Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging And moLecular Analysis 2

Association of activation levels of TIE2 with response to the angiogenesis inhibitor trebananib in HER2+ patients in the I-SPY 2 TRIAL

RI Gallagher[1], JD Wulfkuhle[1], C Yau[2], DM Wolf[2], L Brown-Swigart[2], G Hirst[2], L Esserman[2], D Berry[3], L van't Veer[2], EF Petricoin III[1]
[1]George Mason University; [2]University of California, San Francisco; [3]Berry Consultants, LLC

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Abstract

Background: Trebananib (T), an angiopoietin 1/2 neutralizing peptibody that inhibits interaction with TIE2 receptors, was available to all HR/HER2 subtypes in the I-SPY2 TRIAL. The agent did not achieve the prescribed graduation threshold for any eligible signatures prior to accrual of maximum sample size. We postulated that response to a drug that blocks TIE2 receptor-ligand interaction could be predicted by the measurement of basal TIE2 phosphorylation and downstream signaling in the pre-treatment biopsies.
Methods: Of 267 patients in the T and control arms, 203 patients (T: 128, controls: 73) had reverse phase protein microarray (RPPA) and pCR data available. RPPA data for 33 (phospho- and total) proteins involved in TIE2 signaling were evaluated for association between biomarker and response in the T and control arms alone (likelihood ratio test), and relative performance between arms (biomarker x treatment interaction) using a logistic model (LM). Analysis was also performed adjusting for HR/HER2 status. Markers were analyzed individually; p-values are descriptive and were not corrected for multiple comparisons.
Results: In the TN subpopulation, TIE2 receptor levels (p = 0.037), ERBB3 (p = 0.048), total ERα (p = 0.05) and ERα S118 (p = 0.016) were negatively associated with response to T. In HER2+ patients, phospho-TIE2 Y1119 (p = 0.001) and Y992 (p = 0.0007) were positively associated with T response, as were downstream AKT-mTOR signaling activation proteins such as eIF4G S1108 (p = 0.005), p70S6K T389 (p = 0.011) and T412 (p = 0.038) and FOXO3a S253 (p = 0.041). ERBB2 Y877 (p = 0.028) was negatively associated with response in these patients. TIE2 Y1119, TIE2 Y992, eIF4G S1108, ERBB2 Y877, and FOXO3a S253 all demonstrated a significant treatment interaction by LM. **Conclusions:** While small sample sizes preclude drawing definitive conclusions, our results suggest that activation levels of the TIE2 receptor may be predictive of T efficacy in HER2+ patients and signaling activation downstream of TIE2 such as AKT-mTOR signaling may correlate with response in the HER2+ and TN populations. These results need to be independently validated to determine the significance of these findings.

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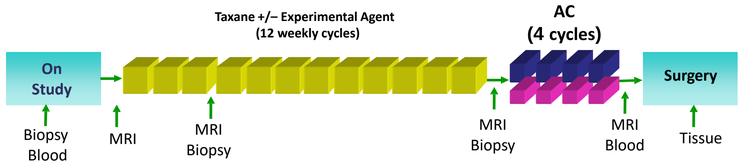
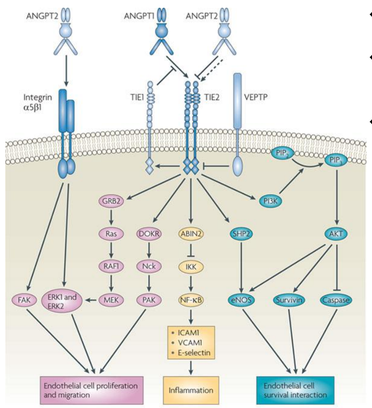
Background

I-SPY 2 TRIAL – A Prototype for Adaptive Design Trials





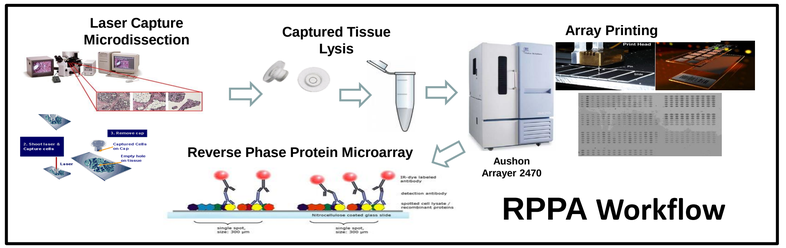
Huang et al. (2010) Nat Rev Cancer. 10:575-585. Nature Reviews | Cancer

- Multicenter Phase II neoadjuvant adaptive design trial for locally advanced breast cancer.
- Patients are assigned to 1 of 10 biomarker signature groups based on HR, HER2 and Mammaprint high or ultra-high risk status.
- Primary goal is to determine predictive possibilities of success in Phase III trials for various targeted therapeutics and determine which patient biomarker signatures respond to various regimens most effectively.

