



HAL
open science

HER2 status in a population-derived breast cancer cohort: discordances during tumor progression

Ulla Wilking, Eva Karlsson, Lambert Skoog, Thomas Hatschek, Elisabet Lidbrink, Goran Elmberger, Hemming Johansson, Linda Lindström, Jonas Bergh

► **To cite this version:**

Ulla Wilking, Eva Karlsson, Lambert Skoog, Thomas Hatschek, Elisabet Lidbrink, et al.. HER2 status in a population-derived breast cancer cohort: discordances during tumor progression. *Breast Cancer Research and Treatment*, 2010, 125 (2), pp.553-561. 10.1007/s10549-010-1029-2 . hal-00555617

HAL Id: hal-00555617

<https://hal.science/hal-00555617>

Submitted on 14 Jan 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**HER2 status in a population derived breast cancer cohort – discordances
during tumor progression**

Authors: Ulla Wilking (1), Eva Karlsson (1+3), Lambert Skoog (1), Thomas Hatschek (1+2), Elisabet Lidbrink (1+2), Goran Elmberger (1), Hemming Johansson (1), Linda Lindström (1), Jonas Bergh. (1+4)

(1) Dept of Oncology-Pathology, Karolinska Institutet & Cancer Center Karolinska, Stockholm Sweden

(2) Radiumhemmet, Karolinska University Hospital, Stockholm Sweden,

(3) Dept. of Oncology, Karlstad Hospital, Karlstad Sweden.

(4) Manchester University at Paterson Institute, Christie Hospital, Manchester, UK

Key words: Breast Cancer, Biomarkers, HER2/neu,

Running title: HER2 status is not stable during tumor progression.

Corresponding Author:

Ulla Wilking c/o Charlotte Larsson

Dept. of Oncology-Pathology, Karolinska Institutet, SE170 77 Stockholm Sweden

e-mail: ulla.wilking@telia.com

Phone: +46733381117

Fax: +468343168

Abstract

Purpose

This retrospective study investigates the correlation of intra-individual HER2 status between primary breast cancers and corresponding recurrences in a population derived cohort.

Materials and methods

The REMARK criteria were used as reference. In 151 breast cancer patients primary tumors were analysed for HER2 status on histopathology sections using immunohistochemistry, IHC, confirmed by Fluorescence In Situ Hybridization (FISH) for IHC 2+ and 3+. Recurrences (loco regional and distant) were investigated by aspiration cytology, using HER2 Immunocytochemistry (ICC), or FISH (ICC in 84 patients and FISH in 102 patients).

Results

In the 151 patients, sites of recurrence were: bone/bone marrow 30%, liver 16%, local recurrence 18%, lung/pleura 10%, axillary lymph nodes 9%, skin (non-local) 7%, supra clavicular lymph nodes 5%, and other sites 7%. In 15 patients (10%) HER2 status changed, seven of 108 patients (6%) from HER2 negative to HER2 positive, and in eight of 43 (19%) from HER2 positive to HER2 negative. Intra-patient agreement in HER2 status was 76% (95% CI 64-87%), and the disagreement was 10% (95% CI 5-15%).

The multivariable Cox analysis showed a significantly increased risk of dying in the patient group with changed HER2 status compared to patients with concordant

positive HER2 status. Overall survival HR 5.47 (95% CI 2.01-14.91) and survival from relapse HR 3.22 (95% CI 1.18-8.77).

Conclusion

The unstable status for HER2 in breast cancer is clinically significant and should motivate more frequent testing of recurrences.

Introduction

Knowledge of the Human Epidermal Growth Factor Receptor 2 HER2/neu (HER2) status is required for correct management of breast cancer patients, as the HER2 status is an independent prognostic factor [1,2] and a predictor of response to treatment with the HER2 monoclonal antibody trastuzumab. In HER2 positive Metastatic Breast Cancer, MBC, response rates are 50-61% in combination with chemotherapy, resulting in significant prolonged Time to Progression, and Overall Survival [3,4]. In Early Breast Cancer, the three year results showed 12% absolute overall survival benefit for trastuzumab treatment. The hazard ratio (HR) for death after 5 years follow-up was 0.63 for the trastuzumab treated group compared to controls, and the absolute disease free survival gain at five years was 9% [5].

In the early studies, approximately 30% of primary tumors showed HER2 gene amplification. This high percentage was most probably due to the fact that data were derived from patients with high-risk disease, patients with metastatic disease, or deceased patients [2]. Recent, population based data (in a mammography screening environment) have shown that 14% of primary tumors have positive HER2 status [6]. HER2 status has generally been presumed to be stable during disease progression, even though this question has in previous studies not been properly addressed. Some studies have revealed intra-individual discordance in HER2 status between primary tumors and sites of relapse [7,8]. A large retrospective study of triple negative disease, based on 789 patients, showed intra-individual discordance regarding HER2 status of 13.6% [9]. One study showed good correlation (97%) in HER2 status between primary breast cancer and circulating tumor cells, collected at

the same time point. That study also reported that, out of 24 patients with HER2 negative primary tumors and circulating tumor cells, nine later developed HER2 gene amplification in circulating tumor cells during tumor disease progression [10]. Despite these previous observations, HER2 status from primary cancers is generally used in the management of metastatic disease, assuming stability of HER2 status during tumor progression.

HER2 status is determined by immunohistochemistry (IHC) or immunocytochemistry (ICC). There are several FDA approved HER2 kits (Dec 2008: Ventana Durham NC USA, DAKO Glostrup Denmark, Chromavision San Juan Capistrano CA USA, Applied Imaging Santa Clara CA USA, Bayer Tarrytown NY USA). The fluorescence in situ hybridization (FISH) method is considered the reference method. Today, FISH is used in selected cases only (IHC 2+ and 3+ in Sweden), mainly because it is more expensive and time consuming compared to IHC. One study demonstrated sensitivity of IHC 2-3+ of 92.6%, and a specificity of IHC 3+ of 98.8%, compared with FISH [11].

