Breast Cancer Subtypes and Prognosis: Answers to Subgroup Classification Questions, Identifying the Worst Subgroup in Our Single-Center Series

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Research Article

Keywords:

Posted Date: March 18th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1447957/v1

Abstract

Background: Breast cancer (BC) remains the most common cancer in women, and it is the second leading cause of cancer-related death in women. Because of advances in treatment, long life is now possible even in patients with metastatic BC, whereas certain groups of patients survive for a very short time despite being diagnosed at an early stage. In many studies, the triple negative BC subgroup is stated to have the worst prognosis, as such patients are deprived of antihormonal therapy and trastuzumab therapy. While HER2 overexpression was interpreted as a poor prognostic factor before trastuzumab treatment, it was reported to be the worst prognostic subgroup of TNBC in posttrastuzumab publications. In the current study, we aimed to find the worst prognostic subgroup as far as we could by capturing the biodiversity in our series of BC patients, and for this purpose, we compared the treatment results in patients grouped according to their receptor status.

Methods: We reviewed the records of patients with BC who were admitted to our department between July 1999 and December 2019. We grouped the patients into four main groups (Luminal A, Luminal B, triple negative, and HER2 enriched) and we recorded patient and treatment characteristics and oncological results. Survival curves were generated using the Kaplan–Meier method, and the significance of survival differences among the selected variables was compared by using the log-rank test. Univariate Cox regression analysis was used to estimate hazard ratios. Then, multivariate Cox regression analysis with the backward elimination method was used to estimate hazard ratios and to identify independent prognostic factors.

Results: A total of 2474 patients with BC and after exclusions, statistical analysis was performed on 2017 patients with BC. The HER2 positivity rate was 23.7% and the TNBC patient rate was 11.7% (n = 236). The distribution of the four main groups was 47.1% for Luminal A, 34.1% for Luminal B, 7.1% for HER2 enriched, and 11.7% for the TN subgroup. Age (<35 years); no axillary surgery; Ki67≥15; high tumor grade; high mitotic index; the presence of skin infiltration; advanced T/N stage; the presence of metastasis; nontreatment with chemotherapy; less than 5 years of using TMX or AI; and being in the HER2-enriched subgroup were determined to be negative factors for overall survival as a result of multivariate analysis.

Conclusions: The HER2-enriched subgroup had the worst prognosis despite receiving targeted therapy. However, treatment with trastuzumab increased survival 1.5-fold over that of the HER2-enriched subgroup that did not receive it.

Introduction

Breast cancer (BC) remains the most common cancer in women, and it is the second leading cause of cancer-related death in women after lung cancer [1]. However, because of advances in treatment, long life is now possible even in patients with metastatic BC, whereas certain groups of patients survive for a very short time despite being diagnosed at an early stage [2]. Every day, we are getting closer to understanding this differential clinical course of BC, and we have the opportunity to define heterogeneity in BC, thanks to the detection of molecular receptors that play a role in breast carcinogenesis and the detection of pathways indicative of rapid proliferation [3–6].

Immunohistochemical (IHC) staining and in situ fluorescent hybridization (FISH) methods are currently used methods for identifying tumor subtypes to achieve better treatment choices and survival. Since the St. Gallen International consensus panel in 2011, four main robust subtypes (Luminal A, Luminal B, triple negative, and HER2 enriched) have proven to be a good classification scheme. According to the presence or absence of receptors, in the classification of BC, four different molecular subtypes have been defined:

- Luminal A (ER and PR positive, HER2-negative, Ki67 low),
- Luminal B (ER and/or PR positive, HER2-positive or Ki67 high),

- HER2 enriched (Hormone (ER and PR) receptor-negative and HER2-positive)
- Triple-negative (TN) (ER- and PR- and HER2-negative).

Each subtype exhibits different oncological results and different treatment strategies (4).

Retrospective data help to identify prognostic factors as well as to measure the effectiveness of treatments and test their effects on subgroups. It is also possible to determine the best subgroup with a good prognosis and to predict the clinical course [7, 8]. However, determining the subgroup with the worst prognosis and predicting the clinical course is still unclear and confusing when the literature is evaluated.

In many studies, the TNBC subgroup is stated to have the worst prognosis, as such patients are deprived of antihormonal therapy and trastuzumab therapy. Additionally, the main systemic treatment is chemotherapy alone in most BC patients with TN who have a poor prognosis [9–14]. The main published result in the years before targeted therapies were placed into routine clinical use was that the HER2-enriched subgroup was the worst prognostic subgroup. However, while HER2 overexpression was interpreted as a poor prognostic factor before trastuzumab treatment, it was reported to be the worst prognostic subgroup of TNBC in posttrastuzumab publications [15–19]. It is clearly known according to several clinical outcomes that Luminal A is the best prognostic subgroup [7–10].

In the current study, we aimed to find the worst prognostic subgroup as far as we could by capturing the biodiversity in our series of BC patients, and for this purpose, we compared the treatment results in patients grouped according to their receptor status.

Material And Method

Following the approval of the Institutional Review Board, we reviewed the records of patients with BC who were admitted to the Radiation and Medical Oncology Department of Trakya University between July 1999 and December 2019. The Human Research Ethical Committee of the Trakya University Medical Faculty Hospital approved (TUTF-BAEK 2021/406) the use of these patients' information for the study.

We grouped the patients into four main groups (Luminal A, Luminal B, triple negative, and HER2 enriched) according to the St Gallen International Consensus Panel and five subgroups according to the receptor status (Table 1). We recorded patient characteristics, such as age, body mass index (BMI), age at menarche, age at menopause, menstruation status, number of births, family history, breastfeeding, hormone replacement status, histological type, localization area in the breast, tumor quadrant, surgical type, axillary surgery type, tumor size, lymph node metastasis, TNM stage, grade, mitotic index, estrogen receptor (ER) status, progesterone receptor (PR) status, Human Epidermal Growth Factor Receptor 2 (HER2) status, Ki-67 level, lymphovascular invasion (LVSI), perineural invasion (PNI), extensive intraductal component (EIC), surgical margin status, skin involvement, whether or not they received chemotherapy, chemotherapy type, whether or not they received radiotherapy, radiotherapy type, tamoxifen (TMX) usage time, aromatase inhibitor (AI) usage time, and luteinizing hormone-releasing hormone (LHRH) usage time. The staging of the tumor was based on The American Joint Committee on Cancer 2013 System.

Table 1
Table showing the step-by-step parameters according to which the groups are created

Groups Name	How is the classification made?	Group Branches
Subtyping 1	Subtype Triple-Negative	Triple-Negative
		None-Triple Negative
Subtyping 2	Original Subtype	Triple-Negative
		Luminal A
		Luminal B
		HER2 enriched
Subtyping 3	Subtype HER2 enriched could get Herceptin	Triple-Negative
		Luminal A
		Luminal B
		HER2 enriched could get Herceptin
		HER2 enriched did not get Herceptin
Subtyping 4	Subtype HER2 positive-negative	Triple-Negative
		Luminal A
		Luminal B HER2 positive
		Luminal B HER2 negative
		HER2 enriched could get Herceptin
		HER2 enriched did not get Herceptin
Subtyping 5	Subtype could get Herceptin	Luminal B could get Herceptin
		Luminal B did not get Herceptin
		HER2 enriched could get Herceptin
		HER2 enriched did not get Herceptin
		HER2 negative

Histopathologic Evaluation

In our pathology department, receptor status assessments are made as follows:

- Primary Novocastra monoclonal antibodies (clone 6F11 for ER and clone 1 A6 for PR) were used to determine the estrogen receptor (ER) and progesterone receptor (PR) status. A positive nuclear reaction was considered “receptor-negative” in less than 1% of tumor cells.
- Immunohistochemical analyses were performed using HER2/neu (Clone 10A7, Novocastra) as the primary antibody. The DAKO Herceptest scoring system, which is also referred to in some national and international guidelines, was used. Tumors showing 3 + membranous staining immunohistochemically (IHC) for HER2/neu antibody or positive gene amplification by fluorescent in situ hybridization (FISH) were considered positive.
- Ki67 was analyzed in paraffin sections by an immunohistochemical method using the MIB-1 antibody. Our pathologist examined the stained section using a standard light microscope with a 40x objective and 10x10

graticule and defined the Ki67 score as the percentage of the total number of tumor cells with nuclear staining. This required counting at least 1000 tumor cells with nuclear staining in ten high-power fields (× 40).

