Cancer Research Foundation.

I-SPY2 trial