Identifying breast cancer molecular phenotypes to predict response in a modern treatment landscape: lessons from ~1000 patients across 10 arms of the I-SPY 2 TRIAL

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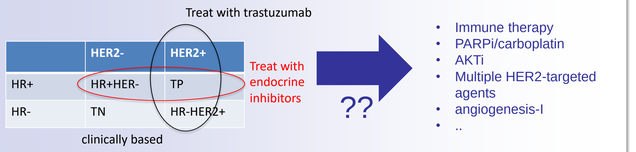
1. Background

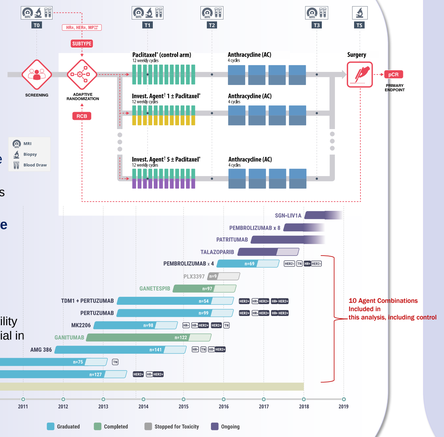
The explosion in new treatment options targeting immune checkpoints, HER signaling, DNA repair deficiency, AKT, and other pathways calls for updated breast cancer subtypes beyond HR and HER2 status to predict which patients will respond to which treatments.



Here we leverage the I-SPY 2 TRIAL biomarker program over the past 8 years across 10 treatment arms to elucidate a minimal set of biomarkers that may improve response prediction in a modern treatment context, and to investigate which new patient phenotypes are identified by these response-predictive biomarkers.

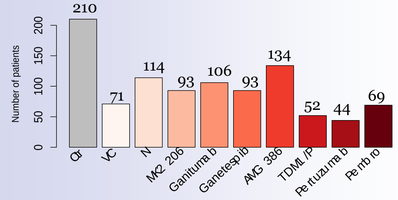
2. THE PATIENTS: I-SPY 2 TRIAL Standing Platform

- **Phase II, adaptively-randomized neoadjuvant trial**
- **Shared control arm**
  Standard neoadjuvant chemotherapy
- **Simultaneous experimental arms**
  Up to four
- **Primary endpoint:** pathologic complete response (pCR)
  No residual invasive cancer in breast or nodes
- **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 300 pt phase III trial in the most responsive patient subset



- ***Biomarker component:*** evaluate biomarkers associated with mechanism of action of each agent, along with the pre-defined subsets

3. DATA/METHODS: Predictive expression biomarkers across 10 arms



Data from 986 patients were considered in this analysis. Treatments included paclitaxel alone (or with trastuzumab (H) in HER2+) or combined with investigational agents: veliparib/carboplatin (VC); neratinib; MK2206; ganitumab; ganetespib; AMG386; TDM1/pertuzumab (P); H/P; and pembrolizumab (Pembro).

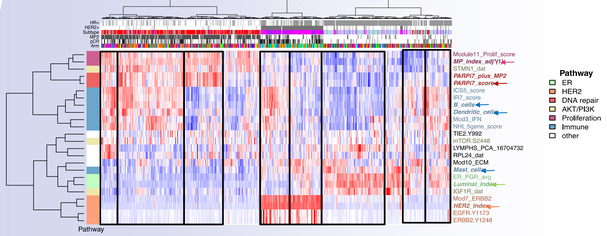
Predictive mechanism-of-action biomarkers