Statistical Analysis

Numerical results are expressed as the mean ± standard deviation, and categorical results are shown as n (%). Survival curves were generated using the Kaplan–Meier method, and the significance of survival differences among the selected variables was compared by using the log-rank test. Univariate Cox regression analysis was used to estimate hazard ratios. Then, multivariate Cox regression analysis with the backward elimination method was used to estimate hazard ratios and to identify independent prognostic factors. All reported p values are two-sided, and a value below 0.05 was considered to indicate statistical significance. Data analysis was performed using SPSS version 20.0 (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

Results

A total of 2474 patients with BC who were treated between July 1999 and December 2019 were evaluated. Patients who did not have the examined parameters were excluded from the study. A total of 131 patients with ductal carcinoma-in-situ and lobular carcinoma-in-situ, 9 patients with phyllodes tumors, and 244 patients whose ER, PR, HER2, and Ki67 information could not be completely obtained were excluded from the analysis. After exclusions, statistical analysis was performed on 2017 patients with BC (Fig. 1). The mean age was 52.07 years, the mean menopausal age was 48.35 years, and the mean menarche age was 13.15 years. The mean BMI was 29.9. The HER2 positivity rate was 23.7%.

The TNBC patient rate was 11.7% (n = 236), and there were no statistically significant differences between the two groups when comparing DFS (190.37 ± 7.19 (176.27-204.46) for TNBC, 218.23 ± 3.68 (211.01-225.44) for NTNBC, p = .739) and OS (221.68 ± 7.92 (206.14-237.21) TNBC and 231.77 ± 3.29 (225.32-238.22) p = .252) (Table 2, Fig. 2a, b).

Table 2
DFS and OS times, comparative Log-rank test, p-value values obtained using Kaplan-Meier method of Triple-Negative Breast Cancer and None-Triple Negative Breast Cancer subgroups forming Subtyping 1

		Subtyping 1		p-value
		Triple-Negative	**None-Triple Negative**	(Log-rank test)
DFS	**Mean ± SD**	190.3 ± 7.1	218.2 ± 3.6	0.739
	95% Confidence Interval	176.2-204.4	211.0-225.4	
OS	**Mean ± SD**	221.6 ± 7.9	231.7 ± 3.2	0.252
	95% Confidence Interval	206.1-237.2	225.3-238.2	

DFS: Disease free survival, OS: Overall survival, SD: Standard deviation, CI: Confidence Interval

The distribution of the four main groups was 47.1% for Luminal A, 34.1% for Luminal B, 7.1% for HER2 enriched, and 11.7% for the TN subgroup. The worst prognostic main group comprised HER2-enriched patients, with 113.70 ± 7.17 months DFS and 125.45 ± 3.03 months OS (Table 3, Fig. 3a, b).

Table 3

DFS and OS times, comparative Log-rank test, p-value values obtained using Kaplan-Meier method of TNBC, Luminal A, and Luminal B and HER2 enriched subgroups forming Subtyping 2

	Subtyping 2	Mean ± Std. Error	95% Confidence Interval		p values (Log-rank test)			
		(Months)	**Lower Bound**	**Upper Bound**	**Triple Negative**	**Luminal A**	**Luminal B**	**HER2 enriched**
DFS	Triple-Negative	190.3 ± 7.1	176.2	204.4				
	Luminal A	226.7 ± 4.3	218.3	235.2	0.139			
	Luminal B	168.3 ± 4.3	159.8	176.9	0.971	0.016		
	HER2 enriched	113.7 ± 7.1	99.6	127.7	**< 0.001**	**< 0.001**	**< 0.001**	
OS	Triple-Negative	221.6 ± 7.9	206.1	237.2				
	Luminal A	237.4 ± 3.8	229.9	244.9	**0.002**			
	Luminal B	180.2 ± 4.0	172.3	188.2	0.160	0.450		
	HER2 enriched	125.4 ± 3.0	112.0	138.9	**< 0.001**	**< 0.001**	**< 0.001**	

DFS: Disease-free survival, OS: Overall survival

The DFS was 101.50 ± 6.4 (88.77-114.23), the OS was 118.14 ± 6.16 (106.06-130.22) in the Herceptin group, and the DFS was 92.79 ± 18 (57.44-128.13); the OS was 94.44 ± 15.23 (64.58–124.30) in the non-Herceptin group (Table 4). The HER2-enriched Herceptin subgroup did not have the lowest DFS and differed from the TNBC, Luminal A, Luminal B subgroups at the level of statistical significance. However, DFS did not differ statistically significantly between HER2-enriched patients who received Herceptin and HER2-enriched patients who did not receive Herceptin. OS was the lowest survival time at the statistical significance level among the TNBC-, Luminal A-, Luminal B-, and HER2-enriched Herceptin subgroups (Table 4, Fig. 4a, b).

Table 4
DFS and OS times, comparative Log-rank test, p-value values obtained using Kaplan-Meier method of TNBC, Luminal A and Luminal B and HER2 enriched could get Herceptin, HER2 enriched did not get Herceptin subgroups forming Subtyping 3

	Subtyping 3	Mean ± Std. Error (Months)	95% Confidence Interval		Log-rank (Mantel-Cox) Chi-Square (Sig)				
			Lower Bound	**Upper Bound**	**Triple Negative**	**Luminal A**	**Luminal B**	**HER-A**	**HER-B**
DFS	Triple-Negative	190.37 ± 7.19	176.27	204.46					
	Luminal A	227.02 ± 4.31	218.57	235.46	2,085 (.149)				
	Luminal B	168.12 ± 4.36	159.57	176.67	0.000 (.977)	5.224 (**.022**)			
	HER2 enriched (HER-A) could get Herceptin	101.50 ± 6.49	88.77	114.23	9.262 (**.002**)	28.443 (**< .001**)	13.935 (**< .001**)		
	HER2 enriched (HER-B) did not get Herceptin	92.79 ± 18.00	57.44	128.13	10.318 (**.001**)	17.954 (**< .001**)	10.409 (**.001**)	1.665 (.197)	
OS	Triple-Negative	221.68 ± 7.92	206.14	237.21					
	Luminal A	237.44 ± 3.83	229.92	244.97	3.100 (.078)				
	Luminal B	180.29 ± 4.04	172.37	188.22	2.122 (.145)	.387 (.534)			
	HER2 enriched (HER-A) could get Herceptin	118.14 ± 6.16	106.06	130.22	4.548 (**.033**)	21.267 (**< .001**)	16.439 (**< .001**)		
	HER2 enriched (HER-B) did not get Herceptin	94.44 ± 15.23	64.58	124.30	16.092 (**< .001**)	32.866 (**< .001**)	30.357 (**< .001**)	5.602 (**.018**)	

The DFS was 163.796 ± 5.78 (152.45-175.13) months, and the OS was 178.95 ± 5.15 (168.83-189.06) months in Luminal B HER2-positive patients. The DFS was 101.23 ± 2.35 (96.61-105.86) months, and the OS was 114.16 ± 2.01 (110.20-118.11) months in Luminal B HER2-negative patients. The Luminal B HER2-positive subgroup had a longer DFS and OS than the Luminal B HER2-negative subgroup. However, this difference was not statistically significant (Table 5, Fig. 5a, 5b). There was a statistically significant difference in the DFS and OS times with HER2 enrichment in the Herceptin, triple negative, Luminal A, Luminal B-HER2-positive, and Luminal B-HER2-negative groups (Table 5, Fig. 5a, b).

Table 5

DFS and OS times, comparative Log-rank test, p-value values obtained using Kaplan-Meier method of TNBC, Luminal A and Luminal B HER2 positive, Luminal B HER2 negative and HER2 enriched could get Herceptin, HER2 enriched did not get Herceptin subgroups forming Subtyping 4.

	Subtyping 4	Mean ± Std. Error (Months)	95% Confidence Interval		Log-rank (Mantel-Cox) Chi-Square (Sig)					
			Lower Bound	**Upper Bound**	**Triple Negative**	**Luminal A**	**Luminal B HER2 Positive**	**Luminal B HER2 Negative**	**HER-A**	**HER-B**
DFS	**Triple-Negative**	190.37 ± 7.19	176.27	204.46						
	Luminal A	227.02 ± 4.31	218.57	235.46	2.085 (.149)					
	Luminal B HER2 Positive	163.79 ± 5.78	152.45	175.13	0.266 (.606)	6.980 (**,008**)				
	Luminal B HER2 Negative	101.23 ± 2.35	96.61	105.86	0.431 (.512)	0.941 (.332)	1.387 (.239)			
	HER2 enriched (HER-A) could get Herceptin	101.50 ± 6.49	88.77	114.23	9.262 (**.002**)	28,443 (**< .001**)	8.157 (**.004**)	15.406 (**< .001**)		
	HER2 enriched (HER-B) did not get Herceptin	92.79 ± 18.00	57.44	128.13	10,318 (**.001**)	17,954 (**< .001**)	7.883 (**.005**)	13.455 (**< .001**)	1.665 (.197)	
OS	**Triple-Negative**	221.68 ± 7.92	206.14	237.21						
	Luminal A	237.44 ± 3.83	229.92	244.97	3.10 (.078)					
	Luminal B HER2 Positive	178.95 ± 5.15	168.83	189.06	1.061 (.303)	0.582 (.446)				
	Luminal B HER2 Negative	114.16 ± 2.01	110.20	118.11	2.699 (.100)	0.018 (.892)	0.259 (.611)			
	HER2 enriched (HER-A) could get Herceptin	118.14 ± 6.16	106.06	130.22	4.548 (**.033**)	21.267 (**< .001**)	11.391 (**.001**)	13.703 (**< .001**)		

HER2 enriched (HER-B) did not get Herceptin	94.44 ± 15.23	64.58	124.30	16.092 (**< .001**)	32.866 (**< .001**)	25.708 (**< .001**)	30.967 (**< .001**)	5.602 (**.018**)

HER2 positivity was separated according to the status of negativity and treatment or nontreatment with Herceptin or not. While the HER2-negative subgroup showed the best survival, it did not differ statistically from the survival of the TNBC, Luminal A, and Luminal B subgroups. However, the HER2-negative subgroup had a significantly better time than the HER2-enriched subgroup in terms of both DFS and OS times. The HER2-negative subgroup had a significantly better outcome than the HER2-enriched subgroups in terms of both DFS and OS times. The best DFS and OS were detected in the HER2-negative subgroup. Additionally, while the HER2-negative subgroup showed the best survival, the survival of the Luminal B Herceptin subgroup was not statistically different from that of the Luminal B non-Herceptin subgroup (Table 6, Fig. 6a, b).