Signature	Estimated pCR Rate (95% probability interval)		Probability Trebananib is Superior to Control	Predictive Probability of Success in Phase III
	Trebananib	Control		
HR-/HER2-	37% (21-53%)	20% (10-30%)	99%	77%
HR-/HER2+	44% (15-74%)	24% (7-42%)	93%	74%
HR+/HER2-	14% (4-24%)	11% (3-19%)	76%	25%
HR+/HER2+	20% (1-39%)	13% (1-26%)	77%	41%

Table 1. Estimated pCR rates and predictive probabilities for trebananib and control arms. No biomarker signatures achieved prescribed probability threshold for graduation of trebananib from the trial.

Materials and Methods



- I-SPY 2 TRIAL pre-treatment biopsy specimens were subjected to LCM to procure tumor epithelium for RPPA analysis. Approximately 10,000 cells were captured for each of 203 samples in the AMG 386 treatment. (128 AMG treatment arm, 73 current control and 43 historical control samples were included in the analysis).
- RPPA data were collected for 33 qualifying biomarker endpoints related to the TIE2 cell signaling pathway.
- Statistical analyses: RPPA data was evaluated for association between biomarker and response in the treated and control arms individually (likelihood ratio test). Relative biomarker performance between arms (biomarker x treatment interaction) was assessed using a logistic model (pCR~ treatment + biomarker + treatment x biomarker). Analysis was also performed adjusting for HR/HER2 status (pCR~treatment + biomarker + treatment:biomarker + HR status + HER2 status). P values are descriptive only and not generalizable. In addition, no adjustments for multiple comparison were made.

Biomarkers identified as predictors of response in HER2+ treated patients

Table 2. Qualifying Biomarker Endpoint Analysis

Endpoint	HER2+			TN		
	AMG386 LR p	Control LR p	Biomarker x treatment int:pval	AMG386 LR p	Control LR p	Biomarker x treatment int:pval
AKT.S473	0.919	0.463	0.736	0.0575	0.259	**0.0289**
AKT.T308	0.891	0.811	0.79	0.178	0.774	0.326
BAD.S136	0.917	0.767	0.79	0.549	0.0701	0.399
EGFR.total	0.0687	0.963	0.0902	0.376	0.157	0.226
EGFR.Y1173	0.986	0.342	0.563	0.0734	0.334	**0.0423**
eIF4E.S209	0.559	0.416	0.366	0.193	0.443	0.78
eIF4G.S1108	**0.00505**	0.798	**0.0142**	0.927	0.707	0.811
ERBB2.Y1248	0.548	0.365	0.993	0.0834	0.474	0.0706
ERBB2.Y877	**0.028**	0.336	**0.0169**	0.729	0.679	0.997
ERBB3.total	**0.036**	0.428	**0.025**	**0.0482**	0.993	0.139
Estrogen.Rec.α.S118	0.152	0.707	0.166	**0.0159**	0.962	0.0789
Estrogen.Rec.α.total	0.536	0.524	0.394	**0.0496**	0.125	0.529
FOXO1.S256	0.47	0.844	0.5	0.121	0.256	0.0585
FOXO1.T24.FOXO3a.T32	0.506	0.706	0.446	0.571	0.728	1
FOXO3a.S253	**0.0414**	0.98	0.0601	0.448	0.221	0.945
GSK3aB.S21.S9	0.46	0.593	0.815	0.0734	0.73	0.463
mTOR.S2448	0.272	0.963	0.362	0.337	0.33	0.898
mTOR.total	0.0965	0.894	0.106	0.295	0.0732	0.772
NFkB.p65.S536	**0.023**	0.384	**0.015**	0.623	0.739	0.946
p27.T187	0.664	0.635	0.551	0.205	0.0747	0.539
p70S6K.S371	0.147	0.42	0.215	0.289	0.209	0.105
p70S6K.T389	**0.0115**	0.476	0.163	0.614	0.294	0.497
p70S6K.T412	**0.0383**	0.181	0.28	0.755	0.693	0.901
PI3K.p85.Y485.p55.Y199	0.582	0.923	0.633	0.588	0.872	0.81
PTEN.S380	**0.0229**	0.605	0.0576	0.576	0.938	0.77
PTEN.total	**0.036**	0.319	0.232	0.784	0.208	0.211
S6RP.S240.S244	0.233	0.451	0.394	0.569	0.878	0.871
SGK.S78	0.738	0.504	0.525	0.337	0.176	0.106
TIE2.total	0.131	0.702	0.147	**0.0374**	0.94	0.192
TIE2.Y1119	**0.00109**	0.919	**0.00184**	0.429	0.0508	0.35
TIE2.Y992	**0.000711**	0.706	**0.00199**	0.805	0.0896	0.186
Tuberin.TSC2.Y1571	0.304	0.332	0.584	0.0597	0.606	0.173
X4EBP1.S65	0.0584	0.529	**0.0484**	0.56	0.899	0.617

