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

Registration Number: NCT01042379

Abstract # 12099

Phosphorylation of AKT kinase substrates predicts response to the AKT inhibitor MK2206 in the I-SPY 2 TRIAL in both HER2- and HER2+ patients

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Abstract

Background: In the I-SPY 2 TRIAL, the allosteric AKT inhibitor MK2206 was available to all HR/HER2 subtypes and graduated in the HR-/HER2+ signature. Qualifying biomarker analysis was performed on 26 proteins/phosphoproteins in the HER-AKT-mTOR pathway to identify candidate proteins correlated with pCR in the HER2+ and HER2- populations treated with MK2206. We postulated that response to MK2206 could be predicted by the relative level of phosphorylation of AKT kinase substrates.

Methods: Of 151 patients in the MK2206 and control arms, 138 patients (MK2206: 87, controls: 51) had RPPA and pCR data. Data for 26 (phospho) proteins involved in HER-AKT-mTOR signaling were assessed for association between biomarker and response in the MK2206 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. Markers were analyzed individually; p-values are descriptive and were not corrected for multiple comparisons.

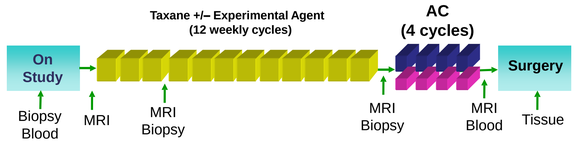
Results: In the HER2+ cohort, phosphorylation of the AKT kinase substrates mTOR S2448 (p = 0.004), GSK3 S21/9 (p = 0.009), FOXO1 S256 (p = 0.007), FOXO1 T24/FOXO3a T32 (p = 0.026), S6RP S240/S244 (p = 0.036), Tuberin/TSC1 Y1571 (p = 0.043) and eIF4G S1108 (p = 0.047) were associated with response. In the HER2- population, AKT S473 (p = 0.012), AKT T308 (p = 0.011), Estrogen Receptor alpha (p = 0.013), mTOR (p = 0.04), NFkB S536 (p = 0.017) and Tuberin/TSC2 Y1571 (p = 0.03) were negatively associated with MK2206 response. FOXO S253 (p = 0.031) and ERBB2 Y877 (p = 0.04) were both positively associated with response.

Conclusions: While our sample size is too small to draw definitive conclusions, our results suggest that the measurement of AKT kinase substrate phosphoproteins could be predictive of MK2206 clinical activity in both HER2+ and HER2- tumors regardless of HR status. These results will need to be validated in independent study sets in order to judge the significance of these initial findings.

The authors would like to acknowledge Gateway for Cancer Research (G-16-900) and the Side-Out Foundation for supporting this work, and to sincerely thank the patients, advocates and investigators for their participation in the I-SPY 2 TRIAL.

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

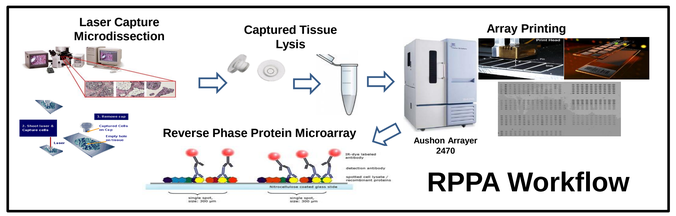
-determine which patient biomarker signatures respond to various regimens

-MK2206 is an orally bioavailable, allosteric, small molecule inhibitor of the serine/threonine protein kinase AKT

-MK2206 was administered to both HER2- and HER2+ patients; pathologic complete response (pCR) was the primary endpoint

Signature	Estimated pCR Rate		Probability MK2206 is Superior to Control	Predictive Probability of Success in Phase III
	MK2206 (N=93)	Control (N=56)		
HR-/HER2-	40.2%	22.4%	96.8%	75.9%
HR-/HER2+	64.1%	35.7%	**97.3%**	**87%***
HR+/HER2-	17.1%	13%	72.7%	31.7%
HR+/HER2+	35.8%	22.4%	85.4%	61.3%

Table 1. Estimated pCR rates and predictive probabilities for MK2206 and control arms



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 138 samples in the MK2206 treatment and concurrent control arms. (87 MK2206 treatment arm, 51 concomitant control samples).
- RPPA data were collected for 26 qualifying biomarker endpoints related to the HER/AKT/mTOR cell signaling pathways.

