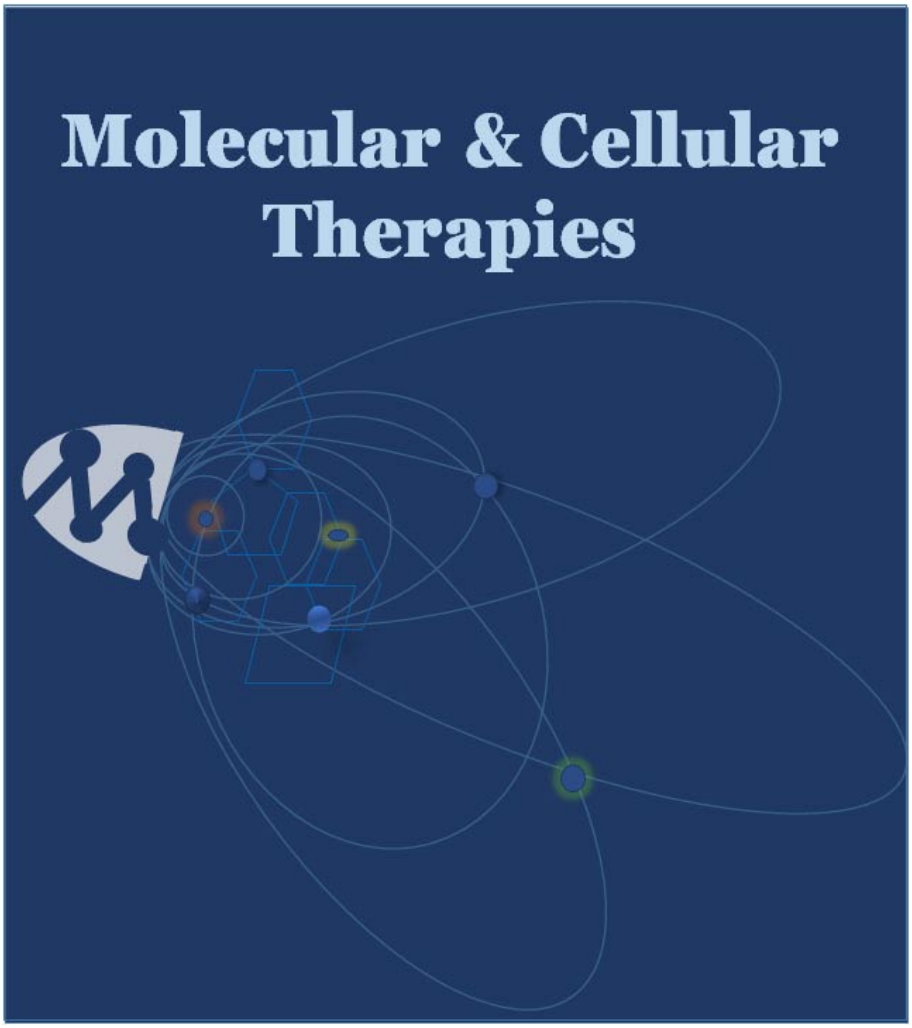

Molecular and Cellular Therapies (2018) 6:2

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Original Research

Assessment of efficacy of trastuzumab (Herceptin) comprising adjuvant therapy of HER2+ breast cancer patients determined based upon statistical analysis of overall survival (OS) and disease-free survival (PFS)

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Abstract

Introduction

In patients suffering from breast cancer, adjuvant radiation, chemotherapy, or immunotherapy, which immediately follow the surgery as the first line therapy, greatly improve overall (OS) and disease-free survival (DFS). Various regimens of adjuvant therapy for these patients have been tested contingent upon the clinical staging. Inclusion of adjuvant immunotherapy is particularly promising.

Specific aim

The aim of this study was to assess efficacy of trastuzumab(Herceptin) -comprising adjuvant immunotherapy in terms of overall and disease-free survival as compared to other adjuvant therapies.

Patients

All patients were presented with the Patient Bill of Rights and have provided the Patient Informed Consent to participate in this study. Eligible patients include those with primary tumors initially staged at the clinical stages: I-T1c N0, II-T0-2, N0-1,or IIIA-T3 N1, or patients for whom neoadjuvant chemotherapy provides the possibility to remove surgically a tumor at the stage IIIA T0-3 N2. Of 9,058 patients enrolled in the Breast Cancer Treatment Program between 2008 and 2015, 6,832 fulfilled the inclusion criteria.

Statistical Analysis

The effects of clinical and demographic factors on overall survival (OS) and disease-free survival(DFS) were assessed using Cox' s proportional hazards regression models. OS and DFS were evaluated with *Kaplan-Meier* calculations. The study was meeting the criteria for a controlled, open-access clinical trial.

Results

OS rates for years 1-7 were, respectively, 99.42%, 97.26%, 94.57%, 92.41%, 90.48%, 88.63%, and 88.23%; thus with the 5-year survivalat 90.48%. The corresponding data for DFS were 96.17%, 84.07%, 77.26%, 72.57%, 68.59%, 65.04%, and 63.05%, respectively; thus with the 5-year DFS at 68.59%. Adverse effects, with the exception of cardiac complications, occurred in 1194 (17%), while causing withdrawal of 421 (6%) patients. Most of other adverse events were related to hepatotoxicity 1755 (25%) and fatigue 681 (9.7%).

Conclusion

These results demonstrate the great benefits of inclusion of immunotherapy as the adjuvant

component as currently the best overall therapeutic strategy for the patients suffering breast cancers. As this strategy greatly exceeds any other therapeutic options available for practicing oncologists at the present time, it definitely justifies allocation of all needed public resources, while assuring highest quality health service for the patients in Poland.

Keywords
breast cancer, trastuzumab, Herceptin, radiotherapy, chemotherapy, immunotherapy, hormonal therapy, adjuvant therapy, human epidermal growth factor receptor – 2, estrogen receptor, progesterone receptor, overall survival, disease-free survival

Introduction

Breast cancer was the most frequently diagnosed malignancy in women in Poland in 2013. It was also thesecond highest cause of cancer-related death, after lung-cancer. The National Cancer Registry reported annual prevalence of breast cancer at 17,200 in 2013 with the standardized incidence rate of 52/100,000. Approximately 5,800 women died of the disease with the standardized mortality rate of 14.5/100,000). What became troubling, that within the last two decades, incidence of breast cancer has increased by about 10,000, although a slow decline in the mortality rate suggests the benefits of early detection and more effective therapies. [1]

Based on combined analyses of randomized clinical trials data, it is apparent that adjuvant treatment with chemotherapy and trastuzumab-comprising immunotherapy in HER2-positive breast cancer patients, versus adjuvant chemotherapy alone, may reduce the risk of recurrence as much as 50% and mortality by 30%. [4]

The real challenge for practicing physicians is confronting the therapeutic efficacy with financial affordability. In Poland, public healthcare funding comprises a national health insurance-based system, which is funded by the Polish Government controlled budget, while with contributions from health insurance. Any therapeutics' recommendations are assessed by the Polish Health Technology Assessment Agency and, if approved according to very rigorous criteria, recommended for being financed for eligible patients and administered at the designated cancer centers. [2,3]

Specific aim

The aim of this study was to assess efficacy of trastuzumab (Herceptin) - comprising adjuvant therapy as compared to other therapeutic options, in terms of overall (OS) and disease-free survival (DFS).

