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

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Abstract #

Prediction of complete pathologic response to veliparib/carboplatin plus standard neoadjuvant therapy in HER2 negative breast cancer: Exploratory protein pathway marker results from the I-SPY 2 TRIAL

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Abstract

Background: In the I-SPY 2 TRIAL, HER2- patients were randomized to receive standard chemotherapy or chemotherapy plus the oral PARP inhibitor veliparib in combination with carboplatin (V+C), which graduated in the HR-/HER2- arm. Exploratory analysis of protein signaling was performed to identify biomarker candidates that correlated with pCR in the HER2- population. We evaluated 110 key signaling proteins using reverse phase protein microarray (RPPA) data from pre-treatment LCM purified tumor epithelium.

Methods: Of 115 patients, 97 (V+C: 61 controls: 36) had RPPA and pCR data. RPPA data was correlated to pCR in both the treated and control patients using parametric (t-test) or non-parametric (Wilcoxon) statistical analysis, depending on data distribution. Only analytes whose pre-treatment levels were associated with response in the V+C but not the control arm were identified (P<0.05). Markers are analyzed individually; p-values are descriptive and were not corrected for multiple comparisons.

Results: 13 protein/phosphoprotein markers were significantly associated with pCR in the V+C arm but not control. Two were positive predictors of response: YAP S127 p= 0.03 and LC3B p=0.04. Negative predictors of response included Cyclin D1 p=0.001, and a number of phosphorylated RTKs: ROS Y2274 p=0.03, IGF1R Y1135/Y1136-IR Y1150/Y1151 p=0.03, ERBB4 Y1284 p=0.002, total HER2 p=0.04, and total IGF1R p=0.01. Moreover, a number of AKT-mTOR pathway proteins were found to be negative predictors of V+C response: ACC S79 p=0.005, p70S6K S371 p=0.01, and B-RAF S445 p=0.01.

Conclusion: Our sample size is too small to draw definitive conclusions and the results are exploratory. Coordinated RTK-mTOR pathway activation appears to be a hallmark signature of lack of response to veliparib in HER2- tumors. We also found that HER2 levels were correlated paradoxically with lack of response in this HER2- population, suggesting potential added clinical value of quantitative HER2 measurement techniques. Such exploratory results merit evaluation in larger trials with HER2- breast cancer patients.

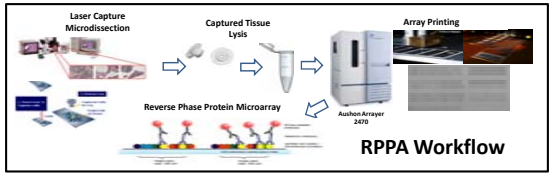
Background

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

- multicenter Phase II neoadjuvant adaptive design trial for locally advanced breast cancer
- patients assigned to 1 of 10 biomarker signature groups based on HR, HER2 and Mammaprint high or ultra-high risk status
- **Goal:** to determine predictive probabilities of success in Phase III trials for various targeted therapeutics
- determine which patient biomarker signatures respond to various regimens
- HER2- patients were randomized to receive standard chemotherapy or chemotherapy plus veliparib and carboplatin (V+C)
- Veliparib + carboplatin graduated in the triple negative (HR-/HER2-) signature

Signature	Estimated pCR Rate (95% probability interval)		Probability V+C is Superior to Control	Predictive Probability of Success in Phase III
	V+C	Control		
All HER2-	33% (22-43%)	22% (10-35%)	91%	53%
HR+/HER2-	14% (4-27%)	19% (6-35%)	28%	8%
HR-/HER2-	52% (35-69%)	26% (10-21%)	99%	88%

Table 1. Estimated pCR rates and predictive probabilities for Veliparib/Carbo and control arms. Accrual stops once the graduation threshold is reached based on MRI response over time and available pCR results. Final probabilities are re-calculated once pathology assessment is complete for all patients in the arm and their concurrent controls.