-Statistical analyses: In our pre-specified analysis plan, logistic regression was used to assess association with pCR in the control and MK2206 treated populations individually. 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. Bayesian analysis was completed using the MCMC simulation based on I-SPY 2 data with the following model: pCR ~ HR + HER2 + biomarker + treatment + treatment*HR + treatment*HER2 + treatment*biomarker. No multiple comparison adjustments were applied; p-values are descriptive. Small sample sizes and/or low numbers of pCRs in the control groups within receptor subsets precluded analysis (CTRL for HER2+: 3/9 pCR; TN: 3/22 pCR).

-For additional analysis within receptor subsets, the Wilcoxon rank sum test was used to assess association of protein/phospho-protein endpoints with pCR in the control and MK2206 treated populations individually. Analytes whose levels were associated with response only in the M and not the control arm were selected for further analysis. Markers were analyzed individually; p-values are descriptive and were not corrected for multiple comparisons. Receiver-operating characteristics (ROC) curves were generated for endpoints significantly associated with pCR in the MK2206 treatment arm of the trial to identify potential cut points for biomarker positivity.

Subtype	MK2206 treat (N=87)	Control (N=51)
HR+/HER2-	19% (5/27)	15% (3/20)
HR-/HER2-	41% (13/29)	13% (3/24)
HR-/HER2+	71% (12/17)	50% (2/4)
HR+/HER2+	36% (5/14)	20% (1/5)

Table 2. pCR rates for RPPA data set of MK2206 treated and control arms of the I-SPY 2 TRIAL

4EBP1 S65	FOXO3a S253
AKT S473	GSK3αβ S21/S9
AKT T308	mTOR total
eIF4E S209	mTOR S2448
eIF4G S1108	NFkB p65 S536
Estrogen Rec α total	p70S6K T389
Estrogen Rec α S118	p70S6K T412
ERBB2 total	p90RSK S380
ERBB2 Y1248	PI3K p85 Y458/p55 Y199
ERBB2 Y877	S6RP S240/S244
ERBB3 total	SGK S78
FOXO1 S256	SHC Y317
FOXO1 T24/FOXO3a T32	Tuberin/TSC2 Y1571

Table 3. Qualifying biomarker endpoints assessed for the MK2206 arms of the I-SPY 2 TRIAL

AKT Kinase Substrate Activation Levels are Associated with Response in MK2206-treated Patients

HER/AKT/mTOR signaling axis phosphoproteins	HER2+ subset (N=31, MK2206 treat)		TN subset (N=29, MK2206 treat)		HR+/HER2- subset (N=27, MK2206 treat)	
	MK2206 arm: OR/unit inc	MK2206 arm: LR p	MK2206 arm: OR/unit inc	MK2206 arm: LR p	MK2206 arm: OR/unit inc	MK2206 arm: LR p
4EBP1 S65	2.55	0.25	0.441	0.148	0.891	0.885
AKT S473	1.04	0.904	0.147	**0.021**	0.293	0.19
AKT T308	1.23	0.696	0.125	**0.011**	0.288	0.193
eIF4E S209	1.29	0.45	0.669	0.3	3.2	0.14
eIF4G S1108	2.22	**0.047**	0.406	0.0666	1.04	0.96
ERBB2 total	0.606	0.333	0.37	0.733	2.18	0.695
ERBB2 Y1248	1.74	0.0783	3.23E-05	0.0773	0.0107	0.512
ERBB2 Y877	0.626	0.22	2.66	**0.0496**	1.68	0.369
ERBB3 total	1.7	0.287	0.596	0.487	1.37	0.589
Estrogen Rec α total	1.15	0.765	0.34	0.0579	0.599	0.265
FOXO3a S253	0.524	0.243	5.21	**0.0126**	1.24	0.693
FOXO1 S256	11.2	**0.0072**	0.687	0.709	0.616	0.655
FOXO1/3a T24/T32	2.9	**0.0258**	0.749	0.361	2.26	0.501
GSK3α/β S21/S9	2.84	**0.00937**	0.77	0.524	1.09	0.825
mTOR S2448	3.84	**0.00425**	0.39	**0.0379**	1.82	0.166
mTOR total	1.17	0.76	0.396	0.0612	0.805	0.708
NFkB p65 S536	1.55	0.533	0.126	**0.00327**	0.571	0.557
p70S6K T389	1.25	0.393	0.249	0.0867	2.33	0.386
p70S6K T412	1.55	0.23	0.476	0.155	2.25	0.231
p90RSK S380	1.76	0.281	0.579	0.259	1.23	0.702
PI3K p85 Y485/p55 Y199	1.52	0.185	0.319	**0.0394**	1.26	0.7
S6RP S240/S244	2.05	**0.036**	0.267	0.0956	1.18	0.835
SHC Y317	1.41	0.18	0.871	0.898	2.11	0.758
Tuberin/TSC2 Y1571	3.19	**0.0434**	0.14	**0.00607**	0.77	0.76
SGK S78	1.01	0.979	0.126	**0.00352**	1.49	0.534
Estrogen Rec α S118	1.78	0.22	0.184	**0.00988**	0.803	0.735

Table 4. Protein/phosphoprotein biomarker association with pCR in HR/HER2 patient subsets treated with MK2206. OR columns: Red=positive association, blue=negative association with pCR.

