

## RESEARCH ARTICLE

# Patients with HER2-positive Early Breast Cancer Receiving Adjuvant Trastuzumab: Clinicopathological Features, Efficacy, and Factors Affecting Survival

Arife Ulas<sup>1\*</sup>, Tugba Kos<sup>2</sup>, Nilufer Avci<sup>3</sup>, Erdem Cubukcu<sup>4</sup>, Omer Fatih Olmez<sup>4</sup>, Nilufer Bulut<sup>4</sup>, Mustafa Degirmenci<sup>4</sup>

### Abstract

**Background:** The aim of the present study was to evaluate clinicopathological characteristics of our early stage breast cancer patients who are epidermal growth factor receptor 2 (HER2) overexpressed/ amplified (HER2+), the efficacy of trastuzumab treatment and survival results. **Materials and Methods:** Patients with HER2- positive early stage breast cancer receiving adjuvant trastuzumab were investigated retrospectively. Clinicopathological features of 210 patients and treatment outcome were analysed. To evaluate survival rates, the Kaplan-Meier method was used. Univariate and multivariate analyses were conducted with the Cox regression model. **Results:** Mean age of the patients was 51.8, 71.9% being postmenopausal. Some 37.6% of patients were node negative, and 31% had T1 tumor size and 52.4% were positive for estrogen receptor. Of 210 patients, 89.5% completed planned 52 weeks adjuvant trastuzumab treatment. The median follow up was 27.5 months (6.0-86.0). Relapse free survival (RFS) was 68.0 months (95% CI: 62.1-74.0) and overall survival (OS) was 74.8 months (95% CI: 69.5-80.1). The 3 year OS for all patients was 92.0% and RFS was 79.6%. During follow up, relapse was detected at the rate of 14.3%. Trastuzumab associated cardiotoxicity was found at the rate of 3.3%. In univariate analyses, larger tumor size and grade III were significantly associated ( $p < 0.05$ ) with RFS. Multivariate analyses of covariates displaying  $p < 0.05$  identified grade III as an independent prognostic factor. **Conclusions:** In the present study, it was established that trastuzumab had a satisfactory safety profile and treatment efficacy as in other clinical studies and that among clinicopathological factors evaluated, only being grade 3 had a significant effect on RFS. The occurrence of relapse with adjuvant trastuzumab makes it necessary to identify molecular predictors, which will define this group better and help explain resistance to anti HER2 based therapies.

**Keywords:** Adjuvant chemotherapy - trastuzumab - HER2 - breast cancer - grade III

*Asian Pac J Cancer Prev*, 16 (4), 1643-1649

### Introduction

Breast cancer is a heterogenous disease. Gene expression studies have identified five distinct molecular subtypes of breast cancer. The use of gene expression profiling is currently limited in many clinical settings, and hence histopathological markers such as estrogen receptor (ER), progesterone receptor (PR), and HER2 are used as surrogates for the molecular subtypes, which divide breast tumors into distinct phenotypes with distinct outcomes (Perou et al., 2000).

The gene for the human epidermal growth factor receptor2 (HER2) encodes for a transmembrane tyrosine kinase receptor protein. HER2 has both prognostic and predictive importance for invasive breast cancer. In approximately 15-25% of patients with breast cancer, HER2 gene is amplified and is associated with increased

locoregional recurrence and breast cancer mortality (Slamon et al., 2001; Gabos et al., 2010).

Trastuzumab is a humanized monoclonal antibody developed against extracellular domain of HER2. It has been reported that HER2 blockage made with the addition of trastuzumab to standard chemotherapy regimes increases time to progression and overall survival (OS) significantly in patients with metastatic breast cancer (Salamon et al., 2001; Abe et al., 2013; Zhu et al., 2013). Major studies carried out subsequently on adjuvant trastuzumab demonstrated that, in over 13.000 participants, it decreased disease recurrence and improved disease free survival (DFS) and OS (Baselga et al., 2006). Results of the Herceptin Adjuvant (HERA) trial at 2 years of median follow-up demonstrated that 1-year adjuvant trastuzumab treatment significantly improved rates of DFS and OS in comparison to observation group, with a significant benefit

<sup>1</sup>Department of Medical Oncology, <sup>1</sup>Ankara Atatürk Training and Research Hospital, Ankara, <sup>2</sup>Faculty of Medicine, Duzce University, Duzce, <sup>3</sup>Balikesir Government Hospital, Balikesir, <sup>4</sup>Ali Osman Sonmez Oncology Hospital, Bursa, Turkey \*For correspondence: drarifeulas@hotmail.com

still observed at 4-year and 8-year median follow-up (Smith et al., 2007; Gianni et al., 2011; Goldhirsch et al., 2013). These findings confirm that adjuvant trastuzumab administered sequentially to chemotherapy is associated with a persistent benefit and remains an appropriate treatment modality in patients with HER2-positive early breast cancer. This effect was not observed only in one study (Spielman et al., 2009). Adjuvant trastuzumab was accepted as standard management in HER2 positive breast cancer patients from 2006.

