

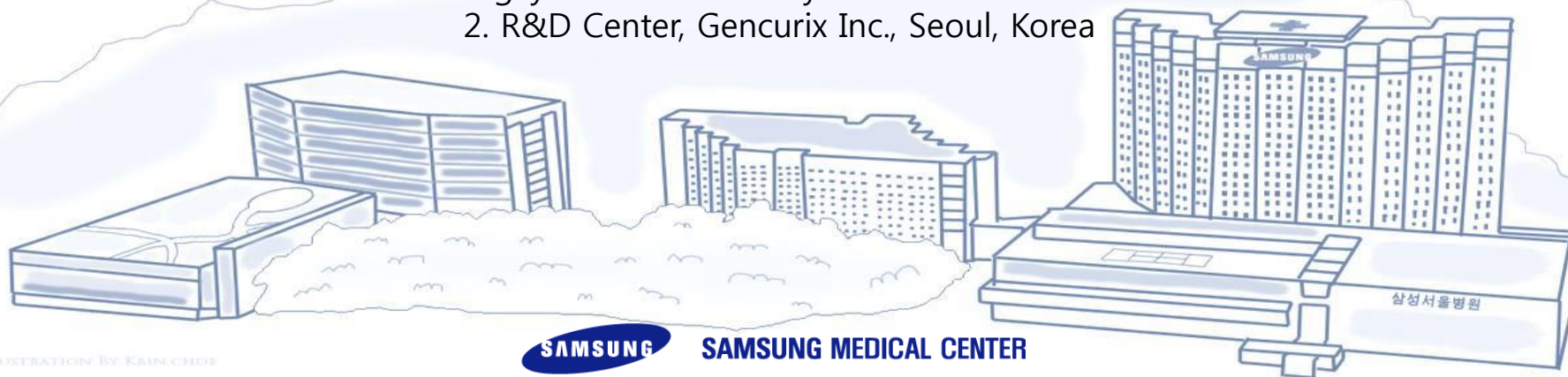
# Only Estrogen receptor “positive” is not enough to predict the prognosis of breast cancer

Running head: Revisiting estrogen positive tumors in 8th AJCC staging era

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## COI

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- JK and BK are employees of Gencurix.
- The other authors have no competing interests to declare.

# Estrogen receptor (ER)

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- One of the most important predictive and prognostic biomarkers in breast cancer
- ER positive tumors are associated with better survival than ER negative tumors

# Definition of ER and PR

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- $\geq 1\%$  of cells stained considered positive for ER & PR
- Multiple results always use positive results
  - If biopsy and resection specimens are tested, and one is positive, while the other is negative, then use the positive results to assign the study group

AJCC Level of Evidence: I



# ER positive tumors in 8<sup>th</sup> AJCC staging

When T/N is...	7 <sup>th</sup> Stage	And Grade is..	And HER2 status is...	And ER status is..	And PR status is...	8 <sup>th</sup> Stage Group is..
T0/T1, N1mi	<b>IB</b>	G1-G3	(+/-)	positive	(+)	<b>IA</b>
T0/T1, N1mi	IB	G3	(-)	positive	(-)	IB
T0N1, T1N1, T2N0	<b>IIA</b>	G1-G3	(+/-)	positive	(+)	<b>IB</b>
T2N1, T3N0	<b>IIB</b>	G1-G3	(+)	Positive	(+)	<b>IB</b>
T2N1, T3N0	<b>IIB</b>	G1-2	(+)	positive	(-)	<b>IIA</b>
T0N2, T1N2, T2N2, T3N1, T3N2	<b>IIIA</b>	G1-2	(+/-)	Positive	(+)	<b>IIA</b>



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# Multigene panels incorporated into 8<sup>th</sup> AJCC staging

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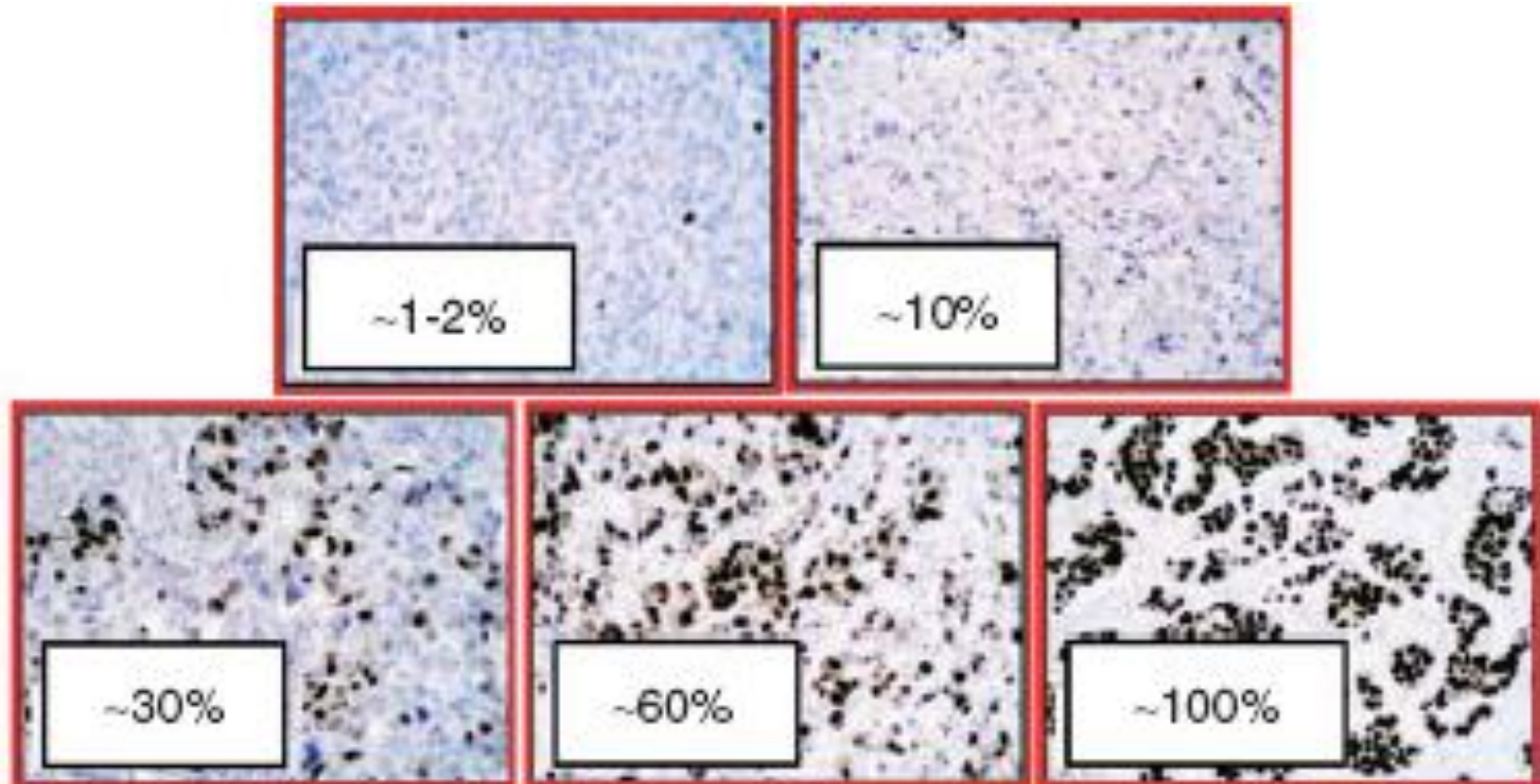
- Patients with
  - ER/PR-positive, HER2-negative and N(-) tumors
  - Size less than or equal to 5 cm (T1-2)
  - Combined with any of the following multigene panels
    - Oncotype Dx<sup>®</sup>: score less than 11
    - Mammaprint<sup>®</sup>: low-risk score
    - Endopredict<sup>®</sup>: low-risk score
    - PAM50<sup>®</sup>: ROR score in the low range
    - Breast Cancer Index (BCI): low-risk range

