BluePrint Luminal subtype predicts non-response to HER2-targeted therapies in HR+/HER2+ I-SPY2 breast cancer patients

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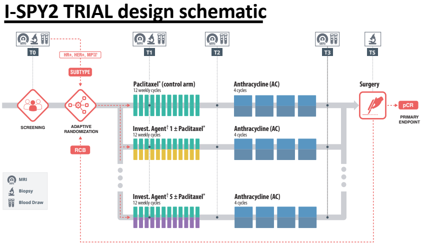
Introduction

- BluePrint molecular profile determines the mRNA levels of 80 genes that discriminate between 3 breast cancer subtypes based on functional molecular pathways: Luminal, HER2 and Basal.
- Previous studies suggest that within the HR+/HER2+ breast cancer subtype, patients classified as BluePrint (BP) Luminal subtype are more responsive to pertuzumab and trastuzumab (P/H) as opposed to trastuzumab (H) alone.
- In the I-SPY2 TRIAL (NCT01042379), HER2-targeted treatment arms include H, P/H, neratinib (N), T-DM1/pertuzumab (P), MK2206/ H and AMG386/H; and patients were classified by BP molecular subtyping in addition to conventional receptors.

Can BluePrint subtype predict response to HER2-targeted agents in I-SPY2 HR+/HER2+ breast cancer patients? What are the pathway differences between the BP subtypes?

Study Cohort

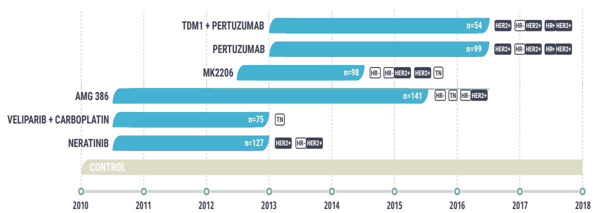
I-SPY2 TRIAL design schematic



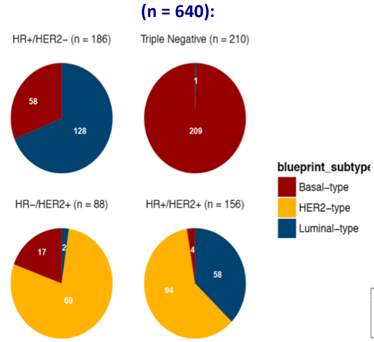
*HER2-positive participants also receive Trastuzumab. An investigational agent may be used instead of Trastuzumab.

- Phase II adaptively-randomized neoadjuvant trial
- Primary endpoint: pathologic complete response (pCR)
- Match therapies with most responsive breast cancer subtypes

Timeline of I-SPY2 Investigational Agents



Distribution of BluePrint molecular subtypes within conventional IHC/ FISH receptor groups (n = 640):



640 patients enrolled in I-SPY 2 TRIAL with BluePrint assignment available

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156 IHC/FISH HR+/HER2+ patients
With pretreatment expression array & pCR data available

↓

156 patients received HER2-targeted agents
- 19 received trastuzumab + paclitaxel (H)
- 42 received neratinib + paclitaxel (N)
- 29 received pertuzumab + trastuzumab + paclitaxel (P/H)
- 35 received T-DM1 + pertuzumab (T-DM1/P)
- 16 received MK2206 + trastuzumab + paclitaxel (MK2206/H)
- 15 received AMG386 + trastuzumab + paclitaxel (AMG386/H)

→ **4** patients classified as BP Basal-type

↓

152 patients classified as BP Luminal/ BP HER2-type
H (n =18); N (n = 40); P/H (n = 29); TDM-1/P (n = 34); MK2206/H (n = 16); AMG386/H (n = 15)

↓

94 patients classified as BP HER2-type
H (n = 5); N (n = 31); P/H (n = 17); T-DM1/P (n = 18); MK2206/H (n = 12); AMG386/H (n = 11)

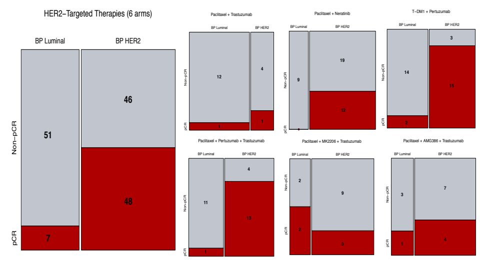
58 patients classified as BP Luminal-type
H (n = 13); N (n = 9); P/H (n = 12); T-DM1/ P (n = 16); MK2206/H (n = 4); AMG386/H (n = 4)