Materials and Methods

PARP total	Cyclin D1 total	MET Y1234/Y1235	AMPKa1 S485	GSK3ab S21/S9	Rb S780
PARP, cleaved D214	E-cadherin total	mTOR S2448	AMPKb1 S108	Heregulin total	RET Y905
EGFR total	eIF4E S209	mTOR total	A-RAF S299	Histone H3 S10	RON Y1353
EGFR Y1068	eIF4G S1108	NFkB p65 S536	ATM Y1981	IGF1R total	ROS Y2274
EGFR Y1148	eNOS S113	p27 T187	ATR S428	IGF1R Y1131/IR Y1146	RTK-ROR1 total
EGFR Y1173	eNOS-NOSIII S116	p38 MAPK T180/Y182	Aurora A T288-B T232-CT198	IGF1R Y1135/Y1136-IR Y1150-Y1151	SRRP S240/S244
EGFR Y992	ERBB3 total	p53 S15	BAD S136	IGFBP5 total	SHC Y317
ERBB2 total	ERBB3 Y1289	p70S6K S371	B-RAF S445	IkBa S32/S36	SRC Y527
ERBB2 Y1248	ERBB4 total	p70S6K T389	Caspase 7, cleaved D198	IR beta total	STAT1 Y701
ERBB2 Y877	ERBB4 Y1284	p70S6K T412	Caspase 9, cleaved D330	IRS1 S612	STAT3 S727
4EBP1 S65	ERK1/2 T202/Y204	p90 RSK S380	CHK1 S345	JAK1 Y1022/Y1023	STAT3 Y705
ABL T735	Estrogen Receptor alpha total	PDGFRa Y754	CHK2 S33/S35	JAK2 Y1007	STAT5 Y694
ACC S79	FADD S194	PDGFRb Y751	cKIT Y703	Ki67 total	Survivin total
AKT S473	FAK Y576/Y577	PI3K p85 Y458-p55 Y199	Cofilin S3	LC3B total	TIE2 S1119
AKT T308	FOXSa S253	PLK1 T210	CREB S133	MCSFR Y732	TIE2 Y992
ALK Y1586	FOXO1 S256	PTEN S380	Cyclin A total	MEK1/2 S217/S211	VEGFR2 Y996
ALK Y1604	FOXO1/FOXO3a T24/T32	PTEN total	Cyclin B1 total	MET total	YAP S127

Table 2: Analytes assessed in exploratory analyses of veliparib + carboplatin and control arms of I-SPY 2 TRIAL. RPPA data was collected for 102 signaling endpoints, including the intact and cleaved forms of PARP which are the targets of veliparib (red text).

-ISPY-2 TRIAL pre-treatment biopsy specimens were subjected to LCM to procure tumor epithelium for RPPA analysis. Approximately 10,000 cells were captured for 116 samples in the veliparib + carboplatin treatment and concurrent control arms. Of these 116 patients, 97 (61 V+C arm, 36 controls) had both RPPA and pCR data.

-RPPA data were collected for 102 exploratory biomarker endpoints related to various cell signaling pathways (see Table 2).

-Statistical analyses: RPPA data was correlated to pCR in both the treatment and control patients using non-parametric (Wilcoxon) statistical analysis. Analytes whose pre-treatment levels were associated with response in the V+C but not the control arm were identified (P<0.05). Markers were analyzed individually; p-values are descriptive and were not corrected for multiple comparisons.

A Number of Signaling Proteins are Associated with pCR in Veliparib +Carboplatin Treated Patients

All Patients

Endpoint	P-value		Higher or Lower in pCR YES Group
	Control	V + C	
ACC S79	0.6045	0.0042	↓
ALK Y1604	0.4989	0.0450	↓
B-RAF S445	0.4674	0.0121	↓
Cyclin D1 total	0.7959	0.0015	↓
ERBB2 total	0.6338	0.0387	↓
ERBB4 Y1284	0.5269	0.0019	↓
IGF1R total	0.7647	0.0127	↓
IGF1R Y1135/Y1136-IR Y1150/Y1151	0.7832	0.0318	↓
LC3B total	0.725	0.0443	↑
mTOR total	0.5758	0.0463	↓
p70S6K S371	0.8803	0.0139	↓
ROS Y2274	0.2188	0.0356	↓
YAP S127	1	0.0265	↑

Table 3: Endpoints Significantly Associated with pCR in HR-/HER2- V+C Treated Patients.

HR-/HER2- Patients

Endpoint	P-value		Higher or Lower in pCR YES Group
	Control	V + C	
AMPK β1 S108	0.6593	0.0327	↓
BAD S136	0.7692	0.0275	↓
Cyclin B1 total	1.0000	0.0197	↓
MEK1/2 S217/S221	0.5549	0.0082	↓

Table 4: Endpoints Significantly Associated with pCR in HR-/HER2- Veliparib/Carboplatin Treated Patients. Mean comparison by Wilcoxon Rank Sum Test resulted in 4 treatment-specific endpoints of 102 tested with p < 0.05.

HR+/HER2- Patients

Endpoint	P-value		Higher or Lower in pCR YES Group
	Control	V + C	
ALK Y1586	0.8571	0.0022	↓
Cyclin D1 total	0.3948	0.0006	↓
E-cadherin total	0.2214	0.0122	↓
ERBB2 total	0.5233	0.0083	↓
IGF1R total	0.9922	0.0129	↓
IκBα S32/S36	0.9818	0.0148	↓
MET total	0.3558	0.0156	↑
p53 S15	0.7181	0.0434	↑
p70S6K S371	0.9922	0.0325	↓

Table 5: Endpoints Significantly Associated with pCR in HR+/HER2- Veliparib/Carboplatin Treated Patients. Mean comparison by Wilcoxon Rank Sum Test resulted in 9 treatment-specific endpoints of 102 tested with p < 0.05.