Patients

All patients were presented with the Patient Bill of Rights. All physicians interacting with the patients in this study were licensed to practice medicine in EU and sworn with Hippocratic Oath. All the procedures were compliant with the Declaration of Helsinki and Good Clinical Practice Guidelines. All therapeutics were acquired from the manufacturers, who guarantee compliance with the GMP requirements and approved by EMA. The study was approved by the Institutional Review Board - Bio-ethics Committees of the Maria Sklodowska-Curie Memorial Cancer Centre and the Institute of Oncology, Warsaw KB/9/2011). Eligible patients include those with primary tumors initially staged at I-T1c N0, II-T0-2 N0-1,or IIIA-T3 N1, or patients for whom initial chemotherapy provides the possibility to surgically remove a stage IIIA T0-3 N2 tumor.

All the patients were tested for HER2, estrogen, and progesterone receptors to qualify for targeted therapy. Of 9,058 patients enrolled in the Breast Cancer Treatment Program between 2008 and 2015, 6,832 fulfilled those inclusion criteria.

Methods

Chosen statistical methods aimed at analyses of the overall survival (OS) and disease free survival (DFS), correlated with demographic and clinical parameters. The data were obtained from the National Health Fund database, inclusive of the Central Insurance Inventory, the Therapeutic System Monitoring Program, and the Patient Treatment Costs System. Clinical data included the time and type of surgery, histopathological diagnosis, receptor status, adjuvant chemotherapy, immunotherapy (Herceptin), radiation therapy, hormonal therapy, and, as well as assessment of potential adverse effects.

Diagnostics

Immunohistochemistry (IHC) and Fluorescent In Situ Hybridization (FISH)

Qualification of the patients for therapy was conducted according to ASCO and EMA Guidelines. [21,22] According to the current recommendations in Poland, HER2 by IHC is determined exclusively in tissue (biopsy, tissue cut). Assessment of HER2 display uses 4-step scale (HER2 0, 1+, 2+, 3+) using IHC. From the clinical management point of view, scores 0 and 1+ are defined as negative and 3+ are positive. Expression 2+ in the IHC study has an ambiguous value and requires an evaluation of the number of copies of the HER2 gene by in situ hybridization (FISH), chromogenic in situ hybridization (CISH). Specifically, HER2-positive status is defined, while observing within an area of tumor that amounts to > 10% of contiguous and homogeneous tumor cells evidence of protein overexpression (IHC). Likewise, gene amplification of HER2 copy number or HER2/CEP17 ratio by FISH or CISH is based on counting at least 20 cells within the area.

Immunohistochemistry methods were used to define HER2 receptor status for all patients and if found equivocal, then HER 2 gene amplification by the FISH method was measured. FISH amplification means at least 6 copies of the gene (up to 4 copies negative and 4-6 results are ambiguous). The FISH results are most often

expressed as the ratio of the number of copies of the HER2 gene to the number of copies of the chromosome 17. In the tumor cell; then the value above 2.2 means amplification. About 15-20% of breast cancers identified by the IHC method as HER2 2+ show amplification of the HER2 gene and are treated as HER2 positive tumors.

The internal quality control of IHC preparations is based on the evaluation of each preparation by two specialized pathologist evaluators and by a divergent evaluation by three evaluators. The external control consists of sending a specified number of preparations to the Polish central institution - Oncology Center - M.C.Skłodowska Institute in Warsaw and re-evaluating them. The percentage of compliance should exceed 90%.

The internal quality control of FISH results relies upon evaluation of each test with the ambiguous score by two or three evaluators.

The external control of FISH tests is based on the known HER2 gene status, while verified by being sent to the center. The rating should be more than 90% compliant.

Adjuvant Therapies

Radiotherapy

Indications for radiotherapy after breast amputation included the presence of metastases in at least 4 axillary lymph nodes and the presence of "positive" (<1 mm) surgical margins of the tumorsat the stage T3. Indication for radiotherapy after breast amputation in cases metastasis of 1-3 axillary lymph nodes with coexistence of other risk factors (young age, ER- or G3 trait, tumor cell abnormalities in vessels) were assessed individually. In this group, postoperative radiotherapy included:

- the chest wall area - 50 Gy in fractionation of 2 Gy per day using energy-efficient electrons (usually 6-9 MeV) or 4-6 MV photons (tangential field technique).
- lymph node fields - total dose of 50 Gy in fractionation of 2 Gy per day using photons of 4-6 MV energy.

Postoperative radiotherapy after was administered in all patients treated. In this group, the entire dose was 50 Gy (25 fractions of 2 Gy in 5 weeks) or 40 Gy in 15 fractions. In most cases, a 10-15 Gy boost (electron beam, photon beam or brachytherapy) was added to the 1-2 cm margin cut.

Chemotherapy

Adjuvant systemic chemotherapeutics included anthracyclines (Doxorubicin) and taxens (Paclitaxel, Docetaxel). The decision to use systemic adjuvant therapy was based on the assessment of the likelihood of benefits associated with individual treatment methods and the individual risk of recurrence (based on assessing prognostic factors). In addition, the risk of undesirable systemic actions was taken into account, based on the performance state, coexisting diseases as well as the preferences of the patients. Adjuvant chemotherapy was usually performed within 3 months of surgery. Treatment duration was 3 to 6 months within 4-8 cycles. The specific choice of treatment regimen and the drug was dependent on the clinical assessment and the reimbursement availability.

Hormonal therapy

Choice of adjuvant hormonal therapy was contingent upon pre- versus post-menopausal state of the patients and the reimbursement status of a particular medicinal product. Competitive receptors inhibitors (Tamoxifen) were the first line chemotherapeutics administered to the pre-menopausal patients.Aromatase inhibitors (Anastrozole) was recommended for the post-menopausal patients.

For treatment with Tamoxifen a daily dose of 20 mg was administered. All patients with ER+ and / or PR+ expression were eligible regardless of age and menopausal status. The standard duration of treatment with tamoxifen is 5 years.