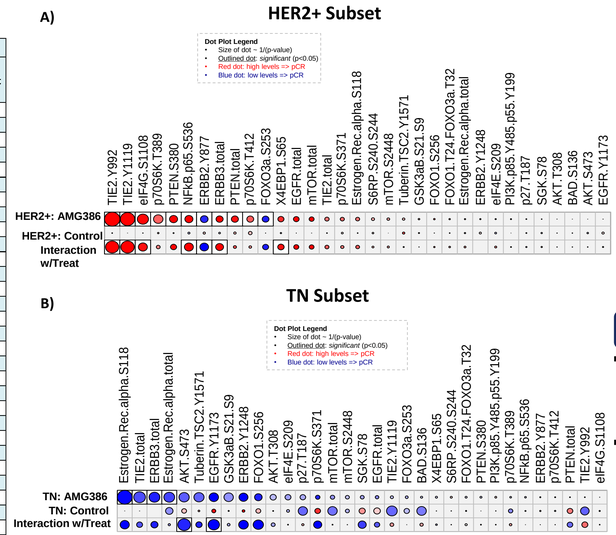


Figure 1. Association dot plots for RPPA endpoints. A) HER2+ patients treated with trebananib showed significant positive association (red-outlined dots) of TIE2 Y992 and TIE2 Y1119 proteins with pCR. **B)** Treated triple negative (TN) population showed significant low association levels (blue-outlined dots) of proteins ERα S118 and total TIE2 with pCR.

Phospho-TIE2 is associated with pCR in treated HER2+ patients

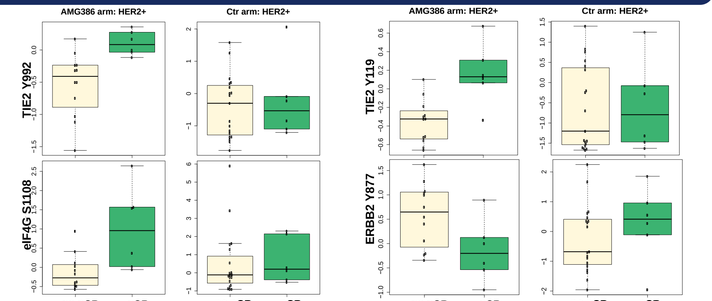
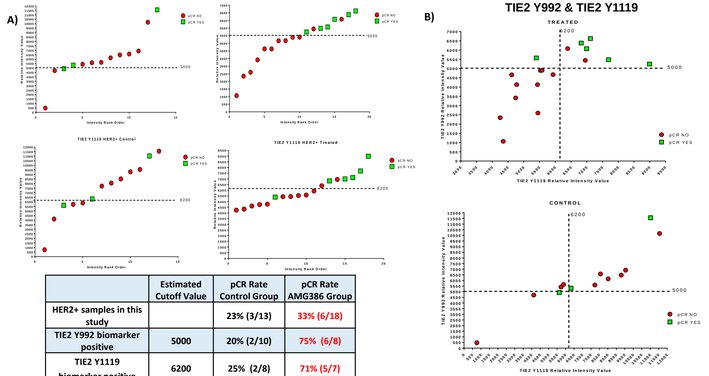


Figure 2. Box plots of RPPA endpoints in the HER2+ subset . Tie2 Y992, Tie2 Y119, and eIF4G S1108 showed association with pCR in the trebananib arm and not in the control arm.

Estimated cutpoints for biomarker discrimination of pCR in trebananib-treated HER2+ patients



	Estimated Cutoff Value	pCR Rate Control Group	pCR Rate AMG386 Group
HER2+ samples in this study		23% (3/13)	33% (6/18)
TIE2 Y992 biomarker positive	5000	20% (2/10)	75% (6/8)
TIE2 Y1119 biomarker positive	6200	25% (2/8)	71% (5/7)
TIE2 Y992 & TIE2 Y1119 biomarkers positive	5000	25% (2/8)	71% (5/7)

Table 3. Biomarker pCR rates in HER2+ population

Figure 3. A) Rank order plots of HER2+ population. B) Two way scatter plot of relative levels of phosphorylated TIE2 in treated and control HER2+ population

Conclusions

- Our results show drug target activation in the HER2+ population with TIE2 Y992 and TIE2 Y1119 associating independently with pCR in trebananib treated HER2+ patients with significant biomarker x treatment interaction. Phospho-eIF4G, p-NFkB, and total ERBB3 were also identified as positive predictors of response within the HER2+ subset.
- Surprisingly the triple negative subtype had only biomarkers negatively associated with response to treatment. This may be a reflection of the molecular heterogeneity of TN tumors compared to HER2+ breast cancer. Consequently, this finding may be a result of mixed population effects and heterogeneous signaling drivers in the absence of overt HER2 overexpression in this patient subset.
- Using an estimated cut point for phosphorylated Tie2, a pCR rate of approximately 70% was achieved in the treatment arm and 20% in the control arm. While these numbers are too small to draw any definitive conclusions, they compare favorably to the pCR rate of approximately 20-30% in the HER2+ population in both the control and treatment arms.

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