

# A Radiosensitivity Gene Signature and PD-L1 Predict Clinical Outcome of Patients with Invasive Breast Carcinoma in The Cancer Genome Atlas (TCGA) Dataset

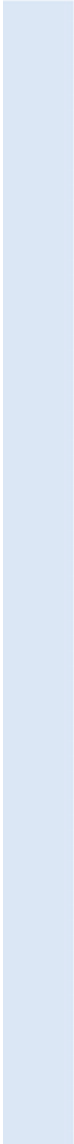
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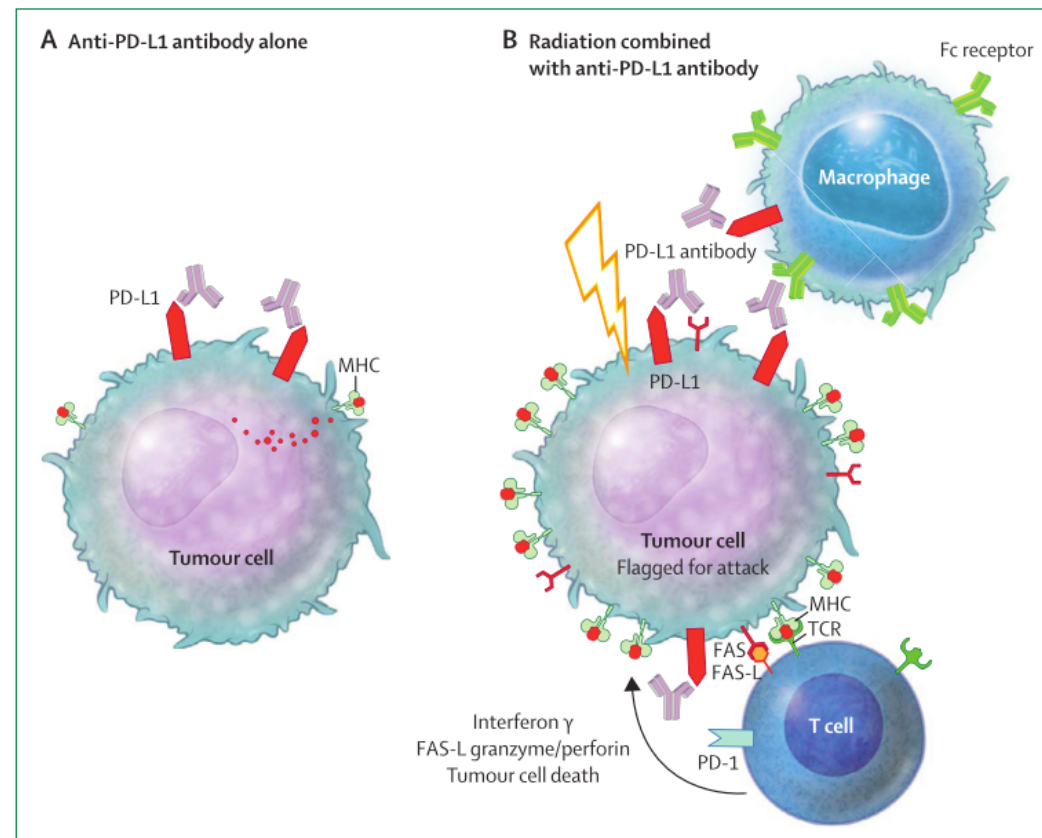


# Introduction (1)

- 
- **Radiation therapy causes**
    - Immuno-suppressive effect
      - Damages to lethal DNA of tumor and normal cells
    - Immune-stimulating effect
      - Enhancing dendritic cells to antigen presentation
      - Promoting recruitment CTLs into tumor microenvironment
  - **Tumor**
    - Immune evading mechanisms
      - PD-L1 transmembrane ligand → T-cell apoptosis

# Introduction (2)

- **Anti-PD-1/PD-L1 Blockade**
  - Clinical efficacy in melanoma, NSCLC, RCC
  - Response rate 20–30 %

