

Table SI. Primer and probe sequences for the 21 genes in the 21-gene recurrence score assay.

Gene	Primer/probe name	Sequence (5'→3')
ACTB (Ref)	S0034/B-acti.f2	CAGCAGATGTGGATCAGCAAG
	S4730/B-acti.p2	AGGAGTATGACGAGTCCGGCCCC
	S0036/B-acti.r2	GCATTTGCGGTGGACGAT
BAG1	S1386/BAG1.f2	CGTTGTCAGCACTTGGAATACAA
	S1387/BAG1.r2	GTTCAACCTCTTCTGTGGACTGT
	S4731/BAG1.p2	CCCAATTAACATGACCCGGCAACCAT
Bcl2	S0043/Bcl2.f2	CAGATGGACCTAGTACCCACTGAGA
	S4732/Bcl2.p2	TTCCACGCCGAAGGACAGCGAT
	S0045/Bcl2.r2	CCTATGATTTAAGGGCATTITTTCC
CCNB1	S1720/CCNB1.f2	TTCAGGTTGTTGCAGGAGAC
	S1721/CCNB1.r2	CATCTTCTTGGGCACACAAT
	S4733/CCNB1.p2	TGTCTCCATTATTGATCGGTTTCATGCA
CD68	S0067/CD68.f2	TGGTCCCAGCCCTGTGT
	S4734/CD68.p2	CTCCAAGCCCAGATTTCAGATTTCGAGTCA
	S0069/CD68.r2	CTCCTCCACCCTGGGTTGT
CEGP1	S1494/CEGP1.f2	TGACAATCAGCACACCTGCAT
	S1495/CEGP1.r2	TGTGACTACAGCCGTGATCCTTA
	S4735/CEGP1.p2	CAGGCCCTCTTCCGAGCGGT
CTSL2	S4354/CTSL2.f1	TGTCTCACTGAGCGAGCAGAA
	S4355/CTSL2.r1	ACCATTGCAGCCCTGATTG
	S4356/CTSL2.p1	CTTGAGGACGCGAACAGTCCACCA
ER	S0115/EstR1.f1	CGTGGTGCCCCCTCTATGAC
	S4737/EstR1.p1	CTGGAGATGCTGGACGCCC
	S0117/EstR1.r1	GGCTAGTGGGCGCATGTAG
GAPD (Ref)	S4738/GAPDH.p1	CCGTTCTCAGCCTTGACGGTGC
	S0374/GAPDH.f1	ATCCACCCATGGCAAATTC
	S0375/GAPDH.r1	GATGGGATTTCCATTGATGACA
GRB7	S0130/GRB7.f2	CCATCTGCATCCATCTTGT
	S4726/GRB7.p2	CTCCCCACCCTTGAGAAGTGCCT
	S0132/GRB7.r2	GGCCACCAGGGTATTATCTG
GSTM1	S2026/GSTM1.r1	GGCCCAGCTTGAATTTTCA
	S2027/GSTM1.f1	AAGCTATGAGGAAAAGAAGTACACGAT
	S4739/GSTM1.p1	TCAGCCACTGGCTTCTGTGCATAATCAGGAG
GUSB (Ref)	S0139/GUS.f1	CCCACTCAGTAGCCAAGTCA
	S4740/GUS.p1	TCAAGTAAACGGGCTGTTTTCCAAACA
	S0141/GUS.r1	CACGCAGGTGGTATCAGTCT
HER2	S0142/HER2.f3	CGGTGTGAGAAGTGCAGCAA
	S4729/HER2.p3	CCAGACCATAGACACTCGGGCAC
	S0144/HER2.r3	CCTCTCGCAAGTGCTCCAT
Ki67	S4741/Ki-67.p2	CCACTTGTCGAACCACCGCTCGT
	S0436/Ki-67.f2	CGGACTTTGGGTGCGACTT
	S0437/Ki-67.r2	TTACAACTCTTCCACTGGGACGAT
MYBL2	S3270/MYBL2.f1	GCCGAGATCGCCAAGATG
	S3271/MYBL2.r1	CTTTTGATGGTAGAGTTCCAGTGATTC
	S4742/MYBL2.p1	CAGCATTGTCTGTCTCCCTGGCA
PR	S1336/PR.f6	GCATCAGGCTGTCATTATGG
	S1337/PR.r6	AGTAGTTGTGCTGCCCTTCC
	S4743/PR.p6	TGTCCTTACCTGTGGGAGCTGTAAGGTC
RPLP0 (Ref)	S0256/RPLP0.f2	CCATTCTATCATCAACGGGTACAA
	S4744/RPLP0.p2	TCTCCACAGACAAGGCCAGGACTCG
	S0258/RPLP0.r2	TCAGCAAGTGGGAAGGTGTAATC
STK15	S0794/STK15.f2	CATCTTCCAGGAGGACCACT
	S0795/STK15.r2	TCCGACCTTCAATCATTTCA
	S4745/STK15.p2	CTCTGTGGCACCCCTGGACTACCTG
STMY3	S2067/STMY3.f3	CCTGGAGGCTGCAACATACC
	S2068/STMY3.r3	TACAATGGCTTTGGAGGATAGCA

