Mechanism of action biomarkers predicting response to AKT inhibition in the I-SPY2 breast cancer trial

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	Genes	Population as a whole with gene expression (n=150: 94 in MK2206, 56 in Ctr)							HER2+ subset (n=34 in MK2206)*		TN subset (n=32 in MK2206)*		HR+HER2- subset (n=28 in MK2206)*	
		MK2206 arm: OR/unit inc	MK2206 arm: LR p	MK2206 arm: LR p (adjusting for HR and HER2)	Cont arm: OR/unit inc	Cont arm: LR p	Biomarker x treatment int (LR p)	Biomarker x treatment int (adjusted for HR and HER2)	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
AKT/MTOR signaling axis	AKT1	0.949	0.803	0.855	0.944	0.887	0.967	0.899	0.986	0.97	0.586	0.175	0.912	0.849
	EGFR	1.23	0.305	1.02	1.01	0.985	0.66	0.664	1.02	0.949	1.21	0.698	0.884	0.806
	EGFR	1.23	0.305	1.09	1.01	0.985	0.66	0.664	1.02	0.949	1.21	0.698	0.884	0.806
	ERBB2	1.51	**0.0464**	1.15	1.47	0.329	0.964	0.927	1.32	0.644	1.17	0.836	0.88	0.916
	ERBB3	1.01	0.977	0.854	0.596	0.155	0.226	0.237	1.02	0.963	1.19	0.74	1.27	0.648
	IGF1R	0.628	0.0678	0.924	0.779	0.522	0.656	0.88	0.259	**0.0356**	2.03	0.2	0.722	0.52
	MTOR	0.905	0.652	1.33	1.69	0.157	0.144	0.213	1.09	0.807	0.747	0.462	0.781	0.659
	NRG1	1.37	0.153	1.2	0.742	0.444	0.182	0.17	0.765	0.626	1.79	**0.0457**	0.166	0.0592
	PIK3CA	1.15	0.481	1.14	1.99	0.18	0.428	0.267	0.857	0.568	2.62	**0.0303**	1.18	0.782
	PTEN	1.06	0.774	1.32	1.03	0.952	0.914	0.84	0.917	0.821	1.43	0.318	1.08	0.89
	STMN1	1.28	0.226	0.855	1.28	0.552	0.982	0.816	1.78	0.155	1.04	0.922	0.888	0.823
Phase 1b study genes	ALDH4A1	1.03	0.898	0.962	0.802	0.578	0.619	0.441	1.23	0.588	0.443	0.0937	1.3	0.643
	G3BP1	0.663	**0.0481**	0.78	0.979	0.963	0.429	0.725	0.611	0.151	0.999	0.998	0.692	0.479
	MIEF1	1.2	0.381	1.08	0.887	0.753	0.491	0.436	1.14	0.661	0.54	0.185	2.84	0.0892
	PHEX	0.809	0.274	0.7	0.795	0.681	0.974	0.71	0.608	0.122	0.654	0.232	0.894	0.827
	PRODH	1.44	0.067	1.3	1.04	0.921	0.499	0.584	1.39	0.287	0.97	0.949	1.43	0.662
	SELENBP1	0.614	0.0558	0.768	0.629	0.147	0.908	0.534	0.668	0.411	0.771	0.502	0.52	0.409
	STARD3	1.52	**0.0427**	1.15	1.71	0.174	0.785	0.553	1.37	0.504	0.131	0.15	0.651	0.773
	TCTEX1D2	1.34	0.161	1.47	0.831	0.65	0.304	0.255	1.05	0.907	1.71	0.151	1.68	0.265
	TM7SF2	0.971	0.888	0.723	0.444	0.0659	0.117	0.313	0.983	0.965	0.363	0.0642	0.564	0.487