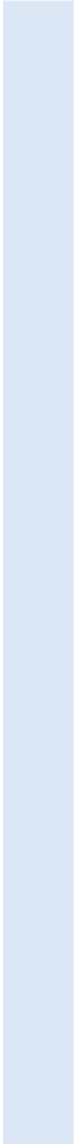


# Introduction (3)

- Prediction of response to RT is important
- In the era of precision medicine
  - *Radiosensitivity Index* (Eschrich et al, 2009)
  - ***“31-gene signature”*** (Kim et al, 2012)
    - Based on SF2 & microarray data of NCI-60 cell lines
    - Not yet validated in clinical data for breast cancer



# Purpose

- 
- **To validate 31-gene signature in TCGA breast cancer dataset externally**
  - **To analyze relationship between PD-L1 and radiosensitivity:  
Radiosensitive (RS) vs. Radioresistant (RR)**
  - **To propose a group potentially benefited from combination with radiotherapy and anti-PD-1/PD-L1 inhibitor**

# Methods (1)

## TCGA Data and Study Population

- **TCGA breast invasive carcinoma (BRCA) data set**
  - Level 3 mRNAseq using the UCSC Cancer Genomics Browser
  - Exclude metastatic (n=7), normal tissue samples (n=113)
  - Exclude Male (n=11) or unidentified gender samples (n=19)
  - Exclude patients with no survival data (N=20)
- **Final N=1,045 patients**

# Methods (1)

## Consensus Clustering & PD-L1 Assessment

- **The 31-gene signature\***

*ACTN1, ANXA2, ANXA5, ARHGDIB, CAPNS1, CBR1, CCND1, CD63, CORO1A, CXCR4, DAG1, EMP2, HCLS1, HTRA1, ITGB5, LAPTM5, LRMP, MYB, PFN2, PIR, PKM2, PTMS, PTPRC, PTPRCAP, PYGB, RAB13, RALB, SCR1, SQSTM1, TWF1, WAS*

\*related signaling pathways: **Integrin, VEGF, MAPK, p53, JAK-STAT, Wnt**

- **Consensus Clustering**

- $K=2$ , Radiosensitive (RS) vs. Radioresistant (RR)

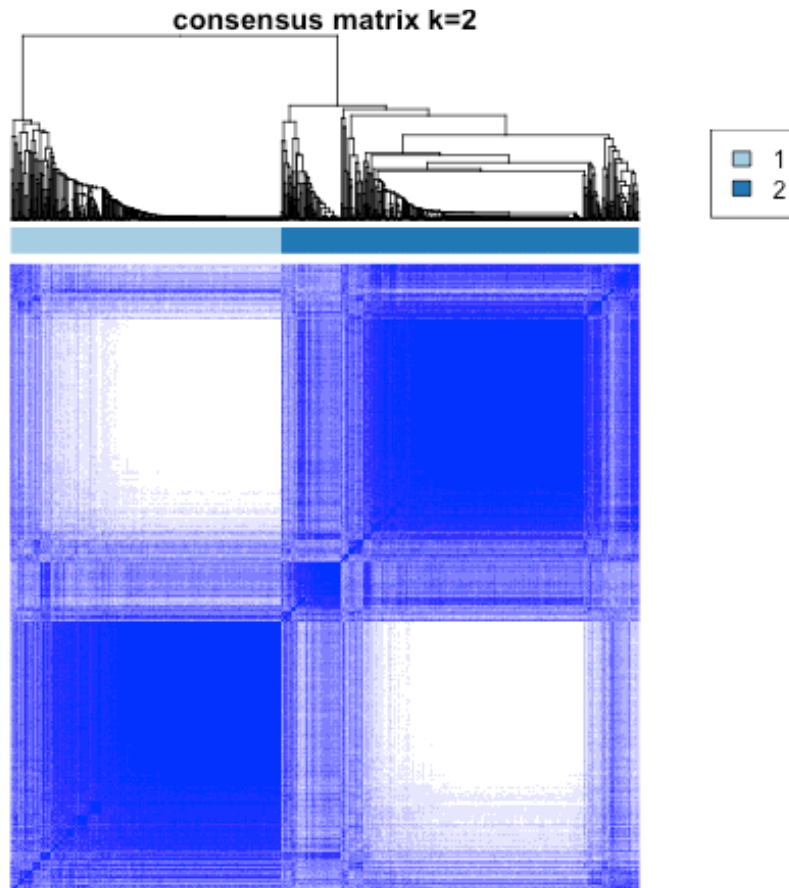
- **PD-L1 is translated by *CD274* gene**