Table 6

DFS and OS times, comparative Log-rank test, p-value values obtained using Kaplan-Meier method of TNBC, Luminal A, Luminal B could get Herceptin, Luminal B didn't get Herceptin and HER2 enriched could get Herceptin, HER2 enriched didn't get Herceptin subgroups forming Subtyping 5.

	Subtyping 5	Mean ± Std. Error (Months)	95% Confidence Interval		Log-rank (Mantel-Cox) Chi-Square (Sig)				
			Lower Bound	**Upper Bound**	**HER2 Negative**	**Luminal B Herceptin Positive**	**Luminal B Herceptin Negative**	**HER-A**	**HER-B**
DFS	**HER2 Negative**	225.237 ± 3.89	217.60	232.86					
	Luminal B could get Herceptin	126.33 ± 5.10	116.32	136.34	3.006 (.083)				
	Luminal B did not get Herceptin	171.69 ± 4.90	162.09	181.30	1.945 (.163)	0.162 (.688)			
	HER2 enriched (HER-A) could get Herceptin	101.50 ± 6.49	88.77	114.23	26.736 (**< .001**)	8.466 (**.004**)	12.783 (**< .001**)		
	HER2 enriched (HER-B) did not get Herceptin	92.79 ± 18,03	57.44	128.13	16.902 (**< .001**)	7.133 (**.008**)	11.736 (**.001**)	1.665 (.197)	
OS	**HER2 Negative**	235.49 ± 3.48	228.66	242.33					
	Luminal B could get Herceptin	148.32 ± 4.17	140.14	156.50	0.607 (.436)				
	Luminal B did not get Herceptin	178.20 ± 4.91	168.56	187.84	0.386 (.534)	0.999 (.317)			
	HER2 enriched (HER-A) could get Herceptin	118.14 ± 6.16	106.06	130.22	18.218 (**< .001**)	14.578 (**< .001**)	11.466 (**.001**)		
	HER2 enriched (HER-B) did not get Herceptin	94.44 ± 15.23	64.58	124.30	30.150 (**< .001**)	30.660 (**< .001**)	25.569 (**< .001**)	5.602 (**.018**)	

The worst subgroup for DFS and OS was the HER2-enriched subgroup, whether receiving Herceptin or not (Table 7). In all pairwise comparisons, only the DFS duration in the HER2-enriched subgroup was not statistically significant. In comparison with all other subgroups, both DFS and OS times were significantly different. In pairwise comparisons of the HER2-enriched subgroup, the strongest difference was found in the order of Luminal A, Luminal B, and TNBC.

Table 7
Comparison of HER2 enriched subgroup with other subgroups

			DFS		OS	
			Pearson Chi-Square	Asymptotic Significance (2-sided) *P value*	Pearson Chi-Square	Asymptotic Significance (2-sided) *P value*
Subtyping 1			0.207	0.649	1.375	0.252
Subtyping 2		HER2 enriched-Luminal A	39.820	**< 0.001**	39.518	**< 0.001**
		HER2 enriched-Luminal B	19.845	**< 0.001**	29.819	**< 0.001**
		HER2 enriched-TNBC	12.876	**< 0.001**	9.715	**0.002**
Subtyping 3	Could get Herceptin	HER2 enriched-Luminal A	26.563	**< 0.001**	17.540	**< 0.001**
		HER2 enriched-Luminal B	12.484	**< 0.001**	12.787	**< 0.001**
		HER2 enriched-TNBC	8.652	**0.003**	3.437	**0.064**
	Did not get Herceptin	HER2 enriched-Luminal A	17.954	**< 0.001**	32.866	**< 0.001**
		HER2 enriched-Luminal B	10.271	**0.001**	29.516	**< 0.001**
		HER2 enriched-TNBC	10.391	**0.001**	16.155	**< 0.001**
Subtyping 4	Could get Herceptin	HER2 enriched-Luminal A	24.648	**< 0.001**	17.784	**< 0.001**
		HER2 enriched-Luminal B HER2 positive	9.208	**0.002**	10.882	**0.001**
		HER2 enriched-Luminal B HER2 negative	9.410	**0.002**	9.072	**0.003**
		HER2 enriched-TNBC	7.911	**0.005**	3.544	0.060
		HER2 enriched-HER2 enriched didn't get Herceptin	1.805	0.179	6.231	**0.003**
	Did not get Herceptin	HER2 enriched-Luminal A	17.954	**< 0.001**	32.866	**< 0.001**
		HER2 enriched-Luminal B HER2 positive	9.068	**0.003**	26.983	**< 0.001**
		HER2 enriched-Luminal B HER2 negative	13.214	**< 0.001**	27.485	**< 0.001**
		HER2 enriched-TNBC	10.391	**0.001**	16.155	**< 0.001**
Subtyping 5	Could get Herceptin	HER2 enriched-HER2 negative	22.721	**< 0.001**	14.808	**< 0.001**
		HER2 enriched-Luminal B could get Herceptin	6.715	**0.010**	12.398	**< 0.001**
		HER2 enriched-Luminal B didn't get Herceptin	10.613	**0.001**	9.278	**0.002**

			DFS		OS	
			Pearson Chi-Square	Asymptotic Significance (2-sided) *P value*	Pearson Chi-Square	Asymptotic Significance (2-sided) *P value*
	Did not get Herceptin	HER2 enriched-HER2 negative	16.761	**< 0.001**	29.920	**< 0.001**
			6.904	**0.009**	30.298	**< 0.001**
			11.633	**0.001**	25.437	**< 0.001**
		HER2 enriched-Luminal B could get Herceptin				
		HER2 enriched-Luminal B didn't get Herceptin				
		HER2 enriched could get Herceptin	1.805	0.179	6.333	**0.012**
		HER2 enriched didn't get Herceptin				

In univariate analysis, age (< 35 years); early age at menarche; postmenopausal status; advanced T/N stage; no surgery on the breast and/or axillary node; high tumor grade; high mitotic index; the presence of skin infiltration; multifocal tumor; ER, PR negativity and HER2 positivity; positive EIC, positive LVI; Ki6 ≥ 15 levels; the presence of metastasis; nontreatment with chemotherapy and radiotherapy; less than 5 years of using TMX or AI; less than 2 years' use of LHRH; and being in the HER2 enriched subgroup were determined to be negative factors for OS. No axillary surgery, T and N stage, not receiving radiotherapy, using TMX less than 5 years, and LHRH for less than 2 years were statistically significant negative factors for OS in multivariate analysis (Table 8).

