I-SPY 2 Qualifying Biomarker Evaluation (QBE): The Challenge and Opportunity for Interrogating Predicted Pathways in an Adaptive Design Biomarker Rich Trial

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Body: I-SPY 2, a multicenter phase 2 neoadjuvant trial in high-risk breast cancer, uses adaptive randomization within biomarker subtypes to evaluate novel agents added to standard chemotherapy. In addition to efficiently evaluating agent/signature pairs, I-SPY 2 is a biomarker rich trial, where samples are profiled for gene expression, protein levels, and mutation status. Biomarkers are classified as established, qualifying, or exploratory. Established biomarkers are those used clinically (HR/HER2 status) or FDA cleared (MammaPrint), and used for adaptive randomization to generate the 10 signatures from which a drug can graduate. Qualifying biomarkers (QB) represent evidence-based, biologic pathway markers (e.g.cell line predictors, known drug targets). QB analyses must be pre-specified and performed under CLIA. Exploratory markers are for discovery and may allow integration of data from different technologies.

The QBE goal is to (1) evaluate biomarkers related to an agent's mechanism of action to identify promising candidates for testing/patient selection in future trials, and (2) create a resource to elucidate biological mechanisms of response. The wealth of biomarker data is both a boon and a challenge. Our small size limits the generalizability of our findings. There are multiple genes in each pathway measured on multiple platforms, creating the problem of multiplicity, which is compounded by the evaluation of multiple proposals. Biomarkers may correlate with HR/HER2/MP subtypes. The adaptive randomization may increase the prevalence of biomarker positive subsets and bias our findings. These challenges limit definitive conclusions, so our statistics are descriptive rather than inferential, and are intended to avoid adding to the false positive biomarker literature.

Methods: Three filters are applied: 1-The difference in biomarker performance in the experimental vs control arm (biomarker x treatment interaction) is evaluated using a logistic model under a pre-specified analysis plan 2-Biomarkers with a treatment interaction are dichotomized. The QB-High group is added to the graduating subtype to define a novel signature and the treatment effect in this group is evaluated 3-If the treatment effect is comparable to the graduating signature, and the prevalence is increased, the I-SPY 2 Bayesian model is modified to include the QB to assess the novel signature.

QBE to date: Veliparib in combination with carboplatin (V/C) and neratinib (N) are the first two agents to graduate from I-SPY 2. For V/C, we have completed initial evaluation for 5 biomarker proposals, including BRCA1/2 germline mutations and expression signatures associated with DNA repair deficiencies. For N, 6 biomarker proposals, including HER family protein signaling markers, have been assessed. Evaluation of the best candidates from these initial analyses in the I-SPY 2 Bayesian framework is ongoing. Mutational analyses are pending.

Conclusions: We have developed a rigorous approach for QB analysis. A small number of QB warrant further assessment. However, I-SPY 2 QB require validation, and should be considered preliminary efforts to effectively screen QB candidates for evaluation in ongoing and future trials.

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Evaluation of HER family protein signaling network as a predictive biomarker for pCR for breast cancer patients treated with neratinib in the I-SPY 2 TRIAL

Background: We hypothesize that response to the pan-ERBB inhibitor, neratinib (N), may be predicted by pre-treatment HER2-EGFR signaling. In the I-SPY 2 TRIAL, N graduated in the HR-/HER2+ signature. All patients received at least standard chemotherapy. For HER2+ patients, N was administered in place of trastuzumab. We evaluated 18 HER family signaling proteins as biomarkers of N response using reverse phase protein microarray (RPMA) data from pre-treatment LCM purified tumor epithelium.