→ "Stage IA"

: same category as T1-2 N0 M0 with ER(+) HER-2 (-)

# Heterogeneity of ER positive

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## Positive

≥ 1% cells

Quantify results

Endocrine Therapy

Expect ~75% ER and 65% PgR

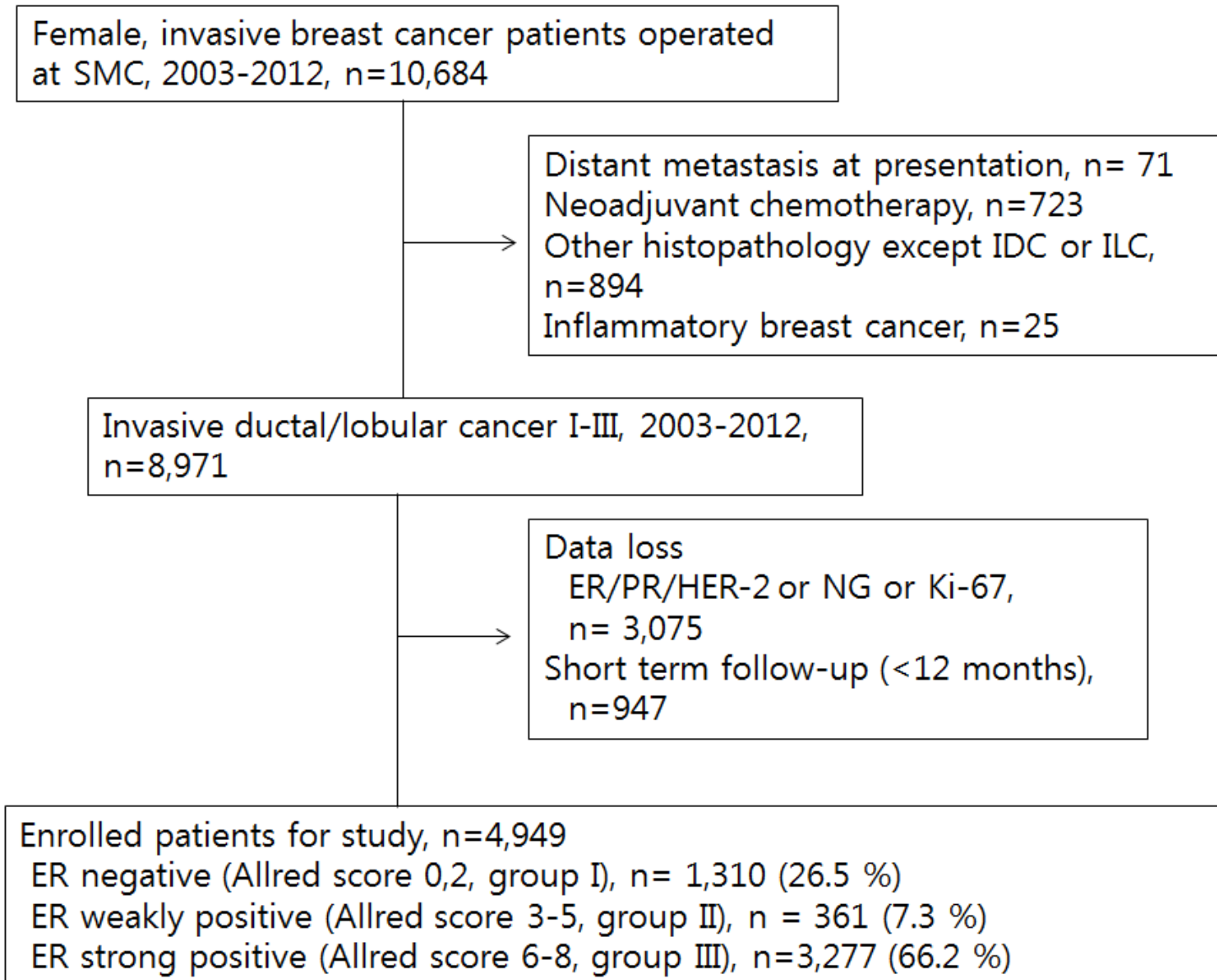


# Purpose

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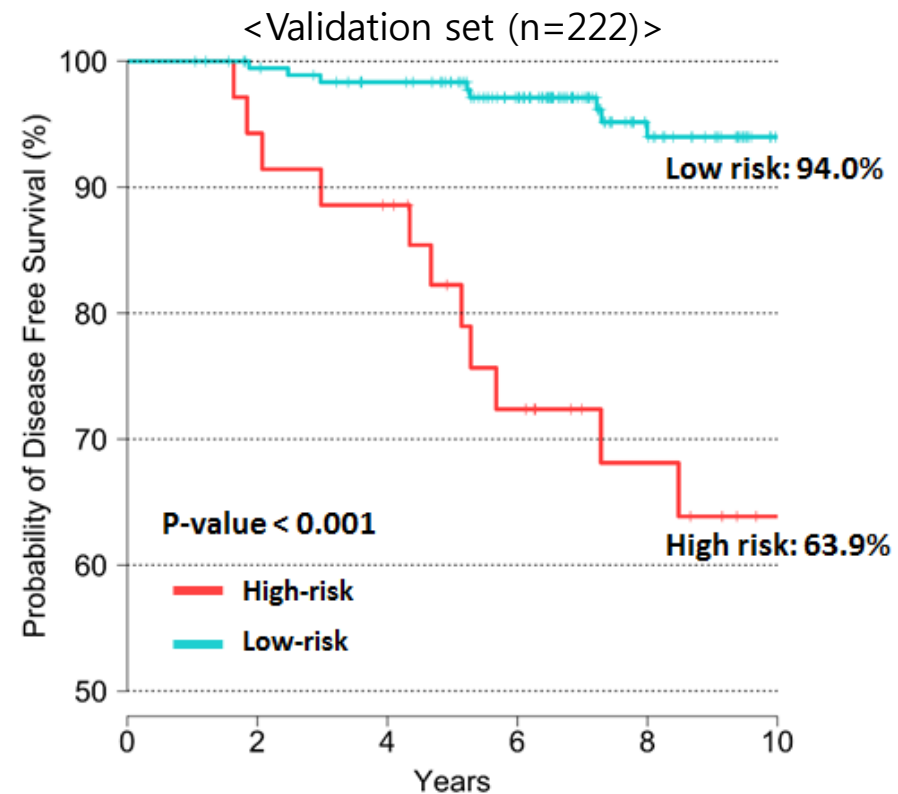
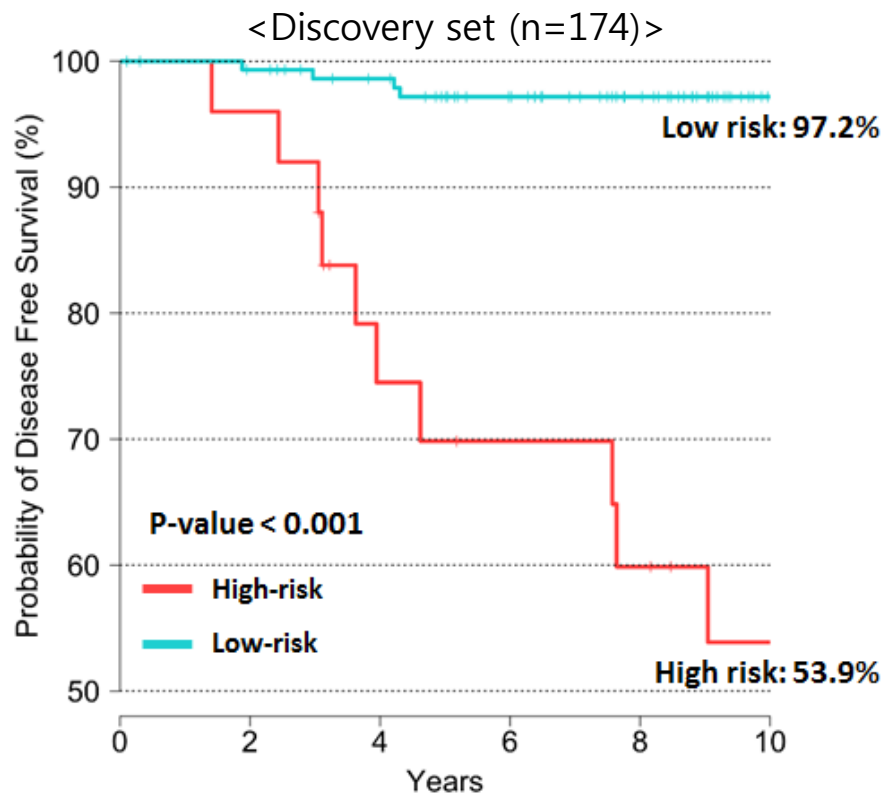
- We hypothesized that the level of ER expression could affect the prognosis and the risk score of multigene panel.
- We analyzed the prognosis and examined multigene panel based on the levels of ER expression.

# Schematic diagram



# BCT score (multigene panel)

- New developed prognostic model for predicting the risk of distant metastasis in patients with HR+/HER2- , pT1-2, pN0-N1 breast cancer
- The patients were categorized as the high risk or low risk group according to a pre-specified cutoff BCT score of 4.