Table 8
Univariable and multivariable analysis of BC survival using Cox's proportional hazards model within DFS

		Univariate analysis		Multivariate analysis	
Patients Descriptions	**Events/Total (%)**	**Hazard ratio (95% CI)**	p	**Hazard ratio (95% CI)**	p
Age group	23/84 (27.3)	1 (Reference)	**.038**	1 (Reference)	.429
< 35 years	138/756 (18.2)	0.627 (0.403–0.974)	.381	1.218 (.747-1.986)	.182
35–50 years	254/1177 (21.5)			1.466 (.836 – 2.570)	
> 50 years		0.826 (0.539–1.266)			
BMI	73/373 (19.5)	1.059 (0.823–1.364)	.656		
< 25	342/1644 (20.8)				
≥ 25					
Menopause Age (mean)	260/2017 (12.8)	1.010 (0.984–1.037)	.470		.
Events 48.36 years					
None Events 48.30 years					
Menstruation Age (mean)	415/2017(20.5)	0.915 (0.850–0.986)	**.019**	.959 (.851 – 1.080)	.486
Events 13.04 years					
None Events 13.17 years					
Menstruation situation	149/787 (18.9)	1.221 (.998-1.495)	.052	1.053 (.746-1.487)	.769
Premenopause	260/1217 (21.3)				
Postmenopause					
Number of births	38/162 (23.4)	1 (Reference)	.435		
No birth	255/1320 (19.3)	.834 (.593-1.173)	.296		
1–2 birth	115/520 (22.1)	.928 (.643-1.339)	.690		
3 and more					
Family History	115/632 (18.1)	.850 (.685-1.054)	.138		
Positive	300/1385 (21.6)				
Negative					
Breast-feeding	229/1192 (19.2)	.869 (.716-1.055)	.156		
Positive	186/825 (22.5)				
Negative					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Breast site	205/1013 (20.2)	1 (Reference)	.939		
Left	186/935 (19.8)	.000 (.000-6.51)	.935		
Right	24/69 (34.7)	< .001 (.000-2.31)			
Bilateral					
Location	391/1948 (20)	1 (Reference)	.061		
Unilateral	17/46 (36.9)	1.590 (.978-2.586)	.135		
Metacron	7/23 (30.4)	1.770 (.838-3.739)			
Sencron					
Tumor Quadrant	80/402 (19.9)	1 (Reference)	.962	1 (Reference)	.583
Inner	234/1205 (19.4)	.994 (.771-1.281)	.657	.924 (.697-1.225)	.096
Outer	54/259 (20.8)	1.081 (.766-1.527)	**.001**	.713 (.479-1.062)	.143
Periareolar	47/150 (31.3)	1.819 (1.269–2.608)		.721 (.466-1.117)	
Multifokal					
Histopathologic Type	344/1652 (20.8)	1 (Reference)	.646		
IDC	26/122 (21.3)	.954 (.640-1.422)	.817		
ILC	45/243(18.5)	.864 (.633-1.179)	.356		
Other					
Surgical Type	122/1016 (12)	1 (Reference)	**< .001**	1 (Reference)	.184
BCS	226/930 (24.3)	1.978 (1.586–2.466)	**< .001**	.834 (.634 – 1.090)	.343
MRM	67/71(94.3)	27.941(20.296–38.465)		1.444 (.676-3.087)	
No surgery					
Axillary Surgery Type	37/451(8.2)	1 (Reference)	**< .001**	1 (Reference)	**.037**
SLND	304/1477(20.5)	2.210 (1.569–3.111)	**< .001**	.645(.427-.975)	.900
AD	74/89(83.1)	21.759 (14.573–32.487)		1.056 (.455-2.448)	
No axillary surgery					
Stage	23/415 (5.54)	1 (Reference)	**< .001**		
I	96/881 (10.8)	1.960 (1.244–3.090)	**.004**		
II	158/583 (27.1)	5.447 (3.517–8.436)	**< .001**		
III	138/138 (100)	95.570 (60.478-151.023)	**< .001**		
IV					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
T stage	67/670 (10)	1 (Reference)	**< .001**	1 (Reference)	**.029**
T1	220/1048 (20.9)	2.207 (1.679–2.902)	**< .001**	1.426 (1.037–1.962)	**.044**
T2	38/155 (24.5)	2.438 (1.637–3.631)	**< .001**	1.579 (1.012–2.464)	**.032**
T3	89/143 (62.2)	11.090 (8.050-15.279)		1.794 (1.053–3.057)	
T4					
Positive Axillary Node Count	78/861 (9.06)	1 (Reference)	**.001**	1 (Reference)	.263
0	80/531 (15.07)	1.668 (1.221–2.278)	**< .001**	.811 (.561-1.171)	.119
1–3	145/402 (36.07)	5.000 (3.795–6.587)	**< .001**	1.338 (.928-1.929)	**.012**
4–9	111/222 (50)	7.384 (5.524–9.870)		1.644 (1.116–2.423)	
≥10					
Metastasis site	25/1627 (1.53)	1 (Reference)	**< .001**	1 (Reference)	**< .001**
None	142/142 (100)	156.760 (102.099-240.686)	**< .001**	158.568(100.278.250.742)	**< .001**
Bone	25/25 (100)	139.613 (79.878-244.018)	**< .001**	131.993 (72.208-241.278)	**< .001**
Lung	15/15 (100)	171.002 (89.530-326.613)	**< .001**	133.403 (64.540-275.738)	**< .001**
Liver	21/21 (100)	173.699 (96.477-312.733)	**< .001**	129.981 (68.258-247.517)	**< .001**
Brain	185/185 (100)	164.232 (107.535-250.822)		126.654 (79.530-201.699)	
Multiple organs					
Skin infiltration	80/152 (52.6)	4.664 (3.642–5.974)	**< .001**	1.249 (.783-1.991)	.351
Positive	335/1865 (18)				
Negative					
Surgical margin	69/367 (18.8)	1.004 (.775-1.301)	.975		
Positive	346/1650 (21)				
Negative					
Grade	23/304 (7.5)	1 (Reference)	**< .001**	1 (Reference)	.198
1	155/987 (15.7)	2.290 (1.478–3.550)	**< .001**	.712 (.424-1.195)	.950
2	237/726 (32.6)	5.417 (3.528–8.317)		1.017 (.595-1.739)	
3					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Mitotic index	97/775 (12.5)	1 (Reference)	**.003**	1 (Reference)	.851
1	98/637 (15.3)	1.546 (1.164–2.053)	**< .001**	1.033 (.734-1.455)	.982
2	218/591 (36.8)			.996 (.726-1.367)	
3		4.303 (3.369–5.497)			
ER receptor	300/1598 (18.7)	.648 (.523-.804)	**< .001**	.838 (.403 – 1.350)	.324
Positive					
Negative	115/419 (27.4)				
PR receptor	240/1337 (18)	.640 (.526-.777)	**< .001**	1.029 (.769-1.376)	.847
Positive	175/680 (25.7)				
Negative					
Ki67	204/1130 (18)	1.758 (1.443–2.143)	**< .001**	1.062 (.811-1.389)	.662
<15	210/885 (23.7)				
≥15					
HER2	125/478 (26.1)	1.646 (1.333–2.032)	**< .001**	1.077 (.730 – 1.590)	.708
Positive	290/1539(18.8)				
Negative					
EIC	96/334 (28.7)	1.646 (1.310–2.069)	**< .001**	**1.175 (.895-1.542)**	.247
Positive	319/1683 (18.9)				
Negative					
LVI	211/954 (22.1)	1.190 (.981-1.443)	.077	1.077 (.832-1.394)	.574
Positive	204/1063 (19.1)				
Negative					
PNI	103/437 (23.5)	1.145 (.917-1.431)	.233		
Positive	312/1580(19.7)				
Negative					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Chemotherapy	40/324 (12.3)	1 (Reference)	**< .001**	1 (Reference)	.604
None	78/235 (33.1)	3.202 (2.186–4.690)	**< .001**	1.137 (.699-1.851)	.301
Neoadjuvant	297/1458 (20.3)			.840 (.563-1.252)	
Adjuvant		1.553 (1.116–2.161)			
Chemotherapy Protocol	40/324 (12.3)	1 (Reference)	**.002**		
None	54/166 (32.5)	1.858 (1.255–2.750)	.447		
FAC	34/273 (12.4)		**.001**		
AC + TXT	279/1235 (22.5)	1.429 (.569-3.592)			
Other		1.694 (1.247–2.301)			
Radiotherapy	295/1757 (16.7)	.302 (.244-.374)	**< .001**	.470 (.352-.626)	**< .001**
Positive	120/260 (46.1)				
Negative					
Radiotherapy Type	120/260 (46.1)	1 (Reference)	**< .001**		
None	44/587 (7.5)	.127 (.090-.179)	**< .001**		
Breast alone	51/1170(4.3)	.389 (.312-.484)			
Locoregional					
Tamoxifen period	130/652 (19.9)	1 (Reference)	**.014**	1 (Reference)	.896
No TMX	9/107 (8.4)	.769 (.623-.949)	**< .001**	1.022 (.733-1.425)	**.030**
TMX ≤ 5 years		.283 (.146-.551)		.425 (.196-.922)	
TMX > 5 years					
AI period	191/937 (20.3)	1 (Reference)	**.042**	1 (Reference)	.317
No AI	32/265 (12)	.812 (.664-.992)	**< .001**	.861 (.643-1.154)	.408
AI ≤ 5 years		.404 (.278-.589)		.817 (.505-1.319)	
AI > 5 years					
LHRH	8/35 (22.8)	1 (Reference)	.544	1 (Reference)	**.037**
None LHRH	57/343 (16.6)	1.242 (.616-2.504)	.048	2.426 (1.057–5.568)	.353
≤2 years		.754 (.570-.998)		1.225 (.798 – 1.880)	
>2 years					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Subtyping 2	51/142 (35.9)	1 (Reference)	**< .001**	1 (Reference)	.447
HER2 enriched	51/236 (21.6)	.479 (.325-.707)	**< .001**	.794 (.438-1.438)	.817
TNBC	182/952 (19.1)	.380 (.278-.520)	**< .001**	.891 (.336-2.362)	.706
Luminal A	131/688 (19.0)	.488 (.353-.675)		1.157 (.543-2.463)	
Luminal B					
HER2 enriched could get Herceptine	40/120 (33.3)	1.515 (.777-2.954)	.223		
HER2 enriched did not get Herceptine	11/22 (50)				

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

In univariate analysis, age (< 35 years); postmenopausal status; advanced T/N stage; no surgery on the breast and/or axillary node; high tumor grade; high mitotic index; the presence of skin infiltration; multifocal tumor; ER, PR negativity and HER2 positivity; the presence of metastasis; positive EIC; positive LVI; Ki67 ≥ 15; positive surgical margin; nontreatment with chemotherapy and radiotherapy; less than 5 years of using TMX or AI; less than 2 years' use of LHRH; and being in the HER2-enriched subgroup were determined to be negative factors for OS. Age (< 35 years); no axillary surgery; Ki67 ≥ 15; high tumor grade; high mitotic index; the presence of skin infiltration; advanced T/N stage; the presence of metastasis; nontreatment with chemotherapy; less than 5 years of using TMX or AI; and being in the HER2-enriched subgroup were determined to be negative factors for OS as a result of multivariate analysis (Table 9).