Methods: 168 patients (N: 106, concurrent controls: 62) had RPMA and pCR data. 18 biomarkers relating to HER family signaling were evaluated: AKT S473, AKT T308, EGFR, EGFR Y1068, EGFR Y1148, EGFR Y1173, EGFR Y992, ERBB2, ERBB2 Y1248, ERBB3 total, ERBB3 Y1289, ERK1/2 T202/Y204, Heregulin, mTOR, mTOR S2448, PI3K p85 Y458/p55 Y199, PTEN S380, and SHC Y317. We assessed association between biomarker and response in the N and control arms alone (likelihood ratio test), and relative performance between arms (biomarker x treatment interaction) using a logistic model. Analysis was also performed adjusting for HR/HER2 status. In an exploratory analysis, we selected the marker with the greatest interaction (phosphorylated EGFR (Y1173)) to dichotomize patients optimally based on the data and assessed it in the context of the graduating signature by adding the EGFR Y1173-High patients to the HR-/HER2+ subtype and evaluating the treatment effect in this 'biomarker-positive' group. Our study is exploratory with no claims for generalizability of the data and does not account for multiplicities. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content).

Results: 7 HER pathway markers (EGFR Y1068, EGFR Y1173, EGFR Y992, ERBB2 total, ERBB2 Y1248, ERBB3 Y1289, SHC Y317) are associated with response in the N but not the control arm. However, the difference in performance between arms did not reach significance by permutation testing. Adjusting for HR/HER2 status, EGFR Y1173 shows a significant biomarker x treatment interaction (p = 0.049). In an exploratory analysis, we dichotomized patients by their EGFR Y1173 levels and evaluated the distribution of pCR rates (Table 1).

	Neratinib (n=106)		Control (n=62)	
	EGFR Y1173 Low (n=31)	EGFR Y1173 High (n=75)	EGFR Y1173 Low (n=29)	EGFR Y1173 High (n=33)
HR-HER2+ (n=28)	0 / 4	12 / 18	1 / 1	1 / 5
Not HR-HER2+ (n=140)	3 / 27	24 / 57	5 / 28	5 / 28

OR between EGFR Y1173 groups in the N relative to control arm is 10.1. When EGFR Y1173 High patients are added to the graduating HR-/HER2+ subset, the OR associated with treatment

is 3.2 and is comparable to that in the HR-/HER2+ signature (OR: 2.1), while increasing the prevalence of biomarker-positive patients by ~50%. Evaluation of EGFR Y1173 under the I-SPY 2 Bayesian model is pending.

Conclusion: Our sample size is too small to draw definitive conclusions. Our exploratory analysis reveals that HER family phosphoproteins associate with response to N, but only phosphorylated EGFR Y1173 appears to add value to the graduating signature. Given that this biomarker would expand the patient population that may benefit, it merits evaluation in other ongoing trials of neratinib.

Abstract P3-06-29: MammaPrint High1/High2 risk class as a biomarker of response to neratinib plus standard neoadjuvant therapy for breast cancer in the I-SPY 2 TRIAL

Christina Yau, Denise M Wolf, Ashish Sanil, Jo Chien, Anne Wallace, Judy Boughey, Doug Yee, Debu Tripathy, Angela DeMichele, Rita Nanda, Steven Chiu, Claudine Isaacs, Kathy Albain, Hank Kaplan, Stacey Moulder, Rebecca Viscusi, Donald Northfelt, Kirsten Edmiston, Anthony Elias, Toncred Styblo, Barbara Haley, Lamorna Brown-Swigart, Susan Flynn, Gillian L Hirst, Meredith Buxton, Nola Hylton, Melissa Paoloni, W Fraser Symmans, Laura Esserman, Don Berry, Minetta C Liu, John W Park, and Laura van 't Veer

Background: Further stratification of the 70-gene MammaPrintTM signature into 'high' and 'ultra-high' risk groups may help predict chemo-sensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/MP2 classification was added to HR and HER2 to define the cancer subtypes used in the I-SPY 2 adaptive randomization engine. Neratinib (N), one of the experimental agents evaluated in I-SPY 2, graduated in the HR-HER2+ signature. All patients received at least standard chemotherapy (paclitaxel followed by doxorubicin/cyclophosphamide; T->AC). HER2- patients were randomized to receive N+T- >AC vs. T->AC. For HER2+ patients, neratinib was administered in place of trastuzumab (N+T->AC vs. H+T->AC). Here, we assess the performance of MP1/MP2 class as a specific biomarker of neratinib response.

