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Integrating Whole Genome Sequencing Data With RNAseq, Pathway Analysis, and Quantitative Proteomics to Determine Prognosis After Standard Adjuvant Treatment With Trastuzumab and Chemotherapy in Primary Breast Cancer Patients

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Background

- Despite improvements in the treatment of HER2-positive breast cancer, a high proportion of patients develop resistance to HER2-targeted agents.
- A number of mechanisms of resistance have been proposed, including:
  - Mutations in *PIK3CA*
  - Lack of antibody-dependent cellular cytotoxicity
  - Low levels of HER2 expression
- Disease recurrence in a subpopulation of HER2-positive patients suggests that approaches are needed to accurately assess HER2 status and/or identify potential resistance markers to identify all patients who may benefit from anti-HER2 therapies.
- Here we report a comprehensive panomics approach that integrates whole genome sequencing (WGS), RNA sequencing (RNAseq) and quantitative proteomics to determine associations between tumor molecular profiles and prognosis among patients with HER2-positive breast cancer.

Patients and Methods

Patients

- Patients were enrolled in the SUCCESS A,[1] SUCCESS B,[2] and PRAEGNANT[3] studies.
- SUCCESS A and SUCCESS B include high-risk breast cancer patients after primary surgery. All HER2-positive patients received a standard chemotherapy, including 3 cycles of FEC followed by 3 cycles of docetaxel or docetaxel plus gemcitabine. Trastuzumab was given to all patients for a total of 12 months.
- PRAEGNANT is a registry of metastatic breast cancer patients. All patients selected for this study received a standard adjuvant chemotherapy, including anthracyclines and taxanes. Trastuzumab was given to all patients for a total of 12 months.
- Patients selected for analysis were divided into Cases and Controls:
  - Cases (nonresponders): all patients who recurred or developed a metastasis within 5 years after diagnosis.
  - Controls (responders): all patients with no recurrence or metastasis within 5 years after diagnosis.

Genomic Analysis

- Matched tumor-normal samples (FFPE tumors and whole blood) underwent WGS; provenance testing was done to ensure specimen purity.
- WGS data were processed using Contraster.[4]

Transcriptomic Analysis

- RNAseq of matched tumor-normal samples was performed to confirm the presence of gene mutations and was used to identify mutational and transcript abundance.

Proteomic Analysis

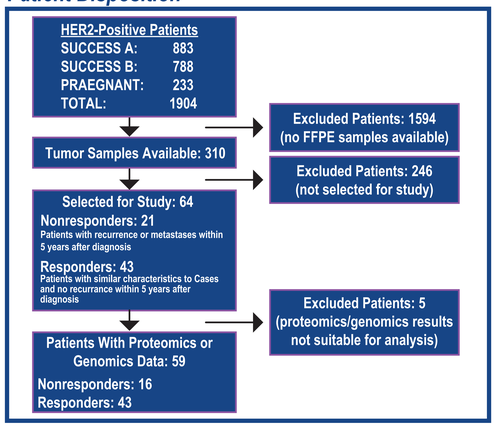
- Proteomics analysis was performed using a quantitative, multiplexed, selected reaction monitoring-mass spectrometry (SRM-MS) assay comprising a panel of 52 proteins.
- Tumor areas from FFPE tissue sections were laser microdissected, solubilized, and enzymatically digested.
- Absolute quantitation of proteins was accomplished through the simultaneous detection of endogenous targets and identical, synthetic, labeled heavy peptides; protein levels were normalized to total protein extracted from each sample.

References

1. Widschwendter P, Friedl TW, Schwentner L, et al. *Breast Cancer Res*. 2015;17:129.
2. Andergassen U, Neugebauer J, Janni W, et al. *Breast*. 2011;20:S86-S86.
3. Fasching PA, Brucker SY, Fehm TN, et al. *Geburtshilfe Frauenheilkd*. 2015;75:41-50.
4. Sanborn JZ, Salama SR, Grifford M, et al. *Cancer Res*. 2013;73:6036-6045.

Results

Patient Disposition



Patient Characteristics

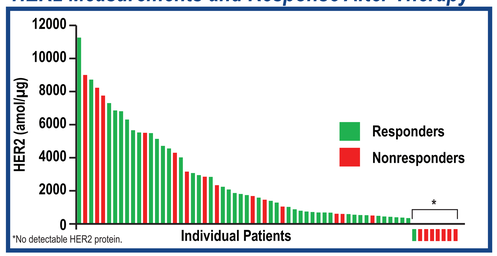
Characteristic	Category	Nonresponders (n=21)	Responders (n=43)
Age, mean (SD)		55.4 (±10.2)	56.9 (± 11.6)
pT, n (%)	1	10 (47.6)	14 (32.6)
	≥ 2	11 (52.4)	29 (67.4)
pN stage, n (%)	Negative	10 (47.6)	20 (46.5)
	Positive	11 (52.4)	23 (53.5)
Grade, n (%)	1	0 (0)	2 (4.7)
	2	10 (47.6)	22 (51.2)
	3	11 (52.4)	19 (44.2)
Hormone receptor status, n (%)	Negative	5 (23.8)	26 (60.5)
	Positive	16 (76.2)	17 (39.5)