The aim of the present study was to investigate the intra-individual correlation of HER2 status between primary breast cancer tumors and corresponding recurrences.

Materials and Methods

This retrospective study was reported in accordance with the REMARK criteria [12]. Morphological verification of recurrent lesions, mostly using Fine Needle Aspirate (FNA) cytology, is routine at our department, Radiumhemmet, at the Karolinska University Hospital, as verification of all clinical and/or X-ray diagnosed breast cancer recurrences. Whenever possible, the samples are analyzed for HER2 status, in

addition to morphology, hormone receptor status and proliferation (Ki-67). For the present study we use data from The Breast Cancer Registry, established in 1976, for the Stockholm-Gotland region to identify breast cancer patients with relapse (using the individually unique 10-digit id-number given to all newborn individuals in Sweden and to immigrants) during the time period January 1 1997 through to December 31, 2007 (patients diagnosed at Radiumhemmet, The Karolinska University hospital and St Goran hospital).

Primary tumor HER2 analysis was carried out, either on the pre-operative biopsy, or the surgical specimen. FNA cytology was mainly used for tumor cell sampling of recurrent sites. HER2 status results were manually extracted from the database at the Department of Pathology/Cytology at the Karolinska University Hospital in Stockholm, and matched against the data from the Breast Cancer Registry.

IHC/ICC analyses were carried out with three antibodies from 2000 to March 2005. These were CB11 (Ventana) A485 (DAKO) and AB17 (Neomarkers, LabVision Fremont USA). From March 2005 IHC analyses were run with two antibodies CB11 (Ventana, and Novocastra Leica, Wetzal Germany from 2007) and A485 (DAKO). The internal control consisted of four breast cancer cell lines: BT474 3+, MDA453 2+, RT4 1+, and 5637 0.

The protein staining of the membrane was set at four levels, according to the manufacturers' instructions (0, 1+, 2+, 3+). IHC/ICC was positive at the 3+ protein level.

If the pathology reports stated IHC/ICC negative, this was interpreted as 0. If the report stated that there was a low protein increase, this was interpreted as 1+ and if written as “intermediate” in the pathology report, this was interpreted as 2+. If reports stated “positive/strong positive”, this was interpreted as 3+.

According to the Swedish Breast Cancer Group recommendation, FISH should be carried out if the IHC/ICC protein level was 2+ or 3+. FISH analyses were carried out using PatVision (Vysis/Abbott), including a probe to the gene and a centromere to chromosome 17. The cut-off level was set at >2.0 copies per cell, according to the manufacturer’s instructions. If the pathology report described amplification, without stating the actual number of gene copies, this was interpreted as a positive result. If IHC/ICC result was 3+, but the FISH analysis showed no gene amplification, this was interpreted as a negative result. If IHC/ICC was 0, 1+ or 2+, and the FISH analysis showed gene amplification, this was interpreted as a positive result.

The recurrence with HER2 status results was included (mainly the first recurrence).

Results are presented in three groups: unchanged HER2 negative status, unchanged HER2 positive status, and changed HER2 status (both positive to negative and negative to positive from primary tumor to recurrence).

The study was approved by the ethical committee at Karolinska Institutet, Stockholm.

Statistical methods

The Agreement between assessments was calculated as the proportion of exact agreements, or by taking chance into account, by the kappa statistics. Kappa categories according to Landis and Koch were used [13].

Follow-up started at the date of breast cancer diagnosis and continued either until the date of death occurred or until the individual was censored (end of follow-up of December 31, 2007). Univariate analyses of survival (from breast cancer diagnosis to death, or censoring and recurrence to death or censoring) in women by use of the Kaplan-Meier method.

The risk of dying was modeled by use of a multivariable proportional hazards (Cox) model, adjusting for age, year of diagnosis, estrogen and progesterone receptor status and clinical stage. The model was not adjusted for histological grade due to too many missing data (> 70%). See table 2 for missing variables. The proportional hazard assumption for the main exposure variable was assessed using Schoenfeld's test statistics [14]; no significant deviation was noted. An arbitrary level of 5% statistical significance was used, presenting 95% confidence intervals (CI).

Results

Patient related results

The study based cohort consisted of a total of 1181 breast cancers patients with recurrent disease at the Karolinska University Hospital (also diagnosed at St Goran hospital) nreported to the Regional Cancer Registry for the Stockholm-Gotland

Health Care region during the time period 1 Jan 1997 to 31 Dec 2007 (total sample).

At Radiumhemmet at the Karolinska University Hospital FNA samples which included HER2 status were analyzed in 368 patients. In 151 of these patients HER2 status was available on both primary tumor and a recurrent lesion (42 patients with primary tumor diagnosis at St Goran hospital and recurrence diagnosis at Radiumhemmet, the Karolinska University hospital).

Sites of recurrence were; bone/bone marrow 43 (30%), liver 24 (16%), local recurrence 27 (18%), lung or pleura 15 (10%), axillary lymph nodes 13 (9%), skin (not local) 10 (7%), supra clavicular lymph nodes 8 (5%), other sites 11 (7%)

The patient flow is shown in Figure 1, and Table 1 demonstrates distribution of recurrent sites, as well as HER2 status and analysis method, in primary cancers and corresponding recurrences..

Patient and tumor characteristics at first diagnosis are presented in Table 2. Twenty-four percent (24%) of patients in the group with unchanged HER2 negative status, and 6 % of the patients with a change in HER2 status had triple negative primary breast cancer.

IHC analyses were carried out on 144 primary tumors, with 86 verified by FISH. In the recurrence samples the corresponding numbers were 84 for ICC and 102 for FISH. See table 1 for HER2 status and analysis method in primary tumors and recurrences.