Methods: 115 patients in the neratinib arm and 76 concurrently randomized controls had Agilent 44K microarrays and pCR data available for analysis. We assess association between MP1/MP2 and response in the neratinib and control arms alone using Fisher's exact test, and relative performance between arms (biomarker x treatment interaction, likelihood ratio p < 0.05) using a logistic model. This analysis is also performed adjusting for HR status as a covariate, and in receptor subsets. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). Our analyses do not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: There are 133 MP1 patients (neratinib: 74, Control: 59) and 58 MP2 patients (neratinib: 41, Control: 17), 84% (49) of which are Her2-. The distribution of pCR rates among MP1/MP2 dichotomized groups are summarized in Table 1.

	Neratinib (n=115)		Control (n=76)	
	MP1 (n=74)	MP2 (n=41)	MP1 (n=59)	MP2 (n=17)
HER2- (n=105)	0 / 17	15 / 33	7 / 39	5 / 16
HER2+ (n=86)	22 / 57	4 / 8	5 / 20	0 / 1

MP2, one of the 10 eligible signatures, did not meet the graduation threshold; and MP1/MP2 did not show a significant biomarker x treatment interaction (OR in neratinib relative to control arm = 1.25). The MP1/MP2 x treatment interaction remains non-significant after adjustment for HR and HER2 status (p=0.54). In HER2- patients receiving neratinib, 45% (15/33) of MP2 patients achieved a pCR, compared to 0% (0/17) of MP1 patients. In the HER2- controls, there is a 31% pCR rate in MP2 (5/16) vs. 18% in MP1 (7/39) patients (OR=2.14). This difference in performance between treatment arms appears

significant (p=0.041). 90% of HER2+ patients are MP1, thus MP1/MP2 status x treatment interaction within the HER2+ subtype cannot be evaluated.

Conclusion: Within the I-SPY 2 population as a whole, MP1/MP2 stratification does not appear to be a specific biomarker of response to neratinib relative to the control arm. The number of HER2- patients is small and precludes any definitive conclusion, but these data motivate further investigation of the biological mechanisms distinguishing MP1 from MP2 to better understand chemotherapy and/or neratanib responsiveness.

Title: MammaPrint High1/High2 risk class as a biomarker of response to veliparib/carboplatin plus standard neoadjuvant therapy for breast cancer in the I-SPY 2 TRIAL

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Body: Background: Further stratification of the 70-gene MammaPrintTM signature into 'high' and 'ultra-high' risk groups may help predict chemo-sensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/MP2 classification was added to HR and Her2 to define the cancer subtypes used in the I-SPY 2 adaptive randomization engine. HER2- patients were randomized to receive standard chemotherapy or the oral PARP inhibitor veliparib in combination with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) signature, where MP2 was not an eligible signature for graduation. Here, we assess the performance of MP1/MP2 class as a specific biomarker of response to V/C.

Methods: 115 HER2- patients (V/C: 71 and concurrent controls: 44) were considered in this analysis. We assess association between MP1/MP2 and response in the V/C and control arms alone using Fisher's exact test, and relative performance between arms (biomarker x treatment interaction, likelihood ratio p < 0.05) using a logistic model. This analysis is also performed adjusting for HR status as a covariate. To assess MP1/MP2 in the context of the graduating signature, we added the MP2 patients to the graduating TN subset and evaluated the treatment effect in this 'biomarker-positive' group. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). This analysis does not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: In the V/C arm vs. concurrent controls, there were 66 MP1 (V/C: 32, Control: 34) and 49 MP2 patients (V/C: 39, Control: 10), 78% of which are TN. The distribution of pCR rates among MP1/MP2 dichotomized groups are summarized in Table 1.

	V/C (n=71)		**Control** (n=44)	
	MP1 (n=32)	MP2 (n=39)	MP1 (n=34)	MP2 (n=10)
TN (n=59)	3 / 8	19 / 30	3 / 13	2 / 8
HR+HER2- (n=56)	1 / 24	4 / 9	4 / 21	0 / 2

The OR between MP1/MP2 risk groups for predicting pCR is 9.71 in the V/C arm (p=6.63E-05), in comparison to an OR of 0.97 in the control arm (p=1). There is a significant biomarker x treatment interaction (p=0.023), which remains upon adjusting for HR status (p= 0.028). Based on the I-SPY 2 Bayesian model, a Phase III trial with 300 MP2 patients has a 95% predictive probability of success.

