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CLINICAL TRIAL

Effect of adjuvant trastuzumab treatment in conventional clinical setting: an observational retrospective multicenter Italian study

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Abstract Clinical trials have shown the efficacy of trastuzumab-based adjuvant therapy in HER2-positive breast cancers, but routine clinical use awaits evaluation of compliance, safety, and effectiveness. Adjuvant trastuzumab-based therapy in routine clinical use was evaluated in the retrospective study GHEA, recording 1,002 patients treated according to the HERA protocol between March 2005 and December 2009 in 42 Italian oncology departments; 874 (87.23 %) patients completed 1-year trastuzumab treatment. In 128 patients (12.77 %), trastuzumab was withdrawn due to cardiac or non-cardiac toxicity (28 and 29 patients, respectively), disease progression (5 patients) or the clinician's decision (66 patients). In addition, 156 patients experienced minor non-cardiac toxicities; 10 and 44 patients showed CHF and decreased LVEF, respectively, at the end of treatment. Compliance and safety of adjuvant trastuzumab-based therapy in Italian hospitals were high and close to those reported in the HERA trial. With a median follow-up of 32 months, 107 breast cancer relapses were recorded (overall frequency, 10.67 %), and lymph node involvement, estrogen receptor negativity, lymphoid infiltration, and vascular invasion were identified as independent prognostic factors for tumor recurrence, indicating that relapses were associated with advanced tumor stage. Analysis of site and frequency of distant metastases showed that bone metastases were significantly more frequent during or immediately after trastuzumab (<18 months from the start of treatment) compared to recurrences in bone after the end of treatment

This study was conducted on behalf of the GHEA group. The members of the GHEA group are listed in Appendix.

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and wash-out of the drug (>18 months from the start of treatment) (35.89 vs. 14.28 %, $p = 0.0240$); no significant differences were observed in recurrences in the other recorded body sites, raising the possibility that the protection exerted by trastuzumab is lower in bone metastases.

Keywords Breast cancer · HER2 · Trastuzumab · Adjuvant therapy · Distant metastasis

Introduction

The HER2 gene is amplified and/or overexpressed in 15–25 % of breast cancers and is associated with an aggressive disease course [1–4]. Trastuzumab, a humanized monoclonal antibody targeting the extracellular domain of the transmembrane tyrosine kinase receptor HER2 [5], has been shown in phase III clinical trials to reduce the risk of relapse and death in patients with HER2-positive breast cancers when administered along with chemotherapy in an adjuvant setting [6–9]. Results of the Herceptin Adjuvant (HERA) trial at 2 years of median follow-up showed that 1-year adjuvant trastuzumab treatment significantly improved rates of disease-free survival (DFS) [hazard ratio (HR) = 0.63, 95 % CI 0.53–0.75; $p < 0.0001$) and overall survival (HR = 0.63, 95 % CI 0.45–0.87; $p = 0.0051$) [10] respect to observation group, with a significant benefit still observed at 4-year [11] and 8-year [12] median follow-up. These findings confirm that adjuvant trastuzumab given sequentially to chemotherapy is associated with a persistent benefit and remains an appropriate treatment modality in patients with HER2-positive early breast cancer. However, estimates provided by clinical trials for eligible patients may not be entirely applicable to all women receiving trastuzumab-based therapy in community practice, pointing to the need for observational studies as a tool to achieve a complete picture of compliance and safety of trastuzumab-based treatment.

The observational retrospective multicenter Italian study GHEA (Group HErceptin in Adjuvant Therapy) was designed to evaluate the adherence to trastuzumab treatment guidelines and the safety of adjuvant trastuzumab in Italian patients treated in routine clinical practice according to the HERA protocol. Indeed, the protocol of the HERA trial is a key reference for trastuzumab-containing chemotherapy regimens in treating HER2-positive breast cancer mainly carried out in an adjuvant setting in Europe, including Italy. As an additional parameter to estimate trastuzumab benefits in community practice, the GHEA study also sought to determine whether patients relapsing under trastuzumab treatment differ from those relapsing after the end of the treatment with respect to prognostic factors and site/frequency of distant metastases.