Supplementary Table 1. pCR association analysis results for pre-specified mechanism-of-action genes, in the population as a whole and in receptor subsets. P-values < 0.05 are in bold face; red denotes markers where higher levels associate with pCR; and blue denotes markers where higher levels associate with non-response. *The small size and/or low number of pCR in the control group within receptor subsets precluded analysis (Ctr for HER2+: 3/10 pCR; TN: 3/24 pCR; HR+HER2+: 3/22 pCR).
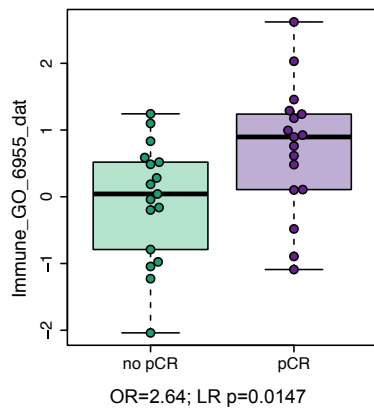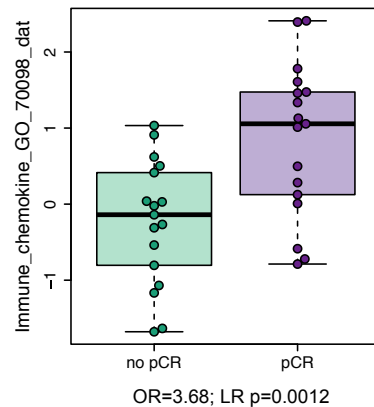
AKT/MTOR signaling axis phospho-proteins	MK2206 arm: OR/unit inc	MK2206 arm: LR p	MK2206 arm: LR p (adjusting for HR and HER2)	Cont arm: OR/unit inc	Cont arm: LR p	Biomarker x treatment int (LR p)	Biomarker x treatment int (adjusted for HR and HER2)	HER2+ subset MK2206 arm: OR/unit inc	HER2+ subset MK2206 arm: LR p	TN subset MK2206 arm: OR/unit inc	TN subset MK2206 arm: LR p	HR+HER2- subset MK2206 arm: OR/unit inc	HR+HER2- subset MK2206 arm: LR p
4EBP1.S65	0.824	0.581	0.491	0.96	0.887	0.737	0.758	2.55	0.25	0.441	0.148	0.891	0.885
AKT.S473	0.747	0.175	0.196	0.963	0.952	0.719	0.737	1.04	0.904	0.147	**0.021**	0.293	0.19
AKT.T308	0.658	0.0684	0.064	1.3	0.814	0.561	0.524	1.23	0.696	0.125	**0.011**	0.288	0.193
eIF4E.S209	1.2	0.407	0.944	0.863	0.716	0.469	0.843	1.29	0.45	0.669	0.3	3.2	0.14
eIF4G.S1108	1.18	0.465	0.983	1.34	0.386	0.748	0.517	2.22	**0.047**	0.406	0.0666	1.04	0.96
ERBB2.total	1.33	0.166	0.35	1.84	0.0996	0.442	0.422	0.606	0.333	0.37	0.733	2.18	0.695
ERBB2.Y1248	1.77	**0.00904**	0.186	1.54	0.222	0.741	0.652	1.74	0.0783	3.23E-05	0.0773	0.0107	0.512
ERBB2.Y877	1.29	0.292	0.446	1.05	0.892	0.622	0.549	0.626	0.22	2.66	**0.0496**	1.68	0.369