## BCT score and ER expression

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- Primary breast cancer operated at SMC
- BCT scores were retrospectively obtained from 386 patients with pT1-T2, pN0-N1, HR+/HER2- breast cancer.
- BCT scores were classified by the levels of ER expression.  
(Allred score 0-2/ 3-5/ 6-8)

# Statistics

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- Categorical variables/ Kruskal-Wallis test or analysis of variance (ANOVA) test
- Categorical variables/ Chi-square or Fisher's exact test
- Kaplan-Meier curves with corresponding results of log-rank tests
  - Disease free survival (DFS), Distant metastasis free survival (DMFS), and Overall Survival (OS)
- Univariate and multivariate analyses for OS
  - Cox regression and proportional hazard model to estimate hazard ratio (HR) and 95% confidence interval (CI)
- All tests were two sided, and  $P < .05$  was considered significant
- SAS version 9.4 (SAS Institute, Cary, NC, USA) and R3.4.0

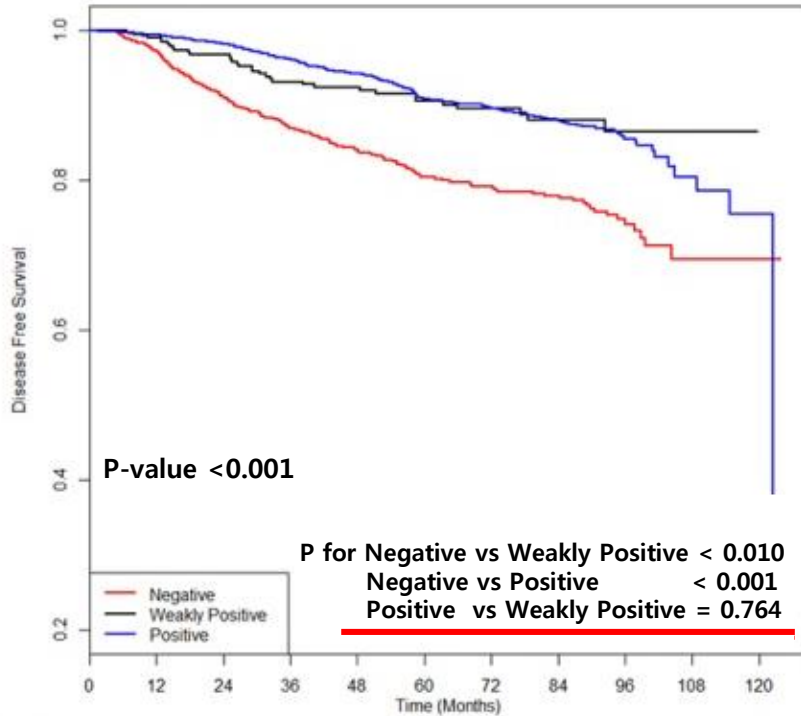
# Baseline characteristics

	ER-negative, n (%)	Weakly ER-positive, n (%)	Strongly ER-positive, n (%)	p-value	
	Group I	Group II	Group III	Group I vs II	Group II vs III
<b>Mean age, ±SD</b>	49.5 ± 9.9	46.5 ± 8.1	48.4 ± 9.0	<.0001	<.0001
<b>Age</b>				<.0001	0.039
≤35	114 (8.7)	21 (5.8)	137 (4.2)		
35-55	858 (65.5)	286 (79.2)	2,489 (76.0)		
≥56	338 (25.8)	54 (15.0)	651 (19.9)		
<b>PR status</b>				<.0001	<.0001
PR negative	1,262 (96.3)	103 (28.5)	182 (5.6)		
PR weakly positive	40 (3.0)	112 (31.0)	486 (14.8)		
PR positive	8 (0.7)	146 (40.4)	2,609 (79.6)		
<b>HER-2 status</b>				0.913	<.0001
Amplification	534 (40.8)	146 (40.4)	348 (10.6)		
Not amplification	776 (59.2)	215 (59.6)	2,929 (89.4)		
<b>Ki-67</b>				<.0001	<.0001
> 20.0 %	197 (15.0)	130 (36.0)	2,013 (61.4)		
≤ 20.0 %	1,113 (85.0)	<b>231 (64.0)</b>	<b>1,264 (38.6)</b>		
<b>Nuclear grade</b>				<.0001	<.0001
Low	18 (1.4)	30 (8.3)	738 (22.5)		
Intermediate	245 (18.7)	123 (34.1)	1,826 (55.7)		
High	1,047 (79.9)	<b>208 (57.6)</b>	<b>713 (21.8)</b>		
<b>LVI</b>				0.065	0.303
Yes	386 (29.5)	125 (34.6)	1,047 (32.0)		
No	916 (69.9)	235 (65.1)	2,220 (67.7)		
<b>Pathologic stage</b>				0.721	0.319
Stage I	526 (40.2)	153 (42.4)	1,519 (46.4)		
Stage II	610 (46.6)	160 (44.3)	1,327 (40.5)		
Stage III	174 (13.3)	48 (13.3)	431 (13.2)		