In phase III studies, it was demonstrated that trastuzumab was well tolerated (Smith et al., 2007; Gianni et al., 2011; Goldhirsch et al., 2013). The main documented adverse event was cardiotoxicity. The risk of cardiotoxicity associated with trastuzumab was addressed in meta analyses (Bria et al., 2008; Dahabreh et al., 2008). In these metanalyses, decreased left ventricle ejection fraction (LVEF) and other cardiac events (including rare cases of symptomatic heart failure) were reported at the rates of 2.2% to 14%.

In our country, the use of trastuzumab as adjuvant treatment in HER2+ breast cancer patients was initiated in 2008. The aim of this retrospective study was to evaluate clinicopathological characteristics HER-2 positive early stage breast cancer patients treated according to HERA protocol in routine clinical practice and the efficacy of adjuvant trastuzumab and survival results in these patients.

## Materials and Methods

### Patients and Methods

The present study was carried out in oncology departments of two centers (Bursa Oncology hospital and Balıkesir public hospital, in Turkey) with the approval of ethics committee. HER2-positive early breast cancer patients diagnosed between January 2009 and January 2014 and who underwent resection and received adjuvant trastuzumab were evaluated retrospectively.

210 female patients who receive early stage adjuvant trastuzumab based treatment and who have immunohistochemically (IHC 3+) HER2 or with fluorescence in situ hybridization (FISH ratio  $\geq 2$ ) or with silver in situ hybridization (SISH), HER2 gene amplification were enrolled in the present study. HER2 was determined by immunohistochemistry (IHC) and scored from 0 to 3+. Scoring of 2+ was assigned when there was weak to moderate complete membrane staining in  $> 10\%$  of tumor cells, whereas scoring of 3+ consisted of uniform intense membrane staining of  $> 10\%$  of invasive tumor cells. SISH or FISH analyses were carried out for all HER2 IHC 2+ samples. HER2 was considered amplified if the ratio of HER2 gene chromosome 17 was  $\geq 2.0$ .

Patients who had metastasis at the time of diagnosis, those who have contralateral breast cancer and cancers other than breast cancer, and the ones who received neoadjuvant treatment were excluded from the study. Demographic characteristics of the patients, their histopathological data, ER and PR status, type of surgery, site of first relapse, and times of relapse, adjuvant chemotherapy and radiotherapy status were recorded.

Staging of the patients was made according to the American Joint Committee on Cancer (AJCC) criteria. Cardiotoxicity developing during treatment process was recorded. Cardiac function was monitored in all patients every 3 months by electrocardiogram, and LVEF was assessed by echocardiography. Cardiotoxicity was defined as a decrease in LVEF of  $> 10\%$  from baseline or a decrease to an LVEF  $< 50\%$  at any time.

Breast cancer first relapse events were registered as local, contralateral, or distant when they occurred, respectively, in the same breast as the first tumor, in the other breast, or in another part of the body (brain, bone,

**Table 1. Baseline Characteristics and Tumour Properties of Patients**

Variables	N (%)
Age (years)	
Mean age $\pm$ SD	51.8 $\pm$ 10.7
$\leq 35$	13 (6.2)
36-65	178 (84.8)
$> 65$	19 (9.0)
Menopause	
Premenopausal	59 (28.1)
Postmenopausal	151 (71.9)
Surgery	
Lumpectomy	57 (27.1)
Mastectomy	153 (72.9)
Stage	
IA	14 (6.7)
IB	8 (3.8)
IIA	75 (35.7)
IIB	51 (24.3)
IIIA	30 (14.3)
IIIB	14 (6.7)
IIIC	18 (8.6)
Histopathology	
Invasive ductal carcinoma	190 (90.5)
Invasive lobular carcinoma	6 (2.9)
Other*	14 (6.7)
Tumour size (mm)	
T1	65 (31.0)
T2	131 (62.4)
$\geq T3$	14 (6.7)
Number of lymph node metastasis	
0	79 (37.6)
1-3	61 (29.0)
4-9	36 (17.1)
$> 9$	31 (14.8)
Unknown	3 (1.4)
Estrogen receptor (ER)	
Negative	100 (47.6)
Positive	110 (52.4)
Progesterone receptor (PR)	
Negative	122 (58.1)
Positive	88 (41.9)
Hormone receptor status	
ER and PR negative	96 (45.7)
ER and/or PR Positive	114 (54.3)
Histologic grade	
1	6 (2.9)
2	77 (36.7)
3	109 (51.9)
Unknown	18 (8.6)