Patients and methods

Patients

The multicenter retrospective observational Italian study GHEA included 1,002 HER2-positive breast cancer patients treated with trastuzumab in an adjuvant setting in 42 Italian oncology departments from March 2005 to December 2009. The database, closed on 16 December 2010, was examined in follow-up. Oncology departments were recruited through a public call for Italian oncologic centers treating at least 50 breast cancers/year and with HER2-positive patients treated with adjuvant trastuzumab from March 2005 to December 2009. Patients who were considered eligible for adjuvant trastuzumab by the oncologist and receiving this treatment for at least one cycle in the indicated period were included. All primary tumors were scored 3+ by immunohistochemistry or 2+/FISH-positive prior to trastuzumab treatment in each institution. As in the HERA trial, trastuzumab was administered, mainly every 3 weeks, in patients with primary breast cancer following surgery, chemotherapy (neoadjuvant and/or adjuvant), and radiotherapy (if applicable). Histopathological data, chemotherapy treatments, type of surgery, site of first relapse, and times of relapse were recorded for each patient. Non-cardiac toxicity data, including haematological, gastrointestinal, respiratory, renal, liver, dermatological, neurological, and infective adverse events, as well as trastuzumab-associated cardiotoxicity, including congestive heart failure (CHF), decrease in LVEF, and alteration of conduction or pace, were registered. Adverse events were assessed clinically and by haematological and biochemical means throughout the treatment in each participating center. Cardiac function was monitored in all patients every 4 months by electrocardiogram, and LVEF was assessed by echocardiography or multiple gate acquisition (MUGA) scan. LVEF decrease of ≥10 percentage points from baseline to <50 % at any time was considered as adverse event. 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 [central nervous system (CNS), bone, liver, lung, visceral, lymph nodes, and other organs]. For patients with multiple synchronous sites of recorded events, the first-relapse site was hierarchically considered as distant, local, contralateral breast cancer, and second primary malignancies. Non-breast second primary tumors were considered as "other neoplasia". Relapses were subgrouped either as "early" when they occurred during the 1-year trastuzumab treatment and in the next 6 months or "late" when they occurred after the 18 months. DFS was defined as the time from start of trastuzumab treatment to the first event.