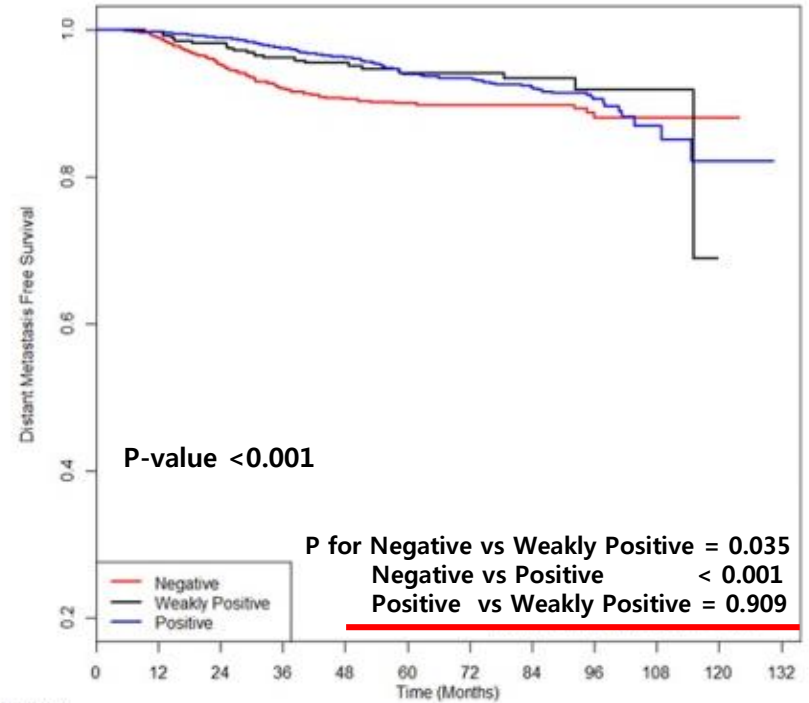
# Treatment characteristics

	ER-negative, n (%)	Weakly ER-positive, n (%)	Strongly ER-positive, n (%)	<i>p</i> -value	
	Group I, n = 1,310 (26.5)	Group II, n = 361 (7.3)	Group III, n = 3,277 (66.2)	Group I vs . II	Group II vs III
<b>Breast Surgery</b>				0.028	<0.0001
BCS	876 (66.9)	219 (60.7)	2,358 (72.0)		
TM	434 (33.1)	142 (39.3)	919 (28.0)		
<b>Axillary Surgery</b>				0.011	0.049
SLNB	627 (47.9)	186 (51.5)	1,697 (51.8)		
ALND	611 (46.6)	143 (39.6)	1,393 (42.5)		
No operation	72 (5.5)	32 (8.9)	187 (5.7)		
<b>Anti-hormonal therapy</b>				<.0001	<.0001
Yes	11 (0.8)	350 (97.0)	3,240 (98.9)		
No	1201 (91.7)	7 (1.9)	16 (1.3)		
Unknown	98 (7.5)	4 (1.1)	21 (0.6)		
<b>Chemotherapy</b>				<.0001	<.0001
Yes	<b>1,105 (84.4)</b>	<b>284 (78.7)</b>	<b>2,257 (68.9)</b>		
No	197 (15.0)	75 (20.8)	1,013 (30.9)		
Unknown	8 (0.6)	2 (0.6)	7 (0.2)		
<b>Radiotherapy</b>				<.0001	<.0001
Yes	<b>969 (74.0)</b>	<b>249 (69.0)</b>	<b>2,581 (78.8)</b>		
No	331 (25.3)	109 (30.2)	681 (20.8)		
Unknown	10 (0.8)	3 (0.8)	15 (0.5)		

# DFS/DMFS and ER expression



No. At Risk	0	12	24	36	48	60	72	84	96	108	120
Negative	1310	1274	1086	861	665	431	348	270	110	36	2
Weakly Positive	361	357	325	272	240	177	136	106	45	7	0
Positive	3277	3256	2956	2421	1860	1126	893	708	252	46	8

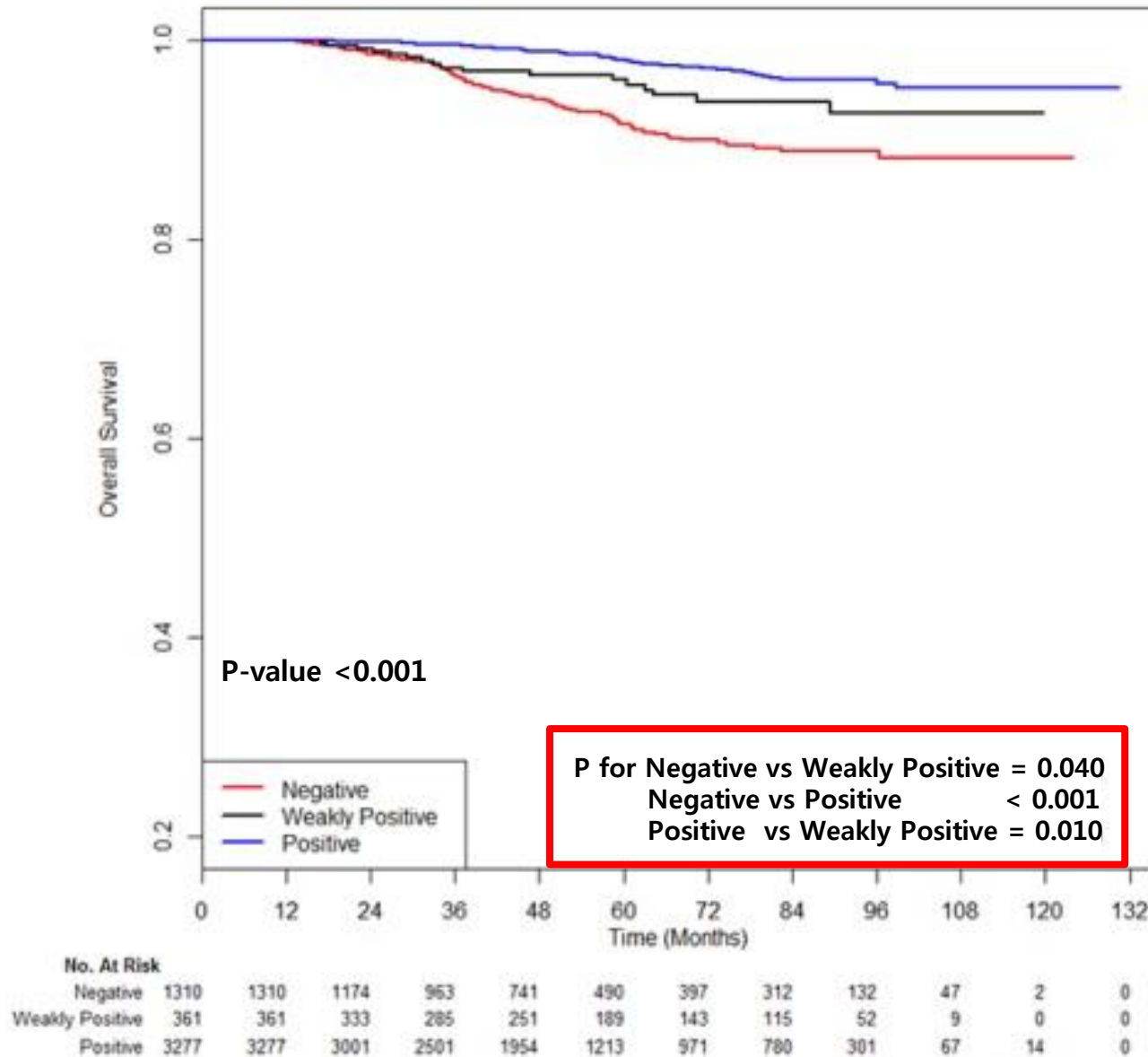


No. At Risk	0	12	24	36	48	60	72	84	96	108	120	132
Negative	1310	1296	1130	906	711	471	384	306	128	44	2	0
Weakly Positive	361	360	329	278	245	181	140	111	49	9	0	0
Positive	3277	3267	2967	2445	1888	1153	919	730	267	51	9	0