Table 9
Univariable and multivariable analysis of BC survival using Cox's proportional hazards model within OS.

		Univariate analysis		Multivariate analysis	
Patients Descriptions	**Events/Total (%)**	**Hazard ratio (95% CI)**	p	**Hazard ratio (95% CI)**	p
Age group	21/84 (25)	1 (Reference)	**.002**	1 (Reference)	.055
<35 years	101/756 (13.3)	.476 (.297-.762)	.550	.598 (.354-1.012)	.914
35–50 years	241/1177 (20.4)	.873 (.559-1.363)		1.033(.569-1.876)	
> 50 years					
BMI	72/373 (19.3)	.919 (.710 – 1.190)	.524		
<25	291/1644 (17.7)				
≥25					
Menopause Age (mean)	244/2017 (12.1)	1.003 (.977 – 1.030)	.832		.
Alive 48.35 years					
Death 48.14 years					
Menstruation Age (mean)	363/2017(18)	.939 (.870-1.015)	.112		
Alive 13.15 years					
Death 13.11 years					
Menstruation situation	114/787 (14.5)	1.582 (1.266–1.976)	**< .001**	.665 (.679-1.403)	.966
Premenopause	244/1217 (20)				
Postmenopause					
Number of births	28/162 (17.3)	1 (Reference)	.745		
No birth	209/1320 (15.8)	.937 (.631-1.389)	.214		
1–2 birth	120/520 (23.1)	1.298 (.860-1.958)			
3 and more					
Family History	87/632 (13.8)	.709 (.557-.902)	**.005**	.902 (.696-1.168)	.434
Positive	276/1385 (20)				
Negative					
Breast-feeding	224/1192 (18.8)	1.156 (.935 – 1.430)	.180		
Positive	139/825 (16.8)				
Negative					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Breast site	185/1013 (18.3)	1 (Reference)	.995		
	178/935 (19)				
	14/69 (20.3)				
Left		.975 (.794-1.198)	.811		
Right					
Bilateral					
Location	363/1948 (18.6)	1 (Reference)	.484		
Unilateral		.778(.401-1.509)	.458		
	9/46 (19.6)				
Metacron		1.524 (.630-3.687)	.350		
	5/23 (21.7)				
Sencron					
Tumor Quadrant	73/402 (18.1)	1 (Reference)	.987	1 (Reference)	.948
Inner	210/1205 (17.4)	1.002 (.768-1.308)	.797	.990 (.734-1.335)	.569
Outer		.951 (.651-1.391)	**.011**	.884 (.577-1.353)	.155
	42/259 (16.2)				
Periareolar		1.659 (1.121–2.456)		.713 (.448-1.136)	
	38/150 (25.3)				
Multifocal					
Histopathologic Type	293/1652 (17.7)	1 (Reference)	.752		
IDC		.937 (.607-1.445)	.768		
	22/122 (18)				
ILC		1.109 (.817-1.504)	.508		
	48/243(19.7)				
Other					
Surgical Type	93/1016 (9.15)	1 (Reference)	**< .001**	1 (Reference)	.279
BCS	221/930 (23.8)	2.344 (1.839–2.987)	**< .001**	1.204 (.873-1.599)	.697
MRM	49/71(69)	19.760 (13.887–28.117)		1.154(.561-2.374)	
No surgery					
Axillary surgery	21/451(4.7)	1 (Reference)	**< .001**	1 (Reference)	.128
SLND	286/1477(19.4)	3.040 (1.950–4.741)	**< .001**	1.466 (.896 – 2.400)	**.004**
AD	56/89(62.9)	22.238 (13.458–36.747)		3.251 (1.451–7.283)	
No aksiller surgery					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Stage	22/415 (5.3)	1 (Reference)	**.001**		
I	108/881 (12.3)	2.199 (1.390–3.477)	**< .001**		
II	150/583 (25.7)	5.085 (3.250–7.954)	**< .001**		
III	83/138 (60.1)	26.548 (16.530-42.638)			
IV					
T Stage	57/670 (8.5)	1 (Reference)	**< .001**	1 (Reference)	**.002**
T1	183/1048 (17.5)	2.110 (1.567–2.841)	**< .001**	1.719 (1.227–2.410)	**.018**
T2	37/155 (23.9)	2.571 (1.699–3.889)	**< .001**	1.749 (1.099–2.786)	**.025**
T3	85/143 59.4()	12.764 (9.091–17.920)		1.843 (1.081–3.143)	
T4					
Infiltrated Axillary Node Count	88/861 (10.2)	1 (Reference)	.228	1 (Reference)	.556
0	69/531 (13)	1.214 (.886-1.664)	**< .001**	.897 (.624-1.289)	.084
1–3	122/402 (30.3)	3.710 (2.819–4.882)	**< .001**	1.390 (.957-2.018)	.657
4–9	83/222 (37.4)	4.563 (3.379–6.161)		1.099 (.726-1.662)	
≥10					
Metastasis site	117/1627(7.19)	1 (Reference)	**< .001**	1 (Reference)	**< .001**
None	73/142 (51.4)	8.934 (6.667–11.972)	**< .001**	5.123 (3.696-7.100)	**< .001**
Bone	15/25 (60)	11.240 (6.562–19.252)	**< .001**	4.350 (2.361–8.015)	**< .001**
Lung	10/15 (66.6)	14.344 (7.513–27.385)	**< .001**	10.520 (5.270-20.999)	**< .001**
Liver	20/21 (95.2)	23.899 (14.826–38.522)		7.798 (4.372–13.909)	
Brain	126/185 (68.1)	15.101 (11.720-19.458)		5.059 (3.710–6.899)	
Multiple organs					
Skin infiltration	83/152 (54.6)	6.585 (5.127–8.459)	**< .001**	2.093 (1.359–3.223)	**.001**
Positive	280/1865 (15)				
Negative					
Surgical margin	73/367 (19.9)	1.427 (1.103–1.846)	**.007**	1.236 (.922-1.656)	.156
Positive	290/1650 (17.6)				
Negative					

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

		Univariate analysis		Multivariate analysis	
Grade	28/304 (9.2)	1 (Reference)	**.004**	1 (Reference)	.074
1	148/987 (15)	1.805 (1.205–2.704)	**< .001**	.656 (.413-1.042)	**.012**
2	187/726 (25.8)	3.484 (2.341–5.185)		.535 (.330-.870)	
3					
Mitotic index	47/775 (6)	1 (Reference)	**< .001**	1 (Reference)	**.006**
1	57/637 (8.9)	2.157 (1.462–3.182)	**< .001**	1.819 (1.182–2.799)	**< .001**
2	256/591 (43.3)	12.288 (8.955–16860)		5.904 (4.086–8.532)	
3					
ER receptor	254/1598 (15.9)	.578 (.462-.723)	**< .001**	.758 (.410-1.404)	.379
Positive	109/419 (26)				
Negative					
PR receptor	213/1337 (15.9)	.641 (.520-.790)	**< .001**	.990 (.711-1.378)	.950
Positive	150/680 (22)				
Negative					
Ki67	183/1130 (16.2)	2.025 (1.636–2.507)	**< .001**	2.627 (1.478–4.670)	**.001**
<15	179/885 (20.2)				
≥15					
HER2	98/478 (20.5)	1.500 (1.188–1.894)	**.001**	1.154 (.729-1.827)	.541
Positive	265/1539(17.2)				
Negative					
EIC	90/334 (27)	1.815 (1.430–2.304)	**< .001**	1.193 (.879-1.621)	.258
Positive	273/1683 (16.2)				
Negative					
LVI	187/954 (19.6)	1.242(1.011–1.527)	**.039**	1.099 (.844-1.431)	.484
Positive	176/1063 (16.5)				
Negative					
PNI	97/437 (22.1)	1.215 (.963-1.533)	.101		
Positive	266/1580(16.8)				
Negative					