Overall survival (OS), from diagnosis to death/censoring date and survival from recurrence (RS) to death/censoring date was analyzed by use of Kaplan-Meier

graphs of the three groups (unchanged positive HER2 status, unchanged negative HER2 status and changed HER2 status). The analyses indicated possible differences between the two HER2 stable groups and the change group (log rank test OS 0.55 and RS 0.04), see **Figure 2 and 3**. One patient was excluded from the survival analyses due to missing relapse date and three patients were excluded due to relapse after censoring date, 2 patients in the stable negative group, 1 patient in the stable positive group and one patient in the change group. To further test this possible difference, in survival by group, a multivariate (Cox) model was used, adjusting for age and year of diagnosis, estrogen and progesterone receptor status. In the adjusted survival model women with changed HER2 status had an increased hazard ratio (HR) for dying compared to women with concordant positive HER2 status, HR 5.47 (95% CI 2.01-14.91) for OS, and HR 3.22 (95% CI 1.18-8.77) for RS **(Table 4)**.

HER 2 results

There were 43 (28%) HER positive primary tumors (IHC 3+ and/or FISH amplified), and 41 (27%) HER2 positive recurrences (IHC 3+ and/or FISH amplified).

In 15 patients (10% of the study population) HER2 had changed during the course of the disease. In seven patients, out of 108 (6% of HER2 negative primary tumors), tumors changed from HER2 negative to HER2 positive, and in eight out of 43 (19% of HER2 positive primary tumors) tumors changed from HER2 positive to HER2 negative **(Table 3)**.

The kappa correlation in HER2 between primary tumors and recurrences was 76% (95% CI 64-87%), and the discordance between primary tumors and recurrences was 10% (95% CI 5-15%).

Trastuzumab treatment

Trastuzumab has been available for treatment of HER2 positive metastatic disease since December 1998, on a named patient basis in Sweden, and was approved for this indication by European Medicines Agency in September 2000. After the adjuvant data on trastuzumab were presented in May 2005 at ASCO, the Swedish Breast Cancer group recommended adjuvant use of trastuzumab in July 2005. Trastuzumab was approved in Europe for adjuvant treatment in April 2006.

Of the 43 patients with HER2 positive primary tumors, 33 patients (77%) received treatment with trastuzumab, four patients in the adjuvant setting and 27 patients for recurrence disease (starting dates of trastuzumab not known for 2 patients).

Of the 42 patients with HER2 positive metastatic disease, 32 patients (76%) received trastuzumab treatment. All patients received trastuzumab for recurrent disease, although for 2 patients the start date for trastuzumab treatment was not known.

Of the 35 patients with unchanged HER2 positive status, 30 patients (86%) received therapy including trastuzumab, four patients in the adjuvant setting, and 23 patients for recurrent disease (starting dates not known for 3 patients receiving trastuzumab).

Of the 15 patients with change in HER2 status, five (33%) received trastuzumab.

See also **Figure 1**, Table 2 **and 3**.

Discussion

Our data show discordance in HER2 status between primary tumor and recurrent sites in 6% of primary HER2 negative tumors and 19% in primary HER2 positive cancers, which reflect a discordance of 10% for the entire study population of 151 individuals with paired samples. More important, the multivariable analysis revealed, despite a small sample size, a significantly increased risk of dying for patients with changed HER2 status, compared to patients with unchanged positive HER2 status. More study patients received adjuvant chemotherapy, compared with the total sample (80% HER2 stable group, 63% in HER2 change group, 57% in HER2 change group and 37% of total sample), indicating more aggressive primary tumors (Table 2). This is in line with a recent publication on neoadjuvant treatment with trastuzumab. In that study, the patients who retained a positive HER2 status had better prognosis compared with patients who changed HER2 status [15]. In the unchanged HER2 negative, unchanged HER2 positive, and in the changed HER2 groups, 55%, 34%, and 56% respectively, received adjuvant endocrine therapy. From these data one cannot conclude that the used adjuvant therapies drive alterations of HER2 status.

In other studies, the discordance in HER2 status between primary tumor and recurrence vary from 50% (in CTC) down to 6% [7-10, 16-18]. However the data from studies based on CTC cannot be compared with standard morphological methods. The numbers of cases included is relatively small in all studies, except for one study published by Liedtke et al. (13.6% discordance in HER2 status). One study concluded that it is unnecessary to take aspirates on metastases, as the discrepancy

between primary tumor and metastasis is below 10%, yet an 18-19% discordance rate between metastatic sites was shown in this study [18]. One small prospective study, based on 16 patients with HER2 positive primary tumors described switch to HER2 negative metastatic disease in 37% of patients [17]. There are other data on change in tumor behavior between primary tumor and recurrence in the same patient. A meta-analysis on studies investigating hormone receptor analyses, showed discrepancies between primary tumor and metastasis in the same patient [19]. Another small study showed interaction between HER2 status and estrogen receptor expression [20]. There are also data showing that HER2 status may change, related to previous hormonal treatment, even if the analyses in that study were carried out on circulating tumor cells [21]. We strongly believe that it is very important to identify these patients, as survival could be prolonged substantially for each patient and unnecessary over treatment might be avoided [3,4].

Technical factors could be one reason behind differences in HER2 results, as IHC analyses are more uncertain compared with FISH, with an increased risk of false positive results [22]. There are several studies showing that the testing technique is very important, as well as the training and skills of the laboratory staff [23,24]. For this study, 86 and 102 of the primary tumor and metastatic samples, respectively, were analyzed with FISH, and this technique has higher sensitivity and specificity compared with IHC/ICC [22]. In order to increase the precision with our IHC/ICC determinations of HER2, they were always run with two or three antibodies. Each run also included cell line controls with well-known HER2 status (BT474 ICC 3+, MDA453 ICC 2+, RT4 ICC 1+, and 5637 0), further enhancing the robustness of the ICC/IMH determinations. In addition, the IHC/ICC and FISH analyses were run

by few individuals, and the FISH slides were read by one physician, with few exceptions. The laboratory at the Karolinska University Hospital participates in the Swedish quality control program for HER2 analyses. Data from this inter laboratory collaboration show relatively stable results between laboratories (kappa correlation in 2006: IHC 0.77 and FISH 0.96) [6]. Taken together, the HER2 analyses have been carried out with optimal techniques and appropriate validation systems, which should enhance the value of the present study.