Table SI. Continued.

Gene	Primer/probe name	Sequence (5'→3')
SURV	S4746/STMY3.p3	ATCCTCCTGAAGCCCTTTTCGCAGC
	S0259/SURV.f2	TGTTTTGATTCCCGGGCTTA
	S4747/SURV.p2	TGCCTTCTTCCTCCCTCACTTCTCACCT
	S0261/SURV.r2	CAAAGCTGTCAGCTCTAGCAAAAG
TFRC (Ref)	S1352/TFRC.f3	GCCAACTGCTTTCATTTGTG
	S1353/TFRC.r3	ACTCAGGCCCATTTCTTTA
	S4748/TFRC.p3	AGGGATCTGAACCAATACAGAGCAGACA

Table SII. Clinicopathological characteristics according to chemotherapy usage in patients with a 21-gene recurrence score of 18-25.

Variable	Total, n (%) (n=374)	Chemo, n (%) (n=115)	Non-chemo, n (%) (n=259)	P-value
Age (years)				0.002
≤50	147 (39.3)	59 (40.1)	88 (59.9)	
>50	227 (60.7)	56 (24.7)	171 (75.3)	
Menstrual status				<0.001
Premenopausal	157 (42.0)	66 (42.0)	91 (58.0)	
Postmenopausal	217 (58.0)	49 (22.6)	168 (77.4)	
Comorbidity				0.003
Yes	157 (42.0)	35 (22.3)	122 (77.7)	
No	217 (58.0)	80 (36.9)	137 (63.1)	
LVI				0.001
No	356 (95.2)	103 (28.9)	253 (71.1)	
Yes	18 (4.8)	12 (66.7)	6 (33.3)	
Tumor size (cm)				0.015
≤2	281 (75.1)	77 (27.4)	204 (72.6)	
>2	93 (24.9)	38 (40.9)	55 (59.1)	
Histological type				0.001
IDC	301 (80.5)	104 (34.6)	197 (65.4)	
Non-IDC	73 (19.5)	11 (15.1)	62 (84.9)	
Tumor grade				<0.001
I/II	268 (71.7)	76 (28.4)	192 (71.6)	
III	40 (10.7)	29 (72.5)	11 (27.5)	
Unknown	66 (17.6)	10 (15.2)	56 (84.8)	
ER status (%)				0.483
<50	7 (1.9)	3 (42.9)	4 (57.1)	
≥50	367 (98.1)	112 (30.5)	255 (69.5)	
PR status (%)				0.008
<20	82 (21.9)	35 (42.7)	47 (57.3)	
≥20	292 (78.1)	80 (27.4)	212 (72.6)	
Ki-67 index (%)				<0.001
<14	209 (55.9)	29 (13.9)	180 (86.1)	
≥14	165 (44.1)	86 (52.1)	79 (47.9)	
Luminal subtype				<0.001
Luminal A-like	153 (40.9)	15 (9.8)	138 (90.2)	
Luminal B-like	221 (59.1)	100 (45.2)	121 (54.8)	
Surgery type				0.803
BCS	172 (46.0)	54 (31.4)	118 (68.6)	
Mastectomy	202 (54.0)	61 (30.2)	141 (69.8)	

Data were analyzed using Fisher's exact test or χ^2 test. Chemo, chemotherapy; LVI, lymphovascular invasion; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor; BCS, breast conserving surgery.