- Cutoff = median RSEM value

- PD-L1-high vs. PD-L1-low

# Results (1)

## Consensus Clustering ( $k=2$ )



Gene Expression Profile from  
The 31-gene signature



Consensus Clustering  
(Unsupervised Machine Learning)



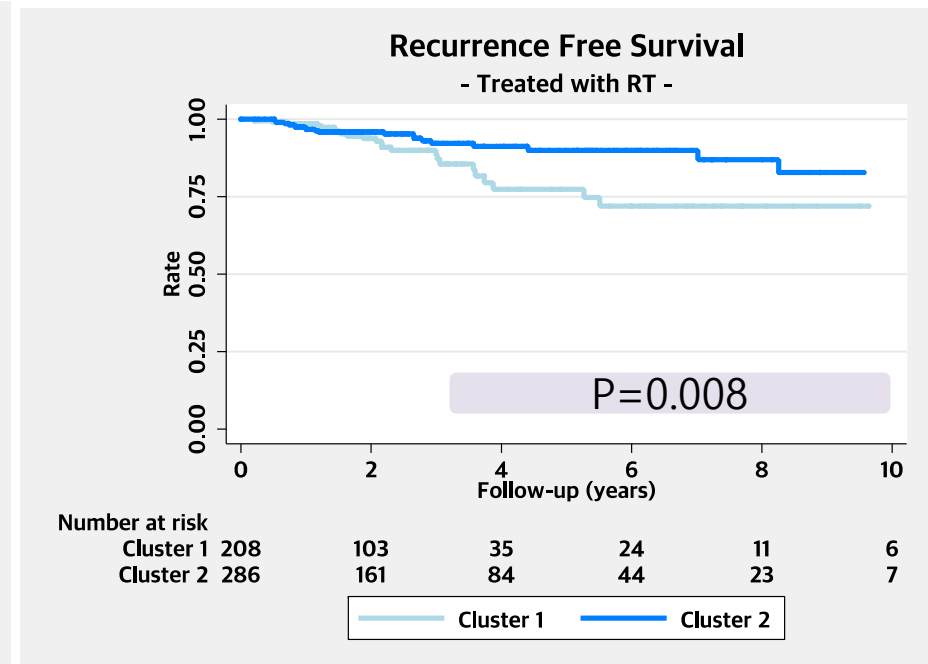
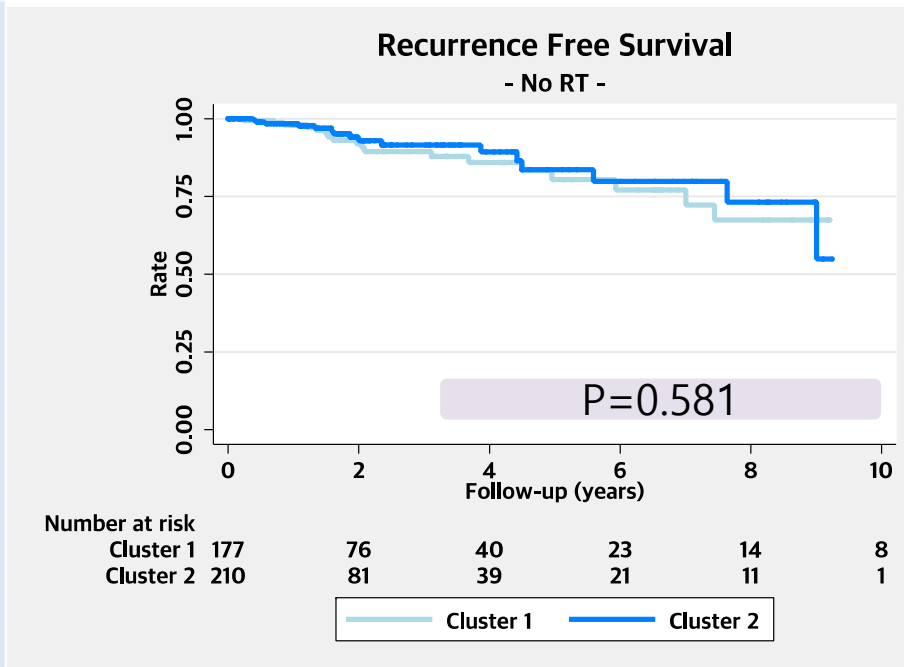
Cluster 1      N = 447 (43%)

