Response to neo-adjuvant chemotherapy and outcomes for I-SPY 1 patients stratified by the 70-gene prognosis signature (MammaPrint) and molecular subtyping (BluePrint)

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Background

Classification of breast cancers into molecular subtypes may be important for the proper selection of therapy for patients as tumors with seemingly similar biology can have strikingly different clinical outcomes. The multi-center neo-adjuvant I-SPY 1 TRIAL (CALGB 150007/150012; ACRIN 6657) showed that breast cancer subtypes as identified by immunohistochemistry or molecular analyses, have distinct clinical outcome[1] (Table 1 and 2). The median follow-up period of the trial is 3.9 years. Here, we present how the 70-gene signature (MammaPrint) now analyzed together with an 80-gene molecular subtyping profile[2] (BluePrint=Basal-type, Luminal-type, HER2-type) stratifies patients into molecular subgroups and show the relation to response to neo-adjuvant chemotherapy and survival for the I-SPY I patients.

Characteristics of I-SPY I patients

Characteristics		I-SPY Trial Evaluable (n=221)	Profiled with Agilent Microarray (n=149)
Age, years	Median (range)	49 (26-68)	48 (27-65)
Premenopausal		48% (106)	49% (72)
Race	Caucasian	75% (165)	76% (114)
	African American	19% (42)	18% (27)
	Asian	4% (9)	5% (7)
	Other	2% (5)	1% (1)
Clinical Tumor Size, cm; median (range)		6 (0-25)	5.5 (0-25)
Tumor longest diameter on baseline MRI, cm; median (range)		6.8 (0-18.4)	6.5 (0-18.4)
Clinically node positive at diagnosis		65% (143)	66% (99)
Histologic Grade (baseline)	Low	8% (18)	7% (10)
	Intermediate	43% (96)	42% (63)
	High	47% (103)	50% (75)
	Indeterminate	2% (4)	1% (1)
Clinical Stage (baseline)	I	1% (3)	2% (3)
	IIA	19% (43)	21% (32)
	IIB	28% (61)	26% (38)
	IIIA	35% (78)	34% (51)
	IIIB	5% (11)	5% (8)
	IIIC	3% (7)	3% (5)
	Inflammatory	8% (17)	7% (11)
	Indeterminate	<1% (1)	1% (1)
Hormone Receptors (baseline)	ER-positive	56% (124)	55% (82)
	PR-positive	46% (102)	44% (66)
	HR-positive (ER or PR)	59% (130)	58% (86)
Her-2 positive (baseline)		30% (67)	30% (45)
HR-negative/Her-2 negative (baseline) (triple negative)		24% (53)	25% (37)
Neoadjuvant Treatment	AC Only	5% (11)	3% (4)
	AC + T	85% (187)	87% (129)
	AC + T+Trastuzumab	9% (20)	9% (14)
	AC + T+ Other	1% (3)	1% (2)
Surgery Type	Mastectomy	56% (123)	57% (84)
	Lumpectomy	41% (92)	40% (60)
	No Surgery	3% (6)	3% (5)
Post-Operative Adjuvant Therapy		58% (128)	56% (84)
	Any hormonal therapy	34% (75)	34% (52)
	Tamoxifen	43% (95)	44% (61)
	Aromatase Inhibitor	12% (27)	12% (20)
	Ovarian Suppression or Ablation	3% (7)	3% (6)
	Trastuzumab	16% (35)	16% (25)

Table 1. Published by Esserman et al.[1]

