Expression-based immune signatures as predictors of neoadjuvant targeted-/chemo-therapy response: Experience from the I-SPY 2 TRIAL of ~1000 patients across 10 therapies

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Background

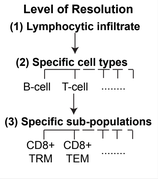
Expression-based signatures have been shown to predict neoadjuvant therapy response; but further studies are needed to deconvolve the contribution of different immune cell types.

Objective: In this study, we compared published T/B cell-related signatures at 3 different levels of resolution as predictors of response in the I-SPY 2 TRIAL

(1) a combined T/B-cell co-expression module, correlated with general lymphocytic infiltrate [Amara 2017]

(2) 14 individual T-cell and a B-cell specific signatures derived from purified immune cells and refined using tumor expression [Danaher 2017]

(3) 9 T cell subpopulation-specific signatures, including a CD8+ T resident memory phenotype (TRM) and a CD8+ T effector memory subset (TEM), generated from microdroplet-based single cell (sc) RNA sequencing of over 6000 tumor associated CD3+ T cells [Savas 2018]



I-SPY 2 TRIAL

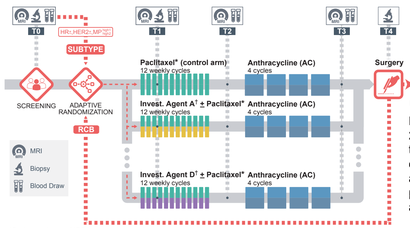
I-SPY 2: Phase 2 trial using response-adaptive randomization within biomarker subtypes to evaluate novel agents when added to standard neoadjuvant therapy for women with high-risk stage II/III breast cancer

Inclusion criteria: Tumor Size ≥ 2.5cm; HR+HER2- MammaPrint (MP) high risk or HR-HER2- or HER2+.

Primary Endpoint: Pathologic complete response (pCR).

Goal: To identify (graduate) regimens that have ≥ 85% predictive probability of increased pCR rate if tested in a neoadjuvant 300-patient phase 3 trial within a (graduating) signature defined by HR, HER2 and MP (Pred.Prob).

Reasons for Regimen Exit: (1) Graduate (Pred Prob≥ 85%); (2) Accrual maximum sample size (Pred.Prob: 10%-85%); (3) Drop for futility (Pred.Prob <10%), (4) Drop for safety issues



*Patients who are HER2+ may also receive tastuzumab (Herceptin)
†An investigational combination of one or more agents may be used to replace all or some of the standard therapy

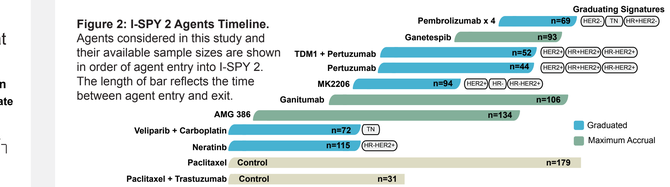
Figure 1: I-SPY2 study schema. 20% of patients are randomized to the shared control arm. Among experimental arms (up to four), adaptive randomization is based on probabilities of achieving pCR within a given subtype for each agent.

Biomarker component: Evaluate ***pre-specified biomarkers*** approved via a proposal process, including biomarkers associated with mechanism of action

METHODS

Pre-treatment expression data available for 989 I-SPY 2 patients from 9 previously reported experimental arms and shared controls

Figure 2: I-SPY 2 Agents Timeline. Agents considered in this study and their available sample sizes are shown in order of agent entry into I-SPY 2. The length of bar reflects the time between agent entry and exit.

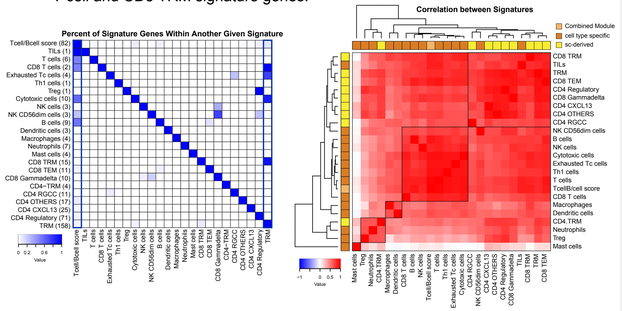


All I-SPY 2 biomarker analyses follow a pre-specified analysis plan. We used logistic modeling to assess each signature as a predictor of pCR within each arm (likelihood ratio test p<0.05). This analysis is also performed adjusting for HR/HER2 status, and within receptor subsets. Our sample size for each arm is small; and our statistics are descriptive rather than inferential. Our analysis is exploratory and does not adjust for multiplicities of other biomarkers outside this study.