Methods

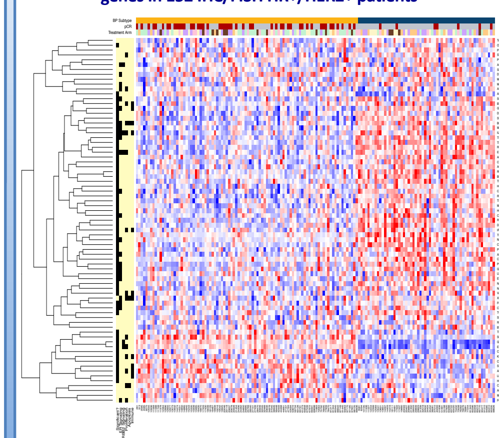
- We used Fisher's exact test to assess association between BP subtypes and pCR
- To identify genes associated with BP Luminal vs. BP HER2 subtype, we applied a Wilcoxon rank sum test and fitted a logistic model, with the Benjamini-Hochberg (BH) multiple testing correction (BH p<0.05).We then performed pathway enrichment analysis using DAVID (ver. 6.8).
- Our study is exploratory and does not adjust for multiplicities of other biomarkers in the trial outside this study

Results

IHC/FISH HR+/HER2+ BluePrint Luminal subtype is associated with lower responses to HER2-targeted agents, with the exception of MK2206/H



Semi-supervised heat map showing the expression of BluePrint genes in 152 IHC/FISH HR+/HER2+ patients

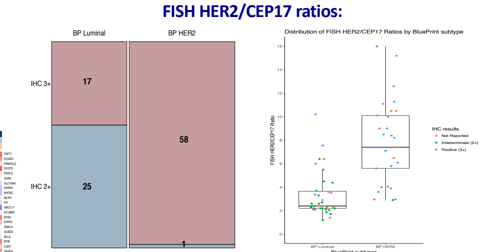


Top 15 up-regulated/ down-regulated genes in BP HER2-type tumors (relative to BP Luminal) in IHC/FISH HR+/HER2+ patients:

Gene symbol	Gene Name	Fold Change	p-value	Adjusted p value
GRB7	Growth Factor Receptor Bound Protein 7	2.50	4.21354E-21	7.25571E-17
ERBB2	Erb-B2 Receptor Tyrosine Kinase 2	1.53	1.10371E-19	9.50294E-16
MIEN1	Migration and Invasion Enhancer 1	2.04	1.53471E-17	8.80926E-14
TCAP	Titin-Cap	1.93	2.21837E-16	9.55006E-13
PGAP3	Post-GPI Attachment to Proteins 3	1.87	3.67058E-16	1.26415E-12
STARD3	StAR-Related Lipid Transfer Domain Containing 3	1.59	9.27484E-15	2.66188E-11
SOX11	SRY-Box 11	1.69	8.12486E-13	1.55456E-09
PNMT	Phenylethanolamine N-Methyltransferase	3.27	1.61532E-12	2.78157E-09
MPHOSPH6	M-Phase Phosphoprotein 6	0.94	9.63829E-11	9.76303E-08
TNFRSF21	TNF Receptor Superfamily Member 21	0.71	2.13716E-10	1.84102E-07
MFSD2A	Major Facilitator Superfamily Domain Containing 2A	0.57	4.45015E-10	3.33181E-07
C15orf39	Chromosome 15 Open Reading Frame 39	0.53	5.53158E-10	3.96891E-07
TGFBR1	Transforming Growth Factor Beta Receptor 1	0.49	1.53988E-09	9.82103E-07
KMO	Kynurenine 3-Monooxygenase	0.82	2.95765E-09	1.75623E-06
DPEP3	Dipeptidase 3	0.67	4.27331E-09	2.29957E-06

