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 (P) (TRP) prior to this combination becoming standard of care, all with trastuzumab as control (C). All three experimental arms graduated, showing improved efficacy over control in one or more receptor subsets (HR+/HER2-, HR+/HER2+, or HER2+).

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

1. Included in 4 Agent Combinations
2. Showing the power of the BluePrint subtyping to define subsets from patients treated with four different regimens.
3. The Power of Subtyping: The relevance of subtyping in breast cancer is illustrated in the I-SPY 2 trial. Patients are stratified into subsets based on HER2, ER, and proliferation biomarkers predicted response to multiple HER2-targeted agents/combinations plus standard neoadjuvant therapy in the I-SPY 2 TRIAL. The diversity of the patient population and the availability of comprehensive biomarker data allows for a detailed analysis of response mechanisms.

3. DATA/METHODS: patients & biomarkers

<table>
<thead>
<tr>
<th>Biomarkers in this study</th>
<th>HER2</th>
<th>ER</th>
<th>proliferation</th>
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<tbody>
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<td>mRNA</td>
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- 10 biomarkers relating to HER2, ER, or proliferation were evaluated in I-SPY 2. These biomarkers include HER2 (n=145), ErbB2, and Ki67. These biomarkers were used to stratify patients into specific subsets for analysis.

4. RESULTS: Biomarkers are correlated by pathway

In the population as a whole, HER2 and HER2 signaling biomarkers are highly correlated (rho=0.805, 95% CI [0.92, 1.00]). HER2 and ER are also highly correlated (rho=0.805, 95% CI [0.92, 1.00]). The correlation between HER2 and ER is strongest in the HR+HER2+ subset (LR p: 0.0012).

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

A. ERBB2 mRNA, protein, phospho-protein associate with pCR

- Higher HER2 levels are associated with response: HER2 IHC 3 status (LR p=0.0032), total ERBB2 protein (LR p=0.035), and ERBB2 phospho-protein levels (LR p=0.001, p=0.001).

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

- In contrast, higher ER and Luminal phenotype expression are associated with non-response to HER2-targeted therapy (LR p=3E-13).

C. Proliferation markers also associate with response

- In addition, quantitative assays of proliferation markers of the total protein (RPPA and ERBB2 phospho-protein) and mRNA (protein signature module 11_Proliferation) levels.

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

- Both HER2 and ERBB2 signaling phenotypes are captured by BluePrint subtyping and are consistent with the individual pathogenic markers. Torsos classified Luminal-type tumors with a lower pCR rate relative to those classified as HER-3 (or Basal-type) (LR p=0.001).

E. No significant associations in HR/HER2+ subset

- These associations remain significant in a model adjusting for HR status and treatment arm, and in the HR+/HER2+ subset.

6. CONCLUSION

High HER2 expression at the mRNA, protein, and phospho-protein levels, and low ER expression are independently associated with resistance to HER2-targeted agents. Proliferation markers may be useful for prioritizing therapies in the HR+/HER2+ subset. Further studies are needed to validate and refine these findings.