When the MP2 patients are added to the graduating TN subset, the OR associated with V/C is 4.36, which is comparable to that of the TN signature (OR: 4.29), while increasing the prevalence of biomarker-positive patients by ~10%.

Conclusion: In our exploratory analysis, MP2 suggests higher sensitivity to V/C combination therapy relative to controls. This observation has prompted an investigation into the biological mechanisms distinguishing the MP1/MP2 subtype that may account for this specificity.

Title: Evaluation of an in vitro derived signature of olaparib response (PARPi-7) as a predictive biomarker of response to veliparib/carboplatin plus standard neoadjuvant therapy in high-risk breast cancer: results from the I-SPY 2 TRIAL

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Body: Background: We developed a 7-gene DNA-repair deficiency signature (PARPi-7) that predicts breast cancer cell line sensitivity to the PARP inhibitor olaparib [PMID: 22875744]. We hypothesized that this signature would also predict response to other PARP inhibitors including veliparib. In the I-SPY 2 TRIAL, HER2- patients were randomized to receive standard chemotherapy or the oral PARP inhibitor veliparib in combination with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) signature. Here we assess the PARPi-7 as a specific biomarker of V/C response.

Methods: 115 HER2- patients (V/C: 71 and concurrent controls: 44) were considered in this analysis. The PARPi-7 signature score is computed from Agilent 44K array data as published using expression levels of BRCA1, CHEK2, MAPKAPK2, MRE11A, NBN, TDG, and XPA. We assess association between PARPi-7 and response in the V/C and control arms alone (Wald $p < 0.05$), and relative performance between arms (biomarker x treatment interaction, likelihood ratio $p < 0.05$) using a logistic model. In an exploratory analysis, we dichotomized patients by the PARPi-7 score using the published in vitro derived cutpoint (0.037). To assess PARPi-7 in the context of the graduating signature, we added the PARPi-7 High patients to the graduating TN subset and evaluated the treatment effect in this 'biomarker-positive' group. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). Our analyses do not adjust for multiplicities of other biomarkers in the trial but outside this study. Results: The PARPi-7 signature associates with patient response in the V/C arm (OR = 3.9, p=0.00056) but not in the control arm (OR = 0.87, p=0.68). There is a significant biomarker x treatment interaction (OR in V/C arm relative to control arm = 4.48, p=0.0028), which remains significant upon adjusting for HR status (p=0.0018). In an exploratory analysis, PARPi-7 dichotomized using the published in vitro derived cutpoint yields 62 PARPi-7 Low and 53 PARPi-7 High patients. 26% of PARPi-7 High patients are not TN. The distribution of pCR rates among PARPi-7 dichotomized groups are in Table 1.

	V/C (n=71)		**Control** (n=44)	
	PARPi-7 Low (n=38)	PARPi-7 High (n=33)	PARPi-7 Low (n=24)	PARPi-7 High (n=20)
TN (n=59)	5 / 13	17 / 25	1 / 7	4 / 14
HR+HER2- (n=56)	2 / 25	3 / 8	4 / 17	0 / 6

When the PARPi-7 High patients are added to the graduating TN subset, the OR associated with V/C is 5.12, which is comparable to that of the TN signature (OR: 4.29), while increasing the prevalence of

biomarker-positive patients by ~12%. Evaluation of PARPi-7 in the context of the graduating signature under the I-SPY 2 Bayesian model is pending.

Conclusion: Our sample size is small. Our pre-specified analysis suggests the PARPi-7 signature shows promise for predicting response to veliparib/carboplatin combination therapy relative to control. If verified in a larger trial, this cell-line derived signature may contribute to the selection criteria of PARP inhibitor trials in the future.