ERBB3.total	1.4	0.25	0.524	0.949	0.863	0.349	0.671	1.7	0.287	0.596	0.487	1.37	0.589
Estrogen.Rec.alpha.total	0.59	**0.0365**	0.185	0.865	0.668	0.379	0.706	1.15	0.765	0.34	0.0579	0.599	0.265
FOXO3a.S253	1.38	0.261	0.274	1.16	0.623	0.688	0.491	0.524	0.243	5.21	**0.0126**	1.24	0.693
FOXO1.S256	1.93	0.163	0.321	0.74	0.379	0.0991	0.14	11.2	**0.0072**	0.687	0.709	0.616	0.655
FOXO1.T24.FOXO3a.T32	1.35	0.125	0.569	1.47	0.518	0.894	0.933	2.9	**0.0258**	0.749	0.361	2.26	0.501
GSK3aB.S21.S9	1.31	0.18	0.17	1.23	0.653	0.908	0.872	2.84	**0.00937**	0.77	0.524	1.09	0.825
mTOR.S2448	1.23	0.346	0.288	1.18	0.634	0.92	0.62	3.84	**0.00425**	0.39	**0.0379**	1.82	0.166
mTOR.total	0.683	0.148	0.296	1	0.998	0.357	0.506	1.17	0.76	0.396	0.0612	0.805	0.708
NFkB.p65.S536	0.552	0.118	0.0604	0.773	0.439	0.537	0.386	1.55	0.533	0.126	**0.00327**	0.571	0.557
p70S6K.T389	1.16	0.467	0.675	1.06	0.904	0.852	0.743	1.25	0.393	0.249	0.0867	2.33	0.386
p70S6K.T412	1.29	0.294	0.644	1.01	0.97	0.528	0.644	1.55	0.23	0.476	0.155	2.25	0.231
p90RSK.S380	1.09	0.742	0.907	0.881	0.724	0.63	0.484	1.76	0.281	0.579	0.259	1.23	0.702
PI3K.p85.Y485.p55.Y199	1.24	0.302	0.792	1.48	0.323	0.701	0.704	1.52	0.185	0.319	**0.0394**	1.26	0.7
S6RP.S240.S244	1.42	0.106	0.432	1.01	0.973	0.43	0.357	2.05	**0.036**	0.267	0.0956	1.18	0.835
SHC.Y317	1.67	**0.0172**	0.252	1.95	0.152	0.76	0.851	1.41	0.18	0.871	0.898	2.11	0.758
Tuberin.TSC2.Y1571	0.859	0.529	0.403	1	0.995	0.779	0.868	3.19	**0.0434**	0.14	**0.00607**	0.77	0.76
SGK.S78	0.836	0.508	0.262	1.15	0.63	0.423	0.308	1.01	0.979	0.126	**0.00352**	1.49	0.534
Estrogen.Rec.alpha.S118	0.795	0.432	0.412	0.963	0.901	0.653	0.723	1.78	0.22	0.184	**0.00988**	0.803	0.735