Cluster 2      N = 598 (57%)



# Results (2)

## Definition of RS vs. RR



Cluster 1 N = 447 (43%)

Cluster 2 N = 598 (57%)



Radioresistant (RR)

Radiosensitive (RS)

# Results (3)

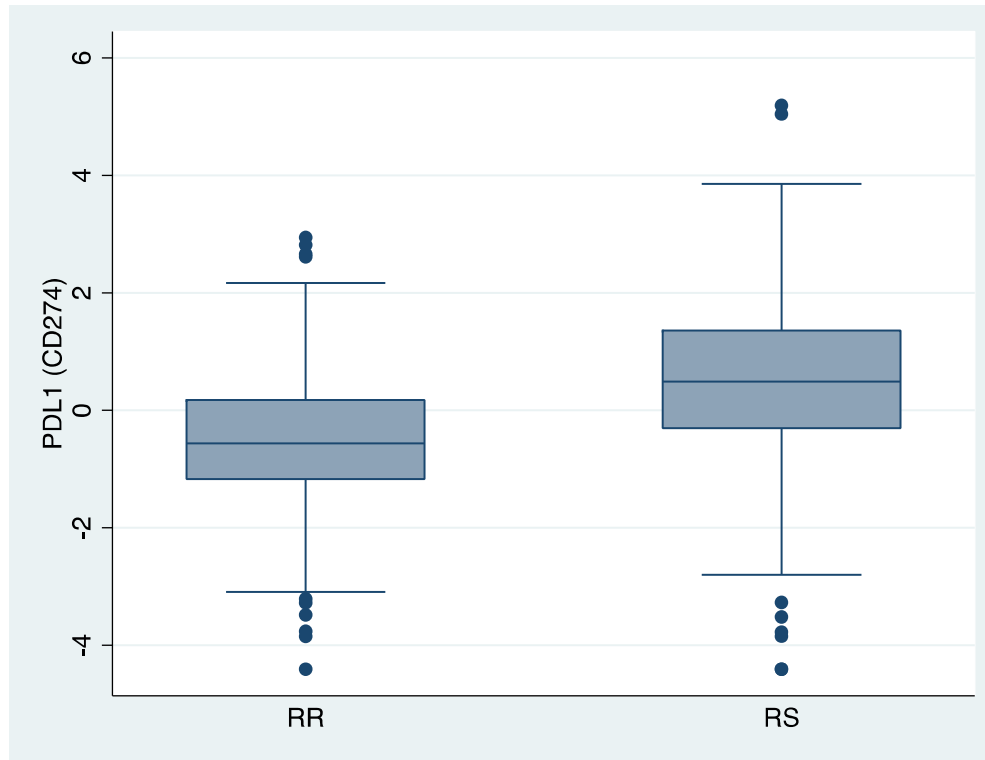
## Patient & Tumor Characteristics by RR vs. RS