\*Mixed tumours, medullary tumours; Histologic grade; 1= Well differentiated; Grade 2= Moderately differentiated; Grade 3= Poorly differentiated

liver, lung, visceral, lymph nodes, and other organs). Relapse Free Survival (RFS) was defined as time from diagnosis to local, regional, or distant relapse, or death from breast cancer. Overall survival was defined as the time relapsing from diagnosis to death or final evaluation in the clinic and calculated accordingly.

#### Statistical analysis

Statistical data were analysed using, SPSS 15.0 software program. Fisher and chi square tests were used for nominal variables and numerical data. Kaplan–Meier analysis was employed for survival rates and comparisons were made with log-rank test. Univariate and multivariate analyses were evaluated with Cox regression model.  $p < 0.05$  value was considered statistically significant.

## Results

#### Patients characteristics

In this retrospective study, 210 HER2 positive early stage breast cancer patients were detected. The number of patients with IHC score 3+ was 156 (74.3%) and FISH analysis was not carried out. The number of patients with IHC score 2+ who were found to be HER2 amplification positive with FISH or SISH analysis was 54 (25.7%). Mean age of the patients was 51.8 and 71.9% was postmenopausal.

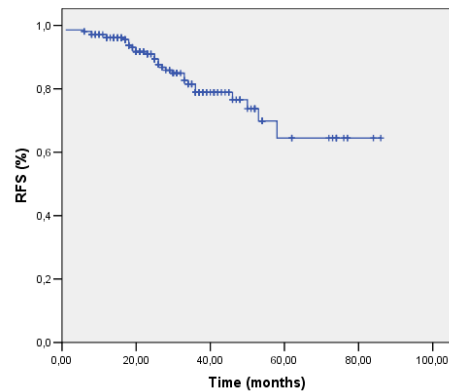
72.9% of the patients underwent radical mastectomy. 37.6% of patients had negative nodes and 31 had T1 tumor size and 52.4% was positive for ER receptor. The rate of either ER or PR positivity was 54.3%. As expected for HER2 positive carcinomas, tumors were mainly grade III (51.9%) and ductal carcinoma (90.5%). Table 1 lists the characteristics of patients and tumors.

#### Adjuvant treatments

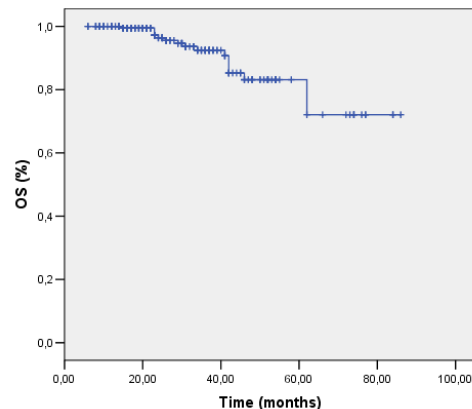
In all patients, adjuvant trastuzumab based treatment was planned for one year at the onset. As in the HERA trial, trastuzumab had been administered, mainly every 3 weeks,

**Table 2. Adjuvant Treatments**

Treatments	N (%)
Chemotherapy	
Antracyclines	51 (24.3)
Taxanes	6 (2.9)
Antracyclines+Taxanes	(72.9)
Hormone therapy	
Tamoxifen	47 (22.4)
Anastrozole	9 (4.3)
Letrozole	58 (27.6)
None	96 (45.7)
Radiotherapy	
Yes	166 (79.0)
No	44 (21.0)
Radiotherapy	
Concurrently with trastuzumab therapy	93 (56.0)
Simultaneous with trastuzumab therapy	73 (44.0)
Duration of Trastuzumab Therapy	
1 years	188 (89.5)
From $\geq 9$ to $< 12$ months	18 (8.5)
From $\geq 3$ to $< 9$ months	4 (1.9)