Mechanism of action Signature/endpoint	Pathway	Type*	Description	Primary pCR predictive arm
Luminal_Index	ER	Gene expression	BluePrint continuous index for Luminal-like (Agendia)	resistance in HR+ in most arms
ER_PGR_avg	ER	Gene expression	ESR1, PGR averaged expression	resistance in HR+ in most arms
HER2_Index	HER2	Gene expression	BluePrint continuous index for HER2-like (Agendia)	HER2-targeted agents
Mod7_ERBB2	HER2	Gene expression	HER2 amplicon expression module [PMID:24516633]	HER2-targeted agents
MP_index_adj*(-1)	Proliferation	Gene expression	MammaPrint risk continuous index*(-1) (Agendia)	most cytotoxics: HR+
IR7_score	Immune	Gene expression	Immune signature [PMID:17683518]	Pembrolizumab
ICS5_score	Immune	Gene expression	Immune signature [PMID:24172169]	Pembrolizumab
NHI_5gene_score	Immune	Gene expression	Immune signature [PMID:21479927]	Pembrolizumab
Dendritic_cells	Immune	Gene expression	Dendritic cell pathway score [PMID:28239471]	Pembrolizumab
B_cells	Immune	Gene expression	B cell pathway score [PMID:28239471]	Pembrolizumab
Mast_cells	Immune	Gene expression	Mast cell pathway score [PMID:28239471]	Pembrolizumab
Mod3_IFN	Immune	Gene expression	IFN immune module [[PMID:24516633]	Pembrolizumab
Module11_Prolif_score	Proliferation	Gene expression	Proliferation module [PMID:24516633]	most cytotoxics: HR+
PARPi7_score	DNA repair	Gene expression	DNA repair deficiency/Olaparib sensitivity [PMID:28948212]	platinum containing (VC)
PARPi7_plus_MP2	DNA repair	Gene expression	PARPi7 and MP_index_adj*(-1) averaged	platinum containing (VC)
ERBB2.Y1248	HER2	RPPA protein/phospo-protein	phospho-ERBB2 level(Wulfkhule/Petricoin) [doi: 10.1200/po.18.00024]	neratinib (pan-HER)
EGFR.Y1173	HER2	RPPA protein/phospo-protein	phospho-EGFR level (Wulfkhule/Petricoin) [doi: 10.1200/po.18.00024]	neratinib (pan-HER)
mTOR.S2448	AKT/PI3K	RPPA protein/phospo-protein	phospho-MTOR level (Wulfkhule/Petricoin)	MK2206 (AKTi)
TIE2.Y992	other	RPPA protein/phospo-protein	phospho-TIE2 level (Wulfkhule/Petricoin)	AMG386 (ANG1/2i)
IGF1R_dat	AKT/PI3K	RPPA protein/phospo-protein	IGF1R expression level	MK2206 (AKTi)
RPL24_dat	other	Gene expression (exploratory)	RPL24 expression level	platinum containing (VC)
Mod10_ECM	other	Gene expression (exploratory)	ECM module [PMID:24516633]	Pembrolizumab
LYMPHS_PCA_16704732	other	Gene expression (exploratory)	Immune/prognostic signature [PMID:16704732]	platinum containing (VC)
STMN1_dat	AKT/PI3K	Gene expression	STMN1 expression level [doi: 10.1200/po.18.00024]	neratinib (pan-HER)

- 24 prospectively defined, mechanism-of-action and pathway-based expression and phospho-protein signatures/biomarkers assayed from pre-treatment biopsies that were previously found to be predictive in a particular agent/arm in pre-specified analysis.
- Here we evaluate these biomarkers in all patients.
- We assessed association between each biomarker and response in the population as a whole and within each arm and HR/HER2 subtype using a logistic model.
- To identify optimal dichotomizing thresholds for select biomarkers, 2-fold cross-validation was repeated 500 times.
- Our analysis is exploratory and does not adjust for multiplicities.