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2014 San Antonio Breast Cancer Symposium

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Title: Detrimental effects of sequential compared to concurrent treatment of Pertuzumab plus T-DM1 in HER2+ breast cancer cell lines

Body: Background. Pertuzumab and T-DM1 are two recently approved monoclonal antibody based therapies targeting HER2+ breast cancer. Pertuzumab interferes with dimerization of HER family members, while T-DM1 binds to HER2 and interferes with its oncogenic function while also specifically delivering a cytotoxic agent (emtansine). One arm of the I-SPY 2 clinical trial is to investigate the efficacy of a combination Pertuzumab plus T-DM1 in HER2+ breast cancer patients. Methods. We performed pre-clinical screening of response to each agent alone and in combination in a set of 21 HER2+ breast cancer cell lines, with an end goal of identifying markers of response to the therapies. There were five treatment regimens employed in the initial screen: i) pertuzumab alone for 72 h; ii) T-DM1 alone for 72h; iii) pertuzumab plus T-DM1 concurrently for 72h; iv) pertuzumab for 24h followed by addition of T-DM1 for 48h more; and iv) T-DM1 for 24h followed by addition of pertuzumab for 48h more. Response was assessed using the Cell Titer Glo assay as a measure of cell viability. To assess the effects of drug

combinations, we used a stringent measure of synergy and antagonism employing the median effect method of Chou and Talalay that included 95% confidence intervals to determine significance. Results. Initial screens showed that concurrent treatment of cells with pertuzumab plus T-DM1 gave significant synergistic interactions in 15/21 cell lines as measured by the median effect method, with combination indices (CI) less than 0.5 (and 95% upper confidence levels less than 1.0) for at least one drug concentration. However, 24h pretreatment with pertuzumab followed by T-DM1 significantly diminished the response of cells to T-DM1, resulting in significant antagonism in 17/21 cell lines test (CI>1.5, lower confidence level greater than 1). Since this could be due to a shorter exposure time to T-DM1, and since patients are scheduled to be treated with pertuzumab first followed by T-DM1 one hour later, we repeated the experiment with one hour between pertuzumab and T-DM1 rather than 24h. While the inhibitory effect was diminished, this treatment regimen still resulted in significant antagonism when T-DM1 was given 1 hour after pertuzumab in 5/5 cell lines tested, in contrast to concurrent pertuzumab plus T-DM1 treatment, which showed synergy. Conclusions. Pertuzumab plus T-DM1 appears to be beneficial when given concurrently, but pretreatment with pertuzumab appears to blunt the efficacy of T-DM1. This has important potential ramifications for patient treatment, and may further elucidate mechanisms of action for both compounds. Further testing will be necessary to determine whether these timing effects are operational in vivo and whether immune effects mitigate the antagonism.

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Disclosures by Author:

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DNA repair deficiency biomarkers identify ER+ breast cancer patients who may benefit from veliparib/carboplatin: Results from the I-SPY 2 trial.

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Abstract Text:

Background: In the I-SPY 2 TRIAL, HER2- patients were adaptively randomized to receive standard chemotherapy or the PARP inhibitor veliparib with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) subtype, and we've previously shown that DNA repair deficiency signatures [BRCAness and PARPi-7] may predict V/C response. Here we combine these signatures into a composite measure of DNA repair deficiency. **Methods**: 115 HER2- patients (V/C: 71 and concurrent controls: 44) are considered in this analysis. BRCA1/2 germline mutation is assessed by Myriad Genetics. The PARPi-7 and BRCAness signature scores are computed from Agilent 44K array data. A patient is predicted DNA repair deficient if carrying a BRCA1/2 mutation or BRCA-like or PARPi7-high. We modify the I-SPY 2 Bayesian model to include DNA repair deficiency status to estimate the predictive probability of V/C demonstrating superiority to control in a 1:1 randomized phase 3 trial of 300 'biomarker-positive' patients. Our study is exploratory with no claims for generalizability and does not adjust for multiplicities of other biomarkers outside this study. **Results:** 15 patients are BRCA1/2 mutation carriers, of which 13 are PARPi7-high or BRCA-like. Comparing PARPi7 and BRCAness (62 PARPi7-low, 53 PARPi7-high; 59 non-BRCA1-like, 56 BRCA1-like) we find only moderate concordance (64%; kappa = 0.29). Altogether, 77 patients are predicted to be DNA repair deficient by one of these measures. 38% (21/56) of HR+/HER2- patients are predicted DNA repair deficient, along with nearly all (56/59) TN. In the V/C arm, 5/13 HR+/HER2- DNA repair deficient patients and 22/38 TN patients had a pCR (vs 0/8 and 5/21 controls respectively). When DNA repair deficient HR+/HER2- patients are added to the TN subset, the probability of phase 3 success is 94%, which is comparable to the graduating TN signature [97% in this model] while increasing patient prevalence. **Conclusions:** Our exploratory analysis suggests that 38% of HR+/HER2- patients in I-SPY 2 are DNA repair deficient and may benefit from V/C. If validated, DNA repair deficiency biomarkers may be used to select HR+/HER2- patients for future PARP inhibitor trials.