**Figure 1. Relapse Free Survival for Patients with HER2- Positive Early Breast Cancer Receiving Adjuvant Trastuzumab**



**Figure 2. Overall Survival for Patients with HER2-Positive Early Breast Cancer Receiving Adjuvant Trastuzumab**

in patients with primary breast cancer following surgery, adjuvant chemotherapy, and radiotherapy (if applicable). Trastuzumab treatment was commenced with an 8 mg/kg loading dose followed by a 6 mg/kg maintenance dose every three weeks for one year duration. 72.9% of the patients received taxane and anthracycline based chemotherapy while 24.3% received only anthracycline based chemotherapy and 2.9% only adjuvant taxane and trastuzumab treatment. 79.0% underwent radiotherapy treatment following mastectomy. 44% of the patients received radiotherapy along with trastuzumab. Finally, 44.3% received hormone treatment ( Table 2).

Cardiotoxicity developed in 7 (3.3%) patients during trastuzumab treatment. Of these patients, 6 were asymptomatic and cardiotoxicity was detected with echocardiography. It was established that cardiotoxicity developed after median 9 cycles (range, 3-15 cycles). When serious symptomatic heart failure was detected in one patient, her treatment was completely discontinued. All patients in whom cardiotoxicity developed were those who previously received anthracycline based chemotherapy.

#### Survival

The median follow up was 27.5 months (6.0-86.0 months). RFS was 68.0 months (95% CI: 62.1-74.0) and OS was found to be 74.8 months (95% CI: 69.5-80.1). The 3 year OS for all patients 92.0% and RFS was 79.6% (Figures 1 and 2).

**Table 3. Univariate and Multivariate Analyses of Parameters for Relaps Free Survival**

Parameters	Univariate analyses			Multivariate analyses		
	HR	95%CI	P	HR	95%CI	P
Menopause (yes vs. no)	0.6	0.3-1.3	0.24			
Surgery (mastectomy vs breast-conserving surgery)	0.6	0.2-1.4	0.25			
Histology (duktal carcinoma vs others*)	0.8	0.2-2.6	0.70			
Tumour size (>T1 vs ≤ T1)	2.8	1.1-8.0	0.05	1.4	0.5-4.2	0.54
Lymph nodes ( positive vs. negative)	2.1	0.9-4.9	0.80			
Estrogen receptor (positive vs. negative )	0.7	0.3-1.3	0.25			
Progesteron receptor (positive vs. negative )	0.2	0.3-1.4	0.26			
Hormone receptor status (positive vs. negative )	0.6	0.3-1.3	0.20			
Grade (III vs. II/I)	2.1	1.1-4.2	0.04	2.1	0.9-4.8	0.07
Anthracycline-taxanes vs. anthracycline-no taxanes	0.9	0.4-1.9	0.86			
Hormone therapy (yes vs no)	0.6	0.3-1.6	0.11			
Radiotherapy ( yes vs no)	0.7	0.3-1.9	0.54			
Concurrently vs. simultaneously with trastuzumab therapy	0.7	0.3-1.8	0.44			

\*HR, hazard ratio, \*Mixed tumors, medullary tumors

In hormone receptor (HR) positive patients (ER and / or PR positive), rate of 3 years OS was 93% and 3 years RFS was 87%. Among HR negative patients (ER neg and PR neg) 3 years OS was 90% and 3 years RFS was 71%. There was no significant difference between HR positive and HR negative patients ( $p < 0.05$ ). In lymph node positive patients, 3 years OS was 88% and 3 years RFS was 73% while in lymph node negative patients, the corresponding rates were respectively, 96% and 89%.

Of overall 210 patients, 188(89.5%) completed planned adjuvant one year trastuzumab treatment. Treatment was interrupted in 21 patients due to relapse during treatment process and in one patient to cardiotoxicity. During median 27.2 months follow up period, metastasis did not develop in 180 (85.7%) patients while local recurrence occurred in 8 (3.8%) patients and metastasis developed in 30 (14.3%) patients. In 14 (6.7%) patients, metastasis developed under trastuzumab treatment. In these patients, the initial site of metastasis was as follows: brain in 7 (3.3%) patients, bone in 7 (3.3%) patients, liver in 5 (2.4%) patients, lung in 5 (2.4%) patients, and skin in 3 (1.4%) patients. In addition, there was also thyroid metastasis concurrent with brain metastasis in one patient. In all patients, time to relapse was median 21.5 months (range; 1.4 to 53.0 months) and in those relapsing during trastuzumab treatment 6.2 months (range, 1.4 to 9.9 months).