As pointed out above, IHC/ICC is a less robust method; 92.6% sensitivity and 98.8% specificity compared to FISH [11]. Thus, we also analyzed the kappa correlation for the separate methods. The results of FISH, IHC/ICC or a mix of both (either FISH or IHC/ICC in primary tumor or recurrence) was 74% (95% CI 59-90) 100% (95% CI 100-100) and 58% (95% CI 27-89), respectively. If the analyses carried out with a mix of either (FISH or IHC/ICC in primary tumor or relapse) were excluded, the kappa correlation was 81% (95% CI 69-93). The kappa correlation of the entire study population was 76% (95% CI 64-87%).

This study is retrospective, as well as most of the other studies, and the level of evidence is therefore regarded as lower compared with prospective data. Biopsy verification of recurrences was previously not standard procedure in the Stockholm-Gotland region at any other hospital. In 1999 we started to morphologically verify recurrences at Karolinska University hospital, Radiumhemmet; thereby we found single patients with non-breast cancer recurrences, and if the material contained enough cells we aimed at analyzing, HER2, estrogen- and progesterone receptors and Ki-67 (MIB-1). There were 1181 metastatic patients reported to the cancer

registry, 368 tumor samples with HER2 status results on recurrences, and there were 151 HER2 status results on primary tumors with corresponding HER2 analysis in any recurrence. Of the total 1181 patients, 669 had the primary diagnosis before 1999, and subsequent testing of selected primary tumors was performed on stored tumor samples. All of this means that there is a selection of the present study sample. The characteristics of the total sample (1021 excluding the study sample) and the study sample (151) are listed in Table 2. Data in this table show that the study population is younger (indicating less frequent morphological confirmation of relapse of older patients), has shorter time to progression, and as expected, a larger proportion of study patients who received adjuvant chemotherapy (more aggressive disease).

Our findings do not indicate that there is a change in HER2 status related to site of relapse (Table 1 and 3). Tumor cell heterogeneity has been observed in some series and could explain discordances in HER2 status between primary tumor and recurrence [25,26]. According to Wu et al primary tumors, as well as recurrences, are heterogeneous, with foci with variable morphology and marker expressions. Our group could not verify this claimed intra-tumor heterogeneity in a study of 78 invasive cancers, although in situ components complicated the picture, and one cancer demonstrated heterogeneity [27]. Based on these somewhat contradictory results, cancers are either heterogeneous for HER2 status, or different clone expansions may explain the changed findings in recurrent lesions compared with the primary cancer. A Danish study shows that ductal carcinoma in situ of comedo type has high HER2 positivity and is homogeneous for HER2. In that study the corresponding invasive cancers have lower levels of HER2 alterations, indicating that some HER2 (in situ) cancers can lose their HER2 characteristics [28]. In addition, we have in a previous

study demonstrated that new and further p53 mutations can occur in the metastatic lesions compared with the primary cancers [29]. More recently, data show that single nucleotide mutational heterogeneity can be a property of low or intermediate grade primary breast cancers and that significant evolution can occur with disease progression (only five of the 32 mutations in the metastasis were found in the primary tumor), and the HER2 gene was amplified in the metastasis, but not in the primary tumor [30].

The conclusion of our study is that a number of patients, who experience a recurrence, will not be managed correctly, if therapy is only based on characteristics of the primary tumor. The oncogene HER lacks stability during tumor progression and this should imply more frequent testing of recurrences.

Acknowledgements:

The Stockholm Regional Oncologic Centre: Jan Adolfsson, Ulla Johansson.

The Local Breast Cancer Tumor Bank: Torsten Hägersten

Karolinska Institutet: Nils Wilking.

Jonas Bergh's research group is supported by grants from:

The Swedish Cancer Society,

The Stockholm Cancer Society,

The King Gustav V Jubilee Fund,

The Swedish Research Council,

The Stockholm City Council,

Karolinska Institutet and Stockholm County Council Research Strategy Committee,

The Swedish Breast Cancer Association (BRO),

Märil and Hans Rausing and

The Karolinska Institutet Research Funds.

REFERENCES

1. Ross SJ, Fletcher JA, Linette GP. The HER-2/neu Gene and Protein in Breast Cancer 2003: Biomarker and Target of Therapy. *The Oncologist* 2003;8:307-325.
2. Slamon DJ, Clark GM, Wong SG, et al. Human Breast Cancer: correlation of relaps and survival with amplification of the HER2/neu oncogene. *Science* 1987;235(4785): 177-82.
3. Slamon, DJ, Leyland-Jones B, Shalk S, et al. Use of chemotherapy plus a monoclonal antibody against HER2/neu/NEU for metastatic breast cancer that overexpresses HER2/neu/NEU. *N Engl J Med* 2001;44(11): 783-92.
4. Marty M, Cognetti F, Maraninchi D, et al. Randomised Phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor-2 positive metastatic breast cancer administered as first line treatment: The M77001 study group. *JCO* 2001;(9): 4265-4274.
5. Slamon D, Eiermann W, Robert N, Pienowski T, Martin M, Rolski J, et al. Phase III Randomized Trial Comparing Doxorubicin and Cyclophosphamide Followed by Docetaxel (AC→T) with Doxorubicin and Cyclophosphamide Followed by Docetaxel and Trastuzumab (AC→TH) with Docetaxel, Carboplatin and

Trastuzumab (TCH) in Her2neu Positive Early Breast Cancer Patients: BCIRG 006 Study. SABCS 2009 abstract 62

6. Ryden L, Haglund M, Bendahl P et al. Reproducibility of Human Epidermal Growth Factor receptor 2 analysis in primary breast cancer - A national survey performed at pathology departments in Sweden. *Acta Oncologica* 2009; 48: 860-866.
7. MacFarlane R, Speers C, Masoudi H, et al. Molecular change in the primary breast cancer versus the relapsed/metstatic lesion from a large population based database and tissue micro array series. *Proc Am Soc Clin Oncol* 2008;44:26(abstract 1000).
8. Lower EE, Glass E, Blau B HER-2/neu expression in primary and metastatic breast cancer. *Breast Cancer Res Treat* 2009;113:301-306.
9. Liedtke C, Broglio K, Moulder S, et al. Prognostic impact of discordance between triple-receptor measurements in primary and recurrent breast cancer. *Annals of Oncology* 2009;July 12 (e-print)
10. Meng S, Tripathy D, Shete S, et al. HER-2 gene amplification can be acquired as breast cancer progresses. *PNAS* 2004;101(25):9393-9398.