2015 San Antonio Breast Cancer Symposium

Title: Residual cancer burden (RCB) with veliparib/carboplatin in the I-SPY2 trial

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Body: Background: I-SPY2 is a multicenter phase 2 trial in high risk stage II/III breast cancer (BC) using adaptive randomization within biomarker subtypes to evaluate novel agents added to standard neoadjuvant chemotherapy. The first regimen to graduate based on the predicted probability of a higher pCR rate within predefined subsets was veliparib/carboplatin + paclitaxel (VC+T→AC vs T→AC) in triple negative BC (TNBC). In TNBC the residual cancer burden (RCB) is prognostic, whether as a continuous index or grouped into classes, with pCR (RCB-0) and RCB-I classes having identical survival. Therefore, we evaluated the use of RCB to further discriminate between investigational and control arms.
Methods: Site pathologists reported RCB for 99% of subjects in the primary efficacy analysis based on pCR (n=114/115). We compared the distribution of RCB reported as a continuous index in each treatment-subset combination to matched concurrently randomized controls using the Wilcoxon rank sum test for RCB index, and Fisher's Exact test for RCB classes (RCB-0/I vs RCB-II/III). The statistics are descriptive rather than inferential, and given the small sample size have no claim on generalizability. We modified the Bayesian model used to compute the estimated probability of success in a future, randomized, phase 3 trial of 300 subjects, if response were defined by either pCR or RCB-I (RCB0/I), or separately if it were defined by pCR alone.
Results: VC+T→AC led to a significantly lower RCB index than T→AC in TNBC (p=0.0021), with a near-significant trend when those with pCR were excluded (p=0.06). There was no significant difference in RCB distributions in the other breast cancer subtypes treated. In TNBC, the odds ratio (OR) for achieving RCB-0/I in the VC+T→AC arm vs control was 8.2 (95% confidence interval (CI): 2.1–35), whereas the OR for achieving pCR was 4.56 (95% CI: 1.25–19.53). The simulations using response information from I-SPY2 to predict the probability of success for VC+T→AC for TNBC in a future phase 3 trial estimated this probability to be 0.99 if modeled using RCB-0/I as the response endpoint, and 0.90 if modeled using pCR as the response endpoint.
Conclusions: Use of RCB index and classes provided additional insight into the effect of adding VC to T, appearing to magnify the improved treatment response that had been observed with pCR rates in TNBC. It will be important to test in randomized trials whether a decrease in the RCB index relative to controls, and/or increased rates of RCB-0/I class, are predictive of survival benefit in TNBC.

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Title: 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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Body: 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: 11 protein/phosphoprotein markers were significantly associated with pCR in the V+C arm but not in controls. 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.

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TN breast cancer patients with multiple sensitivity markers appear more likely to benefit from veliparib/carboplatin: results from the I-SPY 2 TRIAL

Background: In the I-SPY 2 TRIAL, HER2- patients were adaptively randomized to receive standard chemotherapy or the PARP inhibitor veliparib with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) subtype, and we've previously shown that MammaPrint High1/High2 (MP1/2) risk class and the PARPi-7 signature may specifically predict V/C response. Here we evaluate whether combining these signatures can help identify a subset of TN patients especially likely to respond to V/C.