	RR		RS		Total		P-value
	N	%	N	%	N	%	
<b>Age (years)</b>							0.910
≤ 35	14	3.1	18	3	32	3.1	
> 35	433	96.9	580	97	1,013	96.9	
<b>Menopausal status</b>							0.277
Pre	83	18.6	141	23.6	224	21.4	
Peri	16	3.6	21	3.5	37	3.5	
Post	302	67.6	380	63.5	682	65.3	
Unknown	46	10.3	56	9.4	102	9.8	
<b>Stage</b>							0.212
I	83	18.6	98	16.4	181	17.3	
II	242	54.1	348	58.2	590	56.5	
III	99	22.1	136	22.7	235	22.5	
IV	10	2.2	8	1.3	18	1.7	
Unknown	13	2.9	8	1.3	21	2	
<b>Histology</b>							0.003
IDC	339	75.8	416	69.6	755	72.2	
ILC	60	13.4	129	21.6	189	18.1	
Unknown/Other	48	10.7	53	8.9	101	9.7	
<b>Surgery type</b>							0.389
Lumpectomy	110	24.6	156	26.1	266	25.5	
Mastectomy	204	45.6	287	48	491	47	
Other/Not performed	133	29.8	155	25.9	288	27.6	
<b>RT</b>							0.086
No	228	51	273	45.7	501	47.9	
Yes	219	49	325	54.3	544	52.1	
<b>RT technique</b>							0.110
Others*	32	7.2	36	6	68	6.5	
EBRT	187	41.8	289	48.3	476	45.6	
(RT not performed)	228	51	273	45.7	501	47.9	
<b>RT dose (cGy)</b>							0.211
≤6040	114	25.5	185	30.9	299	28.6	
>6040	50	11.2	72	12	122	11.7	
Unknown	55	12.3	68	11.4	123	11.8	
(RT not performed)	228	51	273	45.7	501	47.9	
<b>Adjuvant CTx alone</b>							<0.001
No	402	89.9	396	66.2	798	76.4	
Yes	45	10.1	202	33.8	247	23.6	
<b>Adjuvant CTx + Hx.</b>							0.303
No	306	68.5	427	71.4	733	70.1	
Yes	141	31.5	171	28.6	312	29.9	
<b>PAM 50</b>							<0.001
Basal	14	3.1	131	21.9	145	13.9	
Luminal A	204	45.6	178	29.8	382	36.6	
Luminal B	127	28.4	79	13.2	206	19.7	
Her2	32	7.2	44	7.4	76	7.3	
Unknown/Normal-like	70	15.7	166	27.8	236	22.6	
<b>PD-L1</b>							<0.001
Low	316	70.7	210	35.1	526	50.3	
High	131	29.3	388	64.9	519	49.7	
<b>Total</b>	447		598		1,045		

Abbreviations: RR, radioresistant group; RS, radiosensitive group; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; PD-L1, programmed cell death-ligand 1; RT, radiotherapy; EBRT, external beam radiation therapy; CTx., chemotherapy; Hx., hormone therapy. \*Others RT technique included radioisotope (N=1) and others not specified (N=67).

# Results (4)

## Relation with PD-L1 and RR vs. RS



Mann-Whitney test: p-value < 0.001

# Result (5)

## Factors Associated with "PD-L1 High" status

		Univariate			Multivariate		
		OR	95% CI	P-value	OR	95% CI	P-value
Age	>35 yrs (vs. ≤35 yrs)	1.06	( 0.53 - 2.13 )	0.864			
Menopausal status	Pre (vs. Peri)	1.02	( 0.51 - 2.04 )	0.957			
	pre (vs. post)	0.97	( 0.72 - 1.31 )	0.862			
Stage	Incremental	0.92	( 0.77 - 1.09 )	0.329			
Histology	ILC (vs. IDC)	0.95	( 0.69 - 1.30 )	0.738			
PAM50	Luminal A (vs. Basal-like)	0.70	( 0.48 - 1.03 )	0.072	1.58	( 1.02 - 2.44 )	0.040
	Luminal B (vs. Basal-like)	0.60	( 0.39 - 0.92 )	0.018	1.50	( 0.92 - 2.44 )	0.100
	HER2 (vs. Basal-like)	1.39	( 0.79 - 2.47 )	0.257	2.77	( 1.47 - 5.20 )	0.002
Radiosensitivity	RS (vs. RR)	4.44	( 3.42 - 5.77 )	<0.001	5.81	( 4.19 - 8.05 )	<0.001