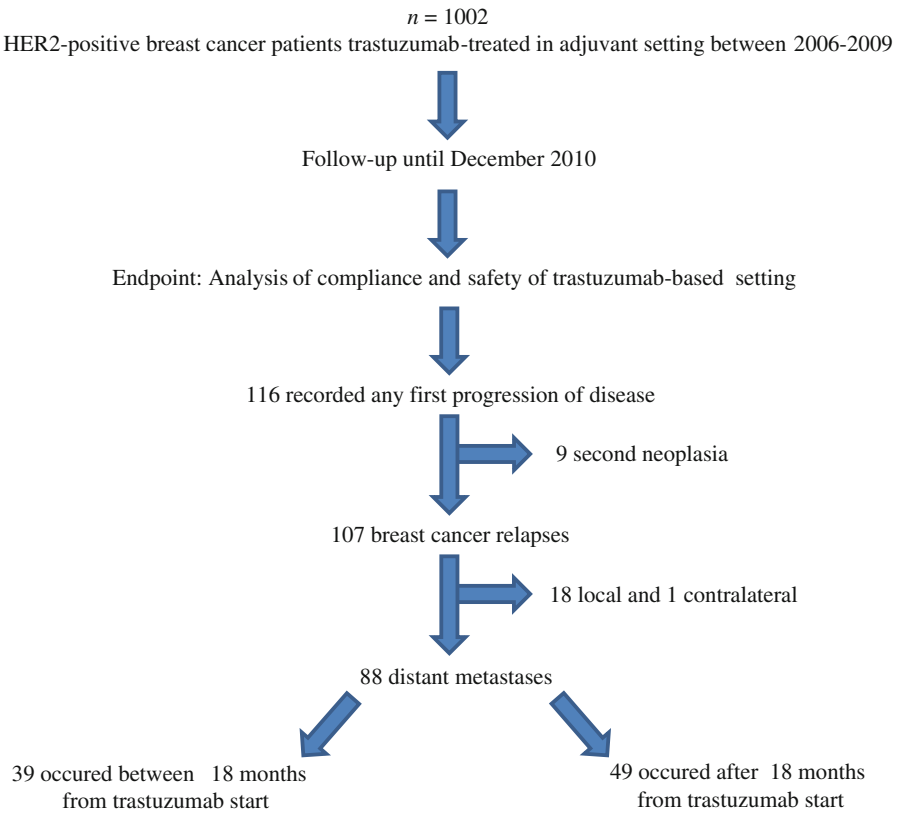

Fig. 1 CONSORT diagram of the Italian retrospective study

Comparison of the prognostic factors an d frequency of first distant metastases in two subgroups

To ensure rapid and homogeneous data-gathering for patients enrolled in the study, a web-based system was specifically developed to record patient clinical and demographical data. Structured forms for data entry using the Microsoft SQL server and with exclusive log-in and password for Internet access to insert and update all data by each participating center were created. The database was accessible to a single administrator who supervised and monitored data accrual in each center since the beginning of the project. At the end of the data entry period, access to the system was restricted for all participating centers. The Independent Ethics Committee of each participant institution approved the observational study.

Statistical analysis

Bivariate analysis was carried out for all variables with respect to relapses, using contingency tables evaluated by Chi square or Fisher's exact test. DFS analysis was carried out based on the life-table method [13] including any types of relapses.

Univariate analysis was carried out using the phreg SAS procedure with univariate Cox proportional hazards regression. All predictors with $p < 0.05$ were used in multivariate analysis with backward elimination, using the Cox regression model and evaluated by Chi square test. Assumption of proportionality was tested for all covariates by including time-dependent covariates in the model using the interaction with log(time). All analyses were conducted using SAS software (SAS Institute Inc., Cary, NC). Two-sided $p < 0.05$ was considered significant.

Results

We analyzed data from 1,002 HER2-positive breast cancer patients treated with trastuzumab in an adjuvant setting. Table 1 lists the characteristics of patients and disease. The median age was 52.9 years, 45.01 % of patients were node-negative, 44.01 % were both ER− and PgR-positive, and 17.16 % were positive for one of the two hormone receptors, and 53.69 % had T1 tumor size (54.85 % of T1 tumors were node-negative). As expected for HER2-positive carcinomas, tumors were mainly grade III (59.99 %) and of ductal histotype (85.43 %). All patients were treated with chemotherapy: 182 received neoadjuvant treatment,