# OS and ER expression

Median follow-up: 57.8 (12.0-136.4) months



# Univariate analysis for OS

	HR for OS (95% CI)	p-value
<b>ER expression</b>		<.0001
ER-negative (Group I)	3.588 (2.612, 4.930)	<.0001
Weakly ER-positive (Group II)	2.051 (1.202, 3.500)	<.0001
Strongly ER-positive (Group III) (ref)		
<b>Pathologic stage</b>		<.0001
Stage I		
Stage II	2.407 (1.584, 3.658)	<.0001
Stage III	7.012 (4.584, 10.724)	<.0001
<b>Nuclear grade</b>		<.0001
Low		
Intermediate	3.345 (1.438, 7.780)	0.005
High	7.372 (3.244, 16.751)	<.0001
<b>Lymphovascular invasion</b>		
Yes	2.982 (2.207, 4.030)	<.0001
No (ref)		
<b>Ki-67</b>		
≤ 20.0 %	0.333 (0.233, 0.475)	<.0001
> 20.0 % (ref)		
<b>PR expression</b>		
Positive	0.299 (0.221, 0.404)	<.0001
Negative (ref)		
<b>HER-2 status</b>		
Amplification	1.079 (0.753, 1.547)	0.677
Not amplification (ref)		

# Multivariate analysis for OS and ER expression

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	Number (%)	Expire ,N (%)	HR (95% CI) <sup>a</sup>	HR (95% CI) <sup>b</sup>	HR (95% CI) <sup>c</sup>
<b>ER expression</b>					
ER-negative	1,310 (26.5)	92 (7.0)	3.617 (2.630, 4.973)	2.943 (2.019, 4.291)	<b>1.868</b> (1.002, 3.481)
Weakly ER positive	361 (7.3)	17 (4.7)	2.035 (1.192, 3,472)	1.757 (1.015, 3.044)	<b>1.773</b> (1.002, 3.137)
Strongly ER-positive (ref)	3,277 (66.2)	65 (2.0)			

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ER, Estrogen receptor; HR, Hazard ratio; CI, confidence interval; ref, reference

a adjusted for Stage

b adjusted for Stage, nuclear grade, lymphovascular invasion, Ki-67

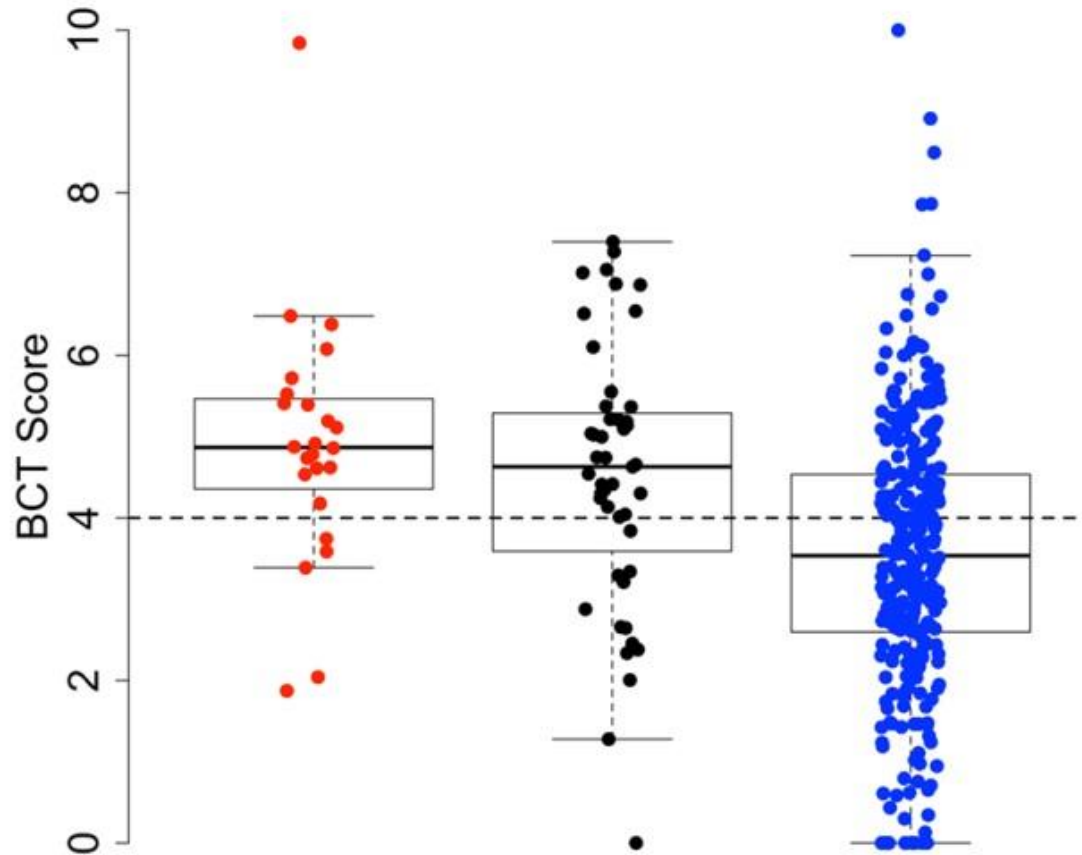
c adjusted for Stage, nuclear grade, lymphovascular invasion, Ki-67, progesterone receptor, HER-2 status