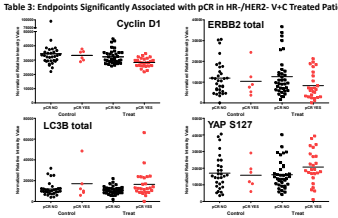


Figure 1. Scatterplots of Selected Endpoints Significantly Associated with pCR in V+C treated patients.

Bayesian Evaluation of total ERBB2 and Cyclin D1 as Biomarkers of Veliparib + Carboplatin Response

	Established RIU Cutoff Value	pCR Rate Control Group	pCR Rate V+C Group
All V+C samples		20.4% (9/45)	38.5% (27/70)
HR-/HER2- graduating signature		23.8% (5/21)	57.9% (22/38)
All V+C samples RPPA study		16.7% (6/36)	41% (25/61)
HR-/HER2- RPPA study		21.4% (3/14)	60.6% (20/33)
Cyclin D1 biomarker negative	30000	9% (1/11)	56.3% (18/32)
ERBB2 total biomarker negative	8900	21% (4/19)	54.8% (17/31)

Table 4: Actual pCR Rates in Biomarker Discriminated Populations

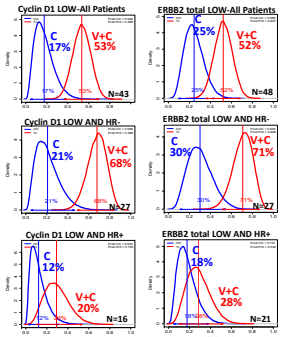


Figure 2: Estimated pCR Rates for Bayesian Evaluations of Cyclin D1 and ERBB2.

Patient Subset	Estimated pCR Rate (95% prob. Interval)		Prob. V+C is Superior to Control	Predictive Prob. of Success in Phase III
	Control	V + C		
All HER2-	22% (10-35%)	33% (22-43%)	91%	53%
ERBB2 total LOW	24.5% (10-44%)	52.2% (36-69%)	98.6%	90%
Cyclin D1 total LOW	17.5% (4-38%)	53.3% (37-70%)	99.6%	93%
HR+/HER2-	19% (6-35%)	14% (4-27%)	28%	8%
ERBB2 total LOW OR HR+/HER2-	21.4% (10-36%)	36.6% (25-49%)	95.5%	69.7%
ERBB2 total LOW AND HR+/HER2-	17.7% (5-38%)	28.5% (10-52%)	79.1%	54.6%
Cyclin D1 total LOW OR HR+/HER2-	17.8% (8-32%)	35.1% (24-48%)	97.3%	78.1%
Cyclin D1 total LOW AND HR+/HER2-	12.1% (2-31%)	29.3% (10-54%)	90.4%	73.5%
HR-/HER2-	26% (10-21%)	52% (35-69%)	99%	88%
ERBB2 total LOW OR TN	25.5% (13-42%)	49% (36-62%)	99%	87.4%
ERBB2 total LOW AND TN	30.1% (11-55%)	70.9% (51-87%)	99.5%	96.6%
Cyclin D1 total LOW OR TN	21.5% (9-39%)	49.3% (36-63%)	99.6%	93.3%
Cyclin D1 total LOW AND TN	20.7% (5-46%)	67.8% (49-84%)	99.8%	98.6%

Table 7: Bayesian estimates of probability for V+C superiority to control incorporating total Cyclin D1 or total ERBB2 levels and comparison to probability estimates for standard biomarker HR/HER2 signatures.

Estimates of probability of Phase III success using these biomarkers for predicting V+C response in more expanded (any HR status) or in HR+ cohorts are comparable to or greater than that observed for the HR-/HER2- graduating subgroup.

Conclusions

- Higher relative measurements of both Cyclin D1 and total ERBB2 expression were identified as negative predictors of response to Veliparib + Carboplatin therapy. Exploratory and retrospective analysis indicated that biomarker stratified and patient populations identified using these two protein markers had substantially increased pCR rates compared to HR/HER2 status alone, either in expanded "all comers" groups or in the HR- or HR+ populations. Verification of this observation in independent study sets is necessary given the small sample sizes analyzed in this study and because no FDR corrections were used in the analysis.
- The observed significance of total ERBB2 expression levels in an IHC/FISH-measured HER2- patient population is intriguing and suggests that more quantitative measurements of HER2 expression than those provided by current technologies provide may improve therapeutic response predictions even in an "off-target" environment.
- Cyclin D1 has a known, kinase-independent role in mediating DNA repair through a direct interaction with RAD51. Decreased expression of Cyclin D1 has been shown to impede homologous recombination-mediated DNA repair mechanisms (Jirawatnotai et al. Nature (2011) 474:230-234). Lower levels of Cyclin D1 may enhance response to PARP inhibition which would be consistent with our observations of lower Cyclin D1 levels being related with response to V+C.

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