Analysis of biomarkers for response and resistance to the AKT inhibitor MK-2206 in the neoadjuvant I-SPY 2 TRIAL for Stage II-III high risk breast cancer

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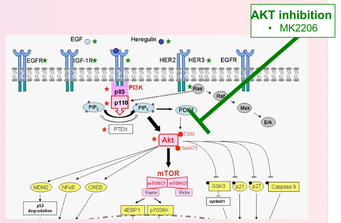
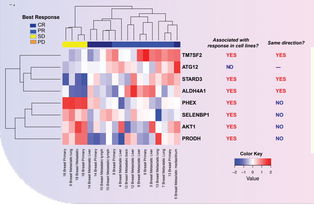
I-SPY2 Trial

1. Background: Do AKT/mTOR pathway genes predict response to AKT inhibition?

- We hypothesized that genes in the AKT signaling axis may specifically predict response to MK2206 and tested expression levels of 10 genes: **AKT1, EGFR, ERBB2, ERBB3, NRG1, IGF1R, PIK3CA, PTEN, STMN1, and MTOR.**

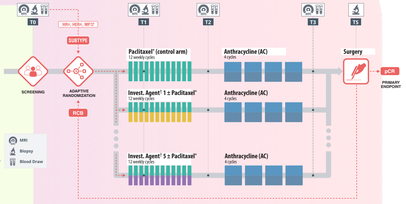




- We also evaluated 9 additional genes previously shown to associate with response to MK2206 *in vitro* and through exploratory analyses in the metastatic setting: **STARD3, TM7SF2, ALDH4A1, PRODH, SELENBP1, G3BP1, SMCR7L, TCTEXD2, and PHEX.**

2. THE PATIENTS: I-SPY 2 TRIAL Standing Platform

- **Phase II, adaptively-randomized neoadjuvant trial**
- **Shared control arm**
  Standard neoadjuvant chemotherapy
- **Simultaneous experimental arms**
  Up to four
- **Primary endpoint:** pathologic complete response (pCR)
  Defined as no residual invasive cancer in breast or lymph nodes
- **Match therapies with most responsive breast cancer subtypes**
  Defined by HR, HER2, and Mammaprint High1/(ultra)High2 (MP1/2) status
- **Agents/combinations "graduate" for efficacy** = reaching >85% predictive probability of success in a subsequent phase III trial in the most responsive patient subset



The AKT inhibitor MK2206 (M) was one of the experimental agents evaluated in I-SPY 2 and graduated in the HER2+, HR-, and HR-HER2+ signatures.

Agent	Graduating Signatures	Estimated pCR rate in Experiment al Arm	Estimated pCR rate in Control arm	Est. Probability of Success in Phase III
MK-2206 (AKT inhibitor)	HR–	46%	26%	88%
	HR-HER2+	62%	35%	91%
	All HER2+	48%	29%	83%

- ***Biomarker component:*** evaluate biomarkers associated with mechanism of action of each agent, along with the pre-defined subsets

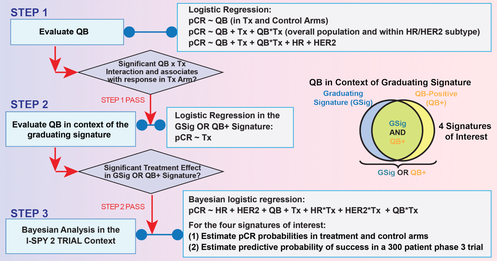
3. DATA: Gene expression microarrays

Subtype	MK2206 arm (N=94)	Control arm (N=56)	Total (N=150)
HR+HER2-	28	22	50
HR-HER2- (TN)	32	24	56
HR-HER2+	18	4	22
HR+HER2+	16	6	22

Data from 150 patients (M: 94 and concurrent controls: 56) were available. Pre-treatment biopsies were assayed using Agilent 44K (32627; n=119) or 32K (15746; n=31) expression arrays; and these data were combined into a single gene-level dataset after batch-adjusting using ComBat.