# Descriptive characteristics of patients with distant metastases

	ER-negative, n=117 n (%)	Weakly ER-positive, n=21 n (%)	Strongly ER-positive, n=170 n (%)
<b>Metastasis site</b>			
Bone	7 (6.0)	2 (9.5)	56 (32.9)
Lung/Pleura	21 (17.9)	6 (28.6)	34 (20.0)
Liver	6 (5.1)	3 (14.3)	12 (7.1)
Brain	8 (6.8)	1 (4.8)	1 (0.6)
Lymph node	4 (3.4)	0 (0)	7 (4.1)
Other sites or combination	71 (60.7)	9 (42.9)	60 (35.3)
<b>DMFI</b>			
≥ 3 years	32 (27.4)	10 (47.6)	110 (64.7)
< 3 years	85 (70.1)	11 (52.3)	60 (35.3)
<b>Stage</b>			
Stage I	19 (16.2)	6 (28.6)	18 (10.6)
Stage II	59 (50.4)	8 (38.1)	77 (45.2)
Stage III	39 (33.3)	7 (33.3)	75 (44.1)
<b>Ki-67</b>			
< 20%	19 (16.2)	5 (23.8)	62 (36.5)
≥ 20%	98 (84.8)	16 (76.2)	108 (63.5)

DMFI, distant metastasis free interval

# BCS score according to ER expression



	ER-negative	Weakly ER-positive	Strongly ER-positive	Total	P-value
n (%)	24 (6.2)	47 (12.2)	315 (81.6)	386	
BCT Score (median)	4.87	4.63	3.54		<b>&lt;0.0001<sup>1)</sup></b>
BCT risk group					<b>&lt;0.0001<sup>1)</sup></b>
Low, n (%)	5 (20.8)	13 (27.7)	192 (61.0)	210 (54.4)	
High, n (%)	19 (79.2)	<b>34 (72.3)</b>	<b>123 (39.0)</b>	176 (45.6)	

<sup>1)</sup>ANOVA test; <sup>2)</sup>Chi-square test



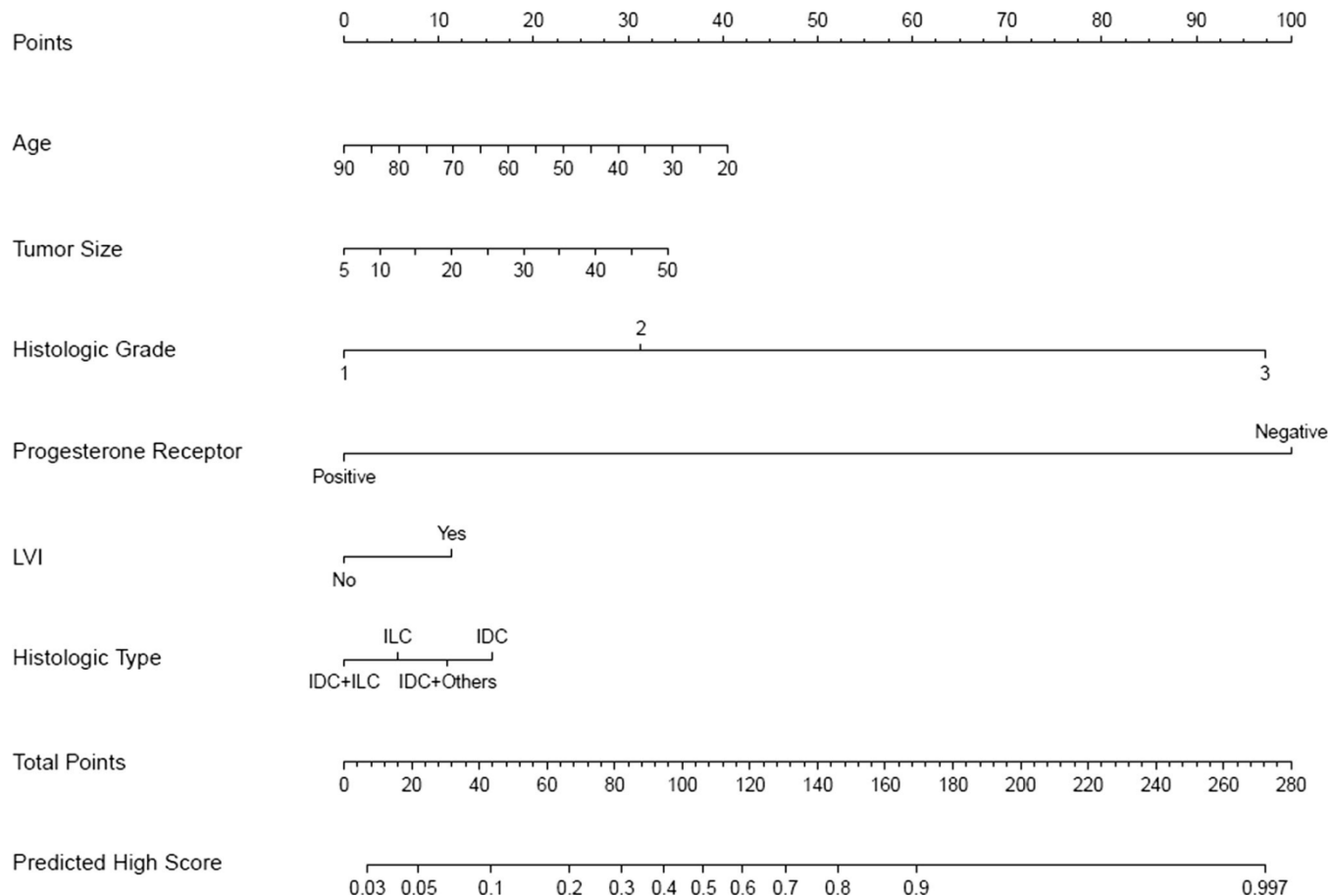
# Risks of underestimation

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- Almost ER positive tumors are down-staged in 8<sup>th</sup> AJCC staging
  - Nomogram to predict Oncotype Dx breast cancer recurrence score
  - ER positive tumors with 1% - 9% by IHC have possible misclassification
- Patients with ER weakly positive breast cancer could be underestimated