11. Yaziji H, Goldstein LC, Barry TS, et al. HER2 amplification ratios in breast cancer using parallel tissue based methods. JAMA 2004;291:1972-1977.
12. McShane, L, Altman D, Sauerbrei W, et al. Reporting recommendations for tumor marker prognostic studies. J Clin Oncol. 2005 Dec 20;23(36):9067-9072.
13. Landis JR, Koch GG. An application of hierarchical kappa-type statistics in the assessment of majority agreement among multiple observers. Biometrics 1977;33(2):363-374.
14. Schoenfeld D (1982) Partial residuals for the proportional hazards regression model. Biometrika 1982; 69: 239-241
15. Mittendorf E, Wu Y, Scaltriti M, et al. Loss of HER2 Amplification Following Trastuzumab-Based Neoadjuvant Systemic Therapy and Survival Outcomes. Clin. Cancer Res 2009;15(23):7381-7388
16. Fehm T, Gebauer G, Jäger W. Clinical utility of serial serum c-erbB-2 determinations in the follow-up of breast cancer patients. Breast Cancer Res Treat. 2002 Sep;75(2):97-106.
17. Pectasides D, Gaglia A. HER2/neu status of primary breast cancer and corresponding metastatic sites in patients with advanced breast cancer treated with trastuzumab-based therapy. Anticancer Res 2006;26(1B9): 647-53.

18. Gancberg D, Di Leo A, Cardoso F, et al: Comparison of HER-2 status between primary breast cancer and corresponding distant metastatic sites. *Ann Oncol* 2002;13:1036-43.
19. Franco A, Col N. Discordance in estrogen (ER) and progesterin receptor (PR) status between primary metastatic breast cancer: a meta-analysis, in Perry M (ed): 40th Annual Meeting of the ASCO. New Orleans, LA, 2004, pp 11, abstract 539
20. Munzone E, Curigliano G, Rocca A, et al: Reverting estrogen-receptor-negative phenotype in HER-2-overexpressing advanced breast cancer patients exposed to trastuzumab plus chemotherapy. *Breast Cancer Res* 2006;8:R4.
21. Lipton A, Leitzel K, Ali SM, et al. Serum HER-2/neu conversion to positive at the time of disease progression in patients with breast carcinoma on hormone therapy. *Cancer*. 2005;104(2):257-63.
22. Pauletti, G, Danekar, Rong H, et al Assessment of methods for tissues-based detection of the HER-2/neu alteration in human breast cancer: direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol* 2000;18(21): 3651-64.
23. Roche PC, Suman VJ, Jenkins RB, et al. Concordance between local and central laboratory HER2 testing in the breast intergroup trial N9831. *J Natl Cancer Inst*

2002;94(11): 855-857.

24. Wolf AC. American Society of Clinical Oncology/College of American Pathologists Guideline <recommendations for Human Epidermal Growth Receptor 2 testing in Breast Cancer. Journal of Clinical Oncology 2007;25 (1):118-145.
25. Wu JM, Fackler MJ, Halushka MK, et al. Heterogeneity of Breast Cancer Metastases: Comparison of Therapeutic Target Expression and Promoter Methylation Between Primary Tumors and Their Multifocal Metastases. Clin Cancer Res 2008;14(7):1938-1946.
26. Cottu PH, Asselah J, Lae M, et al. Intratumoral heterogeneity of HER-2/neu expression and its consequences for the management for advanced breast cancer. Ann. Of Oncol. 2008;19:596-597.
27. Andersson J, Linderholm B, Bergh J, Elmberger G. HER-2/neu (c-erbB-2) Evaluation in Primary Breast Carcinoma by Fluorescent In Situ Hybridization and Immunohistochemistry With Special Focus on Intratumor Heterogeneity and Comparison of Invasive and In Situ Components. Appl Immunohistochem Mol Morphol 2004;Vol 12, No1:14-20
28. Ottesen G, Christensen J, Larsen J, et al. Carcinoma in situ of the breast: correlation of histopathology to immunohistochemical markers and DNA ploidy Breast Cancer Research and Treatment 2000;Vol 60, No 3: 219-226

29. Norberg T, Klaar S, Kärf G, Nordgren H, Holmberg L, Bergh J. Increased p53 mutation frequency during tumor progression--results from a breast cancer cohort. Cancer Res 2001 Nov 15;61(22):8317-21

30. Shah SP, Morin RD, Khattra J, et al. Mutational evolution in a lobular breast cancer tumor profiled at single nucleotide resolution. Nature 2009; Vol 461 8 Oct: 809-913.

Table 1 Distribution of recurrent sites, HER2 status in primary tumours, recurrences and analysis method.