Supplementary Table 2. pCR association analysis results for pre-specified RPPA endpoints, in the population as a whole and in receptor subsets. P-values < 0.05 are in bold face; red denotes markers where higher levels associate with pCR; and blue denotes markers where higher levels associate with non-response. *The small size and/or low number of pCR in the control group within receptor subsets precluded analysis (Ctr for HER2+: 3/9 pCR; TN: 3/22 pCR; HR+HER2+: 3/20 pCR).

A)

Category	Term	Count	PValue	Genes	Fold Enrichment	Benjamini	FDR
MK2206 response pathway enrichments - HER2+ subset							
GOTERM_BP_DIRECT	GO:0002250~adaptive immune response	20	7.77E-07	PIK3CG, GPR183, SIT1, CD1C, KLRK1, CD1B, TNFRSF17, CTSS, SLAMF7, CLEC10A, CD1E, TRAT1, PRKCB, BTK, BTLA, SH2D1A, CD79B, CD79A, CTSH, PAG1	3.9259	0.0019	0.0014
GOTERM_BP_DIRECT	GO:0006955~immune response	35	3.74E-06	C7, GPR183, ENPP2, IFITM2, IFITM3, IL18, CD70, HLA-DMB, IGF1R, CCL23, CXCR5, CCL21, HLA-DOA, THBS1, APLN, LTB, CD27, CR2, LTBR, CMKLR1, IL1RN, CCL19, CTSS, CCL18, CD1E, IGSF6, CCR8, TNFSF10, CCR6, CCL14, CCR2, IRF8, CD79B, TNFAIP1, ADAMDEC1	2.4152	0.0046	0.0066
GOTERM_BP_DIRECT	GO:0070098~chemokine-mediated signaling pathway	13	4.86E-06	CCR8, CCL23, CCL14, CCR6, CXCR5, CMKLR1, CXCR4, CCL21, CCR2, CCL19, XCL1, CCL18, CCL17	5.3194	0.0040	0.0086
GOTERM_BP_DIRECT	GO:0007165~signal transduction	70	5.42E-06	GNA14, PPARG, FST, PDE3B, GJA1, FGF13, CD53, RASSF7, ARHGAP15, CD48, ANK1, PPP1R1A, PITPNC1, GKAP1, DLG5, PRKACB, APLN, NRG2, LTB, PAG1, LTBR, SIT1, TNFRSF17, CLIC2, TRAT1, PLAUR, PRKCB, JUP, CD38, CCR6, PLEKHH3, RIN1, PDE9A, AKAP8, INPP4A, EXT2, TRAF1, INPP1, OSTF1, PLCXD3, SPOCK2, KLRK1, CD70, SPOCK1, MDK, STAT6, IGF1R, TAGAP, CCL23, BCL11B, PDE1A, RASGRP2, HLA-DOA, TRAF4, PIK3R1, HPGDS, MRC1, PHB, DPYSL5, DRG2, SLAMF1, CCL18, TRIM55, P2RX4, LYVE1, TNFSF10, PNOC, CD79B, XCL1, GFRA3	1.7516	0.0033	0.0095
GOTERM_BP_DIRECT	GO:0002407~dendritic cell chemotaxis	7	1.44E-05	PIK3CG, GPR183, CCR6, CXCR4, CCL21, CCR2, CCL19	11.9625	0.0070	0.0253
GOTERM_BP_DIRECT	GO:0019722~calcium-mediated signaling	10	5.26E-05	CCR6, CXCR4, NCALD, PLCG2, HTR2B, LRRK2, RCAN3, SELE, BTK, MCTP1	5.6965	0.0212	0.0925
MK2206 response pathway enrichments - TN subset							
REACTOME_PATHWAY	REACT_7970:Telomere Maintenance	14	5.14E-06	HIST1H2AC, HIST1H2BB, HIST1H2BC, HIST1H2BD, HIST1H2BE, HIST1H2BG, POLE, HIST1H2BH, HIST1H2BO, RFC3, HIST1H2BM, HIST1H2BK, HIST1H2BL, HIST1H2BI, PRIM2, HIST1H2AK, HIST1H2AJ	4.6168	0.0003	0.0051
GOTERM_BP_FAT	GO:0006325~chromatin organization	41	3.15E-05	ING5, MORF4L1, SUPT3H, HIST1H2AC, HP1BP3, SETD1A, VPS72, TSPYL1, HIST1H2BO, CHD8, HIST1H2BM, CHD7, HIST1H2BK, PRMT7, HIST1H2BL, HIST1H2BI, GPX4, PBRM1, ACTL6A, KDM5C, CHD3, SETDB1, HIST1H2BB, HIST1H2BC, HIST1H2BD, HIST1H2BE, C11ORF30, HIST1H2BG, HIST1H2BH, ARID1A, BANP, ARID1B, TAF6L, SUV39H2, DOT1L, RNF8, TAF10, SAP130, SMARCC2, HIST1H2AK, RUVBL1, HIST1H2AJ, RNF20, RNF40	2.0156	0.0844	0.0563