4. METHODS: Qualifying Biomarker Evaluation (QBE)

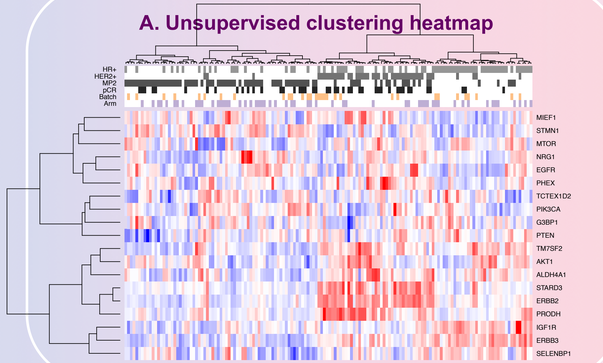
I-SPY 2 qualiing biomarker evaluation is a 3 step filter



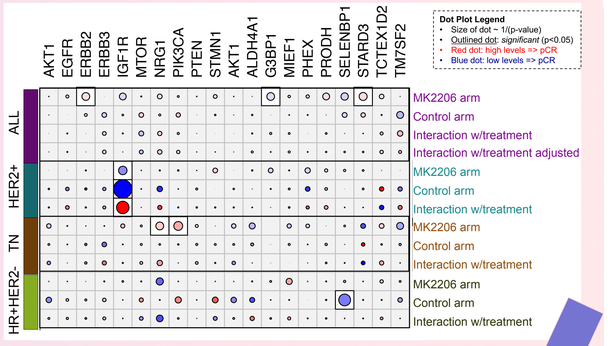
- All I-SPY 2 qualifying biomarker analyses follow a pre-specified analysis plan.
- We used logistic modeling to assess biomarker performance.
- A biomarker is considered a specific predictor of M response if it associates with response in the M arm but not the control arm, and if the biomarker x treatment interaction is significant (likelihood ratio test, p<0.05).
- This analysis is also performed adjusting for HR and HER2 status as covariates, and within receptor subsets, sample size permitting.
- Our statistics are descriptive rather than inferential and do not adjust for multiplicities of other biomarkers outside this study.

5. RESULTS: Association between AKT/mTOR pathway genes and Phase 1b biomarkers, and response to the AKT inhibitor MK2206

A. Unsupervised clustering heatmap

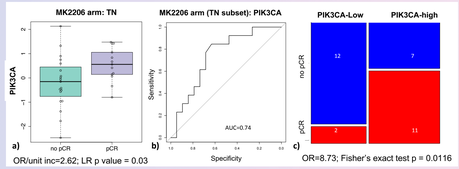


B. Association with response, by arm and receptor subset



C. PIK3CA in the TN subset?

In exploratory analysis that was not pre-specified, using the Youden-optimal threshold in TN in MK2206 to dichotomize PIK3CA:



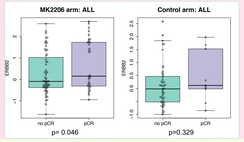
OR/unit inc=2.62; LR p value = 0.03

OR=8.73; Fisher's exact test p = 0.0116

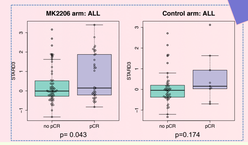
In the TN subset, PIK3CA levels associate with response in the MK2206 arm, but not the Control arm, and dichotomized there is a biomarker x treatment interaction with p<0.05.

RESULTS 3: Within the TN subset, higher levels of NRG1 and PIK3CA, upstream activators of AKT, associate with pCR in the M arm.

ERBB2



STARD3



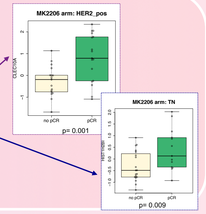
RESULTS 1: Consistent with M graduation in the HER2+ signature, two candidate biomarkers on the HER2 amplicon (ERBB2, STARD3) associate with pCR in the M arm, but not in the control arm. In addition, G3BP1, a component of the RAS signaling pathway, associates with non-pCR in the M arm. However, biomarker x treatment interactions for these genes are not significant, and all three associations to response in M lose significance in a model adjusting for HR and HER2 status.

6. CONCLUSION & COMING ATTRACTIONS

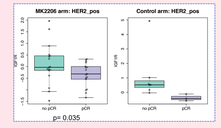
Following our pre-specified analysis, none of the candidate markers tested succeed as specific predictors of response to MK2206 in I-SPY 2. However, several genes in the AKT pathway associate with response to M, and in particular PIK3CA levels within the TN subset may merit further evaluation in future trials.

Coming attractions:

- *Protein/phospho—protein* endpoints promising!
- *Exploratory analysis* in process
  - Overall: **Immune**!!
  - HER2+: Immune and ECM signals
  - TN: histones, DNA repair, and more PIK3
  - HR+HER2-: Ribosomes and Immune



IGF1R in HER2+



RESULTS 2: Within the HER2+ subset, IGF1R is associated with non-pCR in M.