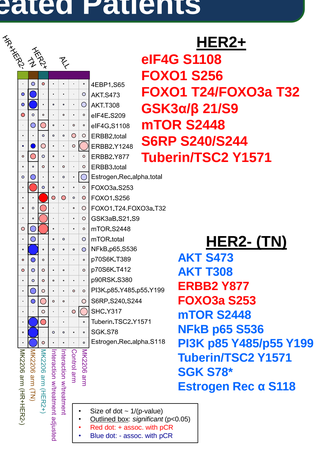


Figure 1. Dot plot of associations shown in Table 4.

FOXO1 S256 Has a Positive Association with pCR in HER2+ Tumors Treated with MK2206

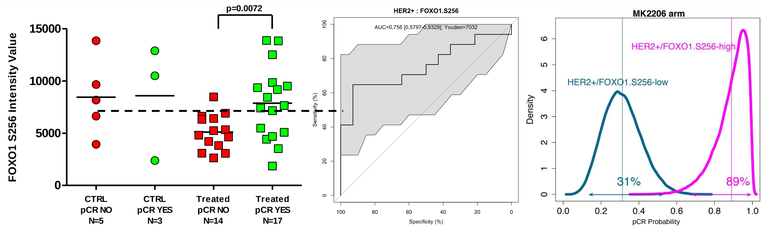


Figure 2. FOXO1 S256 is a positive biomarker of response in HER2+ patients treated with MK2206. Panel 1: One-way scatterplots of FOXO1 S256 expression values by response in HER2+ control and treatment arms for MK2206. Dashed line represents approximate cut point established in ROC analysis. Panel 2: ROC analysis to establish optimal cut point for FOXO1 S256 expression. Panel 3: Bayesian pCR rates in HER2+/FOXO1 high and low expression groups.

-Higher pre-treatment levels of FOXO1 S256 are associated with response in HER2+ tumors

-Establishment of an optimal cut point for pFOXO1 expression demonstrates that biomarker positive patients have significant sensitivity to MK2206 treatment

A Combination of Positive and Negative Biomarkers of Response to MK2206 Treatment Enrich for pCR in TN Patients

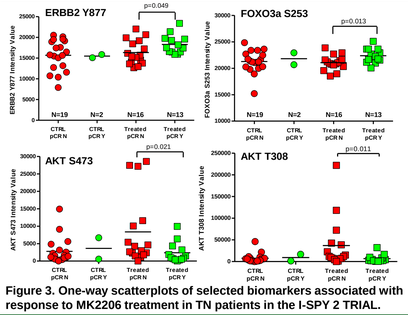


Figure 3. One-way scatterplots of selected biomarkers associated with response to MK2206 treatment in TN patients in the I-SPY 2 TRIAL.

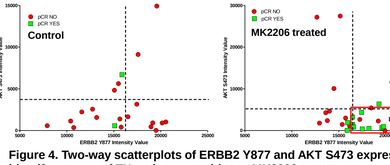


Figure 4. Two-way scatterplots of ERBB2 Y877 and AKT S473 expression values identify a group of TN patients sensitive to MK2206 treatment

-FOXO3a S253 and ERBB2 Y877 are positively associated with pCR in TN patients, while phosphorylated AKT S473 and T308 are negatively associated with response

-a combination of high relative expression of ERBB2 Y877 and low AKT S473 expression identifies a subset of TN patients sensitive to MK2206

Conclusions

-In HER2+ patients, a number of phosphoproteins that are direct kinase substrates of AKT/mTOR protein signaling were positively associated with pCR. These results are not surprising in the context of the hypothesis that, given AKT/mTOR signaling activation is an established HER2-driven downstream event, tumors that are HER2-amplified/overexpressing may be more sensitive to AKT kinase inhibition as a result. Our results support this hypothesis.

-Interestingly, many of the same AKT/mTOR signaling-related proteins had a negative association with pCR in TN tumors. This may be a result of the molecular heterogeneity of TN tumors compared to HER2+ breast cancer and reflects that AKT signaling is regulated by complex feedback systems and activated by diverse upstream events. Consequently, this finding may be due to mixed population effects and heterogeneous signaling drivers in the absence of overt HER2 amplification/overexpression in this patient subset.

-We are working to validate these data in independent study sets to better understand their significance.