# Nomogram to predict ODX RS

- 27,719 Oncotype DX (ODX) Recurrence Score (RS)
  - Female, **ER+**, HER2-, N0, invasive, 6–50 mm tumor size
  - National Cancer Data Base, USA (2010-2012)
- 12,763 ODX-tested patients in 2013 (external validation)





# Misclassification

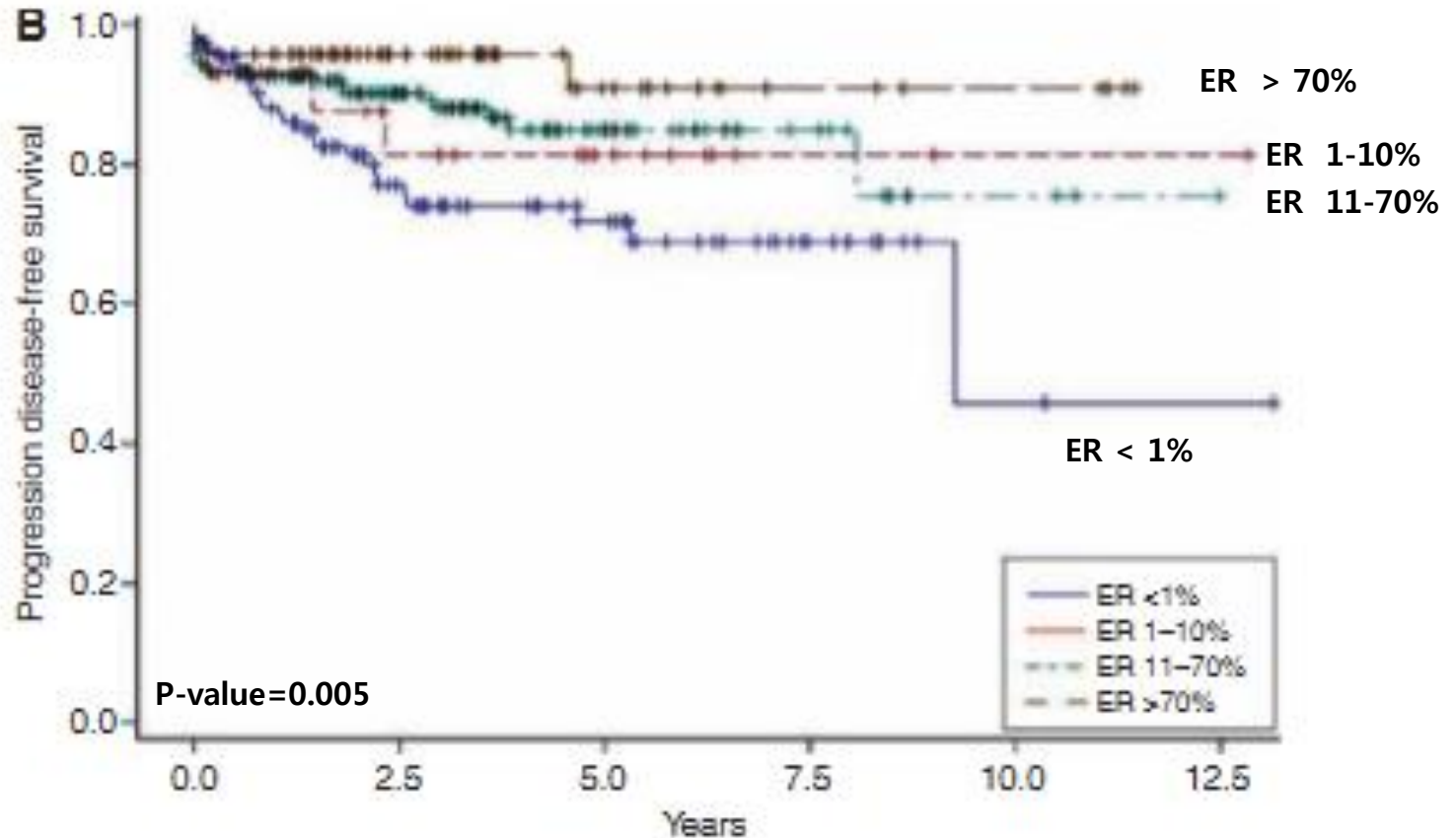
- ER positive tumors with 1% - 9% by IHC may arise from testing artifact (?)

ER IHC		ESR1 m RNA Expression	
		Positive	negative
IHC Level(%)	No. of pts.	No (%)	No (%)
0	183	16 (8.7)	167 (91.3)
1-9	25	6 (24.0)	19 (76.0)
10	6	4 (66.7)	4 (66.7)
>10	251	232 (92.4)	19 (7.6)

IHC		Molecular subtype by PAM 50				
IHC level (%)	No. of Patients	Luminal A	Luminal B	HER-2	Basal	Normal
0	183	2	1	51	111	18
1-9	25	0	2 (8.0%)	8 (32.0%)	12 (48.0%)	3
10	6	2	1	1	1	1
>10	251	120	61	38	16	16

# ER expression and prognosis

- 1,700 invasive breast cancer, 2000-2011, Rochester Medical Center
- As the ER expression is lower
  - More unfavorable pathological features such as NG, PR
  - Worse survival in DFS.



# Conclusion

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- Weakly ER-positive group
  - Worse OS, Higher BCT score and much more high risk group than strongly ER-positive group
- Weakly ER-positive group has significantly higher HR for OS than Strong ER-positive group.
- Only ER “positive” is not enough to predict the prognosis of breast cancer.
  - We should not underestimate in patients with Weakly ER-positive.

# Limitations

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- No central testing for IHC
  - Possibility subjectivity in interpretation
- Grouped by Allred score (Total score=Intensity score+ Proportion score)
  - Possibility of misclassification
- Retrospective study, treatment was not assigned in a randomized method
  - Possibility of affect to prognosis
- Follow-up duration was 57.8 months
  - Relatively short

**Thank you for your attention**

