The ImPrint immune signature identifies high risk early breast cancer patients who may benefit from PD1 checkpoint inhibition in I-SPY2

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Background

- Remarkable increase of novel Immuno-Oncology drugs in many malignancies led to the need for biomarkers to identify who would benefit.
- Various predictive biomarkers have been developed but none have consistently predicted efficacy.
- I-SPY2 qualified several expression-based immune biology related signatures that predict response to PD1/PDL1 immune checkpoint inhibition (ICI)[1,2].
- We assessed whole transcriptome data of patients with high-risk early-breast cancer (EBC) who received ICI within the neoadjuvant biomarker-rich I-SPY2 trial (NCT01042379), aiming to migrate the I-SPY2 research findings into robust clinical grade signature to predict sensitivity to PD1/PDL1 ICI.

Data and Methods

- Whole transcriptome microarray data were available from pre-treatment EBC biopsies of 69 HER2- patients of the I-SPY2 Pembrolizumab (4 cycles) (Discovery set- **Table1A**) and 70 HER2- patients of the I-SPY 2 Durvalumab/Olaparib (Validation set- **Table 1B**) arms. All patients had a High-Risk 70-gene MammaPrint (MP)[3,4] profile, were Luminal- or Basal-type based on 80-gene BluePrint molecular subtyping profile [5,6].
- Pathologic complete response (**pCR**) was defined as no residual invasive cancer in breast or nodes at the time of surgery.
- The most significant predictive genes for pCR (effect size >0.45) were identified by comparing pCR and **RD** (Residual Disease) groups in the Pembrolizumab arm by iteratively splitting the Discovery set in training and test, balancing for Hormonal Receptor (HR) status and using Leave one out cross validation for performance assessment.
- Pathway analysis was performed with gene set enrichment analysis (GSEA) using Molecular Signatures Database/Hallmark gene sets (adjusted p-value ≤0.05).
- Prevalence analysis was performed on a set of 1463 patients enrolled in the I-SPY2 trial (849 HR+HER2-; 614 Triple Negative)

Table 1A

Discovery set	pCR	RD	Total
HR+ HER2-	12	28	40
Triple Negative	19	10	29
Total	31	38	69

Table 1B

Validation set	pCR	RD	Total
HR+ HER2-	20	29	49
Triple Negative	9	12	21
Total	29	41	70

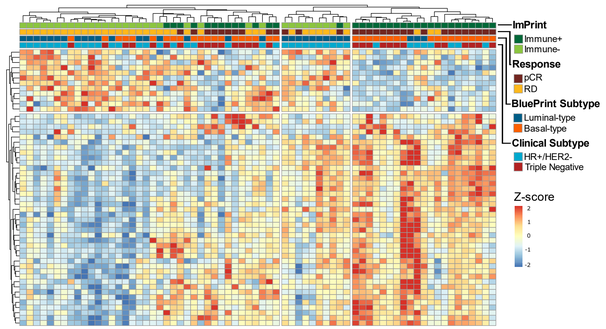


Figure 1. Heatmap of 53 ImPrint genes in Pembrolizumab discovery set (N=69). Distance based on correlation metric and average linkage are applied.

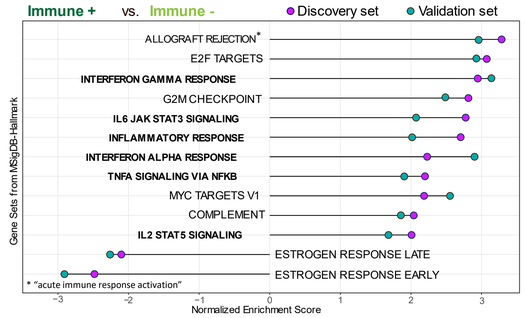
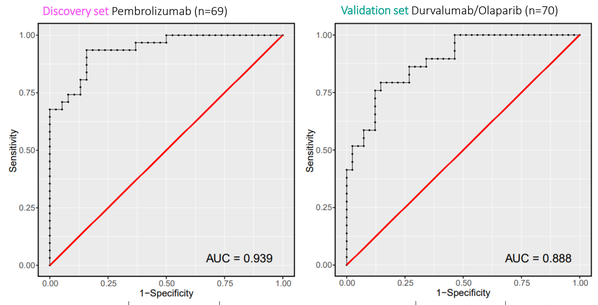


Figure 4. GSEA of ImPrint Immune+ vs. Immune- in the Discovery and Validation sets. In bold are highlighted immune-related pathways.

Results



Discovery set	pCR	RD
Immune+	29	6
Immune-	2	32
Accuracy: 0.88	Sensitivity: 0.94	Specificity: 0.84

Validation set	pCR	RD
Immune+	22	6
Immune-	7	35
Accuracy: 0.81	Sensitivity: 0.76	Specificity: 0.85

Figure 2. Receiving Operating Curves and pCR distribution for the Pembrolizumab Discovery set (**left**) and the Durvalumab/Olaparib Validation set (**right**). AUC=Area Under the Curve

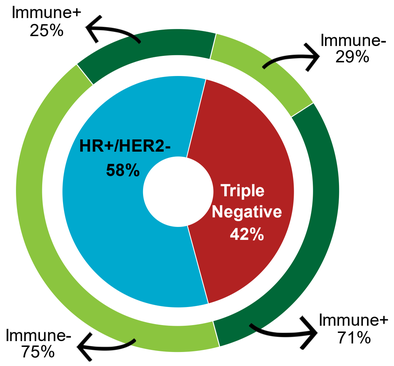


Figure 3. Prevalence analysis of ImPrint (Immune+ vs. Immune-) in the ISPY2 trial high risk patients (n=1463).

The 53-gene signature ImPrint (**Figure 1**), predicts pCR to PD1 inhibition with **94% sensitivity and 84% specificity** in all patients (Discovery set, **Figure 2A**). In Triple Negative, sensitivity and specificity are 100% and 70%, and in HR+HER2- 83% and 89%, respectively. The Positive Predictive Value (PPV) is 77% in the HR+HER2- group. In the Validation set (**Figure 2B**) , ImPrint predicts pCR to PDL1 inhibition with **76% sensitivity and 85% specificity** in all patients, 89% sensitivity and 58% specificity in Triple Negative and 70% sensitivity and 97% specificity in HR+HER2-. Notably, the PPV is 93% for the HR+HER2- group.

ImPrint prevalence analysis on 1463 EBC, shows that majority of Triple Negative EBC is Immune+ (n=438/614, 71%), however a clinically relevant subset of HR+HER2- is also Immune+ (n=213/849, 25%) (**Figure 3**).

Over 90% of the ImPrint genes have known immune response related functions (including PD-L1 and PD-1, as well as immune predictive genes from I-SPY2[2]). GSEA indicates that ImPrint identifies a subset of immune active tumors (Immune+) with enrichment of different immune pathways, such as Interferon-α- and -γ response, known to play key roles in activation of cellular immunity stimulation of antitumor immune-response (**Figure 4**).

Conclusions and Future Directions

- ImPrint predicts pCR to PD1-PDL1 ICI with high sensitivity and specificity in both discovery and validation sets.
- Over 90% of the ImPrint genes have known immune response related functions (including genes that codify PD-L1 and PD-1)
- ImPrint identifies tumors with an immune active phenotype denoted by the enrichment of several immune-related pathways
- ImPrint appears very effective in identifying a subset of HR+HER2- patients who could benefit from immunotherapy.
- Prospective validation of ImPrint will be performed within the I-SPY 2.2. trial.

References

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