Table 1 Baseline patient and tumor characteristics of 1,002 GHEA study patients

Characteristics	No. (%)
Age	
<35 years	35 (3.49)
35–49 years	358 (35.72)
50–59 years	303 (30.23)
>60 years	297 (29.64)
Missing	9 (0.89)
Median age*	52.9 (26–81)
Menopause	
Yes	488 (48.70)
No	298 (29.75)
Uncertain	33 (3.29)
Missing data	183 (18.26)
Nodal status	
N−	451 (45.01)
N+	522 (52.1)
*N*x	14 (1.39)
Missing data	15 (1.50)
Tumor size	
T1	538 (53.69)
T2	351 (35.03)
≥T3	94 (9.38)
Missing data	19 (1.90)
Histological grade	
III	601 (59.99)
II	323 (32.23)
I	13 (1.29)
Missing data	65 (6.49)
HER2 status	
HER2-positive 3+	834 (83.23)
HER2-positive 2+/FISH amplified	168 (16.77)
Hormone receptor status	
ER−/PgR−	377 (37.63)
ER−/PgR+	28 (2.79)
ER−/PgR unknown	2 (0.2)
ER+/PgR+	441 (44.01)
ER+/PgR−	133 (13.27)
ER+/PgR unknown	10 (1.0)
ER unknown/PgR unknown	10 (1.0)
ER unknown/PgR+	1 (0.10)
Surgery for primary tumors	
Mastectomy	406 (40.51)
Quadrantectomy	552 (55.09)
Lumpectomy	32 (3.19)
Missing data	12 (1.20)
Histological type	
Ductal	856 (85.43)
Lobular	52 (5.19)
Mixed	25 (2.50)
Other	63 (6.28)
Missing data	6 (0.6)
Therapy	
Neoadjuvant or adjuvant chemotherapy	
No anthracyclines	157 (15.67)
Anthracyclines-no taxanes	390 (38.92)
Anthracyclines-taxanes	455 (45.41)
Hormone therapy	
Yes	551 (54.99)
Aromatase inhibitors	333/551 (60.44)
Tamoxifen	218/551 (39.56)
Trastuzumab	
Every 3 weeks	980 (97.80)
Weekly	18 (1.80)
Unspecified time of administration	4 (0.40)
Time of trastuzumab treatment	
1-year	874 (87.23)
From ≥9 to <12 months	18 (1.80)
From ≥6 to <9 months	73 (7.28)
From ≥3 to <6 months	21 (2.09)
Less than 3 months	16 (1.60)

* Data are given as year (range)

N axillary lymph nodes, *T* tumor size, *ER* estrogen receptor, *PgR* progesterone receptor

862 received adjuvant treatment, and 42 both. Chemotherapy was mostly anthracycline-based (84.33 %) and 45.41 % of these patients also received taxanes. Hormone therapy was used in 54.99 % of patients, 60.44 %of whom received aromatase inhibitors. Almost all recorded patients (97.80 %) underwent trastuzumab administration every 3 weeks, with 1-year trastuzumab treatment completed in 874 (87.23 %) patients. Of 128 patients (12.77 %) who discontinued trastuzumab, 123 stopped for reasons other than relapse and 5 for relapse during therapy. Treatment withdrawal was due to cardiac and non-cardiac adverse events in 28 (20 for CHF and 8 for decreased LVEF), and 29 patients (10 for dermatological, 6 for haematological, 4 for gastrointestinal, 4 for neurological, 2 for infective, 1 for renal, 1 for liver, and 1 for respiratory adverse events), respectively, and to clinical judgment in 66 (Table 2). Toxicity was the most relevant factor leading to trastuzumab withdrawal in patients treated for <6 months, while the clinical decision was the major motivation to stop treatment in those who received trastuzumab for ≥6 but <12 months (Table 2). Of note, 80.36 % of these patients had T1 and/or N0 tumors. In addition, 156 patients experienced minor non-cardiac toxicities; of 701 patients who were monitored for cardiac toxicities past the end of

Table 2 Treatment compliance

Time of trastuzumab treatment	No. of cases	%	Reasons for withdrawal			
			Progression *N* (%)	Cardiac toxicities *N* (%)	Non-cardiac toxicities *N* (%)	Clinical decision *N* (%)
Less than 3 months	16	1.60	0	9 (56.25)	5 (31.25)	2 (12.50)
From ≥3 to <6 months	21	2.09	1 (4.76)	8 (38.10)	4 (19.04)	8 (38.10)
From ≥6 to <9 months	73	7.28	4 (5.48)	10 (13.70)	18 (24.66)	41 (56.16)
From ≥9 to <12 months	18	1.80	0	1 (5.55)	2 (11.11)	15 (83.34)
1 year	874	87.23	–	–	–	–
Total	1,002	100	5 (0.49)	28 (2.79)	29 (2.89)	66 (6.58)

Table 3 First recorded events

Events	No. (%) $n = 1{,}002$
Other neoplasia	9 (0.90)
Breast carcinoma events	107 (10.70)
Local	18 (1.80)
Contralateral	1 (0.10)
Distant metastases	88 (8.78)

