

Supplementary Information

Supplementary Table S1. Primer sequences used in qRT-PCR

Supplementary Table S2. Demography of the 270 patients with HER2-positive BCs receiving NCTT

Supplementary Table S3. The HER2 RNAscope scores of 270 breast cancer cases were categorized using HER2 IHC and FISH.

Supplementary Table S4. Clinicopathological factors in the four NCTT treatment subgroups

Supplementary Table S1. Primer sequences used in qRT-PCR

Gene	Forward sequence	Reverse sequence
<i>HER2</i>	ACAGT GGCAT CTGTG AGCTG	CCCAC GTCCG TAGAA AGGTA
<i>GAPDH</i>	GAGTC AACGG ATTTG GTCGT	GACAA GCTTC CCGTT CTCAG

Supplementary Table S2. Demography of the 270 patients with HER2-positive BCs

receiving NCTT

	n (%)
Age	
<50y	116 (43.0)
≥50y	154 (57.0)
Histology subtype	
NST	264 (97.8)
non-NST	6 (2.2)
SBR grade	
I	1 (0.4)
II	143 (53.0)
III	126 (46.7)
Pre-NCTT	
cT	
cT1	25 (9.2)
cT2	208 (77.0)
cT3	37 (13.7)
cN	

	cN0	62 (23.0)
	cN1-3	208 (77.0)
Post-NCTT		
ypT		
	ypT0	77 (28.5)
	ypTis	41 (15.2)
	ypT1	
	ypT1mic	14 (5.2)
	ypT1a	26 (9.6)
	ypT1b	24 (8.9)
	ypT1c	24 (8.9)
	ypT2	53 (19.6)
	ypT3	11 (4.1)
ypN		
	ypN0	146 (54.1)
	ypN1-3	85 (31.5)
	ypNx	39 (14.4)
Hormone receptor (HR)		
ER		

	positivity	136 (50.4)
	negativity	134 (49.6)
PR		
	positivity	107 (39.6)
	negativity	163 (60.4)
ER/PR		
	HR positivity	
	ER+/PR+	101 (37.4)
	ER+/PR-	35 (13.0)
	ER-/PR+	6 (2.2)
	HR negativity	
	ER-/PR-	128 (47.4)
HER2		
	IHC2+/FISH+	
	G1	37 (13.7)
	G2	7 (2.6)
	G3	12 (4.4)
	IHC3+	214 (79.3)
Post-NCTT operation		

Partial mastectomy	133 (49.3)
Total mastectomy	137 (50.7)
Post-NCTT lymph node dissection	
SLND	61 (22.6)
ALND	170 (63.0)
Not performed	39 (14.4)
NCTT regimen	
EC(F) to TH(P)	65 (24.1)
TH(P)	43 (15.9)
TH(P) to EC(F)	93 (34.4)
TCarboH(P)	69 (25.6)
Anti-HER2 blockade	
mono	125 (46.3)
dual	145 (53.7)
Pathologic complete response	
Yes	110 (40.7)
No	160 (59.3)

Supplementary Table S3. The HER2 RNAscope scores of 270 breast cancer cases were categorized using HER2 IHC and FISH.

			RNAscope score						
			1	2	3	4	5	Total	<i>p</i> value
HER2 IHC / FISH									
IHC 2+	FISH G1	2 (5.4)	17 (45.9)	8 (21.6)	6 (16.2)	4 (10.8)	37 (100)	0.238	
	FISH G2	0 (0)	4 (57.1)	3 (42.9)	0 (0)	0 (0)	7 (100)		
	FISH G3	1 (8.3)	4 (33.3)	7 (58.3)	0 (0)	0 (0)	12 (100)		
IHC 3+	FISH G1	2 (1.0)	3 (1.5)	27 (13.4)	43 (21.3)	127 (62.9)	202 (100)	< 0.001	
	FISH G3	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)		
	FISH G4	1 (16.7)	3 (50.0)	2 (33.3)	0 (0)	0 (0)	6 (100)		
	FISH G5	1 (20.0)	1 (20.0)	1 (20.0)	2 (40.0)	0 (0)	5 (100)		

The Pearson chi-square test is employed to assess the association between individual FISH groups in cases of HER2 IHC 2+ or 3+ and HER2 RNA scores. The p -value is calculated using the chi-square test.

Bold font indicates statistical significance at the $p < 0.05$ level.

Supplementary Table 4. Clinicopathological factors in the four NCTT treatment subgroups

Factors	EC(F) to TH(P)	TH(P)	TH(P) to EC(F)	TCarboH(P)	<i>p</i> value
Age					0.222
< 50y	31 (47.7)	14 (32.6)	45 (48.4)	26 (37.4)	
≥ 50y	34 (52.3)	29 (67.4)	48 (51.6)	42 (62.3)	
Histology subtype					0.183
NST	64 (98.5)	41 (95.3)	93 (100)	66 (95.7)	
non-NST	1 (1.5)	2 (4.7)	0 (0)	3 (4.3)	
SBR grade					0.777
I/II	35 (53.8)	20 (46.5)	50 (53.8)	39 (56.5)	
III	30 (46.2)	23 (53.5)	43 (46.2)	30 (43.5)	

Pre-NAC

cT

0.884

cT1/T2 58 (89.2) 37 (86.0) 79 (84.9) 59 (85.5)

cT3/T4 7 (10.8) 6 (14.0) 14 (15.1) 10 (14.5)

cN

0.221

cN0 12 (18.5) 6 (14.0) 26 (28.0) 18 (26.1)

cN1-3 53 (81.5) 37 (86.0) 67 (72.0) 51 (73.9)

HR

0.165

Negativity

31 (47.7) 23 (53.5) 36 (38.7) 38 (55.1)

Positivity

34 (52.3) 20 (46.5) 57 (61.3) 31 (44.9)

HER2 IHC / FISH

0.661

IHC 2+ / FISH+	16 (24.6)	9 (20.9)	20 (21.5)	11 (15.9)	
IHC 3+	49 (75.4)	34 (79.1)	73 (78.5)	58 (84.1)	
HER2 RNAscope score					0.704
score 1-4	32 (49.2)	20 (46.5)	52 (55.9)	34 (49.3)	
score 5	33 (50.8)	23 (53.5)	41 (44.1)	35 (50.7)	
Anti-HER2 blockade					< 0.001
mono	43 (66.2)	14 (32.6)	53 (57.0)	15 (21.7)	
dual	22 (33.8)	29 (67.4)	40 (43.0)	54 (78.3)	

The Pearson chi-square test is employed to assess the association between the clinicopathological factors and the four NCTT treatment subgroups. The *p*-value is calculated using the chi-square test.

Bold font indicates statistical significance at the $p < 0.05$ level.