Proteins Assiciated With Response Status

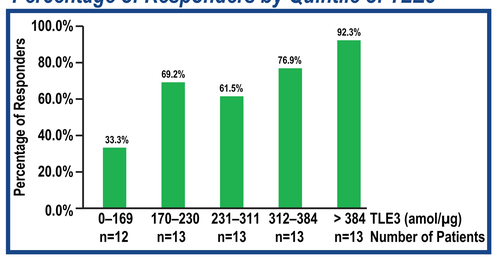
Protein	P-Value	Protein	P-Value	Protein	P-Value
TLE3	0.0027	ERCC1	0.1718	Axl	0.5089
XRCC1	0.0261	HER2	0.2406	P16	0.5622
RRM1	0.0480	TOPO1	0.2709	hENT1	0.5721
MGMT	0.0490	RFC	0.3096	AR	0.6187
Fralpha	0.0749	GART	0.3132	SYP	0.6517
MRP1	0.0749	Ki-67	0.3265	KRAS	0.7738
Ecad	0.0821	TOP2A	0.3895	PTEN	0.9175
ER	0.1331	KRT7	0.4027	SPARC	0.9365
EGFR	0.1524	Vimentin	0.4527	TUBB3	0.9655
PR	0.1524	IDO1	0.4706	DHFR	0.9770
KRT5	0.1717	HGF	0.4853	HER3	0.9906

P-value by Mann-Whitney test.

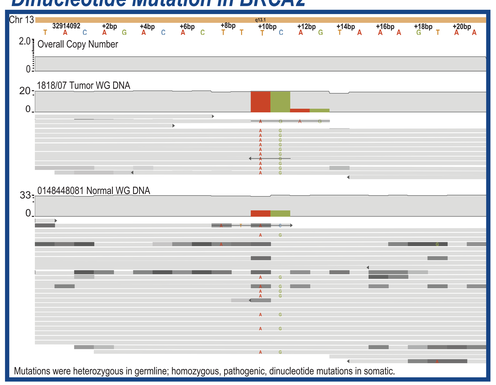
HER2 Measurements and Response After Therapy



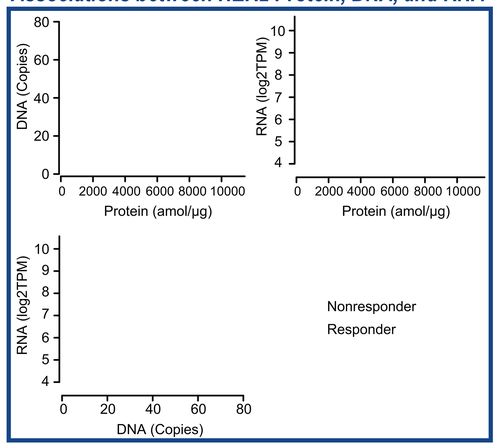
Percentage of Responders by Quintile of TLE3



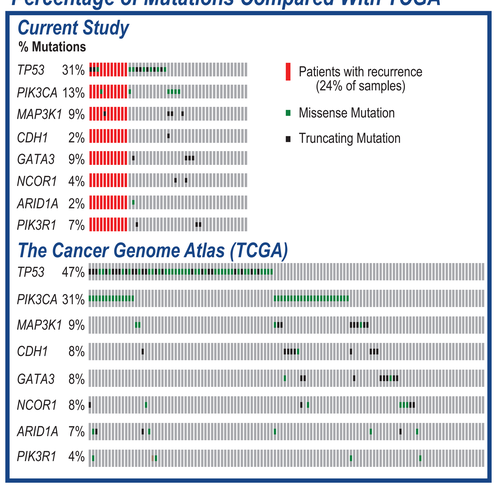
Patient With Pathogenic, Nonsynonymous, Dinucleotide Mutation in BRCA2



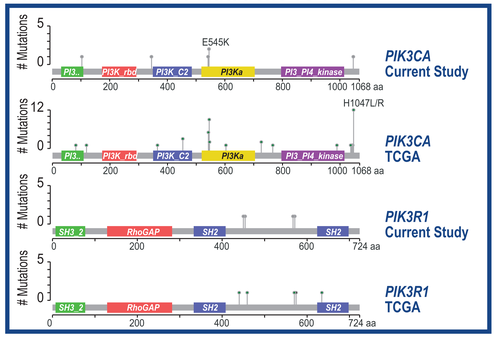
Associations between HER2 Protein, DNA, and RNA



Percentage of Mutations Compared With TCGA



PIK3CA and PIK3R1 Mutations vs TCGA



Conclusions

- In this small, exploratory study of patients treated with chemotherapy and trastuzumab, there appeared to be enrichment for higher response when higher levels of TLE3 protein were detected (92.3% top quintile vs 33.3% bottom quintile).
- Compared with HER2-positive breast cancer patients in TCGA, the rates of *TP53* and *PIK3CA* mutations are different (Fisher's exact test, *TP53* P=0.0794; *PIK3CA* P=0.0279), potentially due to enrichment of patients with metastatic disease.
- A customized genome browser identified a LOH-mediated selection of a pathogenic dinucleotide *BRCA2* variant as a potential driver of disease due to the loss of a section of chromosome 13 encoding for a wild-type *BRCA2* copy.
- RNAseq performed on these archival tissues was successful in > 40% of cases (26/64), but did not produce sufficient numbers for meaningful statistical analysis.
- In this cohort of patients, HER2 expression status was not significantly associated with prediction of recurrence; however, patients with low levels (bottom quintile) of detectable HER2 protein had a notably worse response rate (41.7%) than those with higher HER2 expression (73.1%).
- The treatment regimen, comprising taxanes, anthracyclines, 5-FU, and cyclophosphamide may explain the number of responders with little or no detectable *HER2* amplification or HER2 protein.
- Some patients with high HER2 did not respond to trastuzumab-containing regimens probably due to the complexity of resistance pathways for individual cancer patients.
- Performing comprehensive omics analyses will enable a better understanding of the molecular mechanisms driving recurrence in the real-world setting where patients receive complex treatment regimens.

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