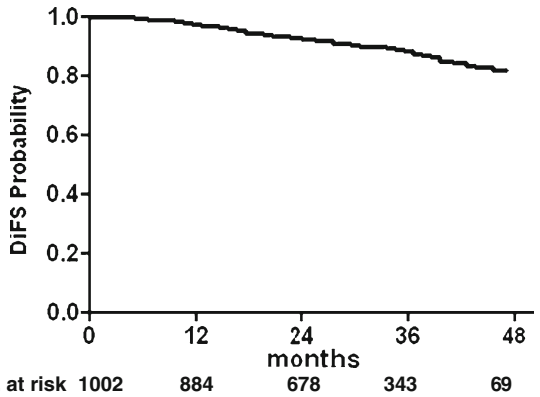

Fig. 2 Disease-free survival from date of first trastuzumab treatment in HER2-positive patients treated in an adjuvant setting

treatment, 10 and 44 patients showed CHF and decreased LVEF, respectively. In the entire series, LVEF was monitored by MUGA in only 20 patients. Non-cardiac toxicities included haematological, gastrointestinal, respiratory, renal, liver, dermatological, and neurological adverse events.

With a median follow-up of 32 months, 107 (10.68 %) breast cancer events, including local (1.80 %), contralateral (0.10 %), and distant recurrences (8.78 %), were registered (Table 3; Fig. 1). In addition, 9 (0.90 %) secondary non-breast primary tumors were observed (one thyroid, one lung, one renal, two endometrial, one leukemia, and three unspecified carcinomas). Figure 2 shows the DFS of our series calculated from the start of trastuzumab treatment. When breast cancer relapse occurring after the end of the treatment was analyzed according to time of trastuzumab treatment, five (13.51 %) recurrences were observed in 37 patients treated <6 months, 6 (6.59) in 91 patients treated from ≥6 to <12 months, and 74 (8.47) in 874 patients who completed the 1-year treatment.

Analysis of the clinical and pathological characteristics listed in Table 1 revealed an association between tumor recurrence and axillary lymph node involvement (*N*+) ($p < 0.0001$), larger tumor size ($p < 0.0001$), absence of estrogen receptors ($p < 0.0001$), and of progesterone receptors ($p = 0.0018$), vascular invasion ($p = 0.0005$), lymphoid infiltration ($p = 0.0023$), grade III ($p = 0.0026$), and chemotherapy regimens ($p = 0.0024$) (Table 4). Univariate analysis indicated that lymph node involvement, grade III, larger tumor size, lymphoid infiltration, vascular invasion, absence of hormone receptors, and treatment with anthracyclines plus taxanes were significantly associated ($p < 0.05$) with DFS (Table 5). A positive significant correlation of the anthracyclines plus taxanes regimen was observed with size and node positivity ($p < 0.0001$), indicating that patients with more advanced tumors received this therapeutic regimen. Multivariate analysis of covariates displaying $p < 0.05$ identified lymph node involvement (HR = 3.633, 95 % CI 1.600–8.249, $p = 0.0020$), presence of estrogen receptors (HR = 0.552, 95 % CI 0.314–0.968, $p = 0.0382$), lymphoid infiltration (HR = 1.912, 95 % CI 1.061–3.444, $p = 0.0310$), and vascular invasion (HR = 1.853, 95 % CI 1.028–3.338, $p = 0.0400$) as independent prognostic factors (Table 5).

Comparison of "early" recurrences (44 cases, including five local relapses) arising during or immediately after trastuzumab treatment (≤18 months from start of treatment) and "late" recurrences (63 relapses, including 13 local and 1 contralateral recurrences) occurring after the end of the treatment and wash-up of the drug (>18 months from start of treatment) revealed no significant differences in clinical and pathological parameters (Table 6).

