HER2, ER, and proliferation biomarkers predict response to multiple HER2-targeted agents/combinations plus standard neoadjuvant therapy in the I-SPY 2 TRIAL

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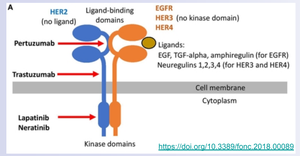
1. Background

A variety of investigational HER2-inhibitors/combinations have been tested in I-SPY 2, including neratinib (N), TDM1 combined with pertuzumab (P) (TDM1/P), and trastuzumab (H) combined with pertuzumab (THP; prior to this combination becoming standard of care), all with trastuzumab as control (Ctr). All three experimental arms graduated, showing improved efficacy over control in one or more receptor subsets (HR+HER2+, HR-HER2+, or/and HER2+).



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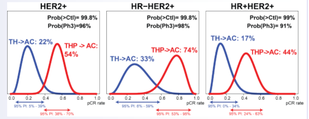
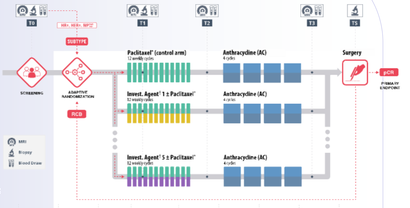
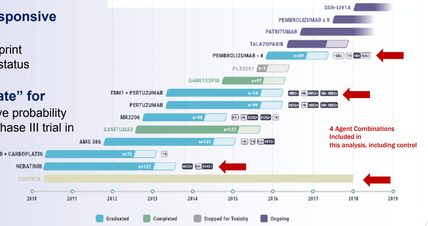


Here we assess 10 biomarkers in the HER2, ER/PR, and proliferation pathways on multiple levels of resolution (expression, protein, phospho-protein) as predictors of response in these four arms, hypothesizing that highly HER2-activated, proliferative tumors may be more sensitive to HER2-inhibition than those that are more luminal and quiescent.

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

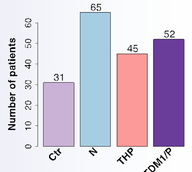




- ***'Qualifying' Biomarker component:*** evaluation of pre-specified biomarkers associated with mechanism of action of each agent, along with the pre-defined subsets

3. DATA/METHODS: patients & biomarkers

193 HER2+ patients were considered in this analysis: (31 Ctr, 65 N, 52 TDM1/P, and 45 THP).



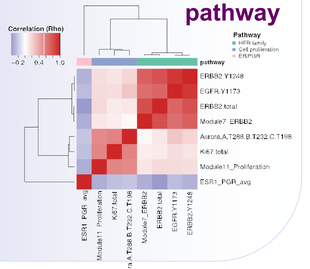
Biomarkers in this study

Mechanism of action Signature/endpoint	Pathway	Type	Description
HER2 IHC	HER2	Standard clinical IHC (CLIA)	HER2 protein level by IHC: +3 , +2, +1
Module7_ERBB2	HER2	Gene expression	HER2 amplicon expression module [PMID:24516633]
ERBB2.total	HER2	RPPA total protein	phospho-ERBB2 level(Wulfkhule/Petricoin)[doi: 10.1200/po.18.00024]
ERBB2.Y1248	HER2	RPPA phospo-protein	phospho-ERBB2 level(Wulfkhule/Petricoin)[doi: 10.1200/po.18.00024]
EGFR.Y1173	HER2	RPPA phospo-protein	phospho-EGFR level (Wulfkhule/Petricoin) [doi: 10.1200/po.18.00024]
ESR1_PGR_avg	ER	Gene expression	ESR1, PGR averaged expression
Module11_proliferation	Cell cycle	Gene expression	Proliferation module [PMID:24516633]
Ki67.total	Cell cycle	RPPA total protein	Total Ki67 protein level(Wulfkhule/Petricoin)[doi: 10.1200/po.18.00024]
Aurora.A.T288.B.T232.C.T198	Cell cycle	RPPA phospo-protein	phospho-AURKA level(Wulfkhule/Petricoin) [doi: 10.1200/po.18.00024]
BluePrint subtype	ER/HER2/Cell cycle	Expression based subtype classifier (CLIA)	Breast cancer subtype classifier by Agendia: Luminal, Her2-type, Basal-type

- 10 biomarkers relating to HER2, ER, proliferation were evaluated: HER2 IHC (n=146), 3 expression signatures (n=192), BluePrint subtype (n=192), and 5 protein/phospho-protein endpoints by RPPA (n=175), all at the pre-treatment time point.
- Each biomarker was tested for association with pCR in the whole population and within each arm using a logistic model.
- This analysis was adjusted for HR status and treatment arm as covariates, and performed within receptor subtypes.
- This analysis does not adjust for multiplicities of other biomarkers.