Treatment with nonsteroidal aromatase inhibitors - Anastrozole (daily dose of 1 mg), Letrozole (daily dose 2.5 mg) - or a steroid inhibitor - Exemestane (daily dose of 25 mg) is only used in the patients, who are after natural menopause, after castration, or in combination with Pharmacological suppression of ovarian function. The ovarian hormone suppression was accomplished by using gonadotropin releasing hormone (GnRH) analogs (Goserelin). For reversible pharmacological suppression use of the GnRH analogue, treatment time for 2 years in pre-menopausal patients with ER / PR expression were eligible. In Poland, two GnRH analogues - Goserelin and Ileuprorelin are available for the treatment of breast cancer. Both drugs are used in the form of implants administered subcutaneously into the abdominal wall. Goserelin is implanted every 28 days at a dose of 3.6 mg and Leuprorelin every 3 months at a dose of 11.25 mg. Considering patients' preferences, they may also be administered intramuscularly. The choice the preparation is contingent upon the physicians' assessments and upon the reimbursement availability.

Immunotherapy

Patients diagnosed with breast cancers overexpressing HER2 were qualified for immunotherapy with trastuzumab (Herceptin). Median number of trastuzumab administrations was 17. The initial loading dose of trastuzumab was 8 mg/kg body weight and a maintenance dose of 6 mg/kg administered at 3 weekly intervals, starting with the loading dose in a 90-minute intravenous infusion. If the drug was delayed 7 days or less for any reason, the dose of 6 mg/kg (without waiting for the next scheduled cycle) was promptly given, and another according to the previous treatment plan. If the drug was delayed by more than 7 days, then the loading dose (8 mg/kg for about 90 minutes) was then administered, followed by subsequent maintenance doses (6 mg/kg) every 3 weeks. The planned duration of trastuzmab adjuvant treatment was 12 months. Trastuzumab treatment was continued until the onset of disease progression or the occurrence of undesirable effects of clinical significance. This adjuvant immunotherapy in Poland is based upon the only breast cancer immunotherapeutic available and reimbursed - Herceptin (Roche).

Study inclusion metrics

Information for the performed procedures (ICD-9), diagnoses (ICD-10), and completed drug prescriptions (drug regulations), enabled us to capture post-treatment relapse data. The study inclusion metrix applicable for treating breast cancer included:

1) A histological diagnosis of breast cancer.

2) HER2 over-expression in cancer cells (i.e. 3+result by IHC), or *HER2* gene amplification (positive (+) outcome by FISH). HER2 status was initially determined by IHC, with equivocal results resolved by FISH.

3) Primary tumor of initial stage I – T1c N0, II – T0-2 N0-1, or IIIA – T3 N1.

4) Post chemotherapy staging, that enables the total surgical removal of a tumor at stage IIIA (T0-3 N2).

5) Radical surgical treatment (mastectomy and axillary lymph node excision), or Lumpectomy/segmentectomy with a normal tissue margin, axillary lymph node removal, with adjuvant radiotherapy of the whole breast (breast conserving therapy).

6) A risk of relapse based on the histology of postoperative material including:

a) the presence of metastases in axillary lymph nodes (pN+).

b) a (largest) tumor diameter >1.0 cm, without metastases in axillary lymph nodes (pN0).

7) Normal heart function based on clinical evaluation, echocardiography, or Multi-gated acquisition scan (MUGA), performed prior to trastuzumab treatment, with a left ventricular ejection fraction (LVEF) of at least 50%.

8) Exclusion of pregnancy.

Disease-free survival (DFS)

Disease-free survival (DFS) was defined as time elapsed from the date of the first administration of Herceptin immunotherapy to the date of relapse, resumption of breast cancer treatment after completing Herceptin treatment, death from any cause without documentation of a cancer-related event , or treatment for another primary tumor.

Overall survival (OS)

Overall survival (OS) was calculated from the date of first administering Herceptin to the date of death, or the date of the last follow-up. It should be emphasized that our patient cohort was homogeneous, with this study meeting the criteria for a controlled, open-access clinical trial.

Statistical analysis

The effects of age, cancer stage, type of treatment (surgery, radiotherapy, chemotherapy, hormonal therapy, immunotherapy), adverse effects on the survival rate were analyzed using non-parametric *Cox's* proportional hazard regression models. Using tests based on these models, effects provoked between factors and by single factors were analysed (layered analysis). The *Kaplan-Meier* method was used to evaluate OS and DFS, with calculations performed using the Statistica 7.1 software (Statsoft; Tulsa, OK).

Results

Clinical outcomes

Between 2008 and 2015, the Breast Cancer Treatment Program enrolled 9,058 patients of whom 6,832 were analyzed in this study. Excluded patients were those with incomplete data and/or those having received less than the full (12-month) treatment. The median follow-up time was 43.7 months (92.9-2.1). In our final cohort, 445 (6.51%) deaths were recorded and 1,674 patients experienced relapse (24.50%). We would like to emphasize that it was impossible to distinguish between local recurrences vs. distant metastases and/or secondary cancers from available public databases. The detailed compilation of the data is presented in the Tables 1 and 2.

The median age of our study group was 56 years (23-84). More than 85% of patientswere aged 40-70. Stage I breast cancer was diagnosed in 23.42% (1,600), stage IIA in 34.85% (2,381), stage IIB in 19.96% (1,364), and stage IIIA in 15.84% (1,082). A primary tumor diameter of between 20-50 mm was found in 50.57% of patients. In 87.54% of patients, invasive cancer was diagnosed, of which 38.36% were malignancy grade G2, and 33.15% were G3; other types of cancer were identified in the remaining patients.

Most patients underwent a modified mastectomy (57.44%) and lymphadectomy (71.52%) as the first line therapy.

Adjuvant radiotherapy was given to 4,548 patients, whereas 6,606 patients received chemotherapy of whom 3,090 were treated with anthracyclines and 3,395 were treated with anthracyclines and taxanes.

Adjuvant immunotherapy was administered with the median number of trastuzumab administrations 17.

OS and DFS were assessed between the first and seventh year of follow-up as presented in the Figures 1 and 2. Factors that were found to significantly influence OS were age, tumor size, lymph node metastasis, type of surgery, adjuvant chemotherapy, adjuvant radiotherapy, cardiotoxicity, comorbidities, time from diagnosis to treatment, and the number of Herceptin administrations as shown in the Table 2. Age, tumor size, lymph node involvement, adjuvant radiotherapy, cardiotoxicity, time from diagnosis to treatment, and the number of trastuzumab administrations were all significantly correlated with a reduced DFS emerge from the Table 2.

Multivariate analysis based of *Cox's* regression models revealed an independent, significant, and adverse impact of being aged above 69 years for OS. For these patients, as well as for those younger than 40 years, there was an increased risk of relapse vs. patients aged 40-69 years. Tumor size, TNM stage (IIB, IIIA, IIIB), and lymph node metastasis were associated with a poor prognosis in terms of OS and DFS.