Abbreviations: OR, odds ratio; RR, radioresistant group; RS, radiosensitive group; yrs, years; IDC, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; HR, hazard ratio; CI, confidence interval

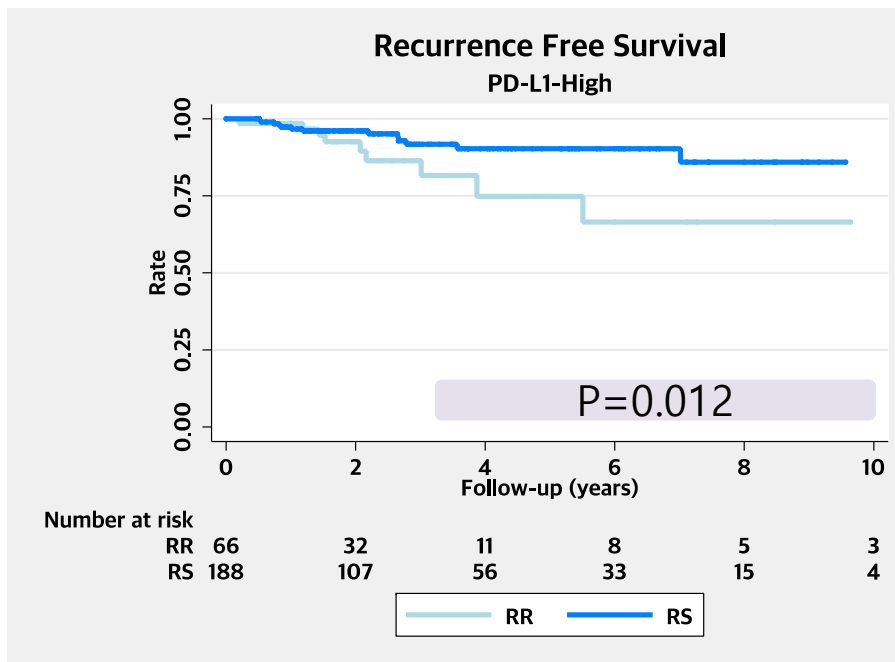
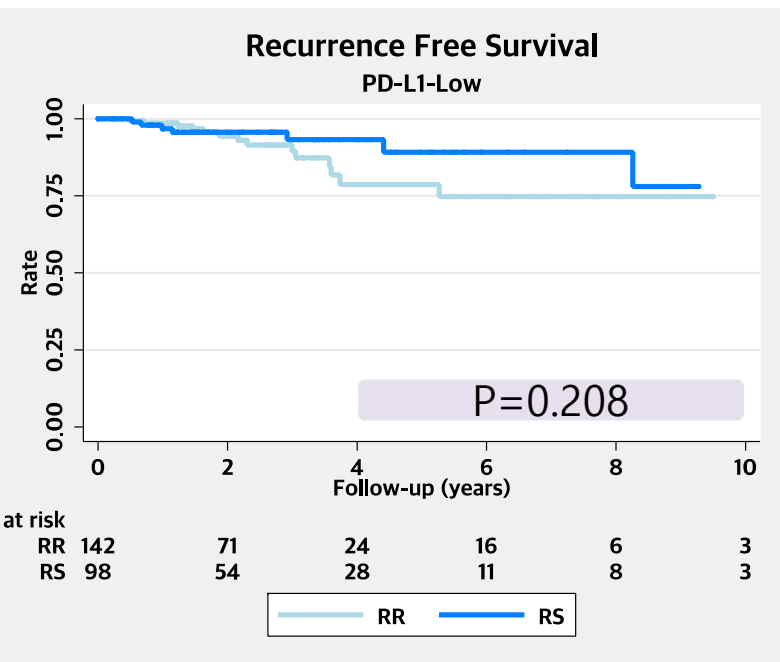
# Result (6)

