

Supplementary Materials

Supplementary Table 1. Contingency table analysis to evaluate patient and tumor characteristics at the time of first and second RS testing in the study cohort.

Parameter			Second BC			* <i>p</i> value
Tumor size ¹			≤2 cm	> 2 cm	Total	
First BC	≤2 cm		35	5	40	0.096
	> 2 cm		13	4	17	
	Total		48	9	57	
Tumor grade ²			Grade 1/2	Grade 3	Total	
First BC	Grade 1/2		30	6	36	0.79
	Grade 3		8	2	10	
	Total		38	8	46	
Histology			IDC	Not IDC	Total	
First BC	IDC		44	6	50	0.75
	Not IDC		4	6	10	
	Total		48	12	60	
Nodal status ³			N0	N1mi/N1	Total	
First BC	N0		38	5	43	0.21
	N1mi/N1		11	3	14	
	Total		49	8	57	
Recurrence			0-25	26-100	Total	
Score category	0-25		33	18	51	0.0015
	26-100		3	6	9	
	Total		36	24	60	

BC breast cancer, IDC invasive ductal carcinoma.

**p* value was calculated using the McNemar's test.

¹ Tumor size information in either or both BC instances was not available for 3 patients.

² Grade information in either or both BC instances was not available for 14 patients.

³ Nodal status information in either or both BC instances was not available for 3 patients

Supplementary Table 2. Contingency table analysis to evaluate the association between clinical characteristics or adjuvant treatments and having a second RS result that is higher than the first.

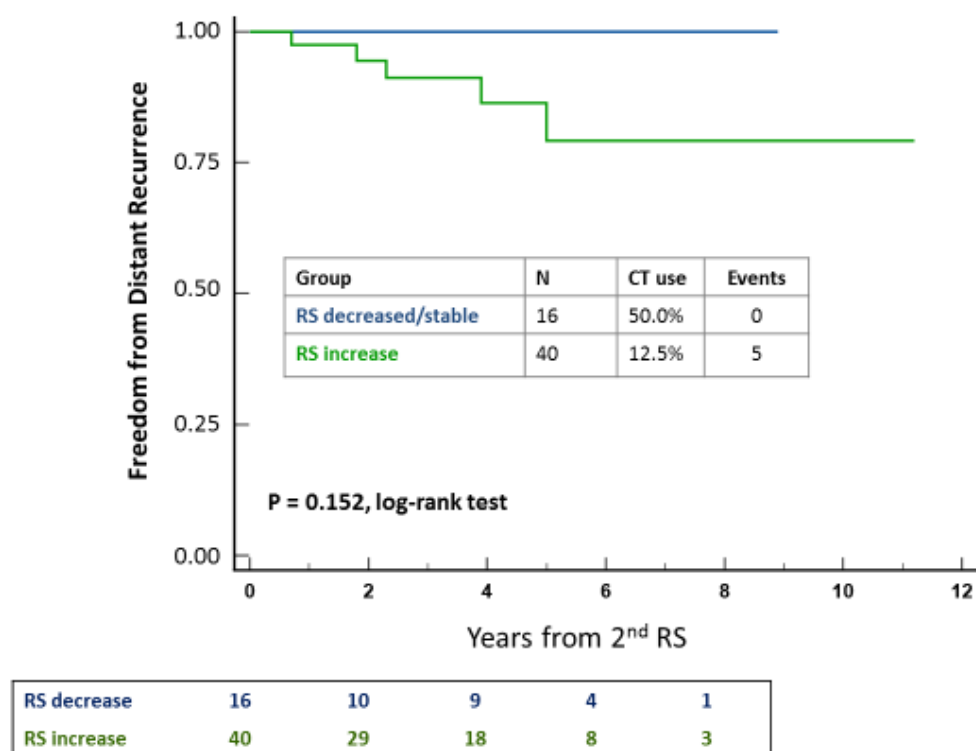
Parameter	Increase in the second RS result, <i>n</i>	No increase in the second RS result, <i>n</i> ¹	Total, <i>n</i>	* <i>p</i> value
Second BC location				
Ipsilateral	32	9	41	0.23
Contralateral	12	7	19	
Total	44	16	60	
Time between the first and second 21-gene testing				
≤5 years	22	6	28	0.40
<5 years	22	10	32	
Total	44	16	60	
Age at the first 21-gene testing				
≤50 years	16	6	32	0.94
<50 years	28	10	38	
Total	44	16	60	
Tumor size at the first 21-gene testing				
≤2 cm	28	14	42	0.11
<2 cm	16	2	18	
Total	44	16	60	
Grade at the first 21-gene testing				
1-2	27	14	41	0.25
3	9	1	10	
Total	36	15	51	
Grade at the first 21-gene testing				
1	6	3	9	1.0
2-3	30	12	42	
Total	36	15	51	
Nodal status at the first 21-gene testing				
Node negative	32	14	46	0.31
Node positive	12	2	14	
Total	44	16	60	
RS category of the first RS result				
0-25	39	12	51	0.23
26-100	5	4	9	
Total	44	16	60	
Surgery type				
Lumpectomy	31	10	41	0.29
Mastectomy	2	2	4	
Total	33	12	45	
Patient received RT after the first RS result				
Yes	36	10	46	0.053
No	6	6	12	
Total	42	16	58	
Patient received RT after the first RS result, to the breast where the second primary BC was eventually identified				
Yes	27	6	33	0.085
No	16	10	26	
Total	43	16	59	

Patient received ET after the first RS result				
Yes	35	13	48	1.0
No	7	3	10	
Total	42	16	58	
Patient received ET within 2 years of the first RS result				
Yes	21	6	27	0.52
No	11	5	16	
Total	32	11	43	
Patient received CT after the first RS result				
Yes	5	4	9	0.24
No	37	12	49	
Total	42	16	58	

CT chemotherapy, ET endocrine therapy, RS Recurrence Score, RT radiation therapy.

*p value was calculated using chi-square test or Fisher's exact test (when at least one subgroup included <5 patients).

¹In one case, the second RS was the same as the first, and in all others, the second RS was lower than the first.



Supplementary Figure 1. KM curves of freedom from distant recurrence for the study cohort by the relationship between the first and second RS (increased vs decreased/stable RS). The box under the graph presents the number of patients at risk at each time point. One-degree of freedom log-rank p values were calculated from all the data. *BC* breast cancer, *CT* chemotherapy, *RS* Recurrence Score.