I-SPY 1 Molecular Subtypes Distribution and Response

	Overall Distribution Available for Analysis, % (n)	pCR, % (n)	pCR vs. not p-value	RCB 0,I % (n)	RCB 0,I vs. II, III p-value	3-year RFS Overall	3-year RFS pCR	3-year RFS NO pCR
Entire Population	**n=215**	**27%** *(58/215)*		**37%** *(74/201)*		**78%** *(168/215)*	**86%** *(50/58)*	**74%** *(118/157)*
IHC /FISH	**n=210**	**27%** *(56/210)*		**37%** *(72/196)*				
HR+/HER2-	**44%** *(n=93)*	**9%** *(8/93)*		**17%** *(1 5/87)*		**87%** *(81/93)*	**100%** *(8/8)*	**86%** *(73/85)*
HR+/HER2+ (w or w/o T)	**16%** *(n=33)*	**33%** *(11/33)*		**50%** *(15/30)*		**75%** *(25/33)*	**61%** *(7/11)*	**82%** *(18/22)*
			<0.001		<0.001			
HR-/HER2+ (w or w/o T)	**16%** *(n=33)*	**58%** *(19/33)*		**72%** *(23/32)*		**75%** *(25/33)*	**89%** *(17/19)*	**57%** *(8/14)*
HR-/HER2-	**24%** (n=51)	**35%** *(18/51)*		**40%** *(19/47)*		**66%** *(34/51)*	**89%** *(16/18)*	**54%** *(18/33)*
HER2+ (w/o any T†)	**14%** (n=29)	**41%** (12/29)		**55%** (16/29)		**75%** (7/29)	**91%** (1/12)	**65%** (6/17)
HR+/HER2+ (w/o any T†)	**55%** (n=16)	**31%** (5/16)		**44%** (7/16)		**81%** (13/16)	**80%** (4/5)	**82%** (9/11)
HR-/HER2+ (w/o any T†)	**45%** (n=13)	**54%** (7/13)		**69%** (9/13)		**67%** (9/13)	**100%** (7/7)	**33%** (2/6)
Population without any Trastuzumab								
Intrinsic Subtype	**n=120**	**n=116**		**n=108**				
Luminal A	**30%** *(n=36)*	**3%** *(1/36)*		**9%** *(3/32)*		**94%** *(34/36)*	**100%** *(1/1)*	**94%** *(33/35)*
Luminal B	**22%** *(n=26)*	**16%** *(4/25)*		**21%** *(5/24)*		**79%** *(20/25)*	**100%** *(4/4)*	**75%** *(16/21)*
Her2-enriched	**9%** *(n=11)*	**50%** *(5/10)*	0.001	**60%** *(6/10)*	0.001	**90%** *(9/10)*	**80%** *(4/5)*	**100%** *(0/5)*
Basal	**36%** *(n=43)*	**33%** *(14/42)*		**41%** *(16/39)*		**58%** *(25/42)*	**85%** *(12/14)*	**45%** *(13/28)*
Normal-like	**3%** *(n=4)*	**33%** *(1/3)*		**66%** *(2/3)*		**100%** *(3/3)*	**100%** *(1/1)*	**100%** *(2/2)*
70-Gene Prognosis MammaPrint								
Low Risk	**9%** *(n=11)*	**0%** *(0/11)*		**18%** *(1/9)*		**100%** *(11/11)*	**NA** *(0/0)*	**100%** *(11/11)*
High Risk	**91%** *(n=109)*	**24%** *(25/105)*	0.12	**31%** *(31/99)*	0.28	**75%** *(80/105)*	**88%** *(22/25)*	**72%** *(72/80)*

Table 2. Data were also analyzed for ROR-S, Wound Healing, p53 Mutation Signature, p53 Gene Chip, MIP Arrays, Ki67 IHC. Published by Esserman et al.[1]

Abbreviations: IHC, immunohistochemistry; FISH, fluorescence-in situ hybridization assay; HR, hormone receptor; T, trastuzumab; RCB is residual cancer burden. RCB 0,1 refers to absence of any invasive cancer (RCB 0) or minimal residual disease (RCB 1); The first four rows of the IHC results show the entire evaluable population, whereas the IHC shaded rows show the population broken down by the use of neoadjuvant trastuzumab (w/T) or without (w/o T).