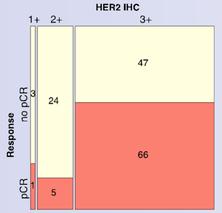
4. RESULTS: Biomarkers are correlated by pathway

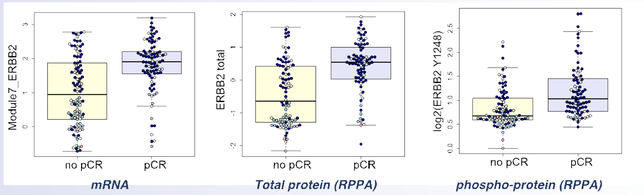
In the population as a whole, HER2 and HER2-signaling biomarkers, evaluated at multiple levels of resolution - IHC, total-/phospho-protein by RPPA, and mRNA (HER2 amplicon module) - are highly correlated (rho=0.8 [0.65-0.92]).



5. RESULTS: Associations with response to HER2-targeted therapy

A. ERBB2 mRNA, protein, phospho-protein associate with pCR
(combined Ctr, N, TDM1/P, THP arms)

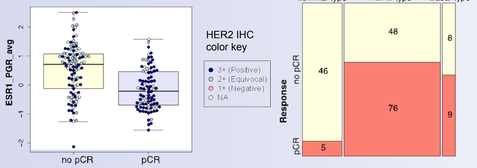




- Higher HER2 levels and activity is associated with response: HER2 IHC 3+ status (LR p=0.00032), total ERBB2 protein (LR p=5.4E-09) ERBB2 phospho-protein levels (LR p=6.58E-06 (pERBB2 (Y1248)) and 9.95E-06 (pEGFR Y1173)), the ERBB2 amplicon expression signature (LR p=2.38E-08).

B. HER2+ with high ER/Luminal phenotype are resistant to HER2-targeted agents

- In contrast, higher average ER/PR expression associated with non-response to HER2-targeted therapy (LR p=4.28E-08).



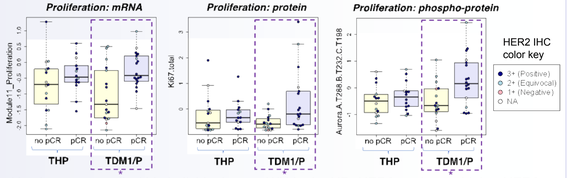
- Both HER2 and ER/PR signaling phenotypes are captured by BluePrint subtyping; and consistent with the individual pathway markers, tumors classified Luminal-type had a lower pCR rate relative to those classified as Her2-type (or Basal-type) (LR p=4.84E-11).

These associations all retain significance in a model adjusting for HR status and treatment arm, and in the HR+HER2+ subset.

C. Proliferation markers also associate with response

- In addition, we quantitatively assessed proliferation markers at the total protein (RPPA: Ki67), phospho-protein (pAURKA) and mRNA (proliferation signature Module11_Proliferation) levels.
- mRNA and pAURK proliferation biomarkers predict response overall; but this association is strongest within the HR+HER2+ subset (LR p: 0.0012 (Module11_proliferation), 0.0036 (pAURK), and 0.045 (Ki67)).

D. Proliferation markers predictive in TDM1/P but not THP



- Numbers are small within individual arms. Within the HR+HER2+ subtype, higher HER2 and lower ER/PR is observed in responders in all experimental arms; but the proliferation markers Module11_Proliferation (LR p=0.0031), Ki67 total protein (LR p=0.0029) and pAURK (LR p=0.006) are associated with response to TDM1/P but not THP, N, or Ctr.

E. No significant associations in HR-HER2+ subset

	Population as a whole (n=192)		Population as a whole, adjusting for HR status and treatment arm		HR+HER2+ subset, adjusting for treatment arm (n=125)		HR-HER2+ subset, adjusting for treatment arm (n=67)	
	OR/unit increase	LR p	OR/unit increase	LR p	OR/unit increase	LR p	OR/unit increase	LR p
ESR1_PGR_avg	0.370	**4.82E-08**	0.331	**3.30E-05**	0.155	**8.49E-08**	1.56	0.42
Mod7_ERBB2	2.47	**2.38E-08**	2.53	**6.25E-08**	4	**3.48E-09**	1.41	0.211
Module11_Proliferation	2.25	**1.62E-05**	1.99	**0.00106**	2.38	**0.00118**	1.54	0.227
ERBB2.total	2.69	**5.41E-09**	2.76	**4.71E-08**	3.79	**4.66E-08**	1.7	0.0815
ERBB2.Y1248	2.49	**6.58E-06**	2.6	**6.69E-06**	3.15	**3.58E-05**	2.01	0.0501
EGFR.Y1173	2.33	**9.95E-06**	2.51	**5.68E-06**	2.77	**3.54E-05**	2.11	0.0599
Ki67.total	1.19	0.27	1.09	0.635	1.77	**0.0448**	0.766	0.247
Aurora.A.T288.B.T232.CT198	1.6	**0.00582**	1.52	**0.0216**	2.05	**0.0036**	1.07	0.797

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. Understanding the implications of predictive biomarkers can give patients an important tool for treatment decision-making*

6. CONCLUSION

High HER2 signaling at the expression, protein, and phospho-protein levels, and low ER signaling, predict response to HER2-inhibition across treatment arms. Proliferation markers may be useful for prioritizing therapies in the HR+HER2+ subset.

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