## Univariate Analysis for RT-treated patients (N = 544, 52%)

	RFS						OS					
	PD-L1-Low			PD-L1-High			PD-L1-Low			PD-L1-High		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
<b>Age (years)</b>												
≤ 35	1.00			1.00			1.00			1.00		
> 35	0.37	0.05- 2.80	0.338	0.72	0.10- 5.39	0.750	0.31	0.04- 2.39	0.263	0.50	0.06- 3.83	0.503
<b>Stage</b>												
I to IV (inc.)	2.03	1.08- 3.81	0.028	1.84	1.02- 3.31	0.043	5.06	2.22- 11.52	0.000	3.88	1.87- 8.05	0.000
<b>Histology</b>												
IDC	1.00			1.00			1.00			1.00		
ILC	1.88	0.66- 5.35	0.236	0.89	0.32- 2.47	0.822	0.82	0.18- 3.66	0.791	2.08	0.78- 5.57	0.145
<b>PAM50</b>												
Basal-like	1.00			1.00			1.00			1.00		
Luminal A	2.92	0.35- 24.14	0.319	1.09	0.36- 3.27	0.881	1.06	0.20- 5.50	0.949	1.01	0.18- 5.66	0.989
Luminal B	5.18	0.60- 44.60	0.134	0.68	0.16- 2.86	0.594	3.13	0.61- 16.15	0.173	3.01	0.57- 15.78	0.192
HER2	8.26E-17	0.00- ∞	1.000	1.05	0.20- 5.49	0.956	3.46	0.29- 41.35	0.327	4.10	0.66- 25.51	0.130
<b>Surgery Type</b>												
Lumpectomy	1.00			1.00			1.00			1.00		
Mastectomy	1.42	0.56- 3.61	0.458	1.20	0.49- 2.95	0.692	1.54	0.54- 4.39	0.424	0.54	0.16- 1.87	0.331
<b>Margin status</b>												
Negative/Close	1.00			1.00			1.00			1.00		
Positive	4.89	1.97- 12.13	0.001	0.50	0.07- 3.71	0.494	2.14	0.61- 7.55	0.237	2.98	0.97- 9.21	0.057

# Result (7)

## Recurrence-Free Survival for RT-treated patients



# Result (8)

## Multivariate Analysis for RT-treated patients (*N* = 544, 52%)

		RFS			OS		
		HR	95% CI	<i>P</i> -value	HR	95% CI	<i>P</i> -value
<b>-L1 Low</b>							
Margin status	Positive (vs. Negative/Close)	4.01	1.43 - 11.28	0.008			
Stage	I to IV (Incremental)	1.65	0.91 - 2.99	0.096	5.06	2.22 - 11.52	0.000
<b>-L1 High</b>							
Radiosensitivity	RS (vs. RR)	0.37	0.16 - 0.87	0.022			
Stage	Incremental	1.87	1.04 - 3.38	0.038	3.88	1.87 - 8.05	0.000

Abbreviations: RFS, recurrence-free survival; OS, overall survival; RR, radioresistant group; RS, radiosensitive group; yrs, years; I, infiltrating ductal carcinoma; ILC, infiltrating lobular carcinoma; HR, hazard ratio; CI, confidence interval; NR, not reported; PD, programmed cell death-ligand 1

# Conclusion

**31-gene signature was validated externally in TCGA breast cancer dataset.**

**Radiosensitivity (RR vs. RS) & PD-L1 status were predictive for clinical outcome of adjuvant RT in patients with breast ca.**

**These could be used for selecting patients potentially get benefit from radiotherapy combined with anti-**



**Thank you for your attention**