The majority of patients (96.69%, 6,606) received chemotherapy. Anthracyclines with or without taxanes were used in 3395 and 3090 patients. While only 121 patients received taxane-containing regimens without

Table-1.Overview of the patients' cohorts

Variable	Patients		Relapsed	
	N	%	*N*	%
	6,832	100.00%		
Age upon initiation of therapy [years]	6,832	100.00%		
-40	495	7.25%	161	9.6
40 - 50	1,381	20.21%	370	22.1
50 -60	2,562	37.50%	606	36.2
60 - 70	1,886	27.61%	408	24.4
70 -	508	7.44%	129	7.7
Metastases to lymph nodes (N)	6,832	100.00%		
0	3,360	49.18%	719	43
1-3	2,454	35.92%	636	38
4-9	870	12.73%	277	16.5
10+	111	1.62%	37	2.2
Undetermined	37	0.54%	5	0.3
Tumor size (mm)	6,832	100.00%		
-20	2,958	43.30%	662	39.5
21 - 50	3,455	50.57%	876	52.4
51+	397	5.81%	126	7.5
Undetermined	22	0.32%	10	0.6
Malignancy (G)	6,832	100.00%		
G1	325	4.76%	79	4.7
G2	2,621	38.36%	646	38.6
G3	2,265	33.15%	554	33.1
GX	1,621	23.73%	395	23.6
Stage (TNM)	6,832	100.00%		
0	3	0.04%	2	0.1
1	1,600	23.42%	33	19.9
2a	2,381	34.85%	53	32
2b	1,364	19.96%	348	20.8
3a	1,082	15.84%	342	20.4
3b	189	2.77%	73	4.4
X	213	3.12%	41	2.4
Receptors' display	6,832	100.00%		
ER- & PGR-	2,689	39.36%	700	41.8
ER- & PGR+	170	2.49%	48	2.9
ER+ & PGR-	860	12.59%	203	12.1
ER+ & PGR+	2,893	42.34%	663	39.6
No data	220	3.22%	60	3.6
Histopathology	6,832	100.00%		
Other malignant neoplasms	180	2.63%	45	2.7
Cancer of special features	154	2.25%	34	2
Carcinoma ductal invasive	5,981	87.54%	1473	88
Carcinoma lobular invasive	246	3.60%	67	4
Cancer invasive mixed	271	3.97%	55	3.3
Surgery 1	6,832	100.00%		
Biopsy without treatment	30	0.44%	12	0.7
Quadrantectomy	1,484	21.72%	292	17.4
Mastectomy simple	928	13.58%	248	14.8
Mastectomy modified	3,924	57.44%	1053	62.9
Wide excission - repeated	30	0.44%	6	0.4
Wide excission	436	6.38%	63	3.8
Surgery 2	6,832	100.00%		
Biopsy of axillary lymph nodes	564	8.26%	139	8.3
Biopsy of sentinel lymph nodes	1,382	20.23%	331	19.8
Lymphadenectomy	4,886	71.52%	1204	71.9
Chemotherapy	6,832	100.00%		
T (taxanes)	121	1.77%	32	1.9
A (anthracyclines)	3,090	45.23%	745	44.5
T + A	3,395	49.69%	827	49.4
No data	226	3.31%	70	4.2

Table-1.Overview of the patients' cohorts (Continued)

Variable	Patients		Relapsed	
	N	%	*N*	%
	6,832	100.00%		
Hormonal therapy	6,832	100.00%		
Administered	4,014	58.75%	943	56.3
Not administered	2,818	41.25%	731	49.7
Radiotherapy	6,832	100.00%		
With trastuzumab	698	10.22%	176	10.5
Without trastuzumab	3,850	56.35%	998	59.6
Not administered	2,284	33.43%	500	29.9
Time span: diagnosis - treatment (days)	6,832	100.00%		
<112	177	2.59%	54	3.2
113-203	2,041	29.87%	507	30.3
204-364	4,107	60.11%	974	58.2
365 <	507	7.42%	139	8.3
Coexisiting diseases	6,832	100.00%		
Yes	5,296	77.52%	1302	77.8
No	1,536	22.48%	372	22.2
Cardiotoxicity	6,832	100.00%		
Yes	324	4.74%	113	6.8
No	6,508	95.26%	1561	93.2
Trastuzumab administration (*N*)	6,832	100.00%		
1-5	214	3.13%	102	6.1
6-9	194	2.84%	94	5.6
10-16	951	13.92%	253	15.1
17-19	5,462	79.95%	1223	73.1
20 +	11	0.16%	2	0.1

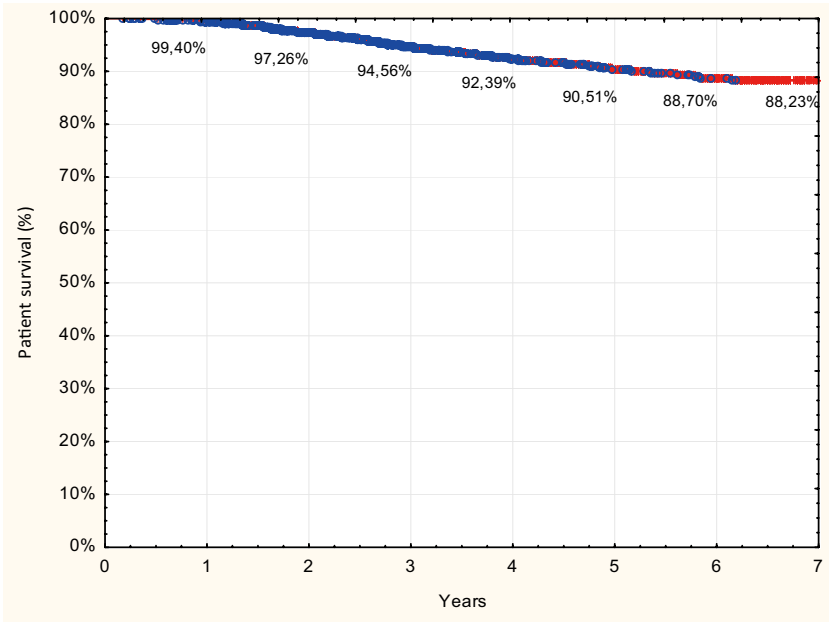

Figure 1. Overall survival (OS) according to specified probabilities of survival for 1-7 full years follow-up (estimated by the *Kaplan-Meier* method)

anthracyclines. There was no evidence that the type of chemotherapy had thesignificant effect on either OS or DFS as evident from the data in the Tables 1 and 2.

Adverse events

The detailed statistical data of the encountered adverse effects are compiled in the Tables 4 and 5.

There were 299 (3.46%) cases of cardiac complication that adversely affected OS and DFS as documented in the Table 3. The respective HR data (OS/DFS) were1.57 (95% CI, 1.11-2.20), and 1.48 (95% CI, 1.23-1.80). The most common cardiac complication was an asymptomatic decrease in ejection fraction and circulatory failure.

Other than cardiac adverse events occurred in 1,186 (13.74%) patientsas shown in the Table 4. In those, hepatotoxicity was the most commonly seen complication

Table-2. Estimated HR (*Cox's* proportional hazards model) with 95% confidence intervals (95%CI) for OS and DFS, determined separately for our cohort's 16 study features. The most common data points were assigned as reference categories (grey rows; HR = 1.00).