In univariate analysis performed for RFS, no statistically significant relation with age, menopausal status, surgical method, histopathology, nodal involvement, estrogen receptor status, progesterone receptor status, chemotherapy regime with taxane or antracycline or with taxane and antracycline, hormonal treatment status, radiotherapy treatment. Univariate analyses indicated larger tumour size and grade III were significantly associated ( $p < 0.05$ ) with RFS. Multivariate analyses of covariates displaying  $p < 0.05$  identified grade III (HR=2.1, 95%CI 0.9 - 4.8,  $p = 0.07$ ) as independent prognostic factor (Table 3).

## Discussion

The treatment of breast cancer has advanced significantly since 1990, the most important advance

being trastuzumab treatment heralding a new era for HER2 positive breast cancer patients. In early stage HER2 positive breast cancer patients, the advent of adjuvant trastuzumab treatment improved outcomes considerably. In the present study, it was found that trastuzumab treatment was feasible and well tolerated in routine clinical practice.

In the present study, median duration of follow up was 27.5 months and, 3 year RFS and OS values were 79.6% and 92.0%, respectively. These survival results are consistent with those of HERA; which is the largest adjuvant trastuzumab study performed in Europe (Smith et al., 2007). All of our patients received trastuzumab treatment every three weeks following locoregional treatment and chemotherapy and 89.5% completed planned one year treatment. Only in one patient, was the treatment interrupted owing to drug associated toxicity. Compliance with treatment and safety was consistent with that reported in the literature (Smith et al., 2007; Palmieri et al., 2011; Campiglio et al., 2013; Tai et al., 2013).

Even though there is significant improvement in survival in HER2 positive patients with adjuvant trastuzumab treatment, relapse may occur quite frequently (Mariani et al., 2009). In the present study, the rate of relapse was 14.3%, which is higher than that reported in the previous studies. For example, in 2 year results of HERA, this figure was reported to be 12% (5). In another adjuvant trastuzumab study carried out with 284 patients, the rate of relapse was reported to be 10.3% and median time to relapse 8.7 months (Krell et al., 2011). In the present study, relapses were established to occur within the first six months.

The most common sites of metastasis were brain and bone in the present study. Sites of distant metastasis, recurrence pattern and CNS involvement were consistent with the findings of previous adjuvant studies (Piccart-Gebhart et al., 2005; Metzger-Filho et al., 2013). In HER2 positive breast cancer patients, CNS disease is an important source of concern. In a metaanalysis performed, significant increase was shown in the risk of brain metastasis (Bria et al., 2008). In patients who received adjuvant trastuzumab, the first event was CNS metastasis while in those who do not receive this treatment, metastasis occurred in other

sites earlier (Romond et al., 2005). Probable explanation may be that the ability of trastuzumab to control systemic disease is stronger in areas other than CNS. The frequency of CNS metastatic disease may be linked to inadequate passage of trastuzumab from blood brain barrier as reported in previous studies (Krell et al., 2011).

In the present study, bone metastasis was more common than that in other studies. In order to assess whether the effect of trastuzumab on bone metastasis is low, penetration of the drug to this tissue may be analysed. Owing to the heterogeneity of the tumor, confirming HER2 expression levels in the tumor cells in the bone may be useful. As reported in four year follow up results of the HERA study, in the present study trastuzumab exerted the most beneficial effect in liver recurrences (Metzger-Filho et al., 2013).

In HER2 positive early stage disease, data on subgroups with high risk of recurrence and death in spite of optimal treatment is not adequate (Dent et al., 2007; Mansell et al., 2009). In the present study, in the analysis of factors influencing the response to treatment and the risk of relapse, unlike other studies, no statistically significant relation was found between RFS and clinical and pathological parameters except for grade III tumor. These results are different from those of previous analyses on factors predicting relapse (Smith et al., 2007; Gianni et al., 2011). In previous studies on adjuvant trastuzumab, lymph node positivity and ER positivity were found to be predictive of DFS (Campiglio et al., 2013; Perez et al., 2011). In the updated analysis of NSABP B-31 and NCCTG N9831 studies for DFS, it was reported that hormone receptor positivity, tumor size over 2 cm and lymph node positivity were predictive of DFS. The combined data also showed the prognostic impact of hormone receptor status on DFS and OS in a multivariate analysis (Perez et al., 2014). Hormone receptor status is the most important discriminator of breast cancer subtypes (Prat and Perou, 2010). Although it is known that trastuzumab treatment is effective on both HR-positive and HR-negative patients, data suggests that HER2 positive disease was influenced from HR status (Vaz-Luis et al., 2013). In the analysis of National Comprehensive Cancer Network Breast Cancer Outcomes database, HER2 positive/HR negative disease had a higher risk of death within 5 years of initial diagnosis compared with patients with HR-positive / HER2-positive disease (Vaz-Luis et al., 2012). In another study, it was demonstrated that clinical benefit lasted longer in HR positive tumors (Vaz-Luis et al., 2013). In the present study, although HR positive patients had longer median RFS and OS compared to HR negative patients, the difference was not statistically significant.