Table 4 Baseline characteristics of non-relapsed or relapsed breast cancer patients

Parameters	Non-relapsed	Relapsed	p[a]
Age (year)	53 (26.81)	52 (30.80)	–
Postmenopause (no./total, %)	428/695 (61.58)	53/83 (63.85)	0.7213
ER-neg (no./total, %)	343/875 (39.20)	63/107 (58.90)	<0.0001
PgR-neg (no./total, %)	436/868 (50.23)	70/105 (66.7)	0.0018
Tumor size (no./total, %)			
T1	496/870 (57.01)	36/104 (34.61)	<0.0001
T2	305/870 (35.06)	42/104 (40.38)	
≥T3	68/870 (7.81)	26/104 (25.00)	
Vascular invasion (no./total, %)	220/655 (33.60)	43/80 (53.75)	0.0005
Lymphoid infiltration (no./total, %)	204/587 (34.75)	38/70 (54.28)	0.0023
Necrosis (no./total, %)	193/637 (30.30)	29/75 (38.67)	0.1481
Grade III (no./total, %)	518/830 (62.41)	77/99 (77.78)	0.0026
$N+$ (no./total, %)	448/873 (51.32)	81/105 (77.14)	<0.0001
Chemotherapy			
Anthracyclines-no taxanes	365/886 (41.20)	29/107 (27.10)	
Anthracyclines-taxanes	375/886 (42.33)	64/107 (59.81)	0.0024
No anthracyclines	146/886 (16.48)	14/107 (13.08)	

For missing data for each variable, see Table 1. Second non-breast primary tumors were excluded

ER estrogen receptor, *PgR* progesterone receptor, *T* tumor size, *N* axillary lymph nodes

[a] Fisher's exact test or Chi square test

Table 5 Univariate and multivariate analyses of parameters for disease-free survival (DFS) in 1,002 patients

Parameters	Univariate analysis			Multivariate analysis		
	HR	95 % CI	p	HR	95 % CI	p
Lymph nodes (pos vs. neg)	2.839	1.800–4.478	<0.0001	3.633	1.600–8.249	0.0020
Grade (III vs. II/I)	2.019	1.257–3.243	0.0037			
ER (pos vs. neg)	0.467	0.317–0.686	0.0001	0.552	0.314–0.968	0.0382
PgR (pos vs. neg)	0.533	0.355–0.799	0.0023			
Size (>T1 vs. ≤T1)	2.286	1.526–3.425	<0.0001			
Menopause (yes vs. no)	1.144	0.731–1.791	0.5566			
Necrosis (yes vs. no)	1.433	0.900–2.280	0.1296			
Lymphoid infiltration (yes vs. no)	2.351	1.468–3.765	0.0004	1.912	1.061–3.444	0.0310
Vascular invasion (yes vs. no)	2.172	1.400–3.372	0.0005	1.853	1.028–3.338	0.0400
Anthracycline-taxanes vs. anthracycline-no taxanes	2.452	1.484–4.058	0.0005			
No anthracycline vs. anthracycline- no taxanes	1.719	0.846–0.0494	0.1345			

HR hazard ratio, p Chi square test

In our series, bone (23.86 %) was the most frequent site of first distant metastasis, followed by CNS (17.05 %). Notably, comparison of the frequency of first distant metastases in the two subgroups (39 early vs. 49 late distant metastases) according to site (Table 7) indicated a significantly higher rate of bone metastases in early than in late recurrences (35.89 vs. 14.28 %, $p = 0.0240$) and a higher, although not statistically significant, incidence of CNS relapses (23.07 vs. 12.24 %). Metastases in the late versus early group were not significantly more frequent in liver (16.32 vs. 10.26 %), lung (18.37 vs. 10.26 %), other viscera (6.12 vs. 0.00 %), or lymph nodes (16.32 vs. 7.69 %); cases with multiple relapses at the time of diagnosis were equally distributed in the two subgroups (14.28 % in early vs. 10.26 % in late metastases).