Methods: 60 TN patients (V/C: 39 and controls: 21) are considered in this analysis. The PARPi-7 and MP1/2 signature scores are computed from Agilent 44K arrays. We stratify TN patients by the number of additional V/C-sensitivity biomarkers (MP2 class, PARPi7-high). We use Bayesian modeling to estimate pCR rates in each arm and the predictive probability of V/C demonstrating superiority to control in a 1:1 randomized phase 3 trial of 300 'biomarker-positive' patients. Our study is exploratory and does not adjust for multiplicities of biomarkers outside this study.

Results: Though 90% of TNs are PARPi7-high or MP2 class, concordance between these biomarkers is only 50%. Compared to the entire TN subgroup, TN patients who are negative for at least one V/C sensitivity marker (PARPi7-low and/or MP1) had lower estimated probability of response to V/C (53% vs. 35%), with a much lower predictive probability of success in phase 3 (39%). In contrast, TN patients with tumors positive for both sensitivity markers (assessed as PARPi7-high **and** MP2) achieved an estimated pCR rate of 79% in the V/C arm vs. 23% in the control arm, with a predictive probability of success in phase 3 of 99.6%.

Conclusion: These exploratory data suggest TN patients who are also MP-High2 and PARPi7-high may be more sensitive to V/C than patients with fewer markers in the 'sensitive' state. If validated, use of multiple V/C sensitivity biomarkers may help refine patient selection.

Biomarker subset within TN	Estimated pCR rate in V/C [95% CI]	Estimated pCR rate in controls [95% CI]	Predictive probability of phase 3 success (300 pt)
Unselected TN (n=60)	53% [39-67]	27% [13-43]	0.904
PARPi7-high **and** MP2 (n=24; 40%)	79% [60-93]	23% [5.8-49]	0.996
PARPi7-low and/or MP1 (n=36; 60%)	35% [17-55]	29% [13-49]	0.386

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Gene and pathway differences between MammaPrint High1/High2 risk classes: results from the I-SPY 2 TRIAL in breast cancer

Background: Further stratification of the 70-gene MammaPrint[TM] prognostic signature into 'high' and 'ultra-high' risk groups may help predict chemo-sensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/2 classification was added to HR and HER2 to define the subtypes used in the I-SPY 2 adaptive randomization engine. The first two experimental agents/combinations to graduate from I-SPY 2 were veliparib/carboplatin (V/C) in the TN subset, and neratinib (N) in the HR-HER2+ subset. MP2 was found to be a sensitivity marker for V/C but not N, whereas MP1 class appears associated with resistance to N within the HER2- subset. Here, we present exploratory analysis to identify the genes and pathways that distinguish MP1 from MP2.

Methods: 263 patients (V/C: 71, N: 115, and controls: 77) with pre-treatment Agilent 44K microarrays and MP1/2 class assessments were considered in this analysis. To identify signature genes associated with MP1 vs. MP2 class, we (1) apply a Wilcoxon rank sum test and (2) fit a logistic model. P-values are corrected for multiple comparisons using the Benjamini-Hochberg (BH) method, with a significance threshold of BH $p<0.05$ from both tests. We then perform pathway enrichment analysis using DAVID. In addition, we perform multivariate analysis adjusting for receptor subtype. Our study is exploratory and does not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: 63% (165/263) of patients are MP1 class and 37% (98/263) MP2. MP1/2 class is associated with receptor subtype (Fisher's exact test: $p<2E-16$), where 71% of TN patients are MP2 and 96% of HR+HER2+ patients are MP1. Of the 70 signature genes, 86% (60/70) differ in expression between MP1 and MP2, with 70% (42/60) expressed at a higher level in MP2, including CDCA7, MELK and CENPA. In a whole transcriptome analysis, 10,500 genes (of ~30,000) appear differentially expressed. Following adjustment for HR and HER2 status, 4368 genes are significantly differentially expressed between MP1 and MP2. By DAVID enrichment analysis, the biggest pathway-level differences are found in cell cycle, proliferation, and DNA repair, with the MP2 set showing higher expression.

Conclusion: MP2 class appears associated with higher expression of cell cycle genes. Association between MP2 class and response to V/C suggests that higher cell cycle activity may contribute to V/C sensitivity.