Recurrent sites	No of patients (%)	HER2 status in primary tumours <i>IHC/FISH</i> ^{1) 3)}		HER2 status in recurrences <i>ICC/FISH</i> ^{2) 3)}	
		Negative	Positive	Negative	Positive
Local	27 (18)	17 <i>8/9</i> ¹⁾	10 <i>2/8</i> ¹⁾	20 <i>8/12</i> ²⁾	7 <i>0/7</i> ²⁾
Axillary lymph nodes	13 (9)	7 <i>4/3</i> ¹⁾	6 <i>1/5</i> ¹⁾	7 <i>1/6</i> ²⁾	6 <i>0/6</i> ²⁾

Supra clavicular lymph nodes	8 (5)	7 3 / 4 ¹⁾	1 0 / 1 ¹⁾	7 0 / 7 ²⁾	1 0 / 1 ²⁾
Skin (non-local)	10 (7)	8 5 / 3 ¹⁾	2 0 / 2 ¹⁾	8 4 / 4 ²⁾	2 1 / 1 ²⁾
Liver	24 (16)	16 8 / 8 ¹⁾	8 0 / 8 ¹⁾	16 0 / 12 ²⁾	8 0 / 8 ²⁾
Bone or Bone marrow	45 (30)	38 18 / 20 ¹⁾	7 0 / 7 ¹⁾	36 15 / 21 ²⁾	9 2 / 7 ²⁾
Lung or pleura	15 (10)	8 7 / 1 ¹⁾	7 3 / 4 ¹⁾	9 6 / 3 ²⁾	6 2 / 4 ²⁾
CNS	3 (2)	1 1 / 0 ¹⁾	2 0 / 2 ¹⁾	1 1 / 0 ²⁾	2 0 / 2 ²⁾
GI	3 (2)	3 3 / 0 ¹⁾	0	3 3 / 0 ²⁾	0
Other	3 (2)	3 2 / 1 ¹⁾	0	3 2 / 1 ²⁾	0
Total	151 (100)	108 59 / 49 ¹⁾	43 6 / 37 ¹⁾	110 40 / 66 ²⁾	41 5 / 36 ²⁾

1) HER2 status based on Immuno Histochemistry, IHC or Fluorescence In Situ Hybridization, FISH (IHC/FISH)

2) HER2 status based on Immuno Cyto Chemistry, ICC or FISH (ICC/FISH)

3) If HER2 status was based on FISH, a previous IHC may have been done, or direct FISH was carried out.

Table 2 Patient and tumor characteristics at diagnosis.

Patient group (total in category)	Median age (range)	Median time to recurrence in months (range)	Clinical stage % 1)				Histopathologic grade (Elston) 2)		ER/PR positive % 3)	Adjuva Chem %
			1	2	3	4	2	3		
HER2 Negative stable (101)	53 (28-85)	28.7 (0-152)	28	45	20	1	8	14	34	57
HER2 Positive stable (35)	51 (28-83)	22 (0-65)	17	60	17	0	3	8	14	80
HER2 change (15)	56 (30-78)	22.3 (7.9-97.9)	25	50	25	0	1	4	25	63
Total sample (excluding study sample) (1021)	56 (23-97)	64.3	44	37	14	1	-	-	34	37

1) 5 patients in the HER2 neg stable group, 2 patients in the HER2 pos stable group, 0 patients in the HER2 change group, respectively had no clinical stage defined.

- 2) 81 patients in the HER2 neg stable group, 24 patients in the HER2 pos stable group, 9 patients in the HER2 change group, respectively had no Elston defined. No patient had Elston 1.
- 3) 28 patients in the HER2 neg stable group, 6 patients in the HER2 pos stable group, 6 patients in the HER2 change group, respectively had no ER/PR result available.

Table 3 Shift in HER2 status from primary tumours to recurrences.

Recurrent sites	No	Primary tumours	Recurrences
Local	27	17 - ¹⁾ 10 + ²⁾ 0 ³⁾	17 - 7 + 3 - (33%) ⁴⁾
Axillary lymph nodes	13	7 - 6 + 4 ³⁾	6 - 1+ (14%) ⁵⁾ 5 + 1 - (17%) ⁴⁾ 0 ³⁾
Supra clavicular lymph nodes	8	7 - 1 + 0 ³⁾	7 - 1 +
Skin (non-local)	10	8 - 2 + 4 ³⁾	8 - 2 +
Liver	24	16 - 8+ 2 ³⁾	13 - 3 + (19%) ⁵⁾ 6 + 2 - (25%) ⁴⁾ 0 ³⁾
Bone or Bone Marrow	45	38 - 7 + 0 ³⁾	35 - 3 + (8%) ⁵⁾ 6 + 1 - (14%) ⁴⁾
Lung or pleura	15	8 - 7+ 1 ³⁾	8 - 6 + 1 - (14%) ⁴⁾ 0 ³⁾
CNS	3	1 - 2 + 0 ³⁾	1 - 2 +
GI	3	3 -	3 -
	3		

Other		3 -	→	3 -

1) Number of patients with HER2 negative primary tumour in the specific recurrence group

2) Number of patients with HER2 positive primary tumour in the specific recurrence group

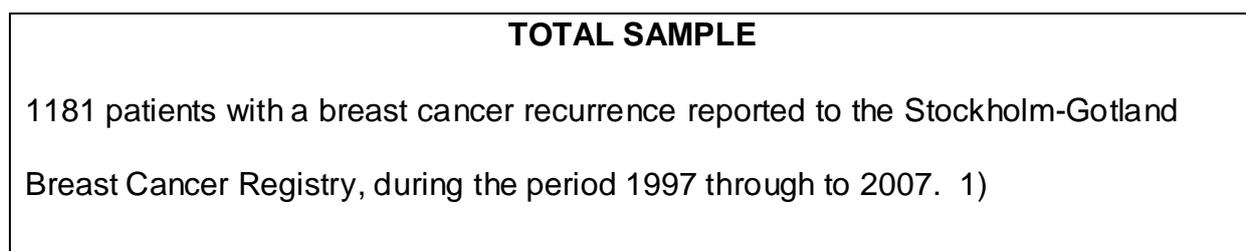
- 3) Number of patients with HER2 positive primary tumour receiving adjuvant trastuzumab
- 4) Percentage changes from primary tumour to recurrence, in the group of patients with HER2 positive primary tumours.
- 5) Percentage changes from primary tumour to recurrence, in the group of patients with HER2 negative primary tumours.