Molecular Subtype Comparison

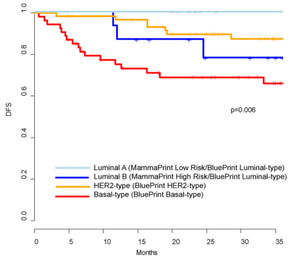
	BluePrint and MammaPrint*		Intrinsic subtyping (PAM50)		Subtyping with IHC/FISH	
	pCR (%)	3yr DMFS	pCR (%)	3yr DMFS	pCR (%)	3yr DMFS
Luminal A	**0/12 (0%) (MammaPrint Low Risk, BluePrint Luminal-type)**	**100%**	**2/42 (5%)**	**97%**	**4/53 (8%)**	**87%**
Luminal B	**8/62 (13%) (MammaPrint High Risk, BluePrint Luminal-type)**	**87%**	**4/31 (13%)**	**82%**		
HER2-type	**9/16 (56%)**	**78%**	**12/23 (52%)**	**86%**	**18/39 (46%)**	**83%**
Basal-type	**19/54 (35%)**	**66%**	**15/46 (33%)**	**58%**	**9/33 (27%)**	**63%**
Total	**36/144 (25%)**		**33/142* (23%)**		**31/125** (25%)**	

*A research version of the 70-gene signature yielded the MammaPrint results

*Data missing for 7 and 24 patients respectively

Table 3. Molecular subclassification is compared for all patients for whom 44K, IHC and pCR was available (n=144) BluePrint and MammaPrint, Intrinsic subtyping (PAM50) and for subtyping with IHC/FISH. This analysis includes patients treated with Trastuzumab.

Survival stratified for subtyping



KM survival analysis for all patients for whom 44K, IHC and pCR was available (n=144). Molecular subclassification was achieved by combining the 70-gene (MammaPrint) signature with BluePrint.

BluePrint development

The 80-gene BluePrint profile was developed in a supervised training method, using samples with concordant ER, PR, and HER2 status by IHC and single-gene readout ensuring the capture of ER/PR/HER2-regulated processes, and development of a more reliable and robust test, than a single-gene read-out by IHC or mRNA measurement. This rational based approach is different from previously defined subtypes based on hierarchical clustering.[2]

Results

The 70-gene MammaPrint signature classified 9% of patients (13/144) as Low Risk, of whom one patient was HER2-type, and the other 12 were Luminal-type (Table 3). None of these patients experienced a pCR. The remaining 131 were classified as 70-gene High Risk (91%). 43% were classified as High Risk Luminal-type (Luminal B) with a pCR rate of 13%, 38% were Basal-type with a pCR rate of 35%, and 11% were HER2-type with a pCR rate of 56%. Patients with BluePrint Basal-type tumors had a 3-year DFS of 66%; HER2-type had a 3-year DFS of 78%; 70-gene High Risk/Luminal-type had a 3-year DFS of 87% and 70-gene Low Risk/Luminal-type showed 3-year DFS of 100%.
The BluePrint/MammaPrint molecular subtype classification shows significant association with intrinsic subtype and clinically assessed receptor status. However, clinically assessed HER2+ patients were distributed across all molecular subtypes, where ER+HER+ are predominantly classified as Luminal-type.

Conclusion

This study was performed with the I-SPY I dataset, which provides a platform to compare, contrast & combine marker signatures to tailor therapy. The molecular subtyping data using BluePrint highly correlates with earlier published I-SPY 1findings. Combining BluePrint, with MammaPrint risk-classification can detect specific groups of patients who are at high risk of recurrence and suggests who would have a higher likelihood to benefit from chemotherapy. MammaPrint Low Risk patients have excellent survival rates, despite the fact that they do not achieve pathological complete response after neo-adjuvant therapy. These findings support the need to investigate whether Low Risk Luminal-type patients could be managed with endocrine therapy alone.

References

1. Esserman et al., *Breast Cancer Research and Treatment*, in press, 2011
2. Krijgsman et al., *Breast Cancer Research and Treatment*, Aug 4, 2011