Variable	Patients		OS		*COX* - HR - OS			DFS		*COX* - HR - DFS			Comments
	N	%	OS	%	HR	CL-95%		DFS	%	HR	CL-95%		
	6,832	100.00%	445	6.51%		min	max	1,674	24.50%		min	max	
Age upon initiation of therapy [years]	6,832	100.00%	445	100.00%				1,674	100.00%				
-40	495	7.25%	19	4.27%	0.60	0.37	0.97	161	9.62%	1.52	1.28	1.81	Min. 24 years
40 - 50	1,381	20.21%	66	14.83%	0.72	0.54	0.95	370	22.10%	1.14	1.00	1.30	
50 -60	2,562	37.50%	168	37.75%	1.00			606	36.20%	1.00			
60 - 70	1,886	27.61%	129	28.99%	1.15	0.91	1.44	408	24.37%	0.96	0.85	1.09	
70 -	508	7.44%	63	14.16%	2.22	1.66	2.96	129	7.71%	1.21	1.00	1.46	Max. 84 years
Metastases to lymph nodes (N)	6,832	100.00%	445	100.00%				1,674	100.00%				
0	3,360	49.18%	135	30.34%	1.00			719	42.95%	1.00			
1-3	2,454	35.92%	166	37.30%	1.59	1.27	2.00	636	37.99%	1.19	1.07	1.32	
4-9	870	12.73%	117	26.29%	3.26	2.54	4.17	277	16.55%	1.53	1.33	1.76	
10+	111	1.62%	24	5.39%	5.32	3.45	8.21	37	2.21%	1.57	1.13	2.18	
Undetermined	37	0.54%	3	0.67%	2.00	0.64	6.29	5	0.30%	0.58	0.24	1.40	
Tumor size (mm)	6,832	100.00%	445	100.00%				1,674	100.00%				
-20	2,958	43.30%	117	26.29%	0.48	0.39	0.60	662	39.55%	0.86	0.78	0.95	
21 - 50	3,455	50.57%	281	63.15%	1.00			876	52.33%	1.00			
51+	397	5.81%	44	9.89%	1.54	1.12	2.12	126	7.53%	1.41	1.17	1.70	
Undetermined	22	0.32%	3	0.67%	1.56	0.50	4.88	10	0.60%	1.92	1.03	3.58	
Malignancy (G)	6,832	100.00%	445	100.00%				1,674	100.00%				
G1	325	4.76%	16	3.60%	0.74	0.44	1.23	79	4.72%	0.95	0.75	1.20	
G2	2,621	38.36%	161	36.18%	1.00			646	38.59%	1.00			
G3	2,265	33.15%	146	32.81%	1.05	0.84	1.31	554	33.09%	0.98	0.88	1.10	
GX	1,621	23.73%	122	27.42%	1.27	1.00	1.60	395	23.60%	1.01	0.89	1.14	
Stage (TNM)	6,832	100.00%	445	100.00%				1,674	100.00%				
0	3	0.04%	1	0.22%	12.10	1.69	86.66	2	0.12%	4.56	1.14	18.29	
1	1,600	23.42%	38	8.54%	0.50	0.34	0.72	333	19.89%	0.95	0.83	1.09	
2a	2,381	34.85%	119	26.74%	1.00			535	31.96%	1.00			
2b	1,364	19.96%	103	23.15%	1.55	1.19	2.01	348	20.79%	1.16	1.02	1.33	
3a	1,082	15.84%	129	28.99%	2.52	1.96	3.23	342	20.43%	1.53	1.34	1.76	
3b	189	2.77%	44	9.89%	4.63	3.27	6.54	73	4.36%	1.80	1.41	2.29	
X	213	3.12%	11	2.47%	0.98	0.53	1.81	41	2.45%	0.80	0.58	1.10	
Receptors' display	6,832	100.00%	445	100.00%				1,674	100.00%				
ER- & PGR-	2,689	39.36%	213	47.87%	1.56	1.26	1.93	700	41.82%	1.09	0.98	1.21	
ER- & PGR+	170	2.49%	14	3.15%	1.60	0.92	2.77	48	2.87%	1.17	0.87	1.57	
ER+ & PGR-	860	12.59%	65	14.61%	1.62	1.21	2.18	203	12.13%	1.05	0.89	1.22	
ER+ & PGR+	2,893	42.34%	138	31.01%	1.00			663	39.61%	1.00			
No data	220	3.22%	15	3.37%	1.17	0.69	1.99	60	3.58%	1.03	0.79	1.34	
Histopathology	6,832	100.00%	445	100.00%				1,674	100.00%				
Other malignant neoplasms	180	2.63%	9	2.02%	0.68	0.35	1.31	45	2.69%	0.98	0.72	1.31	
Special form of cancer	154	2.25%	6	1.35%	0.55	0.24	1.22	34	2.03%	0.86	0.61	1.21	
Carcinoma ductal invasive	5,981	87.54%	396	88.99%	1.00			1,473	87.99%	1.00			
Carcinoma lobular invasive	246	3.60%	19	4.27%	1.14	0.72	1.81	67	4.00%	1.10	0.86	1.40	
Cancer invasive mixed	271	3.97%	15	3.37%	0.85	0.51	1.43	55	3.29%	0.81	0.62	1.06	
Surgery 1	6,832	100.00%	445	100.00%				1,674	100.00%				
Biopsy without treatment	30	0.44%	4	0.90%	1.30	0.48	3.49	12	0.72%	1.50	0.85	2.65	
Quadrantectomy	1,484	21.72%	47	10.56%	0.42	0.31	0.57	292	17.44%	0.77	0.68	0.88	
Mastectomy simple	928	13.58%	46	10.34%	0.63	0.47	0.86	248	14.81%	1.07	0.93	1.23	
Mastectomy modified	3,924	57.44%	334	75.06%	1.00			1,053	62.90%	1.00			
Wide excission - repeated	30	0.44%	0	0.00%				6	0.36%	0.78	0.35	1.74	
Wide excission	436	6.38%	14	3.15%	0.44	0.26	0.75	63	3.76%	0.55	0.43	0.71	

Table-2. Estimated HR (*Cox's* proportional hazards model) with 95% confidence intervals (95%CI) for OS and DFS, determined separately for our cohort's 16 study features. The most common data points were assigned as reference categories (grey rows; HR = 1.00). (Continued)