Discussion

The observational study GHEA shows that trastuzumab treatment is feasible and well-tolerated in routine clinical practice. Indeed, the majority of the patients recorded in the study adhered to trastuzumab administration following locoregional treatment and chemotherapy as in the HERA trial, with injection every 3 weeks in 97.80 % of them and completion of the 1-year trastuzumab therapy in 874

Table 6 Baseline characteristics of relapsed patients according to the time of relapse

Parameters	Early recurrences (0–18 months) $n = 44$	Late recurrences (>18 months) $n = 63$	p[a]
Age (year, range)	52 (30–75)	51 (32–80)	
Postmenopause (no./total, %)	18/29 (62.07)	35/54 (64.81)	0.8150
ER-neg (no./total, %)	26/44 (59.09)	37/63 (58.73)	1
PgR-neg (no./total, %)	28/43 (65.12)	42/62 (67.74)	0.8348
Tumor size (no./total, %)			
T1	20/43 (46.51)	16/61 (26.23)	
T2	14/43 (32.56)	28/61 (45.90)	0.1005
≥T3	9/43 (20.93)	17/61 (27.87)	
Vascular invasion (no./total, %)	15/31 (48.39)	28/49 (57.14)	0.4952
Lymphoid infiltration (no./total, %)	18/33 (54.56)	20/37 (54.05)	1
Necrosis (no./total, %)	12/35 (34.28)	17/40 (42.50)	0.4872
Grade III (no./total, %)	32/40 (80.0)	45/59 (76.27)	0.8065
$N+$ (no./total, %)	32/43 (74.42)	49/62 (79.03)	0.6400
Chemotherapy			
Anthracycline-no taxanes	12/44 (27.27)	17/53 (26.98)	
Anthracycline-taxanes	26/44 (59.09)	38/63 (60.32)	0.9877
No anthracyclines	6/44 (13.64)	8/63 (12.70)	

[a] Fisher's exact test or Chi square test

Table 7 Metastatic sites of first distant relapse in early and late recurrences

Site of distant relapse	Early[a] (0–18 months) $n = 39$ (%)	Late[a] (>18 months) $n = 49$ (%)	p[b]
Central nervous system	9 (23.07)	6 (12.24)	0.2545
Bone	14 (35.89)	7 (14.28)	0.0240
Liver	4 (10.26)	8 (16.32)	0.2927
Lung	4 (10.26)	9 (18.37)	0.3712
Visceral	0 (0)	3 (6.12)	0.2512
Lymph node	3 (7.69)	8 (16.32)	0.3333
Multiple	4 (10.26)	7 (14.28)	0.7484
Unspecified	1 (2.56)	1 (2.04)	1

[a] Data are given as number and percent

[b] Fisher's exact test or Chi squared test

(87.23 %) patients. The treatment discontinuation rate was higher in our series than in the HERA trial [6, 10] due to 6.587 % of patients who stopped trastuzumab not for toxicity but for clinical and/or patient decision, while the frequency of adverse events accounting for trastuzumab withdrawal was similar to that in the HERA trial.

While toxicities were the major reason for treatment withdrawal within 6 months, later withdrawal reflected the clinician's judgment that for patients at low risk of recurrence, the risk of adverse cardiac events could be minimized by reducing the treatment length without compromising efficacy. Indeed, less than 1-year of trastuzumab is reportedly active in decreasing relapse rates [8]. Consistent with this hypothesis, of the 56 patients who stopped the treatment based on the clinician's decision, 80.36 % had T1 and/or N0 tumors. The low aggressiveness of these carcinomas is also demonstrated by the lower frequency of relapses after withdrawal compared with patients who stopped before 6 months of treatment. The decision against completing 1-year trastuzumab treatment is still rare and, in fact, was made for only about 7 % of the patients in Italian clinical practice.

Trastuzumab was withdrawn due to cardiac events in 28 patients (2.79 %), a rate similar to that in the HERA trial [14, 15]. However, another 54 patients experienced cardiac toxicity after the end of treatment, consistent with a recent population-based observational study [16] reporting that the risk of cardiotoxicity associated with trastuzumab following anthracycline appears to increase over time. This underscores the need for long-term surveillance of cardiotoxicity. Since trastuzumab followed anthracycline-based regimens in the majority of patients, clinical practice should include cardiac monitoring past the end of treatment.