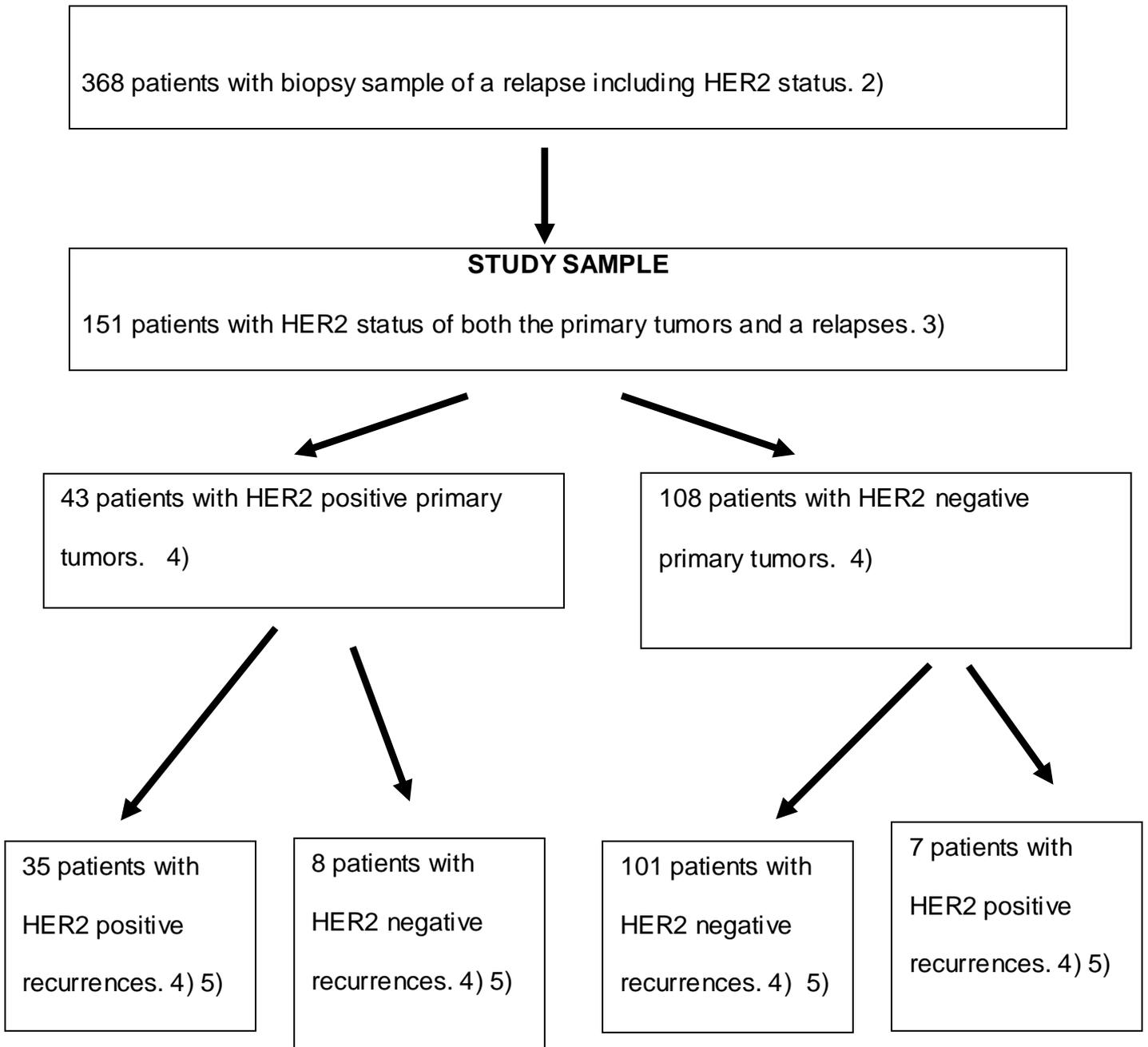
Table 4 Risk of death in breast cancer patients related to intra-individual HER2 status in primary tumour

Intra-individual primary and relapse tumour	Patients		Deaths	Overall survival from breast cancer diagnosis to death or censoring
	Number	Percentage	Overall	Adjusted HR (95% CI)
Her-2/neu status				
Positive	34	23.1	18	1.0 reference
Discordant	14	9.5	10	5.47 (2.01-14.1)
Negative	99	67.3	71	1.85 (0.99-3.44)

*Adjusted for age and year of diagnosis, estrogen receptor status, progesterone receptor status and stage

Figure 1 Patient Flow Chart





1) All Metastatic Breast Cancer patients, reported to the local cancer registry, which were treated at Radiumhemmet, Karolinska University Hospital during the time period 1997-2007. Patients may have had the primary cancer diagnosed at Radiumhemmet Karolinska University Hospital or at St Goran Hospital (excluding patients with metastatic disease before 1997).