Variable	Patients		OS		*COX* - HR - OS			DFS		*COX* - HR - DFS			Comments
	N	%	OS	%	HR	CL-95%		DFS	%	HR	CL-95%		
	6,832	100.00%	445	6.51%		min	max	1,674	24.50%		min	max	
Surgery 2	6,832	100.00%	445	100.00%				1,674	100.00%				
Biopsy of axillary lymph nodes	564	8.26%	31	6.97%	0.83	0.58	1.20	139	8.30%	1.04	0.87	1.24	
Biopsy of sentinel lymph nodes	1,382	20.23%	73	16.40%	0.79	0.62	1.02	331	19.77%	1.01	0.89	1.14	
Lymphadenectomy	4,886	71.52%	341	76.63%	1.00			1,204	71.92%	1.00			
Chemotherapy	6,832	100.00%	445	100.00%				1,674	100.00%				
T (taxanes)	121	1.77%	6	1.35%	0.82	0.36	1.84	32	1.91%	1.05	0.74	1.49	
A (anthracyclines)	3,090	45.23%	214	48.09%	0.97	0.80	1.18	745	44.50%	0.84	0.76	0.93	
T + A	3,395	49.69%	195	43.82%	1.00			827	49.40%	1.00			
No data	226	3.31%	30	6.74%	1.82	1.24	2.68	70	4.18%	1.09	0.85	1.39	
Hormonal therapy	6,832	100.00%	445	100.00%				1,674	100.00%				Administered ~ 4 months.
Administered	4,014	58.75%	217	48.76%	1.00			943	56.33%	1.00			
Not administered	2,818	41.25%	228	51.24%	1.42	1.18	1.71	731	43.67%	1.07	0.97	1.17	
Radiotherapy	6,832	100.00%	445	100.00%				1,674	100.00%				
With trastuzumab	698	10.22%	45	10.11%	1.03	0.75	1.40	176	10.51%	1.23	1.05	1.45	
Without trastuzumab	3,850	56.35%	329	73.93%	1.00			998	59.62%	1.00			
Not administered	2,284	33.43%	71	15.96%	0.34	0.26	0.44	500	29.87%	0.80	0.72	0.89	
Time span: diagnosis - treatment (days)	6,832	100.00%	445	100.00%				1,674	100.00%				
<112	177	2.59%	11	2.47%	0.96	0.52	1.75	54	3.23%	1.37	1.04	1.80	
113-203	2,041	29.87%	99	22.25%	0.73	0.58	0.91	507	30.29%	1.05	0.95	1.17	
204-364	4,107	60.11%	272	61.12%	1.00			974	58.18%	1.00			
365 <	507	7.42%	63	14.16%	1.91	1.45	2.51	139	8.30%	1.16	0.97	1.39	
Coexisiting diseases	6,832	100.00%	445	100.00%				1,674	100.00%				ICD-10: E10-E11; I10-I77; J42-J43
Yes	5,296	77.52%	355	79.78%	1.00			1,302	77.78%	1.00			
No	1,536	22.48%	90	20.22%	0.92	0.73	1.16	372	22.22%	1.03	0.92	1.16	
Cardiotoxicity	6,832	100.00%	445	100.00%				1,674	100.00%				
Yes	324	4.74%	36	8.09%	1.57	1.11	2.20	113	6.75%	1.48	1.23	1.80	
No	6,508	95.26%	409	91.91%	1.00			1,561	93.25%	1.00			
Trastuzumab administration (*N*)	6,832	100.00%	445	100.00%				1,674	100.00%				
1-5	214	3.13%	47	10.56%	5.45	3.99	7.44	102	6.09%	3.05	2.49	3.73	
6-9	194	2.84%	50	11.24%	6.74	4.98	9.14	94	5.62%	3.28	2.66	4.04	
10-16	951	13.92%	98	22.02%	2.77	2.20	3.51	253	15.11%	1.55	1.35	1.77	
17-19	5,462	79.95%	249	55.96%	1.00			1,223	73.06%	1.00			
20 +	11	0.16%	1	0.22%	1.51	0.21	10.74	2	0.12%	0.65	0.16	2.61	

(23.69%), followed by hand-foot syndrome (8.35%). We were unable to define the severity of side effects or complications based on the available data.

Discussion

For years 1-7 of the study follow-up, OS data were, respectively, 99.42%, 97.26%,94.57%, 92.41%, 90.48%, 88.63%, and 88.23%.The data the same years for DFS were 96.17%, 84.07%, 77.26%, 72.57%, 68.59%, 65.04%, and 63.05%. For comparison, in Lombardi study of a population of Italian patients (2,046 patients) treated with trastuzumab and followed for 4 years were 98.7%, 95.4%, 91.5%, and 89.4%, respectively. The respective DFS rates were 93.9%, 85.8%, 79.4%, and 75%. [5] Randomised clinical trials have demonstrated 4-year overall survival rates of 89.3% in the HERA trial. [6] The NCCTG N and NSABP-B31 studies demonstrated rates of 98.31% and 93%. Rates forDFS were 78.6% inthe HERA trial, and 85.7% in the NCCTG N9831i and NSABP-B31 studies. 7] The combined outcomes of the NCCTG N9831 and NSABP-B31 studieswere published in 2014, based on a median follow-up of 8.4 years. OS and DFS data for those trials amounted to 84% and 73.7%, respectively. [8] The SEWCN study reported 3-year OS rates of 98.5%, and DFS rates of 79.4%(OS and DFS times were established

Table-3. Estimated HR (*Cox's* proportional hazards model) for OS and DFS, with 95% confidence intervals (*P*=0.05 (95%CI)), for the 16 qualitative characteristics of the study population.

Lp	OS	DFS	Variables in regression model in *Cox* hazard ratio	Hazard Ratio model OS	OS: CL-95% dla HR		Chi2	Hazard Ratio model DFS	DFS: CL-95% dla HR		Chi2
					min.	max	*P*<0,05		min.	max	*P*<0,05
1	x	x	Age upon initiation of therapy	1.33	1.20	1.47	0.00	0.92	0.87	0.96	0.00
2	x		Metastases to lymph nodes	1.23	1.04	1.47	0.02	1.10	0.99	1.21	0.06
3	x	x	Tumor size	1.39	1.17	1.66	0.00	1.16	1.06	1.28	0.00
4			Malignancy grade (T)	0.96	0.89	1.04	0.31	0.99	0.95	1.03	0.68
5			Stage (TNM)	1.11	0.98	1.27	0.10	1.01	0.94	1.09	0.74
6			Receptors' display	0.95	0.85	1.07	0.41	0.98	0.92	1.03	0.42
7			Histopatholgy	1.06	0.90	1.25	0.48	0.97	0.89	1.06	0.54
8	x		Surgery	1.13	1.02	1.25	0.02	0.99	0.95	1.04	0.82
9			Lymphadenectomy	1.07	0.91	1.26	0.39	0.99	0.92	1.07	0.80
10	x		Chemotherapy	0.82	0.73	0.93	0.00	1.00	0.93	1.08	0.97
11			Hormonothrapy	1.15	0.83	1.58	0.41	1.02	0.86	1.20	0.83
12	x	x	Radiotherapy	0.54	0.45	0.64	0.00	0.83	0.76	0.90	0.00
13	x	x	Time span: DX - RX	1.18	1.01	1.37	0.03	0.91	0.84	0.98	0.02
14	x		Coexisting diseases	1.37	1.07	1.76	0.01	1.01	0.90	1.15	0.82
15	x	x	Cardiotoxicity	2.35	1.61	3.43	0.00	1.60	1.28	2.01	0.00
16	x	x	Number of drugs' administration	0.47	0.43	0.52	0.00	0.58	0.55	0.62	0.00

Table 4.Cardiovascular adverse effects and complications.