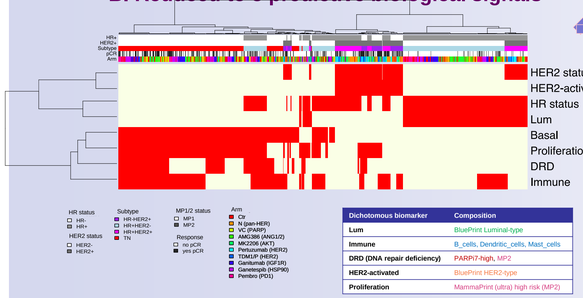
4. RESULTS: Patient subgroups with predicted differential response to I-SPY 2 agents

A. Unsupervised clustering of 24 continuous biomarkers



- Our initial set of 24 predictive biomarkers reflects DNA repair deficiency (n=2), immune activation (n=7), ER signaling (n=2), HER2 signaling (n=4), proliferation (n=2), phospho-activation of AKT/mTOR (n=2), and ANG/TIE2 (n=1) pathways, among others.
- Biomarkers reflecting similar biology are correlated and cluster together

B. Reduced to 5 predictive biological signals

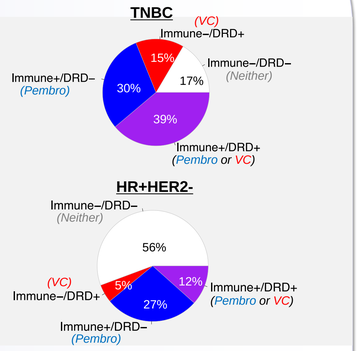


Dichotomous biomarker	Composition
Lum	BluePrint Luminal-type
Immune	B_cells, Dendritic_cells, Mast_cells
DRD (DNA repair deficiency)	PARPi7-high, MP2
HER2-activated	BluePrint HER2-type
Proliferation	MammaPrint (ultra) high risk (MP2)

- We make use of this correlation structure to reduce the dimensionality of the biomarker set to five predictive signals: proliferation, DNA repair deficiency (DRD), immune-engaged (Immune), luminal/ER (lum), and HER2-activated.
- These biomarkers, when dichotomized, identify patient groups with differential predicted sensitivities to I-SPY 2 agents and are present at different proportions within receptor subtypes.

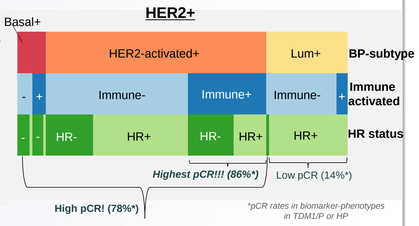
C. Distribution of predictive biomarkers for carboplatin/PARPi and immunotherapy differ in TN and HR+HER2- subsets

- in the HER2- subset, Immune+/DRD+ patients are predicted sensitive to both VC and Pembro, and account for 39% of TN, but only 12% of HR+HER2-.
- On the other end of the spectrum, only 17% of TN are Immune-/DRD-, compared to the majority (56%) of HR+HER2-.
- There are also subsets of patients positive for only one marker, predicted to respond preferentially to VC or Pembro (but not both)



D. Biomarker phenotypes predict differential response to HER2-targeted agents

- For the HER2+ subset, 67% are HER2-activated+, and 25% Lum+
- HER2-activated+ patients are more likely to be Immune+ (44%), vs 23% in lum+.
- HER2-activated+/Immune+ patients have higher predicted sensitivity to HER2-targeted agents than lum+ or Immune- patients.



*pCR rates in biomarker-phenotypes in TDM1/P or HP

5. CONCLUSION

Molecular phenotypes reflecting proliferation, immune engagement, HER2-activation, luminal/ER-signaling, and DNA repair deficiency may provide a roadmap to guide treatment prioritization for emerging therapeutics. In all, these biomarkers predict sensitivity to one or more I-SPY 2 investigational agents for 75% of the ~ 1000 patients.

Advocate perspective: *Providing the right drug for the right patient is not only a hallmark of the I-SPY 2 TRIAL, but also, from an advocate's perspective, critical to avoiding side effects and wasted time from drugs that would not lead to pCR. Determining the best treatment option requires balancing harm - side effects impacting quality of life - with the opportunity for a cure, or at least, extended life span. Understanding the implications of predictive biomarkers can give patients an important tool for treatment decision-making.*

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