An exploratory correlative biomarker analysis of NSABP FB-7, a phase II randomized trial evaluating neoadjuvant therapy with weekly paclitaxel plus neratinib or trastuzumab or neratinib and trastuzumab followed by doxorubicin and cyclophosphamide with postoperative trastuzumab in women with locally advanced HER2-positive breast cancer

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Protocol Overview

BACKGROUND: NSABP FB-7 trial enrolled HER2-positive breast cancer patients (pts) with locally advanced disease randomly assigned to trastuzumab (T) or neratinib (N) or the combination (T+N) with weekly paclitaxel (wP) → standard AC (Fig 1). Overall pathological complete response (pCR) rates yielded greater rates with the dual anti-HER2 therapy (50%) than single-targeted therapies (trastuzumab: 38.1%, neratinib: 33.3%). pCR rates were higher in the HR- than in the HR+ cohort (Jacobs et al, SABCS 2015 abstr #PD5-04).

METHODS: To investigate candidate predictive markers of response to study drugs, pre-treatment core biopsy samples (n=59), and post-treatment surgical samples (n=17) were obtained from a subset of pts. Retrospective correlation with clinical outcome was performed after datalock of Sep 1, 2015. Expression and activation of HER family RTKs, including truncated HER2 (p95) were quantified using the multiplexed microarray Collaborative Enzyme-Enhanced Reactive immuno-assay (CEER)™ platform (Fig 2). pCR data were available in n=51 pts from the biomarker cohort. After excluding low tumor content non-evaluable samples, correlative biomarker analysis was performed in 42 pts with CEER. Expression analysis of pre-treatment biopsy samples and post-treatment residual tumors was done using RNA-Seq analysis. Research grade risk scores were determined and associated with pCR from pre-treatment biopsy samples. Differential gene expression between pre- and post- treatment samples was analyzed.

RESULTS: The CEER data showed that the median levels of pre-treatment phosphorylated HER2 (pHER) and the truncated HER2 (p95) were higher in tumors that achieved pCR than in those that did not in neratinib-containing arms (Fig 3 and 4). However, the small number of samples and the imbalances in ER status in the assayed samples between arms make interpretation difficult. Gene expression profiling of pre-treatment biopsies (n=57) and post-treatment residual tumors (n=12) was assessed with RNA-Seq. MammaPrint scores were determined with microarrays (Agendia) and found to be statistically significantly associated with pCR (p=0.023). Distribution of risk scores across treatment arms are shown in Fig 5.

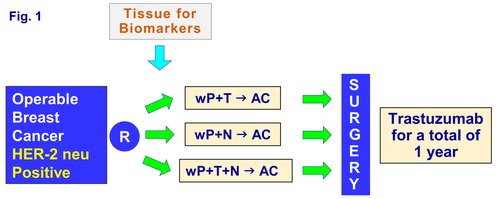
RNA-Seq data was also used to assess gene expression differences in pre- and post-treatment tumors. A cluster dendogram of 7 paired samples (Fig 6) demonstrates that some samples showed little change in gene expression (samples are paired together in the dendogram) and other samples demonstrated larger gene expression differences (samples from same pt are not clustered together). Plots of the gene expression levels of ERBB2, ESR1, proliferation genes, and immune genes are shown (Figs 7 and 8). Many of the proliferation genes show an up-regulation after treatment in all samples. Conversely, the direction and degree of expression levels of HER2 and of several immune genes varies across samples.

One interpretation is that pre- and post-treatment tumors that clustered together were intrinsically resistant to the anti-HER2 therapy. Additionally, tumors with gene expression changes may have acquired resistance; perhaps, as a result of these gene expression changes, and may represent important targets to improve patient outcomes.

SUPPORT: Puma Biotechnology, Inc.