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		Univariate analysis		Multivariate analysis	
Chemotherapy	42/324 (13)	1 (Reference)	.447	1 (Reference)	.340
None	57/235 (24.3)	.816 (.483-1.379)	**.03**	.774 (.458-1.309)	**.02**
Neoadjuvant	264/1458 (57.6)	.648 (.437-.959)		.628 (.424-.930)	
Adjuvant					
Chemotherapy Protocol	42/324 (13)	1 (Reference)	**.048**		
None	55/166 (33.1)	1.483 (1.004–2.192)	**.024**		
FAC	44/273 (16.1)	2.269 (1.113–4.627)	.194		
AC + TXT	216/1235 (17.4)	1.230 (.900-1.682)			
Other					
Radiotherapy	276/1757 (15.7)	.427 (.335-.543)	**< .001**	.885 (.637 – 1.230)	.467
Positive	87/260 (33.4)				
Negative					
Radiotherapy Type	87/1757 (15.7)	1 (Reference)	**< .001**		
No	48/587 (8.2)	.220 (.155-.313)	**< .001**		
Breast alone	228/1170(19.5)	.524 (.409-.672)			
Locoregional					
Tamoxifen period	262/1258 (20.8)	1 (Reference)	**< .001**	1 (Reference)	**.001**
No TMX	96/652 (14.7)	.539 (.426-.683)	**< .001**	.540 (.376–775)	**< .001**
TMX ≤ 5 years	5/107 (4.6)	.146 (.060-.354)		.141 (.075-.367)	
TMX > 5 years					
AI period	169/815 (20.7)	1 (Reference)	.079	1 (Reference)	**.003**
No AI	178/937 (19)	.828 (.671-1.022)	**< .001**	.612 (.442-.848)	**< .001**
AI ≤ 5 years	16/265 (6)	.193 (.116-.323)		.140 (.092-.259)	
AI > 5 years					

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		Univariate analysis		Multivariate analysis	
LHRH	324/1634 (19.8)	1 (Reference)	.765	1 (Reference)	.447
No LHRH		1.121 (.530 – 2.370)	**< .001**	1.402 (.587-3.345)	.987
≤2 years		.430 (.298-.622)		1.004 (.613-1.644)	
>2 years					
	7/35 (20)				
	31/343 (9)				
Subtyping2	45/142 (32)	1 (Reference)	**.001**	1 (Reference)	.751
HER2 enriched	49/236 (20.7)	.493 (.330-.737)	**< .001**	.900 (.471-1.722)	**< .001**
TNBC	178/952 (18.7)	.368 (.266-.510)	**< .001**	10.551 (2.956–37.668)	.548
Luminal A	91/688 (13.2)	.391 (.275-.557)		1.268 (.584-2.755)	
Luminal B					
HER2 enriched could get Herceptine	33/120 (27.5)	2.109 (1.121–3.965)	**.021**		
HER2 enriched did not get Herceptine	14/22 (63.6)				

BMI, body mass index; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; HER-2, human epidermal growth factor receptor 2, LVI, lymphovascular invasion, PNI, perineural invasion, TNM, tumor-node-metastasis staging system based on the system of the American Joint Committee on Cancer, EIC, Extensive intraductal component

Discussion

Retrospective data measure the efficacy of treatments while also helping to test their impact on prognostic factors and subgroups. Similar to old age, its strength comes from experience, from knowing what might happen in the future. The need to group our series in this way and to find the subgroup with the worst prognosis indicated the inconsistency of our patient-specific experiences and the literature information. Patient follow-up in our series was carried out meticulously and regularly by the same physicians. Since the patient-file information was reliable and complete in our study, it is remarkable in terms of its results, although it comprised retrospective data. Although HER2-targeting antagonists have revolutionized the treatment of HER2-overexpressing BC and have produced a better clinical outcome for the HER2-enriched subgroup, it was still identified as the subgroup with the lowest DFS and OS in our series.

Herceptin reduced the risk of the event 1.5 times in the HER2-enriched subgroup, which we determined to be the subgroup with the worst prognosis (p = .223, HR 1.515 (95% CI 777-2.954)). The HER2-enriched subgroup had a 10-fold increased risk of overall survival compared to the Luminal A subgroup.

Foulkes WD et al., in the TNBC article by [9], stated that the subgroup with the lowest survival was TNBC, even though the lowest survival subgroup was seen as HER2 enriched in the survival curve. In the article, it was stated that the HER2-enriched subgroup had the lowest survival rate, and the curve was the subgroup with the lowest survival since these patients did not use targeted therapies. However, the subgroup that showed the lowest survival despite receiving

targeted therapy in our series was the HER2-enriched subgroup. In addition, while there was no statistically significant difference between Luminal A and Luminal B in both DFS and OS times of patients with TNBC in our series, we found that the survival times were significantly better than those of the HER2-enriched subgroup.

Overexpression of HER2 accounts for 20–30% of all BCs. The rate in our series was 23.7%. Activation of the HER2 receptor via tyrosine phosphorylation (20) results in increased proliferation, which is associated with increased relapse rates and increased mortality. Although HER2 expression is a critical event in the etiology of HER2-positive BC, the molecular mechanisms that regulate disease progression and how and why drug resistance develops in a short time are still not fully understood [15–17].

In 1987, Slamon et al. [18] reported that patients with BC in whom HER2 amplification was detected had a significantly shorter relapse and overall survival times [15, 18]. Moreover, amplification was also associated with negative ER or PR status [18].

We know that the estrogen receptor activates the HER2 receptor signaling pathway [17, 20–23]. This may make trastuzumab treatment more effective, as it brings with it the use of antiestrogen (TMX, AI) [22, 23]. In the HER2-enriched subgroup, in which estrogen and progesterone receptors are negative and only HER2 is overexpressed, the efficacy of treatment was limited to only trastuzumab, which may cause the HER2-enriched group to have a worse prognosis. While treatments for HER2 have revolutionized the treatment of HER2-overexpressing BC, the HER2-enriched subgroup still had the lowest survival rate in our series.

In addition, when the parameters used to create the subgroup were taken into the Cox regression analysis one by one, we found that the Ki67 ≥ 15 level negatively affected overall survival; in the multivariate analysis, the HR was 2.627 (1.478–4.670) p = .001, which is consistent with the literature [23, 24]. Another remarkable point in our series is that the use of TMX for more than 5 years reduced both relapse and mortality and the risk of death in AI [25–27].

Currently, as personalized treatments based on the principle that the patient, not the disease, should be treated are discussed and recommended, we can predict that subtyping classifications in BC will assume a much higher place in our future treatment plans and will continue to be a guide for clinicians in the long term. Our results show that subtyping captures most of the biodiversity occurring in BC.

Conclusion

Retrospective data measure the efficacy of treatments while also helping to test their impact on prognostic factors and subgroups. In our series, the HER2-enriched subgroup had the worst prognosis despite receiving targeted therapy. The belief that targeted therapies solve all problems may prevent clinicians from identifying patients with the worst prognosis. However, treatment with trastuzumab increased survival 1.5-fold over that of the HER2-enriched subgroup that did not receive it. Therefore, the HER2-enriched subgroup is a subgroup that needs to be followed carefully, and new treatment options are needed.

Declarations

Ethical Approval and Consent to participate

The study was approved by the Institutional Review Board of our center (TUTF-BAEK 2021/406).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was not supported.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

R.C., A.O., and M.C.U. wrote the main manuscript, N.S. performed statistical tests, E.T. performed pathological evaluation, S.U, I.C., D.N., T.O., S.D., and G.Y. collected the data, R.C., N.S., and A.O. prepared all figures and tables. All authors reviewed the manuscript.

Acknowledgments

Not applicable.