B) MK2206 arm: HER2+ subset

Immune_GO_6955_dat

OR=2.64; LR p=0.0147

C) MK2206 arm: HER2+ subset

Immune_chemokine_GO_70098_dat

OR=3.68; LR p=0.0012

D) MK2206 arm: TN subset

Telomere_histone_REACT_7970_dat

OR=2.65; LR p=0.039
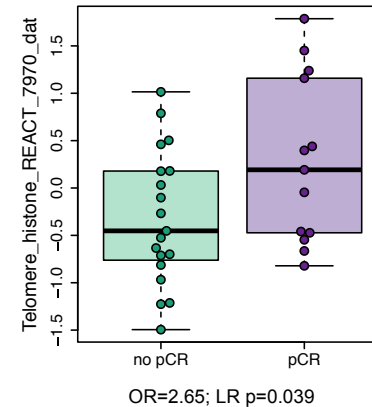
Supplementary Figure 1. Whole genome exploratory pathway enrichment analysis of responders within the HER2+ and TN patient subsets, respectively, in the MK2206 arm. A) Pathway enrichment table of genes associated with pCR in the MK2206 arm using DAVID, top hits, for HER2+ patients (top) and TN patients (bottom). Box plots in (B) and (C) show the mean expression level of genes in the top two enriched (immune) pathways in pCR vs. non-pCR within the HER2+ subset. D) Box plot showing the mean expression level of genes in the top enriched (telomere maintenance/histone) pathway in pCR vs. non-pCR patients within the TN subset.
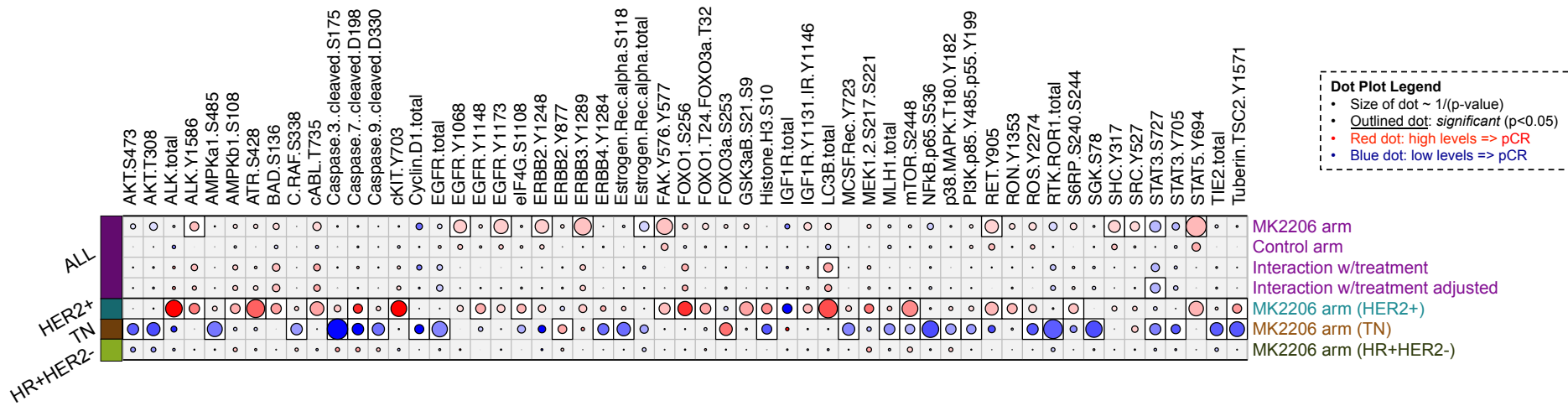
Supplementary Figure 2. Exploratory analysis of 118-endpoint RPPA dataset (115/118 with sufficient data for analysis). This association dot-plot shows the level and direction of association between each protein endpoint (column) and pCR in the MK2206 arm in patient groups as labeled (rows from top to bottom): all patients, HER2+, TN, and HR+HER2-. Key=red/blue dot indicates higher/lower levels ~ pCR; size of dot ~ strength of association (1/p), with dark outline indicating p<0.05. Only endpoints (columns) with at least one association with (uncorrected) p<0.05 are shown.

Supplementary Acknowledgement

We would like to acknowledge the following investigators, staff and sites for their contributions to the I-SPY 2 TRIAL during the period patients were being randomized to MK2206:

Note: Names in bold are site principal investigators, and names that appear in brackets no longer reside at that specific institution.