Immune Signatures Evaluated

Overall, proportions of gene overlap between signatures are small

- But as expected, the T/B-cell module contains a majority of the genes in the T-cell, B-cell and cytotoxic cell specific signatures.
- Interestingly, the larger sc-derived TRM signature also contains most of the CD8 T-cell and CD8 TRM signature genes.



Although the proportions of gene overlap are small, expression levels of most signatures are well correlated.

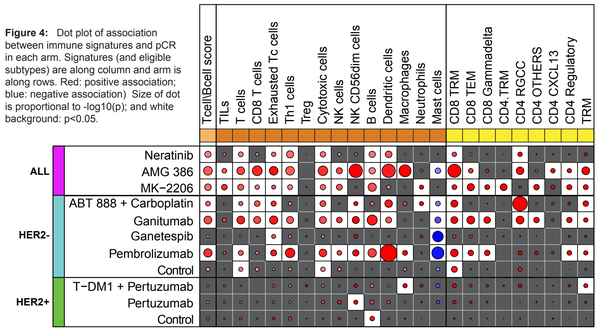
- sc-derived subpopulation specific signatures tend to cluster more closely together
- Mast cell signature is not well correlated with others

Association with Response

In the population as a whole, immune signatures predict response across multiple classes of agents, including the checkpoint inhibitor Pembrolizumab.

- Higher mast cell signature expression is associated with lower pCR rates, as opposed to other immune signatures where higher levels associates with better response.

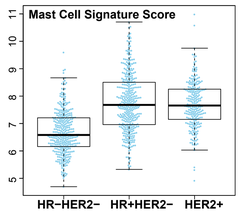
Figure 4: Dot plot of association between immune signatures and pCR in each arm. Signatures (and eligible subtypes) are along column and arm is along rows. Red: positive association; blue: negative association) Size of dot is proportional to -log10(p); and white background: p<0.05.

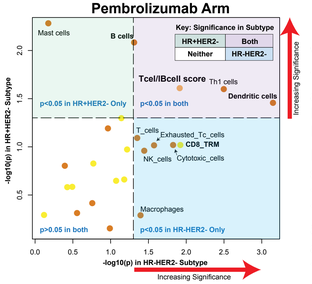


Some immune signature scores may be associated with HR/HER2 subtypes.

- Important to evaluate associations within subtypes

Cell-type and subpopulation-specific signatures most predictive of response vary by subtype and agent





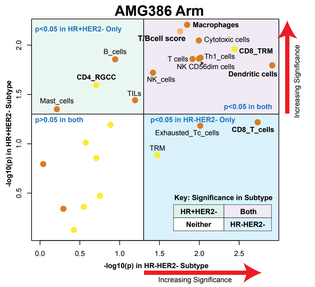
Vignette 1: Pembrolizumab Arm

The T/Bcell co-expression module associates with response to Pembrolizumab in both HR+HER2- and HR-HER2- subtypes.

However, cell-type specific signature with strongest (positive) association:
- **B-cell** in HR+HER2-
- **Dendritic cell** in HR-HER2-

Subpopulation specific signature associated with response:
- **CD8 TRM** in HR-HER2-

Association with Response



Vignette 2: AMG386 Arm

The T/Bcell co-expression module associates with response to the AMG386 arm in both HR+HER2- and HR-HER2- subtypes.

However, cell-type specific signature with strongest (positive) association:
- **Macrophage in HR+HER2-**
- **Dendritic and CD8 T-cell in HR-HER2-**

Subpopulation specific signature associated with response:
- **CD8 TRM and a novel CD 4 signature (CD4 RGCC) in HR+HER2-**
- **CD8 TRM in HR-HER2-**

Vignette 3: HER2 subtype in MK2206 Arm

In the HER2+ subtype, the **T/B-cell module, T-cell** and **B-cell** signatures are associated with response to the AKT-inhibitor MK2206.

Interestingly, among the sc-derived signatures, it is the **CD8-TEM** and **multiple CD4 population-specific signatures,** rather than CD8-TRM, that associate with response.

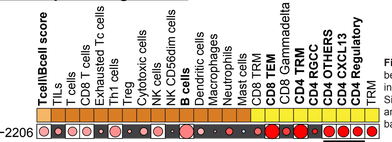


Figure 8: Dot plot of association between immune signatures and pCR in HER2+ patients in the MK2206 Arm. Signature with significant associations are highlighted in bold and with white background.

Conclusions

- Our exploratory study suggests that immune signatures are associated with response to multiple I-SPY 2 experimental agents and implicates different immune cell types as response-predictive within breast cancer subtypes.
- Single cell sequencing derived population specific signatures may help further de-convolute how different immune cell types contribute to therapy responsiveness.

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