Tumor size (T), defined as the largest diameter of the primary breast tumor, was early recognized as an important prognostic factor in breast cancer (Koscielny et al., 1984; Carter, Allen and Henson, 1989). Yet, in the present study, while tumor size was found to be influential on RFS in univariate analysis, its influence was not seen anymore in multivariate analysis.

Compatible with the present study, in NSABP B-31 and NCCTG N9831 studies 70% of the patients were

in grade 3, evaluated as a unfavorable prognostic factor (Perez et al., 2014). Histological grade, shows a very strong correlation with prognosis; patients with grade I tumours have a significantly better survival than those with grade II and III tumours ( $p < 0.0001$ ) (Elston and Ellis, 1991).

In the present study, the occurrence of relapse during adjuvant trastuzumab treatment may be ascribed to many factors. The development of relapse during adjuvant trastuzumab treatment indicates *de novo* trastuzumab resistance, hence trastuzumab treatment may not yield more benefit (Pohlmann, Mayer and Mernaugh, 2009). As reported previously, stronger effect of trastuzumab along with chemotherapy, the change in receptor status with exposure to trastuzumab, HER2 negative phenotype becoming more potential are among the probable causes cited (Mittendorf et al., 2009). In a study reporting relapse risk at the rate of 11% (Campiglio et al., 2013), it is proposed that as the distribution of pathological parameters is the same in early and late relapses, these parameters are not associated with trastuzumab resistance (Gianni et al., 2011; Campiglio et al., 2013). We also believe that lower number of patients in the present study and short duration of follow up may have influenced the results.

In the present study, the rate of cardiotoxicity associated with trastuzumab treatment was found to be 3.3%, which was similar to that reported in the previous meta-analyses (Smith et al., 2007; Suter et al., 2007; Bria et al., 2008; Dahabreh et al., 2008). All of our patients with the development of cardiotoxicity had previously received anthracycline based chemotherapy. The event was reversible in all but one of patients with the development of cardiotoxicity. Compliance with treatment and tolerability was quite satisfactory. In a recent population based observational study, it was reported that the risk in cardiotoxicity was obvious in trastuzumab treatment following anthracycline and it became more marked over time (Bowles et al., 2012). In patients receiving RT concurrent with trastuzumab, no short term cardiotoxicity was observed, as has been reported by others (Shaffer et al., 2009). Since the majority of patients receive trastuzumab following anthracycline based regimen, cardiac monitorization is imperative during treatment in clinical practice. Long term monitorization is necessary in order to evaluate probable long term toxicity.

Retrospective design of the present study, relatively small sample size and short duration of follow up are among its limitations. In addition, it was not possible to reevaluate receptor status in tumor samples. We reported our findings based upon the fact that majority of early adjuvant studies reported two year outcomes. We believe that evaluation of response to treatment and cardiotoxicity in the present study is more reliable, as it included data only in two oncology centers, unlike the previous multicenter studies, and treatment and follow up was carried out at the same center.

In conclusion; as reported in the previous studies of adjuvant trastuzumab, it was demonstrated in the present study that survival results were improved especially in HER2-positive early stage breast cancer, which is especially aggressive and known to recur within a few

years of the diagnosis. Satisfactory safety profile and treatment efficacy in the present study were in keeping with that reported in previous clinical studies. Of all classical prognostic factors, only tumor grade 3 was found to have prognostic significance. The occurrence of relapse with adjuvant trastuzumab clearly indicates the need for determining molecular predictors, which will define this group better, help to avoid unnecessary treatment and explain the resistance to HER2 based treatment better. In the future, larger prospective studies, which will evaluate the molecular characteristics of subtypes with relapse, are required.

## Acknowledgements

The authors have declared no conflicts of interest.

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