Table SIII. Multivariate analysis of factors associated with chemotherapy in patients with a 21-gene recurrence score of 18-25.

Variable	OR	95% CI	P-value
Age (≤ 50 years vs. > 50 years)	1.18	0.40-3.74	0.768
Menstrual status (premenopausal vs. postmenopausal)	2.53	1.38-4.65	0.003
Comorbidity (yes vs. no)	0.56	0.30-1.05	0.071
LVI (yes vs. no)	3.30	0.94-11.56	0.062
Tumor size (> 2 cm vs. ≤ 2 cm)	2.21	1.20-4.09	0.011
Histological type (non-IDC vs. IDC)	1.20	0.15-9.40	0.860
Tumor grade			< 0.001
III vs. I/II	2.75	1.18-6.44	0.019
Unknown vs. I/II	0.29	0.12-0.71	0.007
PR status ($< 20\%$ vs. $\geq 20\%$)	4.36	2.24-8.48	< 0.001
Ki-67 index ($\geq 14\%$ vs. $< 14\%$)	6.90	3.83-12.46	< 0.001
Luminal subtype (luminal B-like vs. luminal A-like)	1.17	0.40-3.47	0.572

OR, odds ratio; CI, confidence interval; LVI, lymphovascular invasion; IDC, invasive ductal carcinoma; PR, progesterone receptor.

Table SIV. Clinicopathological characteristics according to chemotherapy usage in patients with a 21-gene recurrence score ≥ 31 .

Variable	Total, n (%) (n=209)	Chemo, n (%) (n=186)	Non-chemo, n (%) (n=23)	P-value
Age (years)				0.009
≤50	67 (32.1)	65 (97.0)	2 (3.0)	
>50	142 (67.9)	121 (85.2)	21 (14.8)	
Menstrual status				0.009
Premenopausal	67 (32.1)	65 (97.0)	2 (3.0)	
Postmenopausal	142 (67.9)	121 (85.2)	21 (14.8)	
Comorbidity				0.001
Yes	79 (37.8)	63 (79.7)	16 (20.3)	
No	130 (62.2)	123 (94.6)	7 (5.4)	
Surgery type				0.168
BCS	108 (51.7)	93 (86.1)	15 (13.9)	
Mastectomy	101 (48.3)	93 (92.1)	8 (7.9)	
Tumor size (cm)				0.984
≤2	145 (69.4)	129 (89.0)	16 (11.0)	
>2	64 (30.6)	57 (89.1)	7 (10.9)	
Histological type				0.001
IDC	182 (87.1)	167 (91.8)	15 (8.2)	
Non-IDC	27 (12.9)	19 (70.4)	8 (29.6)	
Tumor grade				0.060
I/II	121 (57.9)	109 (90.1)	12 (9.9)	
III	64 (30.6)	59 (92.2)	5 (7.8)	
Unknown	24 (11.5)	18 (75.0)	6 (25.0)	
LVI				0.606
Yes	10 (4.8)	10 (100.0)	0 (0.0)	
No	199 (95.2)	176 (88.4)	23 (11.6)	
ER status (%)				1.000
<50	16 (7.7)	15 (93.8)	1 (6.2)	
≥50	193 (92.3)	171 (88.6)	22 (11.4)	
PR status (%)				0.335
<20	83 (39.7)	76 (91.6)	7 (8.4)	
≥20	126 (60.3)	110 (87.3)	16 (12.7)	
Ki-67 index (%)				0.156
<14	64 (30.6)	54 (84.4)	10 (15.6)	
≥14	145 (69.4)	132 (91.0)	13 (9.0)	
Luminal subtype				0.701
Luminal A-like	19 (9.1)	18 (94.7)	1 (5.3)	
Luminal B-like	190 (90.9)	168 (88.4)	22 (11.6)	

Data were analyzed using Fisher's exact test or χ^2 test. Chemo, chemotherapy; BCS, breast conserving surgery; IDC, invasive ductal carcinoma; LVI, lymphovascular invasion; ER, estrogen receptor; PR, progesterone receptor.