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Figures

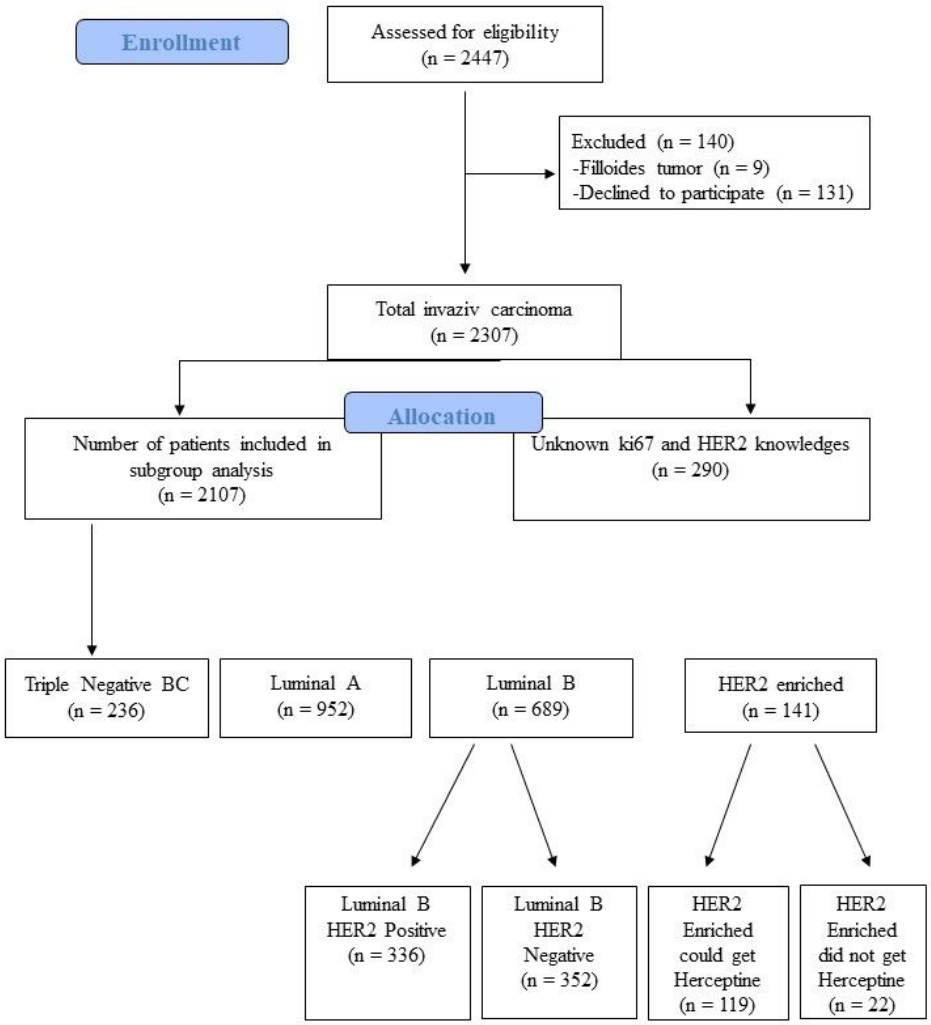

Figure 1

Distribution of BC patients in our series by subtyping

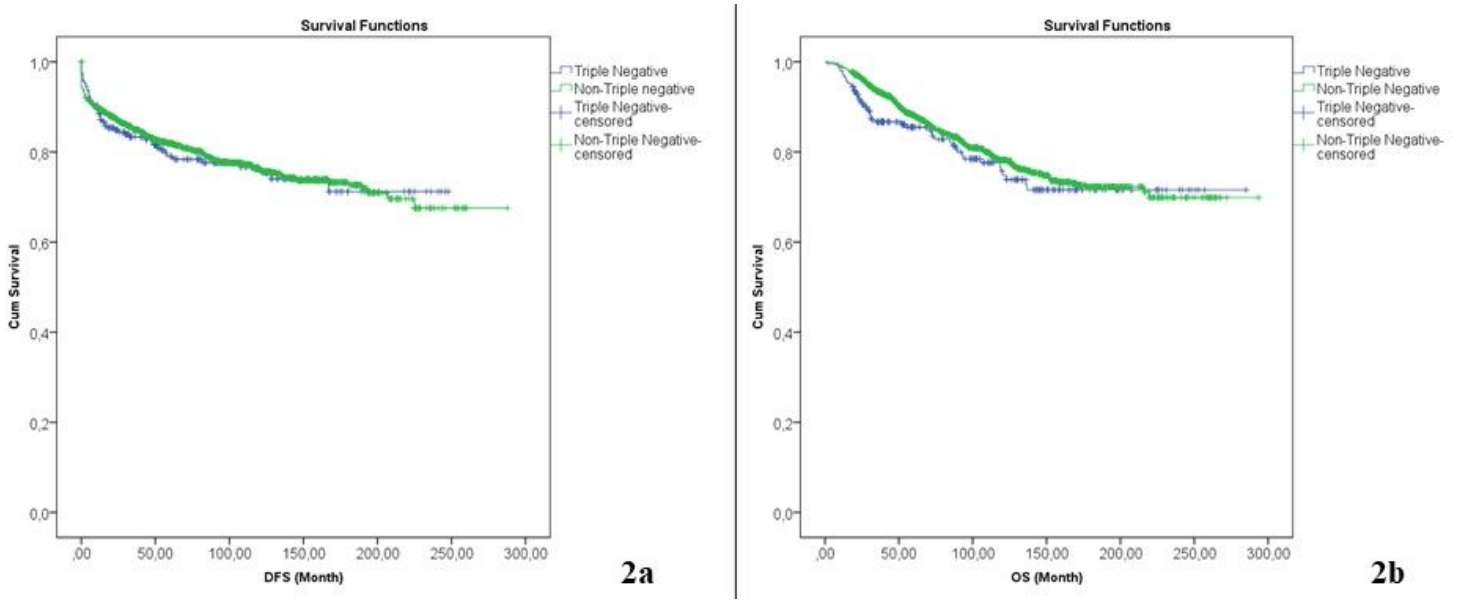

Figure 2

Survival curve of DFS **(a)** and OS **(b)** for the TNBC and NTNBC subgroups producing subtype 1 using the Kaplan–Meier method.

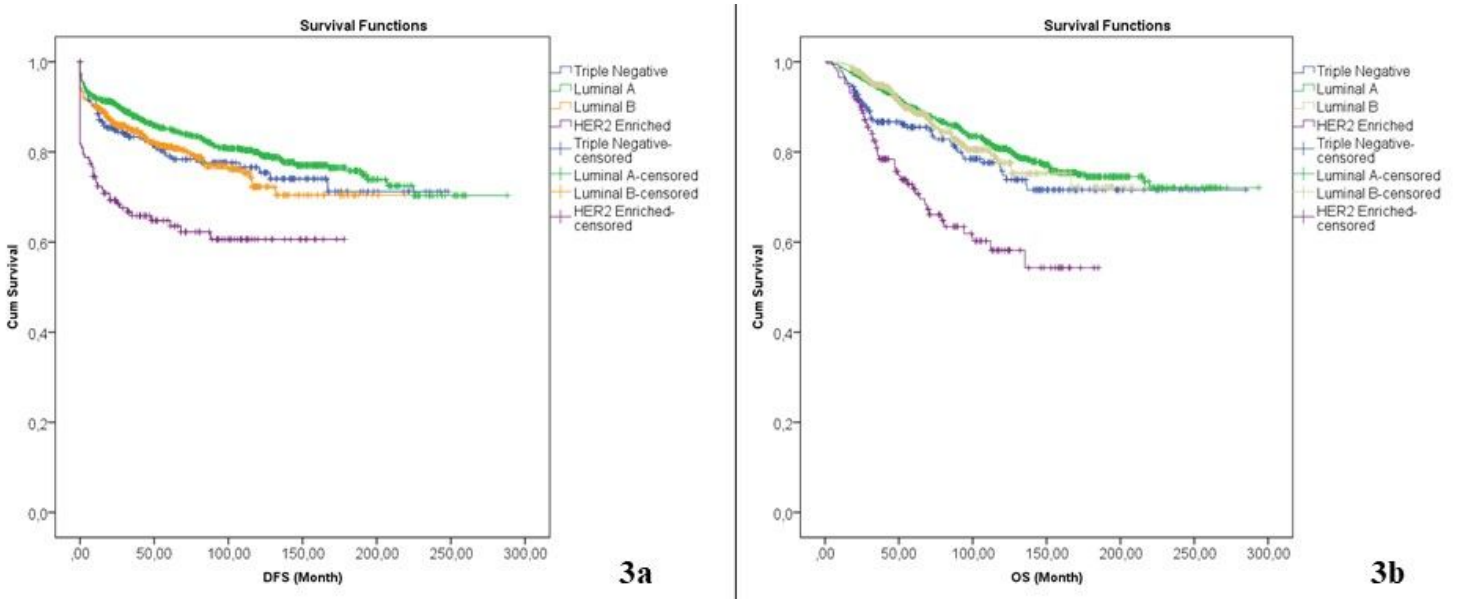

Figure 3

Survival curve of DFS **(a)** and OS **(b)** for TNBC, Luminal A, Luminal B, and HER2-enriched subgroups producing subtype 2 using the Kaplan–Meier method.