Gene symbol	Gene Name	Fold Change	p-value	Adjusted p value
RERG	Ras Like Estrogen Regulated Growth Inhibitor	-1.49	1.00916E-13	2.48253E-10
TBC1D9	TBC1 Domain Family Member 9	-1.21	3.93883E-13	8.47833E-10
AL133644	Protein phosphatase 1J	-1.28	1.23594E-11	1.93481E-08
MAPT	Microtubule Associated Protein Tau	-1.68	1.73626E-11	2.29987E-08
PPM1J	Protein Phosphatase, Mg2+/Mn2+- dependent 1J	-1.16	1.69159E-11	2.29987E-08
ESR1	Estrogen Receptor 1	-1.08	3.72165E-11	4.57763E-08
CCDC74B	Coiled-Coil Domain Containing 74B	-1.56	5.38966E-11	6.18733E-08
ZSWIM5	Zinc Finger SWIM-Type Containing 5	-0.74	6.27664E-11	6.75523E-08
DBNDD2	Dysbindin Domain Containing 2	-1.01	1.41998E-10	1.35844E-07
AGBL2	ATP/ GTP Binding Protein Like 2	-0.49	2.24514E-10	1.84102E-07
PARD6B	Par-6 Family Cell Polarity Regulator Beta	-1.32	2.1905E-10	1.84102E-07
KIAA1324L	KIAA1324 Like	-0.79	2.53895E-10	1.98731E-07
NXNL2	Nuceloredoxin Like 2	-0.69	8.31753E-10	5.72911E-07
SLC39A6	Solute Carrier Family 39 Member 6	-1.37	1.40153E-09	9.28246E-07
SPR	Sepiapterin Reductase	-0.74	2.57399E-09	1.58301E-06

IHC/FISH HR+/HER2+ BluePrint Luminal is associated with lower FISH HER2/CEP17 ratios:



Immune-related biological processes were significantly enriched based on DAVID functional enrichment analysis

- **HR+/HER2+ BP HER2-type patients demonstrated higher expression levels of immune-related genes e.g. CTLA4, ITGB2**

Category	Term	Count	%	p-value	Benjamini
GOTERM_BP_DIRECT	negative regulation of T cell proliferation	15	1	3.60E-07	1.50E-03
GOTERM_BP_DIRECT	inflammatory response	56	3.6	7.30E-06	1.50E-02
KEGG_PATHWAY	Cytokine-cytokine receptor interaction	37	2.4	9.60E-05	2.70E-02
GOTERM_MF_DIRECT	protein binding	748	47.7	4.30E-04	4.30E-01
GOTERM_BP_DIRECT	positive regulation of inflammatory response	16	1	4.80E-04	4.90E-01
GOTERM_BP_DIRECT	neutrophil chemotaxis	15	1	5.30E-04	4.20E-01
GOTERM_BP_DIRECT	adaptive immune response	25	1.6	5.40E-04	3.70E-01
GOTERM_BP_DIRECT	positive regulation of gene expression	37	2.4	7.50E-04	4.10E-01
GOTERM_BP_DIRECT	regulation of G1/S transition of mitotic cell cycle	7	0.4	9.50E-04	4.30E-01
GOTERM_BP_DIRECT	response to lipopolysaccharide	26	1.7	1.10E-03	4.30E-01
GOTERM_BP_DIRECT	response to wounding	14	0.9	1.10E-03	3.90E-01
GOTERM_BP_DIRECT	negative regulation of interferon-gamma production	9	0.6	1.10E-03	3.60E-01
GOTERM_BP_DIRECT	xenobiotic catabolic process	5	0.3	1.10E-03	3.40E-01
GOTERM_BP_DIRECT	apoptotic signaling pathway	15	1	1.10E-03	3.20E-01
GOTERM_MF_DIRECT	chemokine activity	12	0.8	1.20E-03	5.40E-01
GOTERM_BP_DIRECT	chemotaxis	21	1.3	1.30E-03	3.50E-01

Conclusion

- Our analysis suggests that IHC/FISH HR+/HER2+ BP Luminal subtype is associated with lower response rates to HER2-targeted agents , including Pertuzumab/ Trastuzumab, and may need an alternative strategy.
- IHC/FISH HR+/HER2+ BP HER2 subtype appears associated with higher expression of immune-related genes, relative to BP Luminal; and suggests that immune signaling may contribute to HER2-targeted therapy sensitivity.

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