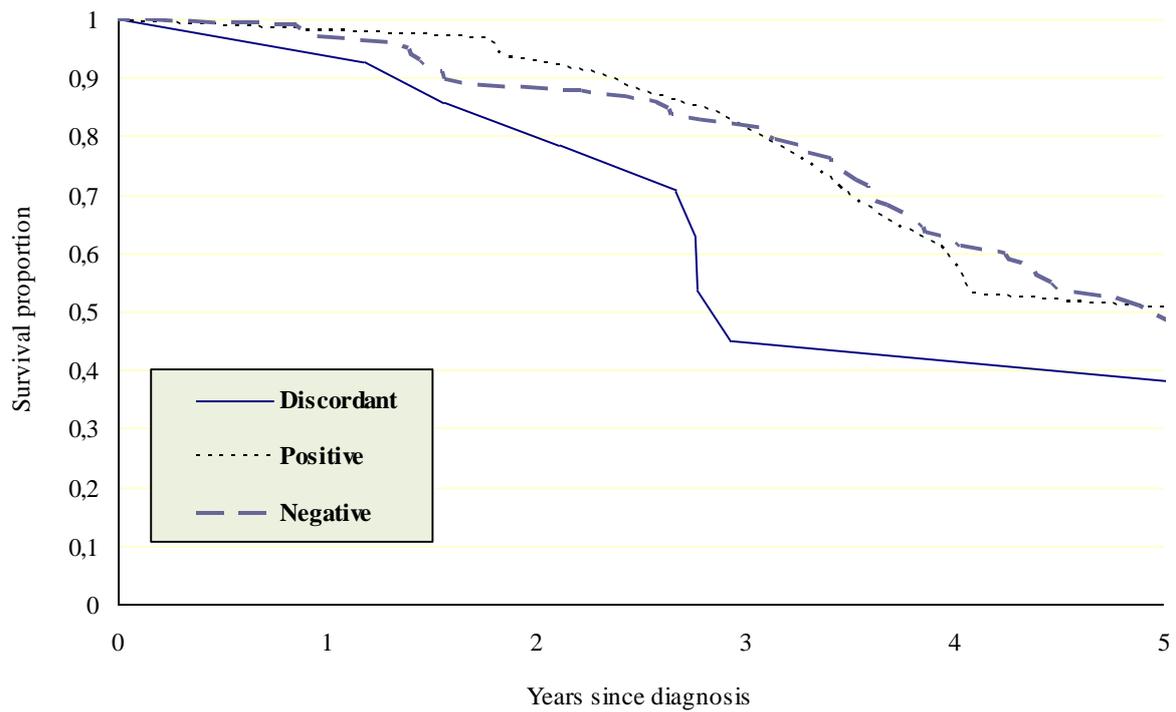
2) Since year 1999/2000 we have aimed at taking a biopsy of all patients with a recurrence to verify diagnosis and to analyze prognostic/predictive factors. In 541 patients a biopsy (mostly FNA, Fine Needle Aspirate) was taken of a clinical or radiological suspected recurrence (~50% of the total sample). During the first years HER2 was not always tested, as trastuzumab was unavailable, or that too few cells were available for analysis, or that the referring clinician did not request a HER2 test.

3) 669 of the patients had the primary tumor before 1999. HER2 analysis was not introduced until 1999/2000 and trastuzumab was approved in Q3 2000 (on a named patient basis during 1999-2000). In 29 of the 151 patients the HER2 status was therefore analyzed on banked tumor samples. Thus, the fewer number of HER2 analyses in primary tumors. The remaining 122 patients had the primary tumor in 1999 or later.

4) See table 1 for HER2 status in primary tumors, analysis method and recurrent sites.

5) See table 3 for changes in HER2 status between primary tumors and relapses.

Figure 2 Overall survival (OS) in breast cancer patients with stable and changed HER2 status.

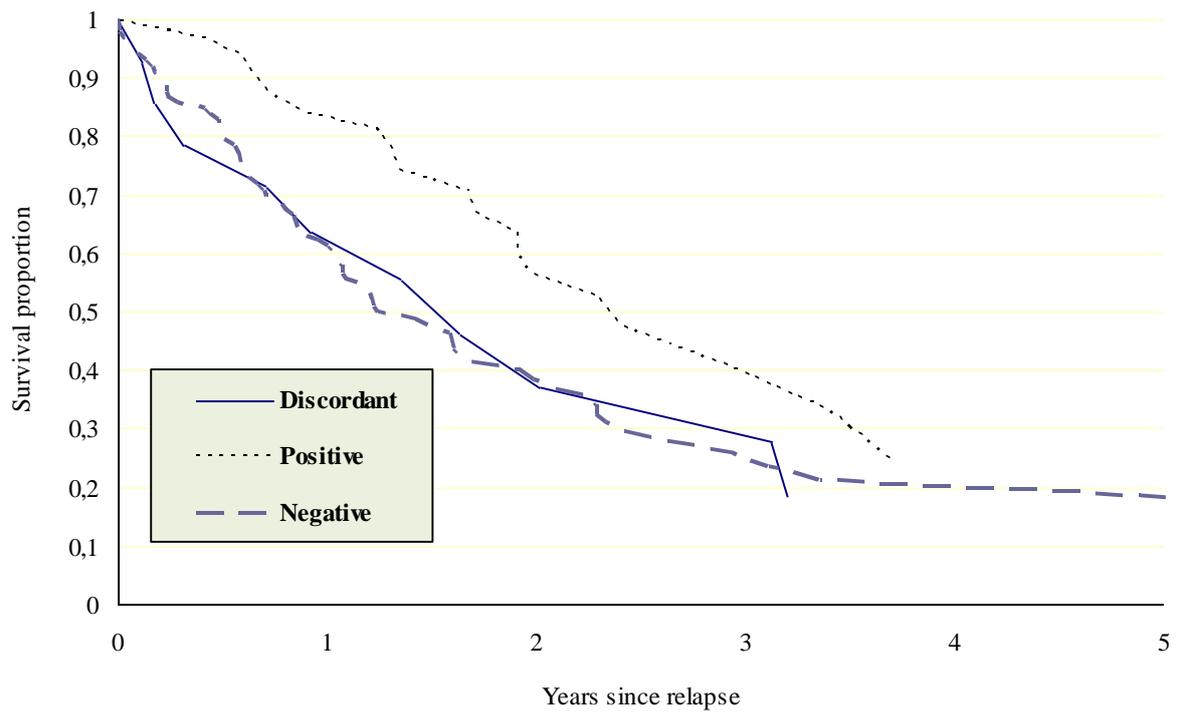


Negative = Patients with stable HER2 negative primary tumour and recurrence

Positive = Patients with stable HER2 positive primary tumour and recurrence

Discordant = Patients with a change in HER2 status between primary tumour and recurrence

Figure 3 Survival from relapse to death or censoring date (RS) in breast cancer patients with stable and changed HER2 status.



Negative = Patients with stable HER2 negative primary tumour and recurrence

Positive = Patients with stable HER2 positive primary tumour and recurrence

Discordant = Patients with a change in HER2 status between primary tumour and recurrence