Table SV. Multivariate analysis of factors associated with chemotherapy in patients with a 21-gene recurrence score ≥ 31 .

Variable	OR	95% CI	P-value
Age (≤ 50 years vs. > 50 years)	4.30	0.90-20.54	0.068
Comorbidity (yes vs. no)	0.30	0.11-0.81	0.018
Histologic type (non-IDC vs. IDC)	0.18	0.06-0.53	0.002

OR, odds ratio; CI, confidence interval; IDC, invasive ductal carcinoma.

Table SVI. Multivariate analysis of factors associated with chemotherapy usage in patients with a 21-gene recurrence score of 26-30.

Variable	OR	95% CI	P-value
Age (≤ 50 years vs. > 50 years)	5.75	2.08-15.90	0.001
Menstrual status (premenopausal vs. postmenopausal)	1.56	0.30-8.20	0.600
Comorbidity (yes vs. no)	0.74	0.34-1.61	0.451
Tumor size (> 2 cm vs. ≤ 2 cm)	2.17	0.92-5.11	0.077
Histological type (non-IDC vs. IDC)	0.39	0.05-2.88	0.358
Tumor grade			0.247
III vs. I/II	3.71	0.74-18.66	0.112
Unknown vs. I/II	0.63	0.08-4.67	0.647
Ki-67 index ($\geq 14\%$ vs. $< 14\%$)	1.06	0.43-2.61	0.906
Luminal subtype (luminal B-like vs. luminal A-like)	7.75	3.28-18.32	< 0.001

OR, odds ratio; CI, confidence interval; IDC, invasive ductal carcinoma.

Table SVII. Clinicopathological characteristics of patients not receiving chemotherapy after multidisciplinary team recommendation.

Patient	RS	Age (years)	Comorbidity	Surgery type	Histological subtype	Tumor stage	Tumor grade	ER (%)	PR (%)	Ki-67 (%)	Luminal subtype	LVI
1	30	41	Yes	Mastectomy	IDC	1	II	80	95	2	Luminal A	No
2	26	39	Yes	Mastectomy	IDC	1	I	80	80	10	Luminal A	No
3	30	60	Yes	BCS	IDC	1	II	95	5	5	Luminal B	No
4	29	54	Yes	Mastectomy	IDC	1	II	95	60	10	Luminal A	No
5	29	74	Yes	BCS	IDC	1	II	95	10	2	Luminal B	No
6	29	57	Yes	BCS	IDC	1	II	95	50	3	Luminal A	No
7	27	76	Yes	Mastectomy	IDC	2	II	95	15	20	Luminal B	No
8	27	57	Yes	Mastectomy	IDC	1	III	95	15	20	Luminal B	No

RS, recurrence score; BCS, breast conserving surgery; IDC, invasive ductal carcinoma; ER, estrogen receptor; PR, progesterone receptor; LVI, lymphovascular invasion.

Table SVIII. Incidence of recurrence in 783 patients according to the 21-gene RS classification and chemotherapy usage.

	All patients			Chemo cohort		Non-chemo cohort		
	Total	Events, n (%)	P-value	Total	Events, n (%)	Total	Events, n (%)	P-value
18-25	374	3 (0.8)	0.183	115	0 (0.0)	259	3 (1.2)	0.227
26-30	200	6 (3.0)		140	4 (2.9)	60	2 (3.3)	0.764
≥31	209	4 (1.9)		186	4 (2.2)	23	0 (0.0)	0.604

RS, recurrence score; chemo, chemotherapy.