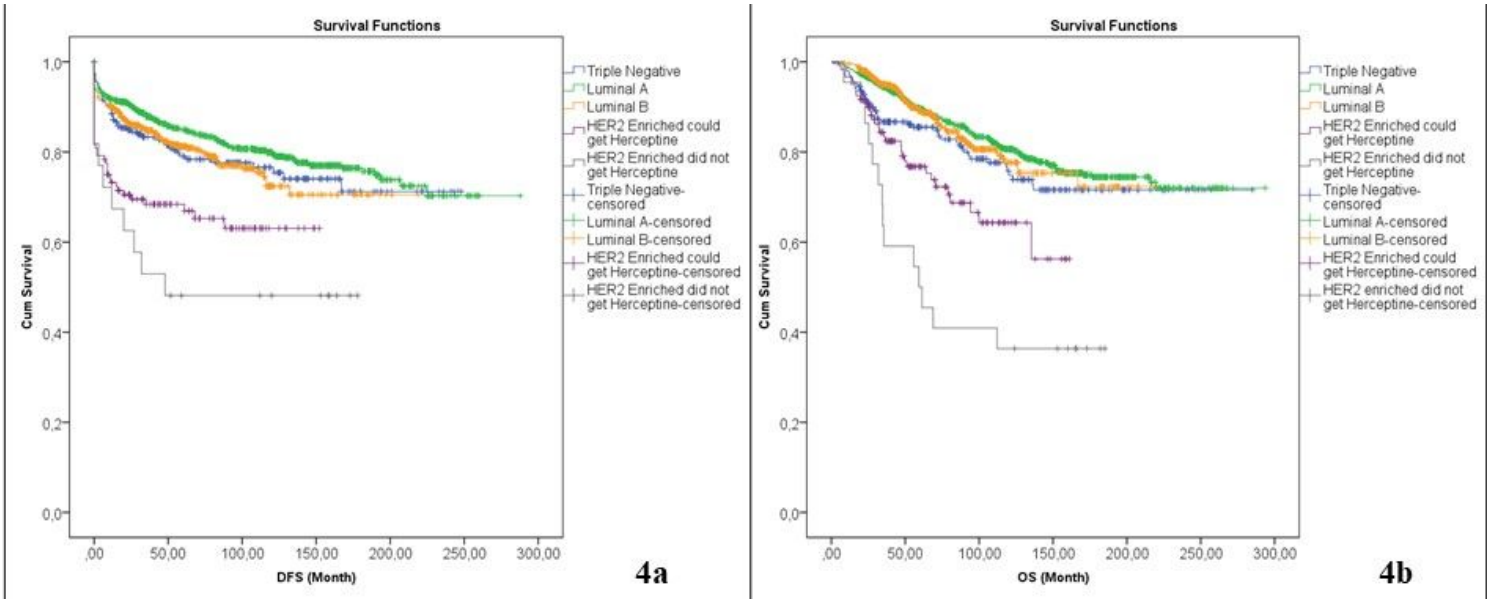

Figure 4

Survival curve of DFS **(a)** and OS **(b)** for TNBC, Luminal A, Luminal B, and HER2 -enriched subgroups that received Herceptin and the HER2-enriched subgroups that did not receive Herceptin producing subtype 3 using the Kaplan–Meier method.

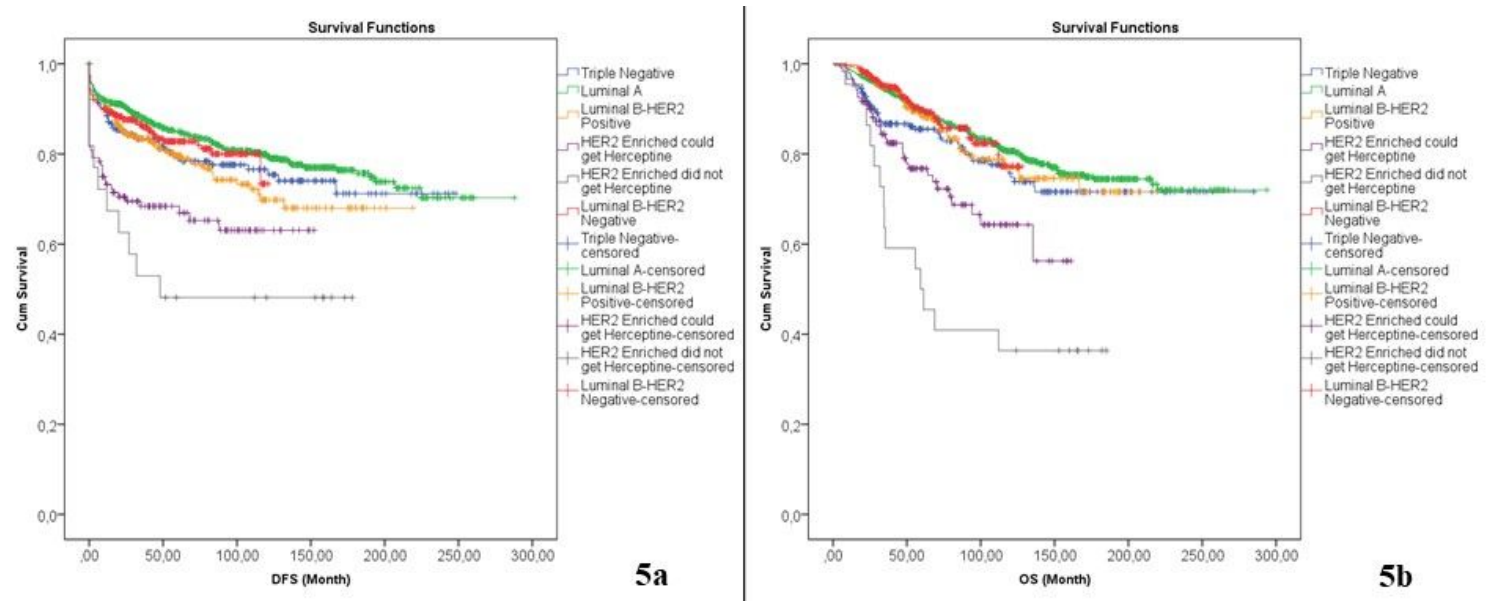

Figure 5

Survival curve of DFS **(a)** and OS **(b)** for the TNBC, Luminal A, and Luminal B subgroups that received Herceptin, the Luminal B subgroups that did not receive Herceptin, the HER2-enriched subgroup that received Herceptin, and the HER2-enriched subgroup that did not receive Herceptin, producing subtype 4 using the Kaplan–Meier method.

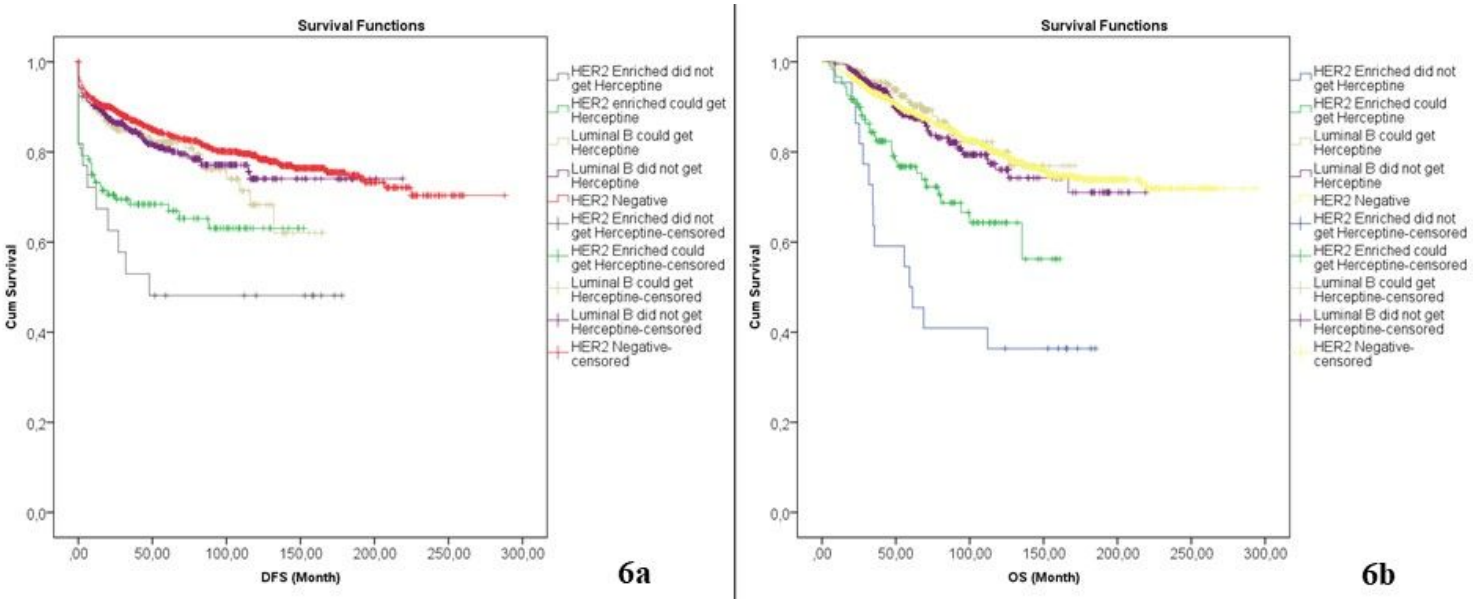

Figure 6

Survival curves of DFS **(a)** and OS **(b)** for the HER2-negative, Luminal B subgroup receiving Herceptin, the Luminal B subgroup that did not receive Herceptin, the HER2-enriched subgroup that received Herceptin, and the HER2-enriched subgroup that did not receive Herceptin, producing subtype 5 using the Kaplan–Meier method.