NSABP FB-7 Schema

Fig. 1



wP=weekly Paclitaxel; T=trastuzumab; N=neratinib

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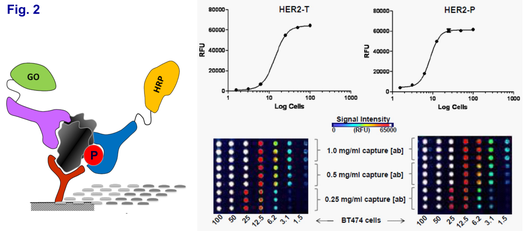
Pathway Activity Can Be Quantitated by CEER

Fig. 2

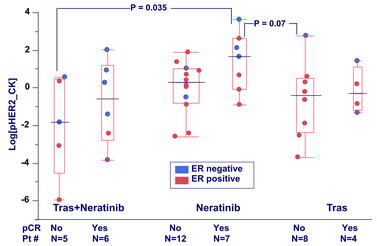


- Proximity mediated immuno-microarray platform
- Zeptomole sensitivity (~10^4 to 10^5 target molecule)
- Extreme specificity due to triplex immuno-complex requirement

- CEER provides 'Quantitative Output' based on reference biological materials.
- Longitudinal monitoring of tumor biology can be achieved and thus real-time treatment adjustment may be possible in clinic.

Phos-HER2 Correlates with pCR to Neratinib

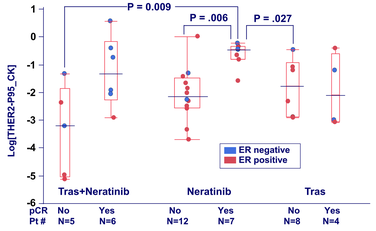
Fig. 3



- Median levels of phos-HER2 were higher in neratinib pCR group than in the no pCR groups.
- This small study does not support the use of CEER based phos-HER2 determination as a biomarker due to the overlap between pCR and no pCR. Also, the imbalances between ER+ and ER- samples across arms make interpretation difficult.

p95-HER2 Correlates with pCR to Neratinib

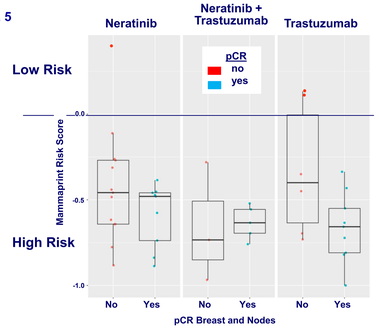
Fig. 4



- Median p95-HER2 levels were higher in samples with pCR within the neratinib arm than in the no pCR samples within any arm.
- CEER based p95-HER2 determination may identify patients with a higher likelihood of achieving pCR to neratinib but small numbers and ER imbalances across arms makes this a provisional conclusion. Additional study maybe warranted.

MammaPrint Score by Treatment Arm

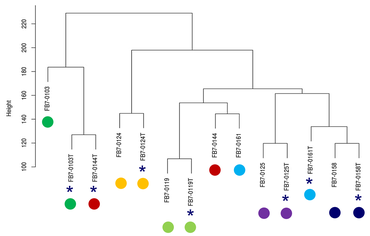
Fig. 5



- The 3 identified low risk patients did not achieve pCR.

Cluster Dendogram of Pre- and Post-* Treatment Samples

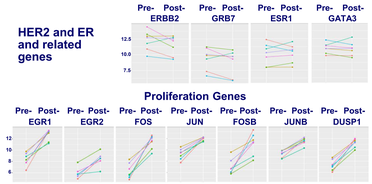
Fig. 6



- Patients with altered gene expression patterns
- Patients with little change in gene expression

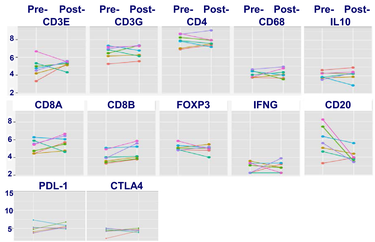
Gene Expression Changes Pre- and Post-treatment

Fig. 7



Changes in Expression of Immune Genes Pre- and Post-treatment

Fig. 8



CONCLUSIONS

- Activation of the HER2 pathway based on p95HER2 and phosphoHER2 as assessed by CEER shows a correlation with neratinib pCR.
- In this subset of FB-7 patients, MammaPrint scores are comparable to patients enrolled into the I-SPY2 trial.
- Cluster analysis pre- and post- treatment (residual disease) shows dramatic change in gene expression in some samples and little change in others, suggesting some samples have intrinsic resistance and others acquired resistance to anti-HER2 therapies.
- The direction and degree of change in the expression of HER2 and several immune genes varied across samples pre- and post-treatment. However, proliferation genes were more uniformly up-regulated.

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