Types of cardiotoxicity	Patient number	(%)
Asymptomatic decrease of LVEF	156	(52.17)
Circulatory insufficiency	18	(6.02)
Symptomatic decrease of LVEF	18	(6.02)
Cardiac arrhythmia	12	(4.01)
Cardiac infarction	4	(1.34)
Thromboembolism	3	(1.00)
Cardiomyopathy	3	(1.00)
Arterial hypertension	3	(1.00)
Pericarditis	1	(0.33)
Coronary heart disease	1	(0.33)
Other unspecified cardiotoxicities	80	(26.76)
Total cardiotoxicity (of total patients)	299	(3.46)

Table 5.Side effects and complications other than cardiovascular

Types of side effect	Patient number	(%)
Hepatotoxicity	281	(23.69)
Hand-foot syndrome	99	(8.35)
Nephrotoxicity	84	(7.08)
Neutropenia	81	(6.83)
Fatigue	77	(6.49)
Neurotoxicity	68	(5.73)
Diarrhea	62	(5.23)
Infection	40	(3.37)
Thrombocytopenia	22	(1.85)
Allergy	16	(1.35)
Deep vein thrombosis of the lower limbs	14	(1.18)
Anemia	12	(1.01)
Vomiting	9	(0.76)
GI bleeding	4	(0.34)
Other unspecified side effects	317	(26.73)
Total	1186/6832	(13.74)

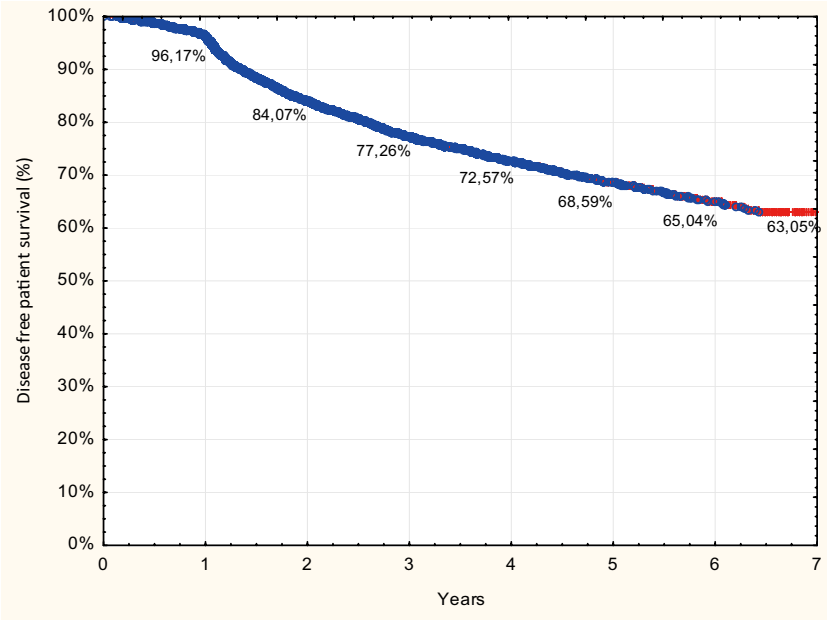

Figure 2. Disease free survival (DFS) according to specified probabilities of survival for 1-7 full years follow-up(estimated by the *Kaplan-Meier* method)

using the date of surgical intervention, not the date of the first administration of trastuzumab, as in other studies). [9]

When comparing results of our analysis within this Polish study, with those cited above, we report similar OS rates, butpoor DFS rates. Currently, we can neither rule out delays in introducing trastuzumab treatments, nor inadequate treatment durations, as contributory factors to the poor DFS rate. Approximately 60% of patients commenced adjuvant trastuzumab therapy within 29-52 weeks of their diagnosis, with 7% beginning treatment at later dates. Further, a 12-month treatment (designated as complete treatment) was achieved for less than 80% patients.

Our analysis of the effect of adjuvant trastuzumab therapy is based on 6,832 HER2-positive breast cancer patients in Poland, and is the largest study of its kind for a clinical group treated on the basis of satisfying uniform inclusion and exclusion criteria while remaining open-access. The phase III HERA study comprised 5,081 patients, of whom 1,964 were treated with trastuzumab after completing neo-adjuvant or adjuvant chemotherapy that contained anthracyclines, taxanes and anthracyclines, or methotrexate (the CMF regimen). The median age of that patient cohort was 49 years; 30% patients were lymph node negative and 49% were estrogen/progesterone (ER+ / PR +) receptor negative. Adjuvant chemotherapy based on anthracyclines was used in 94% cases, with 26% treated solely with taxanes; 76% patients received adjuvant radiotherapy. The mean time between diagnosis and the use of trastuzumab was 8.4 months [6].

A slightly smaller proportion of patients in our study presented with lymph node involvement (50.28%), and received radiotherapy (66.56%). Forty-five percent of our cohort were treated with anthracyclines alone, and almost fifty percent with a combination therapy of anthracyclines and taxanes.

In the BCIRG-006 study on 3,222 patients with early HER2-positive breast cancer receiving adjuvant chemotherapy, with or without trastuzumab, the median follow-up was 65 months, with a 75% rate of five-year DFS in the AC-T arm (anthracycline,cyclophosphamide, docetaxel). In comparison, 84% of patients were disease free when the same regimen was combined with trastuzumab; aDFS rate of 81% was reported for patients receiving non-anthracycline TCH (docetaxel, carboplatin, andtrastuzumab).The estimated OS rates, seen as appropriate for the respective patient groups, were 87%, 92%, and 91%. [10]

Our study population had a slightly higher proportion of five-year survival in patients treated with anthracyclines and taxanes (91.72%) vs. treatment based exclusively on an thracyclines (90.31%). Respectively, five-year time to relapse rates were, 66.56% and 70.79%. An Italian study suggests that the poorer outcome of patients receiving adjuvant taxanes may reflect less favorable prognoses for this patient cohort, although no correlations between severity, the type of combination therapy used,and extent of disease were identified. [5]Nevertheless a *meta*-analysis based on 19 clinical studies clearly indicates that the risks of recurrence or death are reduced in patients treated with adjuvant taxane-based chemotherapy. [11-13]

