I-SPY2 Trial

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

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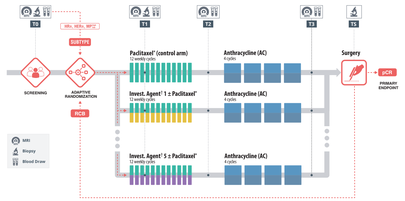
BACKGROUND

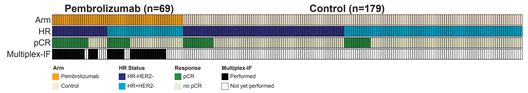
Pembrolizumab (Pembro), an anti-PD-1 immune checkpoint inhibitor, has been approved for the treatment of a variety of cancers including melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, and urothelial carcinoma. Pembro was recently evaluated in HER2$^-$ breast cancer patients in the neoadjuvant I-SPY 2 TRIAL and graduated in the triple negative (TN), HR$^+$HER2$^-$, and HER2$^-$ signatures. HER2$^-$ patients were randomized to receive Pembro+paclitaxel followed by doxorubicin/cyclophosphamide (P+T -> 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 immune microenvironment in these cases via multiplex fluorescence IHC (fIHC) and immune expression signatures will identify biomarkers that predict response to Pembro.

I-SPY2 ADAPTIVE TRIAL: Pembrolizumab



- Multicenter, Phase II, adaptively-randomized neoadjuvant trial
- Shared control arm - Standard neoadjuvant chemotherapy
- Primary endpoint: pathologic complete response (pCR)
- Match therapies with most responsive breast cancer subtypes
  - Defined by HR, HER2, and Mammaprint High1/(ultra)High2 (MP1/2) status
- Agents/combinations "graduate" for efficacy = reaching >85% predictive probability of success in a subsequent phase III trial in the most responsive patient subset

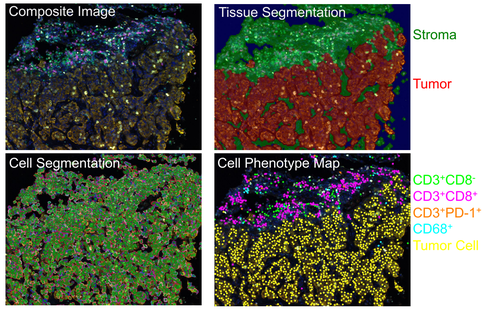
	Estimated pCR Rate (95% PI)		Probability Pembro Superior to Control	Predictive Probability of Success in Phase 3
	Pembro	Control		
HER2-	0.44 (0.33 – 0.55)	0.17 (0.11 – 0.23)	>0.999	0.985
HR-HER2-	0.60 (0.44 – 0.75)	0.22 (0.13 – 0.30)	>0.999	0.996
HR+HER2-	0.30 (0.17 – 0.43)	0.13 (0.07 – 0.19)	0.996	0.834



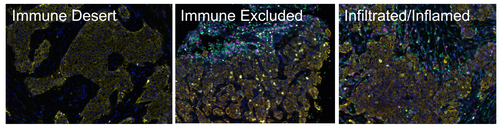
METHODS

Gene Expression: Data from 248 patients (Pembro: 69; controls: 179) were available. Pre-treatment biopsies were assayed using 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 x treatment interaction is significant (likelihood ratio test, $p<0.05$). This analysis is also performed adjusting for HR status as covariates, and within receptor subsets. Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

Multiplex fluorescence immunohistochemistry (fIHC): Pre-treatment FFPE samples were immunostained using Opal reagent kits (Perkin Elmer) on a fully automated Ventana Discovery platform, imaged with a Vectra® 3.0 automated imaging system, and analyzed with inForm® software (Perkin Elmer). The 7-plex panel included CD3, CD8, CD68, PD-1, PD-L1, Ki67, and cytokeratins. An algorithm for tumor/stroma segmentation developed in inForm was used to randomly select 7-10 high power fields (hpfs) for imaging that contained at least 40% tumor. Cell phenotype maps were generated for each of these hpfs for each sample. Cell densities were determined per area of stroma, tumor, or total tissue and averaged across all hpfs for a given case.



Immune Landscapes in Breast Cancer

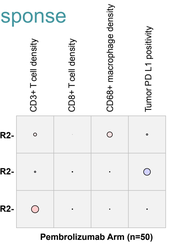


RESULTS

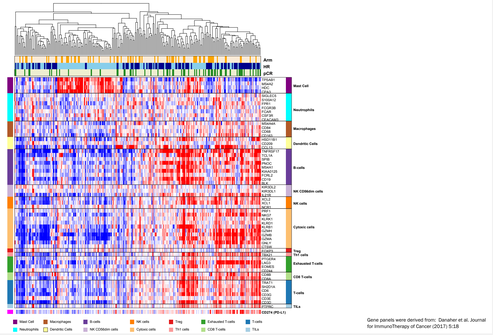
Association of Immune Cell Infiltrates with Response

Total CD3$^+$ T cells, CD8$^+$ T 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).

Color of circle reflects magnitude of coefficient from logistic models (red: positive, blue: negative). Size of circle is proportional to the likelihood ratio test p value.

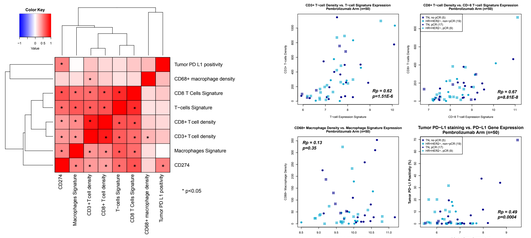


Heatmap of Marker Genes Defining Immune Cell Populations

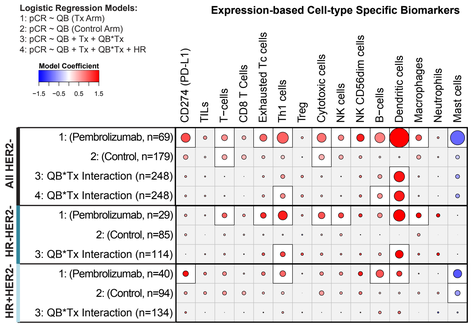


Gene panels were derived from: Danaher et al. Journal for ImmunoTherapy of Cancer (2017) 5:18

Correlations of Immune Cell Gene Signatures with fIHC Results



Association of Immune Cell Gene Signatures with Response



Color of circle reflects magnitude of coefficient from logistic models (red: positive, blue: negative). Size of circle is proportional to the likelihood ratio test p value. White background indicates p<0.05.

SUMMARY

- None of the immune cell types identified by fIHC were significantly associated with response (pCR) to pembrolizumab + chemotherapy.
- T cell gene signatures correlated with T cell infiltrates by fIHC, 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 Th1 cell, B cell, 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-SPY2 operates as a precompetitive consortia, with study sponsors FNIH (2010-2012) and QuantumLeap Healthcare Collaborative (2013-present).

I-SPY2 has received the gracious support of: The Safeway Foundation, Bill Bowes Foundation, Quintiles Transnational Corporation, Johnson & Johnson, Genentech, Amgen, Inc., The San Francisco Foundation, Give Breast Cancer the Boot, Eli Lilly and Company, Pfizer, Inc., Eisai Company Ltd., Side Out Foundation, Harlan Family, The Avon Foundation for Women, Alexandria Real Estate Equities, Inc., and private individuals and family foundations.

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