We observed that patients treated with adjuvant trastuzumab in the Italian clinical practice were at an earlier stage of disease compared with those included in the

HERA trial. Overall, the 11 % of relapse events observed in the GHEA study is similar to the 13 % reported in the 2-year median follow-up of HERA [10], considering the earlier stage of disease of our patients. Recently published results [17] derived from 87 patients treated with adjuvant trastuzumab in a public hospital showed a compliance and safety similar to that of our study and of the HERA trial, further supporting the feasibility of this treatment outside clinical trials.

Our analysis of the role of pathobiological markers in increasing the risk of relapse suggests that the recurrences in trastuzumab-treated patients in clinical practice are associated with a more advanced stage of disease, consistent with the progressively decreased dependence of more advanced tumors on the HER2 signaling pathway [18]. Since maximum trastuzumab activity in clinical trials is reportedly restricted to the time of treatment and a few months thereafter [10], we hypothesize that relapses in this period are "truly" resistant, while later relapses that might have been sensitive when trastuzumab was present develop upon antibody wash-out. Thus, assuming that early relapses recorded in our study are associated with trastuzumab resistance, we speculate that the pathobiologic parameters analyzed are not involved in trastuzumab resistance since their distribution was similar in early and late relapses. This is consistent with other reports showing Forest plots unable to identify parameters predictive of resistance and useful in clinical practice; indeed, no association between response to trastuzumab and hormone receptor status, grade, node involvement, or size has been found [10, 11].

Notwithstanding the observational nature of our study and the limited number of cases of relapses in our series, we noted a significantly higher frequency of bone relapses during the trastuzumab treatment as compared to recurrences at that site after the end of treatment and wash-out of the drug. While the higher frequency of CNS metastatic disease [19] is consistent with the inability of trastuzumab to pass the blood–brain barrier and affect brain metastases [20–22], the lower activity of trastuzumab in preventing bone relapses should be of interest for clinicians to better direct choice of therapies in these patients. Proof of the low activity of trastuzumab in countering bone metastases awaits analysis of bone relapses occurring during and after trastuzumab treatment with respect to the observational HERA patient population. If proven, the basis of this low efficacy of trastuzumab on bone metastases warrants analyses to estimate the drug's penetration in this tissue, although antibodies can localize in bone [23], or to confirm in vivo the reduction of HER2 expression levels in tumor cells homing to the bone, as reported in some studies ([24] and references therein). These mechanisms might impair both cytotoxic and cytostatic effects of trastuzumab (reviewed in [4]), triggering resistance escape mechanisms through upregulation of other receptor activities or through perturbation of the bone microenvironment [25–27].

Our data pointing to low trastuzumab activity in bone relapses are consistent with its higher activity on visceral recurrences observed in the HERA trial with 1-year median follow-up [6], i.e., during or immediately after trastuzumab administration, and also at 4-year median follow-up, when the best trastuzumab-induced benefits were observed for liver recurrences [28].

Among other trials of trastuzumab in the adjuvant setting, only PACS06 reported relapses according to site in the randomized arms [29]; however, the lack of a statistically significant reduction in relapse risk by trastuzumab observed in that study precludes comparison with our results. Moreover, final overall survival analysis of the EGF104900 trial, a phase III randomized multicenter open-label study of lapatinib alone compared with lapatinib plus trastuzumab in patients with HER2-positive metastatic breast cancer, whose disease progressed during prior trastuzumab therapy, showed that patients with visceral but not those with bone disease significantly benefited from the addition of trastuzumab to lapatinib treatment [30].

In conclusion, our data revealed high compliance of adjuvant trastuzumab treatment in Italian oncology clinics, a satisfactory trastuzumab safety profile, and a treatment efficacy close to that observed in clinical trials. Our results indicating a significantly higher frequency of bone metastases in the first 18 months from the start of trastuzumab treatment merit-specific future analyses to address the possibility that trastuzumab has low efficacy in bone metastases.

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Conflict of interest GM has received fees as an invited speaker to oncology meetings from Roche, Celgene, Elsai, Novartis, Glaxo, Agendia and Alphagenetics. All remaining authors have declared no conflict of interest.

Appendix

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