The median age of the Polish study population was 56 years vs. 54 in the Italian study, and 49 years in the HERA trial. The SEER (Surveillance, Epidemiology, and End Results) database reported a median age for patients with HER2-positive breast cancer of 51-52 years [10,14,15,16,17]. Our study shows the adverse effects for OS while receiving the diagnoses at a more advanced age (>69 years) [HR 2.22, (95% CI, 1.68-2.96)]. In younger patients (<40 years), and for those aged above 69 years, there is also an increased risk of cancer relapse compared to those aged 40-69 (<40 years: HR 1.52, (95% CI, 1.28-1.81) vs. aged >70: HR 1.21, (95% CI; 1.00 - 1.46). Similar findings were reported in the Italian study, where the HR for DFS and OS for patients <40 years of age were, respectively, 1.31 (95% CI 0.96-1.76) and 1.62 (95% CI 1.02 - 2.59). For patients aged >70 these data were 1.37 (95% CI 0.97 - 1.94) and 2.59 (95% CI 1.68 - 4.0). [5]In a four-year follow-up of patients treated with adjuvant taking part in the NCCTG N 9831 and NSABP B-31 trials, the HR for OS in patients ≥ 60 years of age was 1.38 (95% CI 1.08 to 1.76) [7]. The literature confirms the unfavorable prognoses for OS or relapse in older patients, and suggests that these findings may reflect high comorbidities or the use of suboptimal drug doses or poor compliance. Breast cancer rates in patients aged <40 years are estimated to be approximately 6.6%, which is comparable to our study (7.25%). Clinical observations also confirm the unfavorable prognosis in this, younger age group. Many studies emphasize that breast cancer at a young age is a negative predictive factor that correlates with a higher risk for local recurrence (up to 35%), and cancer in the contralateral breast. In addition, one cannot exclude other drivers of less favorable prognoses for these patients such as genetic lesions and diagnoses at more advanced disease stages. [18-20]

The Polish study population demonstrated that tumor size, TNM stage (IIB, IIIA, IIIB),as well as lymph node metastases, were associated with an adverse impact on OS and the risk of relapse. Patients with no lymph node involvement demonstrated four and seven-year OS rates of 94.82% and 91.76%, respectively, with relapse rates of 74.97%, and 64.77%. In the NCCTG N 9831 and NSABP B-31 four-year follow-up studies, the 4-year relapse rate in the trastuzumab arm, without lymph node metastasis, was 86.9%, whereas for patients with lymph node involvement, these data ranged from 89.7% (1-3 nodes), to 83.5% (4-9 nodes), falling down to 73% (>10 nodes). [7]

Our study found that patients with tumors greater than 5 cm in diameter had a HRof 1.66 (95% CI 1.22 - 2.26) for OS, whilst for lesions of less than 20 mm, the OS HR was 0.46 (95% CI 0.36 - 0.59). The corresponding seven-year OS rate for the larger tumors (diameter >5 cm) was 79.65%,increasing to 92.88% for lesions of less than 2 cm; DFS rates for small versus large tumors were 64.21% and 54.37%, respectively. In the BCIRG 006 study, patients receiving AC-T with trastuzumab and TC with trastuzumab with a primary lesion of less than 1 cm manifested an 86%

DFS rate after 5 years, whilst in the group receiving AC-T these rates were 72% (AC-T trastuzumab: HR 0.36 (P < 0.03); TCH: HR 0.45 (P < 0.09)). In the case of tumors exceeding 2 cm, rates for thefive-year time to relapse were 82%, 79%, and 71%, respectively. For lesions of between 1and 2 cm there were no statistically significant differences in a 5-year DFS. [10]

Within the time frame of our study, there were recorded deaths 464 (7%). In comparison, the BCIRG 006 study reported348 (11%) deaths for a 3,222 patient cohort, whilst the NCCTG N 9831 and NSABP B-31 131 studies recorded a death rate of 6.5%, inclusive of relapse, secondary tumors, serious adverse events related to treatment, and unrelated to the disease deaths. [8,10]

We report 1194 (17%) adverse events, excluding cardiac complications, for which 6%of patients ceased their treatment (data could not be retrieved for 20% cases). Most of theevents were due to liver injury (25%) and fatigue (9.7%). However, hepatotoxicity due to anthracyclines and/or taxanesincluded into the therapeutic cocktails could not be excluded. In the HERA trial, 5.5% of patients stopped their trastuzumab treatment because of side effects. [6]

Serious cardiac complications during adjuvant trastuzumab treatment are estimated to occur for 0.5% of patients, which includes circulatory insufficiency of 0-3.9%. The most common asymptomatic condition in the Polish study population was decreased LVEF (1.81%). Circulatory insufficiency, independent of severity, occurred in approximately 0.21% of patients, with clinically significant reductions in LVEF reported in 0.21% of patients. In the trastuzumab group of the HERA trial a significant decrease in LVEF was seen in 3.6% of patients, and severe heart failure in 0.8%. [6] It should be noted that it was not possible to establish the severity of side effects or complications from our database.

Accessible data were not containing compliance information. We are in the process of the data acquisition of this critical factor for attaining and assessing efficacy.

Since 2006, adjuvant use of trastuzumab for HER2-positive breast cancer patients has been centrally financed by a dedicated drug program in Poland. Patient eligibility for this program rests with their HER-2 status, together with other specific inclusion/exclusion criteria that generates a relatively homogeneous patient group.

Conclusions

For patients with HER2-positive breast cancer who qualified for adjuvant trastuzumab (Herceptin) treatment, survival was prolonged. This subset of patients (HER2-positive) suffers a particularly aggressive form of the disease, with a poor prognosis for approximately 20%. Trastuzumab significantly extends overall survival and delays relapse, which has led to its adoption as the current standard in care for such cases.

Our study demonstrates high efficacy treatment for breast cancer patients in Poland that achieves therapeutic efficacy comparable to those reported in international clinical trials at the top health care center in the World. As such, our analysis justifies the allocation of public resources for funding this effective immunotherapy trastuzumab(Herceptin) as a permanent part of the adjuvant therapy for the qualified patients.

Declarations

Compliance with Declaration of Helsinki

All physicians interacting with the patients in this study were licensed to practice medicine in EU and sworn with Hippocratic Oath. All procedures were compliant with the Declaration of Helsinki and Good Clinical Practice Guidelines. The study was approved by the Bio-ethics Committees of the Maria Sklodowska-Curie Memorial Cancer Centre and the Institute of Oncology, Warsaw KB/9/2011).

Abbreviations

human epidermal growth factor receptor – 2 (HER2), estrogen receptor (ER), progesterone receptor (PR), immunocytochemistry (ICH), fluorescent in situ hybridization (FISH), overall survival (OS), disease-free survival (DFS).

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Conflict of interest statement

BJ has reported honoraria and reimbursement for travel expenses and honoraria for lectures from Roche and Novartis, Pfizer, Astellas.

KT has reported reimbursements for travel expenses from Novartis and honoraria for lecture from MSD.

PR have received honoraria and was a member of Advisory Board for Novartis, Roche, MSD, BMS, Bayer and Amgen, and has received honoraria for lectures from Pfizer, Roche, BMS, MSD, Novartis.

MK participated in advisory boards, attended symposia, and has received personal payments and travel grants from Roche, Amgen, Bayer, Pfizer and Novartis.

AT, AC, BKS, and MM have declared no conflicts of interest.

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